ASPECTS OF THE PHARMACOTHERAPY OF AIRWAYS DISEASE

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Declaration

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University or College.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of this thesis.

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CONTENTS

SUMMARY

Asthma morbidity and mortality have been increasing in most industrialised countries over the last two decades despite anti-asthmatic medication sales at an all time high. Drug therapy remains the mainstay of treatment in asthma yet there have been concerns that modern drug therapy may be contributing to this increase in morbidity and mortality. The research presented in this thesis sought to examine several issues related to current asthma pharmacotherapy.

In recent years, asthma management guidelines have emphasised the earlier introduction of inhaled corticosteroids and less reliance on β_2-agonists. The prescribing of anti-asthmatic drugs within Tasmania was examined from April 1991 to April 1994 using computerised dispensing records from nearly one-third of all the community pharmacies within the State. Trends in prescribing were compared with national data and regional State differences were examined. It was found that drug utilisation, both nationally and within Tasmania, appeared to be changing in line with current management guidelines with increases in inhaled corticosteroid and sodium cromoglycate usage while β_2-agonists usage remained fairly stable. There was a marked decrease in the ratio of β_2-agonists:inhaled corticosteroids dispensed over the period of the study.

Nebulised ipratropium bromide (an anti-cholinergic drug) is often combined with nebulised β-agonists in the treatment of asthma and chronic obstructive airways disease. To reduce the time that patients spend inhaling nebulised drug, ipratropium bromide is often mixed with β-agonists immediately prior to administration but is sometimes bulk mixed and stored for several days before use. A reversed-phase ion-pair assay using UV detection was developed to study the stability of these bulk mixed nebuliser solutions. The stability of an admixture of proprietary ipratropium bromide and salbutamol nebuliser solution (1:1, v/v) was then examined for 5 days under different storage conditions. It was found that admixtures of ipratropium bromide and salbutamol nebuliser retain greater than 90% of their initial concentrations if stored at or below 22°C for periods of up to 5 days.

Finally, an assay using solid-phase extraction was developed to measure urinary levels of salbutamol in a relatively large group of asthmatic patients using inhaled therapy. Salbutamol levels in 'spot' urine samples were investigated as a potential indicator of over-use. Median levels of unchanged drug were 378 ng/ml (range 0-34.4 µg/ml) and 2.55 µg/ml (range 0-49.8 µg/ml) in community and hospital patients respectively. Even when conceding the limitations of 'spot' urine sampling there were large inter-patient differences in levels corrected for dosage, probably due to differences in technique of administration and pharmacokinetic variability. There were also indications of possible low level saturation of metabolism which may have clinical implications.

ACKNOWLEDGMENTS

CHAPTER 1

General Introduction

Over recent years, asthma morbidity and mortality has been increasing in most industrialised countries despite effective drugs for control of asthma symptoms (Musk et al, 1987; Jackson et al, 1988). Over the same period drug sales have also increased (Jenkins et al, 1988, Klaukka et al, 1991). There have been suggestions that modern drugs used in the treatment of asthma may actually be causing the increase in asthma morbidity and mortality (Barnes and Chung, 1992). Because drug use has been closely associated with rising asthma prevalence, the objective of this study was to examine several current issues in the pharmacotherapy of asthma and related conditions such as chronic obstructive airways disease and emphysema.

Asthma is now regarded primarily as an inflammatory disease rather than simply bronchoconstriction (Barnes, 1989). This has led to changes in the management guidelines of asthma with greater emphasis on earlier introduction of inhaled corticosteroids (Anonymous, 1992; Tse and Bridges-Webb, 1993). In addition, there have been concerns that over-reliance on β_2-agonist therapy could be linked to increasing asthma morbidity and mortality (Sears et al, 1990; van Schayck, 1991).

The cost of asthma to the Australian community has been estimated at up to $720 million per annum (NAC report, 1992). The National Asthma Campaign (NAC) was introduced in 1990 in response to rising morbidity and mortality with the goals of improving diagnosis and management and reducing preventable deaths from asthma.

The impact of the changing asthma management guidelines and the NAC on prescribing of anti-asthmatic drugs and morbidity and mortality within Tasmania was examined with the assistance of computerised pharmacy dispensing records. The background pathology, pharmacotherapy, current controversies and treatment guidelines in asthma are discussed in Chapter 2. Chapter 3 describes the relatively new discipline of pharmacoepidemiology, the study of drug use within a population (Hartzema, 1992; Henry, 1988). Pharmacoepidemiology, as the name suggests, is essentially the combination of pharmacology and epidemiology. Pharmacoepidemiology is used in Chapter 4 to assess the impact of the NAC on the prescribing of anti-asthmatic drugs within the Tasmanian population. Tasmanian prescribing trends were also compared with National prescribing trends.

For patients unable to use metered-dose inhalers (MDIs), inhalation of nebulised respirator solutions is an integral component of the modern treatment of airways disease (Horsley, 1988; Johnson, 1989). Patients with severe asthma or chronic obstructive airways disease are often administered β-agonist drugs in combination with ipratropium bromide, a quaternary anti-cholinergic drug (O'Driscoll et al, 1989). It has been suggested that by mixing the drugs before administration both nursing time and time spent by the patient inhaling drug is reduced (Fry and Williamson, 1991). In addition it has also been suggested that patient compliance may also be increased because of the reduced administration time (Roberts and Rossi, 1993).

This mixing of drugs, however, raises issues of compatibility. Compatibility issues between salbutamol (a commonly used β_2-agonist) and ipratropium bromide have been addressed over short storage periods (Iacono et al, 1987). The compatibility of bulk mixed drugs over a period of days, however, has not been addressed.

A single stability-indicating assay designed to measure concentrations of several common β_2-agonists and ipratropium bromide in nebuliser solution was developed in Chapter 5. This assay was modified slightly in Chapter 6 to determine the stability of salbutamol and ipratropium bromide, such as in bulk mixed nebuliser solutions, over a period of days.

Despite its wide spread use over many years there is surprisingly little published information on the pharmacokinetics of salbutamol, especially after inhalation which is mainly due to difficulties in detection. The pharmacokinetics of salbutamol, with emphasis on inhalation therapy, are examined in Chapter 7.

Following inhalation salbutamol is excreted as free drug and metabolite in urine. (Morgan et al, 1986). Levels of drug and metabolite in urine are typically much higher than plasma due to urine acting as a reservoir for excreted drug. An assay using solid-phase extraction and UV detection was developed in Chapter 8 to allow the determination of salbutamol and indirect determination of the major metabolite in urine following administration of salbutamol by inhalation, either via MDI (metered dose inhaler), DPI (dry powder inhaler), or nebuliser. This assay was used to examine urine levels of drug in a relatively large sample of community and hospital patients in Chapter 9. The possibility of using single 'spot' urine levels as an indicator of possible over-use was examined as well as correlation with reported dosage. Limited pharmacokinetic data were also examined from the urine samples.

The implications of the research presented in this thesis on the pharmacotherapy of airways disease are summarised in Chapter 10.

CHAPTER 2

Asthma Background

2.1 SUMMARY

It is now generally regarded that asthma is an inflammatory disease rather than simply bronchoconstriction. This has changed the rationale for therapy with less reliance on bronchodilator medication and more emphasis on anti-inflammatory medication. The pathophysiology of asthma, the rising morbidity and mortality of asthma, strategies for reducing the morbidity and mortality, asthma pharmacotherapy, and the controversies in therapy are outlined.

2.2 ASTHMA AS AN INFLAMMATORY DISEASE

Asthma is a chronic illness associated with widespread narrowing of the tracheobronchial tree and characterised by symptoms of episodic coughing, dyspnoea, wheezing and chest tightness, alone or in combination (McFadden and Gilbert, 1992). Asthma was once considered a disease associated with bronchoconstrictor mechanisms and possible airway smooth muscle abnormalities. However, in recent years it has been recognised that chronic asthma involves a characteristic inflammatory response in the airways (Barnes, 1989; Alpers 1991). It is now apparent that airways inflammation is present even in patients with mild asthma (Laitinen et al, 1985; Beasley et al, 1989).

Histological changes in asthma include shedding of the airway epithelium, interstitial oedema, and sub-basement-membrane collagen deposition (Jeffery et al, 1992). The unique pathological features of asthma are the lack of neutrophils and the large numbers of eosinophils in the exudative phase, the sparsity of phagocytic-cell infiltrates and the absence of granulation tissue and fibrosis (McFadden and Gilbert, 1992). It has been suggested that asthma can be viewed as a chronic eosinophilic bronchitis (Barnes, 1989). The inflammation in asthma is different to that inflammation seen in bronchiolitis in smokers or during viral infections as the

airways are typically free of fibrosis, apart from collagen deposition below the basement membrane (McFadden and Gilbert, 1992).

The root cause of airways inflammation in asthma still remains uncertain (McFadden and Gilbert, 1992; Frew and Holgate, 1993). The development of bronchial inflammation is complex involving eosinophils, mast cells, platelets and many different inflammatory mediators (Paterson et al, 1995). In addition, bronchial hyperresponsiveness, a characteristic of asthma (Barnes, 1989), is an exaggerated bronchoconstrictor response to many different stimuli and is related to both the extent of inflammation in the airways (Chung, 1986; O'Byrne et al, 1987a) and the severity of the disease (Hargreave et al, 1981; Britton et al, 1988).

In patients with active disease, acute exacerbations can occur on exposure to non-specific stimuli such as physical exertion, cold air, and respiratory irritants (McFadden and Gilbert, 1992). The role of allergens in asthma is still not clear. It has been suggested that virtually all patients with asthma have an atopic component (Burrows et al, 1989; Sears et al, 1991). However, other estimates suggest that as many as one third of all patients with asthma are not atopic (Montogomery Smith, 1988) and that gene mutations may influence underlying asthma, even in the absence of atopy (van Herwerden et al, 1995). In addition, many patients with allergic rhinitis and well defined allergen sensitivity have no clinical evidence of asthma (Britton, 1992).

The bronchospastic response to allergen inflammation typically consists of an early phase, due to the direct effect of mediators on bronchial smooth muscle, and late phase due to mucosal oedema and cellular infiltration 3 to 12 hours after inhalation of the allergen (Frew and Holgate, 1993). Recent research has shown that the CD4+ T-lymphocyte is important in the initiation of asthma and may be activated by exposure to antigens or other agents to release various cytokines

including interleukin 3, 4 and 5 (Kay, 1992; Paterson et al, 1995). The interleukin 3, 4 and 5 cytokines activate B-lymphocytes and the recruitment of a mixed leucocyte infiltrate consisting of inflammatory cells such as eosinophils, macrophages, basophils and neutrophils (Beasley et al, 1989; Paterson et al, 1995). This is shown in Figure 2.1.

The activated B-lymphocytes produce IgE (Kay, 1992). IgE can cause inflammation of the airways by binding with mast cells resulting in the release of mediators (associated with the immediate or early phase asthmatic response due to their direct effect on bronchial smooth muscle) and the recruitment of inflammatory cells typically associated with the late phase response (Djukanovic et al, 1990). These mediators include leukotrienes, histamine, prostaglandins, prostanoids, neuropeptides, endothelin-1, platelet-activating factor, eosinophil-derived cytotoxic peptides and neutrophil- and eosinophil-induced chemotactic factors of anaphylaxis (Wasserman, 1983; Kaliner, 1989; Drazen and Austen, 1987; Paterson et al, 1995). These mediators are responsible for constriction of bronchial smooth muscle, mucosal oedema, mucus production as well as interference in removal of mucus (Wasserman, 1983; Kaliner, 1989; Drazen and Austen, 1987) and may enhance both the immediate and late onset phases via a positive feed back loop (McFadden and Gilbert, 1992). In addition, allergen specific T-cells may be found in allergic persons (Kay, 1992).

The role of mast cells remains unclear. While it is thought that mast cells may be important in the acute bronchoconstrictor response to an antigen (early phase) and possibly to exercise, the role of the mast cell in chronic asthma, particularly the late response and bronchial hyperresponsiveness, remains less certain (Barnes, 1989).

T-lymphocyte
activated by antigen

Cytokines released by T cells

Interleukin 3, 4, 5

B-lymphocyte

IgE

Inflammatory cell activation

Eosinophils, macrophages, basophils, neutrophils

Mast cell

Inflammation

Mediators

Leukotriene C₄
Histamine
Prostaglandin D₂

Mediators

Histamine
Leukotrienes
Prostanoids
Neuropeptides
Endothelin-1
Platelet-activating factor
Eosinophil-derived cytotoxic peptides

Disease

Further inflammation
Epithelial destruction
Mucosal damage and remodelling
Hyperplasia of bronchial smooth muscle
Mucous gland hyperplasia and hypersecretion
Microvascular leakage and oedema
Bronchial hyperreactivity

Bronchoconstriction,
oedema, secretion

Symptoms

Wheeze, dyspnoea,
cough, sputum

Figure 2:1   *Outline of the inflammatory pathophysiology of asthma. Adapted from Paterson et al, 1995.*

In addition to the inflammatory mediators, neurogenic mechanisms have been implicated in asthma (Barnes, 1986a). Non-cholinergic non-adrenergic sensory nerves may release neuropeptides by an axon reflex that may have an important role in asthma (Barnes, 1989; Barnes, 1986b). These neuropeptides include substance P (a potent inducer of microvascular permeability and mucus secretion), neurominin A (a potent bronchoconstrictor) and calcitonin gene-related peptide (an effective and long-lasting dilator of bronchial vessels; Barnes, 1989). The roles of these various mediators in the pathogenesis of asthma will become clearer as more selective mediator antagonists are developed (Barnes, 1989).

2.3 INCREASING ASTHMA MORBIDITY AND MORTALITY AND THE COST TO THE COMMUNITY

There is ample evidence that over the last two decades asthma morbidity and mortality have increased in most industrialised countries (Sly, 1984; Benatar, 1986; Burney, 1986; Evans et al, 1987; Fleming and Crombie, 1987; Barnes, 1988; Jackson et al, 1988; Beasley et al, 1990; Weiss and Wagener, 1990). This is despite large increases in anti-asthmatic medication sales over the same period (Keating et al, 1984; Hay and Higenbottam, 1987; Klaukka et al, 1991).

The same pattern has occurred in Australia (Musk et al, 1987; Jenkins et al, 1988). Australian mortality rates from asthma are higher than England, Wales, Canada and the United States, although lower than New Zealand (Woolcock, 1986). Annual asthma deaths in Australia have risen from 2.3 deaths/100,000 population in 1978 more than doubling to 5.7 deaths/100,000 population in 1989 (derived from data provided by the Australian Bureau of Statistics). There is, however, evidence that the mortality rate from asthma has begun to decline from the 1989 peak shown in Figure 2.2 (source: Australian Bureau of Statistics).
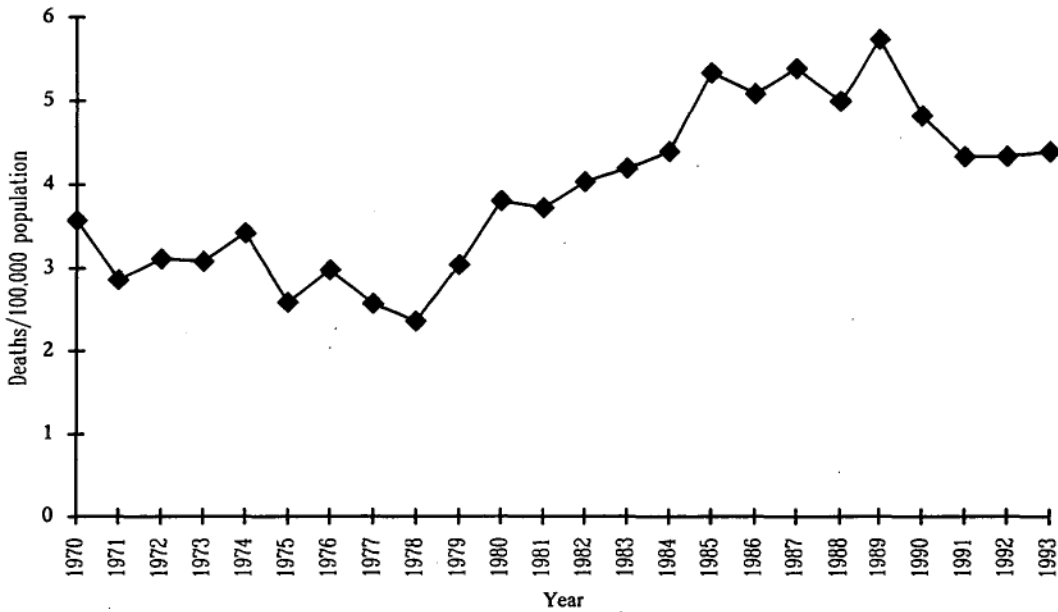
Figure 2.2   *Asthma mortality in Australia represented as asthma deaths per 100,000 population. Source: Australian Bureau of Statistics.*

An estimated 1.4 million Australians (9% of the population) reported experiencing asthma as either a recent or long-term condition in 1989-90 (National Health Survey 1989-90). The proportion of the population experiencing asthma as a long-term condition rose from 2% in 1977-78 to 8% in 1989-90 with respiratory conditions, including the common cold, accounting for 24% of all days lost from work due to illness or injury (National Health Survey 1989-90). The National Health Survey 1989-90 also found that asthma was more prevalent in younger people, with 12% of persons aged less than 25 reporting asthma. The asthma prevalence in children has been noted to be even higher at around 17% (Bauman et al, 1992a).

Given the high morbidity and mortality due to asthma it not surprising that the cost to the community is high. It has been estimated that in 1991, the cost to the Australian community was between $585 to $720 million per annum (NAC report, 1992). This estimate consisted of $320 million in direct medical related

costs such as pharmaceuticals, medical consultations and hospitalisations, and between $265 to $400 million in indirect costs due to lost productivity such as absenteeism and reduced effectiveness at work (NAC report, 1992). Of additional interest is the fact that a person with poorly controlled asthma will incur greater costs than an asthmatic who is well controlled. Based on computer modelling, the more asthmatics who have their disease well controlled, the lower the total cost to the community (NAC report, 1992).

It has been estimated that in Australia in 1990-1991, 4.0% of all encounters to a general practitioner resulted in a prescription for a bronchodilator, preventive agent (such as an inhaled corticosteroid or sodium cromoglycate) or methyl xanthine. Of all prescriptions written by general practitioners, 8.4% were for a bronchodilator, preventive agent or methyl xanthine (Bridges-Webb et al, 1992).

In 1992, the top 10 drug list ranked by DDDs/1000 population/day in Australia included salbutamol with the highest level of community use of all prescription drugs (30.3 DDDs/1000 population/day), beclomethasone dipropionate (only inhaled preparations) in third position (12.3 DDDs/1000 population/day) and budesonide in sixth position (10.4 DDDs/1000 population/day; Anonymous, 1994). The 1992 top 10 drugs by prescription counts included salbutamol with 4,884,371 prescriptions in second position behind amoxycillin (Anonymous, 1994). The 1992 top 10 drug list ranked in order of cost to the government in Australian dollars included salbutamol in fifth position ($45 million), beclomethasone inhaled preparations in seventh position ($32 million), ipratropium bromide in eighth position ($30 million) and budesonide in ninth position ($28 million; Anonymous, 1994).

2.4   STRATEGIES FOR REDUCING MORBIDITY AND MORTALITY IN AUSTRALIA

Recognising the increase in asthma morbidity and mortality, an NHMRC working party (formed in 1987) on asthma-associated deaths released a report in June 1988 with strategies for reducing morbidity and mortality in Australia (NHMRC report, 1988). This report made the recommendations shown in Table 2.1.

Following an asthma awareness campaign in 1988, The National Asthma Campaign, a joint initiative of the Thoracic Society of Australia and New Zealand, the Royal Australian College of General Practitioners, the Pharmaceutical Society of Australia and the State and Territory Asthma Foundations, was established in 1990 to combat the increasing morbidity and mortality from asthma in Australia. The 3 year goals of the National Asthma Campaign were that: (i) most people with asthma will be correctly diagnosed, (ii) most people with asthma will be using a recognised asthma management plan to help them manage their asthma, and (iii) there will be a decline in preventable deaths from asthma.

The NAC and the asthma management plan were based on a publication of the Thoracic Society of Australia and New Zealand (Woolcock et al, 1989) which described a six point approach to asthma management as shown in Table 2.2. The plan provided guidelines for all doctors who treat patients with the hope that improved treatment will reduce morbidity and mortality from asthma in the future. Further guidelines have been published (Anonymous, 1992; Tse and Bridges-Webb, 1993) and it would seem sensible that new guidelines are published regularly in the future as understanding of the disease and therapeutic implications become clearer.

Table 2.1 *Strategies for reducing morbidity and mortality from asthma in Australia. Based on NHMRC report (1988).*

Asthma in Australia

Strategies for reducing morbidity and mortality

(NHMRC working party report, June 1988)

(i) The immediate distribution of information about:
- currently agreed criteria for assessment of severity and management of patients with asthma;
- consumer (patient and parent) responsibility and roles in shared management of asthma as a chronic illness.

(ii) The implementation of community based education programs through appropriate Commonwealth and State mechanisms.

(iii) Having regard to the disincentives in current fee schedules, the Health Insurance Commission be requested to explore methods of facilitating education of patients with asthma by medical practitioners.

(iv) The RACGP, through its Family Medicine Program, be asked to consider making education about asthma a priority for doctors.

(v) Fast lane procedures for admission to emergency care at hospitals be introduced.

(vi) The urgent establishment of a National Asthma Mortality register with the capacity to conduct appropriate investigations.

(vii) Research, aimed at providing better understanding of the causes of asthma morbidity and mortality and their control, be made a priority area within the NHMRC grants scheme.

(viii) Research be directed at defining the environmental factors presumed to act either as inducers of the disease or as triggers of attacks, in the Australian population.

Table 2.2   *Asthma management plan based on Woolcock et al, 1989.*

1. Assess severity of the asthma	Assess when stable and not during an acute attack. Apply guidelines given in plan.
2. Achieve "best" lung function	Use peak flow meter or spirometer and know "predicted" values; treat with bronchodilator and corticosteroid drugs by mouth until "best" value is found.
3. Maintain "best" lung function by:	
Home monitoring	Ensure patient records peak expiratory flow readings and continues to maintain "best" value.
Therapy	Prescribe drugs including aerosol corticosteroid drugs, bronchodilator agents, cromoglycate and theophylline.
Avoiding trigger and aggravating factors	Investigate and minimise (where possible) provoking stimuli and aggravating factors.
4. Write an action plan	Write and discuss a plan, based on peak expiratory flow readings, to increase drug dosages and to gain rapid access to medical care.
5. Educate the patient	Patient and family should understand the nature of the disease, relevant triggers, the aims of treatment, use of drugs and how to implement the action plan.
6. Review regularly	Continue to care for the patient even when the asthma becomes mild.

Table 2.3   *Anti-asthmatic drugs and some common adverse effects.*

Drug class	Common adverse effects
Bronchodilators Salbutamol Terbutaline	Tremor, tachycardia, hypotension, nausea, hypokalaemia, sweating, muscle cramps, headaches, CNS disturbances, paradoxical bronchoconstriction, oral irritation.
Inhaled corticosteroids beclomethasone dipropionate budesonide	Oropharyngeal candidiasis, hoarseness, throat irritation, paradoxical bronchospasm.
Methyl xanthines Theophylline Aminophylline Choline theophyllinate	GI upset, CNS stimulation, tachycardia, palpitations, headache, insomnia, tremor, arrhythmias, convulsions.
Anti-cholinergics Ipatropium bromide	Urinary retention, acute angle glaucoma, dry mouth, throat irritation, cough, visual accommodation disturbances.
Anti-allergics Sodium cromoglycate	Mild throat irritation, cough, transient bronchospasm.

2.5   PHARMACOTHERAPY OF ASTHMA

The pharmacotherapy of asthma relies mainly on 5 classes of drugs, bronchodilators, inhaled corticosteroids, methyl xanthines, ipratropium bromide and sodium cromoglycate. Table 2.3 shows the commonly used anti-asthmatic drugs in Australia, together with possible adverse effects.

2.5.1 β_2-Agonists

In the past, asthma therapy has relied on achieving bronchodilation. Nebulised adrenaline became available in the 1930s (Gandevia, 1975), and was followed by the development of non-selective β-agonists such as isoprenaline in the 1940s (Thompson and Watkins, 1994), and the development of β_2-agonists during the 1960s (Frew and Holgate, 1993). Stimulation of β_2-receptors in the airway smooth muscle in humans results in the activation of adenylate cyclase and an increase in intracellular levels of cAMP. This leads to activation of protein kinase A which inhibits the phosphorylation of myosin and lowers the intracellular ionic calcium

concentration, resulting in smooth muscle relaxation (Barnes, 1989). Airway muscle in humans has only β_2-receptors (Carstairs et al, 1985), hence stimulation of these receptors results in the smooth muscle relaxation.

Mast cells also have β_2-receptors (Butchers et al, 1980), and it has been suggested that β_2-agonists also inhibit the release of mediators from mast cells in the airways (Butchers et al, 1980; Howarth et al, 1985; Church and Hiroi, 1987). β_2-Agonists are known to be more potent mast cell stabilisers than sodium cromoglycate (Church and Hiroi, 1987). β_2-Agonists may also inhibit the release of acetylcholine from postganglionic cholinergic nerves in the airway (Rhoden et al, 1988), reduce microvascular leakage and have beneficial effects on ciliary function and mucus production (Nelson, 1995).

β_2-Agonists are the most effective bronchodilators in current use (Kemp, 1993) and protect against all bronchoconstrictor challenges (Barnes, 1989). Hence the term 'reliever' is often used to describe β_2-agonists due to their ability to relieve bronchoconstrictor challenges. They may be delivered by inhalation, or systemically by oral or intravenous routes (Thompson and Watkins, 1994). Inhaled therapy by either MDI or nebuliser is the most efficient means of delivering high local concentrations of drug to the airways, and minimising systemic side effects (Thompson and Watkins, 1994).

Inhaled β_2-agonists are indicated for the short-term relief of bronchoconstriction and are the treatment of choice for acute exacerbations of asthma (Barnes, 1989). They are highly effective against early phase exercise and allergen induced asthma bringing symptomatic relief (Skorodin, 1993). However, β_2-agonists do not inhibit either the late response to allergens or the subsequent bronchial hyperresponsiveness (Cockcroft and Murdoch, 1987). Because of their symptomatic relief, it is now suggested that there is a danger that β_2-agonists may

be used in worsening asthma to relieve symptoms, masking an underlying deterioration in the disease. Although there are beneficial non-bronchodilator actions of β_2-agonists, for maintenance therapy in all but the mildest cases of asthma, it is essential that β_2-agonists should be used in combination with inhaled corticosteroids or sodium cromoglycate, and if necessary, theophylline or ipratropium bromide (Thompson and Watkins, 1994).

The past few years has seen the development of long acting β_2-agonists including salmeterol, formoterol and bambuterol (Boulet, 1994). These agents have a later peak effect (typically 2-4 hours) and a significantly longer duration of action (typically greater than 12 hours) compared to the short acting β_2-agonists such as salbutamol, terbutaline and fenoterol (peak effect usually 30-90 minutes after inhalation and duration of action 4-8 hours; Boulet, 1994). Longer acting β_2-agonists have particular efficacy in nocturnal asthma and 'morning dippers', as well as reducing the overall β_2-agonist load in comparison to salbutamol in conventional dosage regimens (Dalonzo et al, 1994; Thompson and Watkins, 1994). In a twelve month comparison in moderate asthmatics, patients treated with salmeterol demonstrated a lower incidence of asthma and related events compared to patients treated with salbutamol (Britton et al, 1992).

Long acting β_2-agonists do protect against the late phase response but it has been suggested that this is due not to a clinically significant anti-inflammatory effect, but rather, to a prolonged functional antagonism (Taylor et al, 1992). It is currently recommended that long acting β_2-agonists be used only in patients already receiving inhaled corticosteroids (Rees, 1991; Anonymous, 1992; Goldie et al, 1995). There has been no evidence that long acting β_2-agonists significantly influence the chronic inflammatory response (Barnes and Chung, 1992; Goldie et al, 1995). In addition, there have also been some questions regarding tolerance to protective effects against bronchoconstrictor stimuli in mild asthmatics despite

well maintained bronchodilation (Cheung et al, 1992). It has been shown that patients using regular salmeterol treatment may also require higher doses of salbutamol when used for relief of acute symptoms (Grove and Lipworth, 1995a). The use of short acting β_2-agonists for chronic therapy, not just acute symptomatic relief, has now been scrutinised. It would follow, therefore, that over reliance on long-acting β_2-agonists may also mask an underlying deterioration in a patient's asthma. This will also be discussed in Section 4.5.

2.5.2 Glucocorticosteroids

Corticosteroids are anti-inflammatory agents and have long been used in the treatment of asthma, although their mechanism of action is still disputed (Barnes, 1989). Corticosteroids produce a decrease in inflammatory cells, especially eosinophils, inhibit leucocyte infiltration, inhibit activated T-lymphocytes, improve β_2-adrenergic cell function, stabilise oedema, attenuate mediator release, especially from activated eosinophils, and inhibit mast cell growth (Alpers, 1991; Gibson, 1992). Corticosteroids are the only available therapy which decrease the degree of bronchial hyperresponsiveness and reduce airway inflammation and oedema (Alpers, 1991). A reduction in bronchial hyperresponsiveness may take up to 3 months of continuous therapy (Woolcock et al, 1988).

Corticosteroids also inhibit the late-phase response (Cockcroft and Murdoch, 1987; O'Byrne et al, 1987). In other words, unlike β_2-agonists which provide symptomatic relief and may mask deterioration in the underlying disease, corticosteroids treat the underlying disease process (Paterson et al, 1995). Hence, corticosteroids are often termed 'preventers' in contrast to bronchodilator 'relievers'.

By the 1960s, it was realised that the long term use of oral corticosteroids was associated with significant adverse effects (Frew and Holgate, 1993). The adverse

effects of high dose glucocorticoids are shown in Table 2.4. Inhaled beclomethasone dipropionate was introduced in 1970 and rapidly established itself at the forefront of asthma treatment (Frew and Holgate, 1993). Because of its route of delivery, systemic side effects were reduced with the drug being delivered directly to the site of action. The efficacy and safety of inhaled corticosteroids now enable long term treatment with these drugs (Alpers, 1991).

Systemic adverse effects from inhaled corticosteroids have been a controversial topic. With conventional inhalers, 85-90% of each dose is swallowed, of which some is absorbed. At doses greater than 1000 μg/day for adults (> 400 μg/day for children), biochemically detectable impairment of adrenal responsiveness to corticotrophin has been reported (Frew and Holgate, 1993). In the absence of previous or concomitant treatment with oral glucocorticoids, inhaled glucocorticoids in doses of 1500 μg/day or less in adults and 400 μg/day or less in children have little if any affect on pituitary-adrenal suppression related adverse effects (Barnes, 1995). Spacer devices, a recent development in MDI delivery, significantly reduce systemic absorption and should be used for drug delivery above these dosages (Frew and Holgate, 1993; Barnes, 1995). Fluticasone is a new inhaled corticosteroid which appears to be more potent than other corticosteroids with essentially no oral absorption and rapid hepatic metabolism (Thompson and Bremner, 1993). It is anticipated that this will allow the delivery of higher doses with reduced systemic effects (Thompson and Bremner, 1993; Lipworth, 1993).

Health care providers and usually lay persons are aware that corticosteroids can cause serious side effects (Skorodin, 1993; Hanania et al, 1995). There have been some concerns about the possible side effects of inhaled corticosteroids, especially growth suppression in children (Frew and Holgate, 1993; Kamada, 1994). This has probably led to a reluctance to introduce inhaled corticosteroids into anti-asthmatic regimens in the past, especially in patients with moderate asthma.

Because of the recent questions regarding the safety of long term β_2-agonist use and a greater understanding of the pathogenesis of asthma as an inflammatory disease, there has now been general agreement towards a shift in the management of the disease with greater use of inhaled corticosteroids (Alpers, 1991; Frew and Holgate, 1993; Paterson et al, 1995). Inhaled corticosteroids or sodium cromoglycate are now recommended much earlier in the disease for use as a regular maintenance therapy in all but the mildest cases of asthma (Barnes, 1989; Alpers, 1991; Larsen 1992; Thompson and Watkins, 1994).

Table 2.4   *Adverse effects of high dose glucocorticoids. Adapted from Jackson and Bowman, 1995.*

- Hypertension (occurs in 80% of patients).

- Osteoporosis and resulting fractures.

- Impaired glucose tolerance and diabetes.

- Suppression of immune function.

- Poor wound healing and suppression of fibroblast activity.

- Severe psychological disturbances, predominantly euphoria and depression.

- Muscle wasting and central obesity.

- Peptic ulcer disease.

- Inhibition of linear growth in children.

- Avascular necrosis.

- Cataracts.

- Electrolyte disturbances such as hypokalaemia.

2.5.3   Methyl xanthines

Methyl xanthines include theophylline, aminophylline (theophylline ethylene diamine) and choline theophyllinate. Although theophylline has been used as a mainstay in asthma treatment for 50 years the mechanism of action is still unclear (Persson, 1986). It was originally thought that it caused bronchodilation by

inhibiting phosphodiesterase, leading to an increase in cAMP although this requires concentrations of drugs far exceeding the therapeutic range (Barnes, 1989). Other possible modes of action include adenosine receptor antagonism, although a closely related drug, enprofylline, has greater broncholidator potency but is not an adenosine antagonist (Cockcroft et al, 1989), inhibition of intracellular release of calcium and stimulation of catecholamine release (Barnes, 1989).

Theophylline is a less effective bronchodilator than the β_2-agonists (Barnes, 1989) but unlike β_2-agonists, theophylline also inhibits the late phase response suggesting anti-inflammatory effects (Rogers et al, 1985; Mapp et al, 1987; Barnes, 1989; Alpers, 1991; Jenne, 1994).

Intravenous aminophylline is an effective treatment for bronchospasm in acute severe asthma, however, there has been debate as to whether it has any additional benefits above those of the β_2-agonists. Although there were no differences in spirometric response, Wrenn et al (1991) found that acute severe asthmatics who were treated with intravenous aminophylline in addition to bronchodilators recovered more quickly.

There has been a shift away from the use of theophylline in recent years due to questions over its efficacy, its narrow therapeutic range and drug interactions (Alpers, 1991; Frew and Holgate, 1993; Paterson et al 1995). Continuous long-term therapy requires regular serum monitoring (Alpers, 1991) although xanthines are often prescribed without such monitoring (Rumbak and Self, 1992; Frew and Holgate, 1993).

The therapeutic range of theophylline is 10-20 μg/ml. At serum levels over 20 μg/ml most individuals will experience toxicity consisting of tremor, nausea and CNS excitation (Frew and Holgate, 1993) and at higher plasma concentrations

more serious adverse effects such as cardiac arrhythmias and seizures may occur (Barnes, 1989). Despite these toxic effects, theophylline remains useful for some patients, especially those with nocturnal asthma (Alpers, 1991; Frew and Holgate, 1993). For maintenance therapy, twice daily dosage of a slow release preparation should be used (Barnes, 1989).

2.5.4 Ipratropium bromide

Cholinergic antagonists have been used for many centuries in herbal remedies for asthma but their adverse effects have proved unacceptable (Barnes, 1989). The development of ipratropium bromide, a quaternary ammonium derivative which is not readily absorbed from the gut and does not cross the blood-brain barrier, subsequently reducing the incidence of adverse effects, has renewed interest in this group of drugs (Barnes, 1989).

Ipratropium bromide acts by blocking muscarinic receptors in smooth muscle leading to inhibition of vagal cholinergic tone, resulting in bronchodilation. Cholinergic antagonists provide various levels of protection against bronchoconstrictor challenges, are not particularly effective against allergen challenge, do not block the late phase response and do not inhibit the release of mediators from mast cells (Cockcroft et al, 1978; Howarth et al, 1985; Gross, 1988).

Ipratropium is less effective than adrenergic agonists in the treatment of asthma (Barnes, 1989) and should not be used as a first line drug or sole therapy for asthma (Alpers, 1991). The onset of action is slower than β_2-agonists but the action is more prolonged, lasting up to 8 hours and may be of more benefit to patients with a major component of bronchitic symptoms (Barnes, 1989). The addition of ipratropium bromide to anti-asthmatic therapy may provide better therapeutic

coverage in some patients, especially those with severe acute asthma (O'Driscoll et al, 1989; Rees and Price, 1995a).

2.5.5   Sodium cromoglycate

Sodium cromoglycate has been classified as an anti-allergic agent. This is because of its prophylactic action by suppressing allergic response without possessing anti-inflammatory properties (Paterson et al, 1995). The mechanism of action of sodium cromoglycate is still not clear. Because sodium cromoglycate inhibits the immediate response to allergens and exercise, it was originally thought that this was achieved by inhibiting the release of mediators (Cockcroft and Murdock, 1987).

It has also been suggested that sodium cromoglycate may act on other inflammatory cells such as macrophages or eosinophils because of its ability to inhibit the late response and bronchial hyperresponsiveness, whereas other mast cell stabilising drugs do not (Barnes, 1989). In addition, it has been suggested that sodium cromoglycate may act on sensory nerves in the airway involved in the neural mediation of bronchoconstriction (Barnes, 1989).

There has been considerable recent promotion of sodium cromoglycate, given its anti-allergic properties, the very low incidence of adverse effects, an increase in dosage, and the current understanding of asthma as primarily a disease associated with airway inflammation due to antigen exposure. Sodium cromoglycate is now marketed in a 5 mg inhaler preparation in addition to the original 1 mg inhaler.

Sodium cromoglycate, while having a long history of popularity for use in children with asthma, has now also become a recommended first-line therapy for these patients (Alpers, 1991; Breslin, 1993). Some adults may also benefit, particularly atopic patients and those with exercise induced asthma (McFadden and

Gilbert, 1993; Tse and Bridges-Webb, 1993). Despite its relative safety and efficacy, sodium cromoglycate is not effective in all patients and there seems to be no indicators to predict which patients will respond (Barnes, 1989). A trial of 4-8 weeks is usually needed to determine if the patient will benefit (McFadden and Gilbert, 1993; Rees and Price, 1995a). Nedocromil sodium is closely related to sodium cromoglycate, with similar properties but has no advantages over sodium cromoglycate (Ruffin et al, 1987).

2.5.6   Other drugs and new developments

Other drugs used in the treatment of asthma include those drugs, not specifically anti-asthmatics, that act on the immune system such as methotrexate, gold salts, and troleandomycin, which have been shown to possess corticosteroid sparing activity (Barnes, 1989). Ketotifen, an antihistamine, showed some marginal benefit in a study by Hendy et al (1986) and cetirazine, another antihistamine, has shown some marginal benefit in exercise induced asthma (Ghosh et al, 1991).

Zileuton, a 5-lipoxygenase inhibitor, blocks the synthesis of leukotrienes, mediators in the inflammatory cascade. It has been shown to be effective in improving airway obstruction and preventing associated symptoms (Israel et al, 1993). Other drugs under development include anti-adhesion molecules (inhibit leucocyte recruitment), anti-cytokines (impair leucocyte activation, recruitment and survival) and anti-effector cells that act by temporarily removing T cells (Henahan, 1995).

The search for a mediator antagonist or synthesis inhibitor is unlikely to result in the development of a new singularly effective asthma treatment (Paterson et al, 1995). It is more likely that these new compounds may be combined with existing treatments to tailor medication to individual needs.

2.6   THERAPEUTIC CONTROVERSIES AND THE ASTHMA PARADOX

As discussed earlier, there has been an alarming increase in asthma related morbidity and mortality in most industrialised countries, despite medication sales at an all time high. There have been numerous hypotheses to explain this paradoxical increase in morbidity and mortality. Environmental factors have been implicated, including pollution (Anonymous, 1989; Christie et al, 1992; Rennick and Jarman, 1992; Antó and Sunyer, 1995), occupational exposure to various compounds (Bernstein, 1992), pollens (Bellomo et al, 1992), and it is well documented that house dust mite is a trigger for asthma (Spector, 1991). Other studies have shown that there have been major misconceptions in the community about the disease in the past (Rubinfield et al, 1988) and a major degree of under-recognition and under-treatment of asthma (Anonymous, 1993a; Bauman et al, 1992b; Dales et al, 1992; Gibson et al, 1993a; Gibson et al, 1993b; Marks et al, 1994; Abramson et al, 1995), which could clearly contribute to asthma mortality. There has, however, been a disturbing suggestion that the increase in asthma drug sales, particularly bronchodilators, may not be a result of, but may rather be contributing to, increasing asthma morbidity and mortality (Barnes and Chung, 1992).

During the 1960s, isoprenaline, a non-selective β-agonist, was marketed in several countries in a high dose preparation (Isuprel Forte®). An epidemic of asthma deaths followed. Isoprenaline was implicated (Speizer et al, 1968) and mortality fell after the publicity and withdrawal of the over the counter preparation in Britain (Stolly, 1972). There was, however, a similar rise in mortality of asthma in some countries which was not linked to the introduction of high strength isoprenaline inhalers (Barnes and Chung, 1992).

A second epidemic followed in New Zealand in the 1970s with the introduction of fenoterol. It was suggested that there was a causal association between the

widespread use of fenoterol and asthma deaths (Crane et al, 1989a; Pearce et al, 1990; Grainger et al, 1991) which was supported by a crossover study (Sears et al, 1990) that showed worsening of asthma control with regular fenoterol use. Despite criticisms of these studies being confounded by disease severity, fenoterol use was clearly associated with an increased risk of death from asthma.

It was suggested that fenoterol may have more adverse effects than related compounds due to less β_2 selectivity and may have been marketed at a higher dose than related compounds (Wong et al, 1990). It was originally thought that these deaths were most likely due to cardiac arrhythmias induced by large doses of a less selective β_2-agonist, given its effect of tachycardia after inhalation (Crane et al, 1989b), but there is no direct evidence of this (Barnes and Chung, 1992). It has also been suggested that salbutamol can exert a cardiotoxic effect, evidenced by an increase in CKMB (creatinine kinase isoenzyme), when given by the intravenous route (Chazan et al, 1992). Although it is difficult to exclude arrhythmia as a precipitating cause of sudden asthma deaths (Barnes and Chung, 1992), Molfino et al (1991) found no evidence that arrhythmias are associated with near fatal attacks in patients admitted to hospital.

It is still not clear as to whether fenoterol was responsible for the New Zealand epidemic or whether it was simply a marker, being a drug used in a high risk patient, and whether the potential problem was unique to fenoterol or common to all β_2-agonists, and if so, if this is related to β_2 selectivity or other mechanisms (Rubinfield, 1991). It is still argued that despite the limitations, the New Zealand time trends of asthma deaths are consistent with fenoterol being the main cause of the New Zealand asthma mortality epidemic (Pearce et al, 1995).

Following the fenoterol controversy, Page (1991) hypothesised that the regular use of all β_2-agonists, due to their nature of bronchodilation, permit antigen to

penetrate deeper into the airways. This is suggested to result in histological inflammatory changes and increased bronchial hyperresponsiveness, thus accelerating airways inflammation, leading to increased morbidity and mortality (Page, 1991). Studies have shown that regular treatment with β_2-agonists can lead to reduced protection against constrictor challenges, including antigens and exercise (Cheung et al, 1992; Cockcroft et al, 1993; Grove and Lipworth, 1995b).

After the acute protective effects (decreased bronchial hyperresponsiveness) of β_2-agonist administration have worn off, there may be a rebound increase in bronchial hyperresponsiveness with regular β_2-agonist use (illustrated in Figure 2.3 after a single dose of a β_2-agonist; Wahedna et al, 1993). It has also been suggested that tachyphylaxis may develop due to down regulation of β_2-receptors, but this effect does not appear to occur readily (Barnes and Chung, 1992). Although tolerance to anti-bronchoconstrictor effects has been shown, there is no conclusive evidence as yet, to suggest that tolerance develops to the bronchodilator effects of β_2-agonists (Grove and Lipworth, 1995b). Despite the hypothesis that regular β_2-agonist therapy may worsen asthma control, there is still no convincing evidence explaining how this would happen (Fuller, 1994).

There is no doubt that asthma morbidity and mortality are clearly associated with increased use of β_2-agonists as patients with more severe asthma require greater β_2-agonist rescue (Spitzer et al, 1992; Castle et al, 1993; Fuller, 1994). In a large case-control study using linked health insurance data-bases consisting of a cohort population of 12,301 asthmatic patients, Spitzer et al (1992) reported that increasing use of β_2-agonist was associated with an increased risk of mortality. In retrospect, it has been suggested that the headlines following the study by Spitzer et al (1992) were misleading and were based on an extremely weak association between β_2-agonist use and death (Mullen et al, 1993). In addition, Suissa et al

(1994) showed that increasing use of β_2-agonists in a patient should be regarded as a serious warning sign of an impending life-threatening attack.
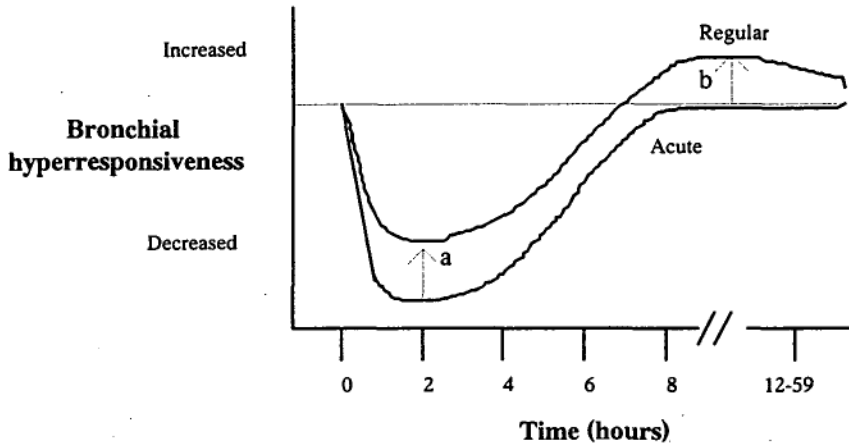
Figure 2.3   *Differences in bronchial hyperresponsiveness after a single dose of β-agonist with regular and acute β-agonist use. (a) Diminished reduction in bronchial hyperresponsiveness (decreased protective effect) after regular administration of a β-agonist. (b) A rebound increase in hyperresponsiveness may occur after the acute protective effect has worn off after regular administration of a β-agonist. (Adapted from Tattersfield, 1994).*

Two possible scenarios to explain the association between increasing β_2-agonist use and increased risk of mortality have been proposed by Suissa et al (1994): the 'β-agonist hypothesis' (β_2-agonist use causing worsening of asthma control) and the 'severity hypothesis' (β_2-agonist use acting as a marker for increasing disease severity). The asthma paradox and hypothesis of a possible causal link between increased β_2-agonist use and increased morbidity/mortality are depicted in Figure 2.4.

The hypothesis of a causal link between β_2-agonist use and an increase in disease severity obviously relies on showing that β_2-agonist use worsens asthma control (Fuller et al, 1994). There have been several studies suggesting that regular β_2-agonist use does worsen asthma control (Sears et al, 1990; van Schayck et al, 1991; Spitzer et al, 1992). These studies, however, are in contrast to those suggesting that

regular β$_2$-agonist therapy does not lead to a deterioration in asthma control or can improve asthma symptoms (Britton et al, 1992; Pearlman et al, 1992; Castle et al, 1993; Chapman et al, 1994; Dalonzo et al 1994). These studies are summarised in Table 2.5. In addition, asthma mortality in the United Kingdom has not increased in the past 10 years despite a doubling in the use of β$_2$-agonists (Tattersfield, 1994).
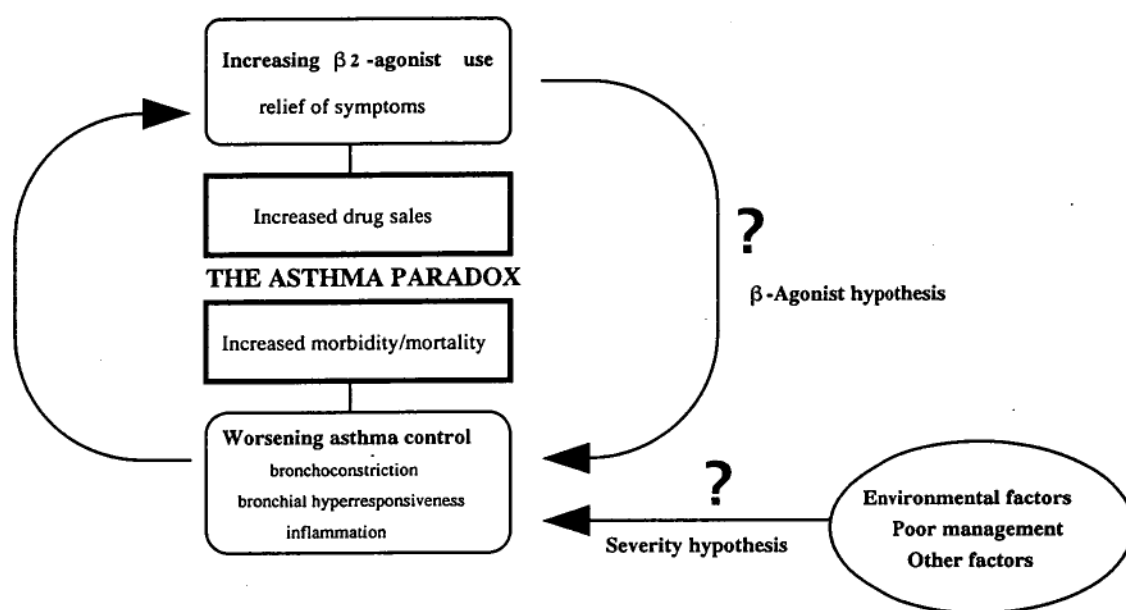
Figure 2.4   *The asthma paradox. Increased sales of anti-asthma drugs, in particular β$_2$- agonists, have not reduced either asthma morbidity or mortality. Have β$_2$- agonists been contributing to worsening asthma control or have the increases in morbidity/mortality been due to other factors such as poor management and the environment? Suissa et al (1994) have described these possible causes as the β-agonist' hypothesis and the 'severity' hypothesis respectively.*

The fact remains that there have been two epidemics of asthma deaths in the last 40 years, both of which were associated with a high dose bronchodilator with less β$_2$ selectivity than related compounds, and both epidemics resolved once the drugs had been withdrawn (Tattersfield, 1994). Whether or not these drugs were solely responsible has never been determined (Barnes and Chung, 1992). What cannot be argued is that increasing β$_2$-agonist use in a patient is closely associated with worsening asthma severity. This indicates the need for intervention in line with

Table 2.5   *Studies examining regular versus as-needed bronchodilator therapy.*

Title	Study Outline	Authors
Regular inhaled beta-agonist treatment in bronchial asthma.	Eighty nine subjects with stable asthma took part in a double-blind, placebo controlled, randomised crossover study over 48 weeks. Patients took either placebo or fenoterol by DPI for 24 weeks before swapping to the alternate DPI as well as supplementary aerosol as necessary. Regular treatment with fenoterol was associated with deterioration of asthma control in the majority of subjects.	Sears et al, 1990.
Bronchodilator treatment in moderate asthma or chronic bronchitis: continuous or on demand? A randomised controlled study.	Two hundred and twenty three patients participated in a two year randomised controlled prospective crossover study in which patients were assigned to receive continuous treatment or treatment on demand. Continuous treatment without anti-inflammatory treatment accelerated decline in ventilatory function.	van Schayck et al, 1991.
The use of β-agonists and the risk of death and near death from asthma.	Health insurance databases were examined in a cohort of 12,301 patients for whom asthma medications had been prescribed between 1978 and 1987. An increased risk of death or near death was associated with the regular use of inhaled β_2-agonists, especially fenoterol.	Spitzer et al, 1992.
A twelve month comparison of salmeterol with salbutamol in asthmatic patients.	Six hundred and sixty seven moderate asthmatics participated in a double-blind, parallel group study taking salbutamol and salmeterol regularly for 12 months (as well as supplementary bronchodilation when necessary). Both treatments were well tolerated throughout the study period with a similar incidence of adverse effects.	Britton et al, 1992.
A comparison of salmeterol with albuterol in the treatment of mild-to-moderate asthma.	Two hundred and thirty four patients were divided into three treatment groups (regular salbutamol, regular salmeterol and placebo) as well as supplementary bronchodilation over a 12 week treatment period. Regularly administered salbutamol was no more or less beneficial than salbutamol used on an as-needed basis.	Pearlman et al, 1992.
Serevent nationwide surveillance study: a comparison of salmeterol with salbutamol in asthmatic patients who require regular bronchodilator treatment.	Treatment over 16 weeks with either salmeterol or salbutamol was not associated with an incidence of deaths related to asthma in excess of that predicted.	Castle et al, 1993.
Regular vs as-needed inhaled salbutamol in asthma control.	Three hundred and fourteen asthma patients were treated in a four-week, randomised, crossover trial of regular and as-needed salbutamol. Regular salbutamol in moderate asthmatics was associated with less frequent asthma symptoms.	Chapman et al, 1994.
Salmeterol xinofoate as maintenance therapy compared with albuterol in patients with asthma.	Three hundred and twenty two asthma patients were treated with salmeterol, salbutamol or placebo for 12 weeks. No deterioration of asthma control was observed with the regular use of salmeterol.	Dalonzo et al, 1994.

current management guidelines (Anonymous, 1992; Tse and Bridges-Webb, 1993) to reduce the reliance on bronchodilator therapy, with greater emphasis placed on early introduction of anti-inflammatory therapy. Figure 2.5 shows the current international consensus toward the stepwise management of asthma.

Clinical features pretreatment [a]

Step 1. Mild Asthma

- Intermittent brief symptoms (< 1-2/week)
- < 1-2 nocturnal symptoms/month
- Asymptomatic between epidsodes
- PEFR or FEV₁ > 80% predicted, variability < 20%

Steps 2 and 3. Moderate Asthma

- > 1-2 exacerbations/week
- Activity and sleep affected
- Nocturnal symptoms (> 2/month)
- Almost daily need for inhaled beta-agonist
- PEFR or FEV₁ 60-80% predicted and variability 20-30%

Step 4. Severe Asthma

- Frequent exacerbations
- Continuous symptoms
- Frequent nocturnal symptoms
- Activities limited
- PEFR or FEV₁ < 60% predicted and variability > 30%

Particularly for
nocturnal symptoms

Therapy (NB. Must include patient education about prevention in addition to symptom control)

- Short-acting inhaled β₂- agonist prn (not more than 3 times/week)

- Inhaled sodium cromo- glycate or short acting β₂-agonist before exercise or allergen exposure

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- Inhaled corticosteroid (200- 500 microgram daily up to 750 microgram daily if necessary) [b]

or

Sodium cromoglycate

+

- Short-acting inhaled β₂- agonist (prn, up to 3-4/daily)

- Inhaled corticosteroid (800- 1000 microgram daily) [b]

+

- Sustained release theophylline

or

- Oral β₂- agonist

or

- Long-acting inhaled β₂- agonist (especially for nocturnal symptoms) [c]

+

- Short-acting inhaled β₂- agonist (prn, up to 3-4/daily)

- Inhaled corticosteroid (800- 1000 microgram daily) [b]

+

- Sustained release theophylline
   and/or
- Oral β₂- agonist

or

- Long-acting inhaled β₂- agonist (especially for nocturnal symptoms)

with or without

- Short-acting inhaled β₂- agonist once daily [c]

+

- Oral corticosteroid (altern- ate day or single daily dose)

+

- Short-acting inhaled β₂- agonist (prn, up to 3-4/daily)

Control not achieved

Control not achieved

Control not achieved

Outcome

Control of asthma

- Minimal (ideally no) chronic symptoms
- Minimal (infrequent) episodes
- No emergency visits
- Minimal need for inhaled β₂-agonists

- No limitations of activities (including exercise)
- (Near) normal PEFR and circadian variation < 20%
- Minimal (or no) adverse effects from medication

NB. Once sustained control achieved, a step-down reduction in therapy may be carefully considered (to identify minimum therapy required to maintain control)

Best possible results

- Least symptoms
- Least need for inhaled β₂- agonists
- Least limitation of activities
- Best PEFR with least circadian variation
- Least adverse effects from medication

[a] One or more features may be present; assign patient to most severe grade id which any feature is present

[b] inhaled corticosteroid dose refers to beclomethasone dipropionate

[c] May consider inhaled anti-cholinergic

Figure 2.5 *Stepwise approach to the management of asthma. Adapted from the International consensus report on the diagnosis and management of asthma (Anonymous, 1992).*

CHAPTER 3

Pharmacoepidemiology Background

3.1 SUMMARY

Pharmacoepidemiology is a relatively new discipline involving the study of the patterns and effects of therapeutic drug use within populations. The background and methodology of pharmacoepidemiology and its applications in improving therapeutic drug use are examined. In addition, the current state of drug utilisation data in Australia is discussed. The role of pharmacoepidemiology in monitoring asthma drug therapy, in light of the asthma paradox and shift in emphasis to preventive (anti-inflammatory and anti-allergic) medication is also discussed.

3.2 BACKGROUND OF PHARMACOEPIDEMIOLOGY

3.2.1 What is pharmacoepidemiology?

Pharmacoepidemiology is a relatively new discipline involving the study of the patterns and effects of therapeutic drug use within populations (Hartzema 1992; Henry, 1988; Wertheimer and Andrews, 1995). Pharmacoepidemiology is essentially the merging of clinical pharmacology (the study of effects of drugs in humans) and epidemiology (the study of distribution and determinants of disease in populations; Strom, 1994).

The methodology of pharmacoepidemiology has a three stage process. Firstly, a sample population of subjects is studied; secondly, the information obtained from this sample is generalised; and thirdly, a conclusion is drawn about the population in general (Strom, 1994). This conclusion is referred to as an association. There are four basic types of associations that can be observed in a pharmacoepidemiology study, as shown in Table 3.1. The basis of pharmacoepidemiological research is to differentiate between these associations (Strom, 1994). This usually involves statistical analyses to determine if the differences in outcomes may have occurred by chance. To find a true causal

association, all three types of errors (random error, bias and confounding) must be excluded (Strom, 1994). In addition, further criteria must exist for a causal association (Table 3.2). No single criteria is sufficient for a causal association. Analogously, it is not necessary to satisfy all criteria for a causal association (Strom, 1994).

Pharmacoepidemiological study design may consist of randomised clinical trials, cohort studies, case-control studies, analysis of secular trends, case series and case reports (Strom, 1994). These studies are briefly described in Table 3.3. Case reports, case series, analyses of secular trends, case-control studies and cohort studies have been referred to as observational study designs (Strom, 1994). In observational studies, investigators do not control drug therapy but observe and evaluate the results of ongoing medical care (Strom, 1994).

Analyses of secular trends can examine trends in disease and exposure over time or between different populations. These types of studies may be particularly useful in the postmarketing surveillance of drugs for observing rare drug effects that would not necessarily be observed in cohort studies due to the number of exposed patients that would be needed (Strom, 1994). For example, if an adverse drug reaction is suspected in 1 in 10,000 users, a sample size of approximately 30,000 patients would be needed to provide 95% confidence in detection (Porta and Hartzema, 1991). A limitation of analyses of secular trends is that confounding variables cannot be controlled. Because these studies only observe groups, they lack data on individuals. Another limitation is that disease trends may not be ideally recorded. When examining trends in disease, there may be (i) trends in how frequently patients are diagnosed with a given disease dependent on factors such as disease awareness at the time of diagnosis, (ii) changes in diagnostic methods, (iii) advances in diagnostic technology allowing a more accurate diagnosis of previously hard to diagnose diseases, (iv) changes in coding systems,

(v) changes in population demographics, and (vi) difficulty in differentiating between incidence of disease and mortality rates (Strom, 1994). The inherent scientific weakness of analyses of secular trends (ecologic studies) must be remembered and should be regarded as being, at best, an hypothesis generating activity (Eisdaile et al, 1987).

Table 3.1   *Types of associations between factors studied (Adapted from Strom, 1994).*

Association type	Nature of association
• none	independent
• artifactual:	spurious or false
random error	unsystematic variation
bias	systematic variation
• indirect	confounded
• causal	direct or true

Table 3.2   *Criteria for the causal nature of an association (Adapted from Strom, 1994).*

- Coherence with existing information (biological plausibility)
- Consistency of the association
- Time sequence
- Specificity of the association
- Strength of the association:
    quantitative
    dose-response relationship
    study design

Table 3.3 *Types of pharmacoepidemiology study designs, their advantages and disadvantages (Adapted from Strom, 1994).*

Study design	Description	Advantages	Disadvantages
Randomised clinical trial (experimental study)	Randomised clinical trials are essentially experimental studies in which the investigator controls the therapy and randomly allocates patients between the study groups. Associations demonstrated in randomised clinical trials are more likely to be causal associations than associations shown in other study types.	Most convincing design. Only design that controls for unknown or unmeasurable confounders.	Most expensive. Artificial. Logistically more difficult. Ethical objections.
Cohort studies	Cohort studies identify subsets of a defined population and observe them over time, generally comparing patients exposed to a particular therapy with unexposed patients. Patients are recruited into cohort studies on the basis of presence or absence of exposure and their subsequent disease course is then studied.	Can study multiple outcomes. Can study uncommon exposures. Selection bias less likely. Unbiased exposure data. Incidence data available.	Possibly biased outcome data. More expensive. May take years to complete if done prospectively.
Case-control	Case-control studies compare patients with a disease with controls without the disease and differ from cohort studies on the basis of patient recruitment. Patients recruited into case-control studies are selected on the basis of presence or absence of disease, prior exposures are then studied.	Can study multiple exposures. Can study uncommon diseases. Logistically easier and faster. Less expensive.	Control selection problematic. Possibly biased exposure data.
Analyses of secular trends	Analyses of secular trends, or ecologic studies, examine trends in an exposure that is a presumed cause and examine trends in a disease that is a presumed effect and test whether the trends coincide.	Can provide rapid answers.	No control of confounding factors.
Case series	Case series are collections of patients, all of whom have single exposure, whose clinical outcomes are then evaluated and described.	Easy quantitation of incidence.	No control group so cannot be used for hypothesis testing.
Case reports	Case reports, as used in pharmacoepidemiology, are usually reports that describe a single patient who was exposed to a drug and experienced an adverse outcome. Case reports are useful for raising hypotheses about drug effects that can be tested with more rigorous studies.	Inexpensive and easy method for generating hypothesis.	Cannot be used for hypothesis testing.

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3.2.2   Applications of pharmacoepidemiological studies

Accurate population-based data on drug usage and effects are required to ensure that drugs are safe, effective and utilised appropriately (Jacobson et al, 1992). Investigation of prescribing patterns can be a powerful tool in rationalising drug use and creating greater economic efficiency in health care (Clark, 1986). Population based data on the usage and effects of drugs can be used for (i) drug utilisation research, (ii) studies of drug efficacy and/or adverse drug reactions, incorporating postmarketing surveillance (PMS), (iii) economic evaluations of drug therapies (Hurley and McNeil, 1988; Hurley et al, 1988) and, (iv) to define optimal therapy or establish prescribing guidelines (MacLeod, 1991). The advantages of using pharmacoepidemiology for PMS include factors such as large populations may be studied over long periods of time, relatively low cost compared to clinical trials, sample populations may include patients not normally included in clinical trials and uncommon adverse drug reactions may be observed (Strom, 1994).

There have been several recent studies which illustrate the benefits of pharmacoepidemiology. These are shown in Table 3.4. Pharmacoepidemiology has identified causal associations between gastrointestinal bleeding with individual non-steroidal anti-inflammatory drugs (Rodriguez and Jick, 1994; Figueras et al, 1994; Traversa et al, 1995) and possible links between oral contraceptive use and protection from Guillain-Barre syndrome (Stricker et al, 1994), inappropriate prescribing practices (Henry et al, 1991; Montanaro et al, 1992; Rosholm et al, 1993) and differences in patterns of benzodiazepine use linked with morbidity and psychosocial problems (Ekedahl et al, 1993; Ruiz et al, 1993). From these examples it is clear that pharmacoepidemiology has already shown its ability to act as a basis to increase the rational use of drugs, and is likely to have an increasing impact on clinical medicine (Strom and Tugwell, 1990).

3.2.3 Problems in therapeutic comparisons: the role of the defined daily dose

To allow any meaningful analysis of observational pharmacoepidemiology research, either within studies or between studies, there must be agreement on standardised units of comparisons for drugs (Cooke, 1991). For example, therapeutic comparisons between different drugs based on units of weight would not take into account factors such as molecular weight, potency, bioavailability and route of administration. The use of units of weight for drug comparison between a relatively potent and selective β_2-agonist, such a salbutamol, and a non-selective β-agonist, such as orciprenaline, becomes difficult.

Recognition of this dilemma of comparison of drugs has led the WHO (World Health Organisation) to recommend the adoption of the defined daily dose (DDD). The DDD is a unit of comparison between drugs and was originally introduced by the Nordic Medicines Commission where DDDs are defined as the average maintenance doses for the main clinical indication of the drug in adult patients. DDDs are not meant to be recommended doses but merely provide a standardised unit for comparative purposes (Cooke, 1991). In Australia in 1988, the Drug Utilisation Subcommittee (DUSC) was formed by the Pharmaceutical Benefits Advisory Committee (PBAC) to investigate ways of providing comprehensive and valid drug utilisation information in Australia (Birkett, 1993). DUSC adopted the now international unit of drug utilisation, the defined daily dose (DDD) per thousand population per day.

Table 3.4   *Some recent pharmacoepidemiology studies.*

Title	Author	Study Outline
Modelling the market uptake of new drugs following listing for subsidy in Australia. A report from the Drug Utilisation Subcommittee of the Australian Pharmaceutical Benefits Advisory Committee.	Birkett and McManus, 1995.	The market uptake of simvastatin, omeprazole, budesonide, fluoxetine and moclobemide were modelled using the sigmoid Emax model for drug-receptor binding. There were substantial differences in uptake rates between drugs and good fits of the model to the data.
Recent trends in the prescribing of cholesterol lowering drugs in Australia.	Henry et al, 1991.	The use of clofibrate increased to a greater extent than the preferred treatment for hyper-cholesterolaemia (cholestyramine and colestipol).
Drug utilisation in general practice: prescribing habits of National Formulary drugs by GPs of Emilia Romagna (Italy) in 1988 and 1989.	Montanaro et al, 1992.	It was found that there was over prescription of well documented drugs such as H_2-antagonists, ACE inhibitors, calcium antagonists and HMG-Co-A-reductase inhibitors. There was frequent inappropriate prescribing of some drugs.
Benzodiazepine prescribing patterns in a high-prescribing Scandinavian community.	Ekedahl et al, 1993.	Higher than national average prescribing of benzodiazepines, possibly due to higher psychiatric morbidity, psychosocial problems and deviant prescribing habits.
Outpatient utilisation of anti-depressants- a prescription database analysis.	Rosholm et al, 1993.	A large number of patients were found to be prescribed anti-depressants in low doses suggesting that the drugs were being used for indications other than depression or that a large number of patients were not given a sufficient dose.
Comparative study on benzodiazepine use in Canada and Chile.	Ruiz et al, 1993.	Although total benzodiazepine use was similar in both countries, there were striking differences in the use of individual benzodiazepines, possibly due to differences in the health care systems. These differences in patterns of use may determine differences in morbidity rates associated with these drugs.
Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs.	Rodriguez and Jick, 1994.	Users of azapropazone and piroxicam had a higher risk of upper gastrointestinal bleeding than patients using ibuprofen, naproxen, diclofenac, ketoprofen and indomethacin. Indomethacin and ibuprofen showed a substantial increase in risk from a low to a high daily dose.
Spontaneous reporting of adverse drug reactions to non-steroidal anti-inflammatory drugs- a report from the Spanish system of Pharmacovigilance, including an early analysis of topical and enteric coated formulations.	Figueras et al, 1994.	The study raised concerns about the relative safety of sustained -release and enteric-coated non-steroidal anti-inflammatory drug preparations in practice.
Gastroduodenal toxicity of different nonsteroidal anti-inflammatory drugs.	Traversa et al, 1995.	The use of ketorolac carried a greater risk of gastrotoxicity than other non-steroidal anti-inflammatory drug preparations.

Table 3.4 *continued.*

A case-control study of drugs and other determinants as potential causes of Guillain-Barre syndrome.	Stricker et al, 1994.	A case-control study was performed to investigate the possible role of drugs and other determinants in the causation of Guillain-Barre syndrome. There was significantly lower use of oral contraceptives in female cases of Guillain-Barre syndrome, suggesting that oral contraceptives may offer a protective effect.

3.3   AUSTRALIAN DRUG UTILISATION DATA

It has been suggested that Australia is falling behind many other countries, particularly in Europe, in the important health care area of pharmacoepidemiology (Henry, 1988). Major deficiencies have existed in the routine sources of Australian pharmacoepidemiology data and in data collection (Hurley et al, 1988) with no single comprehensive source. As noted by Henry (1988), it is even difficult to answer simple questions concerning the number of patients in Australia who have received a particular drug.

The main sources of drug utilisation data collection in Australia include the Pharmaceutical Benefits and Repatriation Pharmaceutical Benefits Schemes (PBS and RPBS), private marketing companies such as Intercontinental Medical Statistics (IMS), and the Australian Health Survey (Hurley et al, 1988). These and other sources are shown in Table 3.5. The lack of systematic methods of examining prescribing patterns has contributed to relatively little drug utilisation research being performed in Australia. In addition, the limited research that has been performed has largely had to rely on increasingly incomplete data (Birkett et al, 1991; Henry et al, 1991; Hurley et al, 1990; Jenkins et al, 1990), data provided by the questionnaires completed during the Australian Health Surveys of 1977-78 and 1983-84 (Lockwood and Berbatis, 1990), on information obtained from private market research companies (Mant et al, 1988; Harvey, 1988) or on the National Survey of Morbidity in General Practice (Bridges-Webb et al, 1992).

The formation of DUSC has addressed 3 major problems in drug utilisation research in Australia: (i) the lack of a uniform drug coding or classification system, (ii) the need for an adequate unit of drug utilisation measurement, and (iii) the problem of obtaining comprehensive and continuing data on drug prescribing, drug dispensing and drug use (Birkett, 1993).

The major limitations of drug utilisation data from the PBS/RPBS is that the data is incomplete. PBS data is only complete for listed drugs prescribed to pensioner or concessional patients, drugs costing more than the maximum patient contribution (presently $16.20) and drugs prescribed to general patients who have spent more than approximately $400 on PBS drugs in a calender year (under the 'safety net' scheme). Drugs listed in the PBS schedule with a dispensed price below the maximum patient contribution are not recorded, such as most of the bronchodilator preparations and many drugs are not listed on the PBS or RPBS, with the patient responsible for full payment. Only about 60% of total prescription sales are currently PBS items (based on extrapolations from 1994 Guild Digest data; Table 4.4) and this would be expected to decrease further as patient contributions increase. RPBS data only encompass patients entitled to treatment because of service in the Australian armed forces, accounting for 7-8% of all community pharmacy prescriptions (Hurley et al, 1988). To correct these deficiencies in PBS/RPBS drug utilisation data, the DUSC has collaborated with the Pharmacy Guild of Australia to obtain survey data on drugs not covered by the PBS or RPBS. Data is collected from computer dispensing data in a stratified random sample of pharmacies throughout Australia and is aggregated by drug code. These data provide information on all PBS and RPBS categories as well as private, general and S3 recordable items (S3Rs) and so is more comprehensive than PBS/RPBS data alone (Birkett, 1993).

Table 3.5   *Some major sources of drug utilisation data within Australia**

Source	Scope	Comments
Pharmaceutical Benefits Scheme (PBS)	• Only drugs listed on PBS - mostly S4 items. • prescriptions dispensed in community pharmacies or by approved doctors in isolated areas. • community or private hospital patients (not public hospital patients).	• no data on number of patients, patient's age or sex, dosages or diagnoses. • data set has diminished with recent increases in patient contribution.
Repatriation Pharmaceutical Benefits Scheme (RPBS)	• drugs on restricted list. • prescriptions dispensed in community pharmacies (not repatriation hospitals). • patients entitled to treatment because of service in the Australian armed forces. • 7-8% of community pharmacy prescriptions.	• no data on number of patients, patient's age or sex, dosages or diagnoses.
National Survey of Morbidity in General Practice.	• GP recorded details of doctor-patient encounters • Stratified random sample of GPs • details recorded such as patient age, sex, reasons for encounter, problems managed and drugs prescribed.	• only performed in 1962-63, 1969-74 and 1990-91. • data represented as percentages rather than DDDs. • not all drugs prescribed are dispensed.
Community Pharmacy Dispensing Records	• all prescriptions dispensed in community pharmacies. • includes recordable S3 items.	• this source has not been investigated in depth. • compatibility of different software may be a problem. • most non-prescription drugs excluded.
Australian Medical Index (AMI) produced by Intercontinental Medical Statistics (IMS)	• quarterly publication with prescribing patterns for diagnosis and utilisation patterns for drugs. • based on Australian Morbidity Survey of GPs. • Data projected to give Australian estimates.	• not all drugs prescribed are dispensed. • co-prescribing data limited, non-prescription drugs excluded. • data availability restricted and may be costly. • no data on number of patients receiving a particular drug.
Australian Pharmaceutical Index (API) produced by IMS	• monthly publication • sales of pharmaceuticals to community pharmacies (not hospitals). • prescription and non-prescription drugs.	• no data on patients, dosages or diagnoses. • data availability restricted.
Strategic Planning Index (SPI)	• quarterly publication with prescribing patterns for diagnosis and utilisation patterns for drugs. • based on survey of GPs. • details of treatment substitutions, additions and commencements	• diary data recording system. • not all drugs prescribed are dispensed. • data not projected.
Hospital pharmacy dispensing records	• all prescriptions dispensed in hospital pharmacies. • mostly S4 items.	• the large number of drug coding systems would make merging of data difficult. • many different computer systems. • computerisation not universal.
Australian Pharmaceutical Marketing Research Group (APMRG) survey	• quarterly report of dollar sales of pharmaceuticals to hospitals. • 80% of manufacturers participate.	• unit sales and individual products not identified. • data availability restricted.

*Adapted from Hurley et al, 1988

3.4   ANTI-ASTHMATIC DRUGS: THE ROLE OF PHARMACOEPIDEMIOLOGY

The benefits of monitoring anti-asthmatic drug consumption within the community are obvious given the history surrounding the epidemics associated with isoprenaline and fenoterol in the 1960s and 1970s. In addition, the Australian Adverse Drug Reaction Committee (ADRAC), through its parent committee, the Australian Drug Evaluation Committee (ADEC), first noted the latest increase in asthma associated deaths during the 1980s (NHMRC report, 1988). The matter was then referred to the NHMRC in 1988. Subsequent to the NHMRC report on the matter, an action plan and the NAC were developed, and it now seems that asthma mortality may be falling.

Following the International Consensus Report on Asthma Management, Toogood (1993) stated that challenges for medical research include epidemiologic studies to critically assess the impact of recommended treatment regimens on therapeutic outcome. The following study examined regional anti-asthma medication sales, morbidity (indicated by hospital admissions) and asthma mortality (asthma deaths) in Tasmania. The objective of the study was to examine if anti-asthma medication sales were changing in line with current therapeutic guidelines (i.e. greater emphasis on anti-inflammatory and anti-allergic therapy), if there were any regional differences within the State and if there was any association between anti-asthmatic medication sales and asthma morbidity and mortality.

CHAPTER 4

Anti-Asthmatic Pharmacoepidemiological Trends in Tasmania from April 1991 to April 1994

4.1 SUMMARY

Pharmacoepidemiological data on the use of anti-asthmatic medications in Tasmania were examined following the recent shift in emphasis to preventive therapy, and compared with national prescribing trends. The prescribing of anti-asthmatic drugs in Tasmania was studied at six-monthly intervals using data retrospectively obtained from computerised dispensing systems from almost one-third of all community pharmacies within the State. The data collection included all prescriptions dispensed in the pharmacies, irrespective of supply under the Pharmaceutical Benefits Scheme, the Repatriation Pharmaceutical Benefits Scheme, as a private prescription or as a recordable S3 item.

Results of the pooled data were quantified by using the Defined Daily Doses (DDDs) dispensed. Drugs were grouped into β-agonists, inhaled corticosteroids, methyl xanthines, ipratropium bromide and sodium cromoglycate, then converted to Defined Daily Doses (DDDs)/1000 population/day by extrapolation to the entire Tasmanian population on the basis of prescription volume. National anti-asthmatic prescribing data was provided by the Drug Utilisation Subcommittee (DUSC), and compared with Tasmanian prescribing trends.

Tasmanian prescribing was generally similar to the national data, with large increases in the prescribing of inhaled corticosteroids (an increase of 61% to 22.8 DDDs/1000 population/day) and ipratropium bromide (an increase of 138% to 7.2 DDDs/1000 population/day), a decrease in theophylline usage (a decrease of 43% to 6.6 DDDs/1000 population/day) and an increase in sodium cromoglycate prescribing (an increase of 52% to 1.5 DDDs/1000 population/day) over the period of the study. The prescribing of β-agonists remained fairly stable over the period of the study although there was a large decrease of 36% in the ratio of β-agonists:inhaled corticosteroids DDDs dispensed.

Recent trends in the prescribing of anti-asthmatic medications were found to be similar to national trends. Tasmanian prescribing of anti-asthmatic medications appear to be changing in line with current management guidelines with greater emphasis on the prescribing of inhaled corticosteroids.

4.2   INTRODUCTION

As discussed earlier in Chapter 2, there has been an alarming increase in asthma related morbidity and mortality in most industrialised countries, despite medication sales being at an all time high. There has been a disturbing suggestion that the increase in asthma drug sales, particularly bronchodilators, may not be a result of, but rather may be contributing to, increasing asthma morbidity and mortality (Barnes and Chung, 1992). There are two hypotheses to explain this paradoxical increase in morbidity with increasing use of bronchodilators. The 'severity hypothesis' suggests that increasing bronchodilator sales are an indicator of increasing disease severity whereas the 'β-agonist hypothesis' suggests that β-agonists are responsible for the adverse asthma outcomes independent of disease severity (Suissa et al, 1994). Current management guidelines suggest a reduction in the reliance on bronchodilator therapy with greater emphasis placed on the early introduction of anti-inflammatory therapy.

Pharmacoepidemiology is a relatively new discipline involving the study of the patterns and effects of therapeutic drug use within populations (Hartzema 1992; Henry, 1988) and may be used to observe and evaluate the results of ongoing medical care (Strom, 1994). In this study analyses of secular trends were used to examine the asthma paradox and regional differences in prescribing of anti-asthmatics within Tasmania. In addition, prescribing trends were examined to see whether prescribing habits were changing as recommended by current guidelines (Anonymous, 1992; Tse and Bridges-Webb, 1993) with a greater emphasis on preventive (anti-allergic and anti-inflammatory) medication.

4.3   METHOD

4.3.1   Collection of computerised pharmacy dispensing data

Tasmania lends itself to pharmacoepidemiology studies well with a relatively stable population of around 470,000. The trends in anti-asthmatic medication prescribing within Tasmania were examined using community pharmacy computer based dispensing records. The majority of pharmacy dispensing computer systems used within the State are either Amfac-Chemdata III and IV (Canberra, ACT), or Tasmanian Pharmacy Computer Users Group (TPCUG; Lindisfarne, Tas) systems. Both of these systems offer a drug summary facility whereby all drugs dispensed over a designated time period can be counted from the stored computer records and printed out in a drug summary list. This list contains all PBS/RPBS, general, private and S3 recordable drugs (by brand name) and the number of unit quantities dispensed (an example is shown in Figure 4.1 for both Amfac-Chemdata and TPCUG systems). This computer generated drug summary list is far more comprehensive than PBS/RPBS data alone as only concessional, pensioner and 'safety net' MDI prescriptions are recorded on PBS/RPBS databases. The method for obtaining drug summary lists from both the Amfac-Chemdata and TPCUG pharmacy dispensing computer systems are shown in Figures 4.2 and 4.3 respectively.

All pharmacies dispensing PBS items within the State were sent a letter in May 1992 explaining the project (Appendix 1), an outline for obtaining drug summary lists from both the Amfac-Chemdata and TPCUG pharmacy dispensing computer systems (identical to Figures 4.2 and 4.3 respectively), and a return postage paid envelope. The letter invited pharmacists to participate by sending monthly dispensing data (retrieved from their dispensing computer systems via the drug summary facility) for the months of April 1991, October 1991 and April 1992 in a return postage paid envelope to the Tasmanian School of Pharmacy. The drug

summary lists were then examined for the anti-asthmatic medications shown in Table 4.1 with the drugs and quantities entered into a spreadsheet for further analysis. This is discussed further in Sections 4.3.2 and 4.3.3. A virtually identical letter was sent out to all pharmacies dispensing PBS items within the State in May 1993 which invited pharmacists to contribute October 1992 and April 1993 data. A notice asking for contributing pharmacies was also included in the TPCUG newsletter (Appendix 2). A final letter was sent out in May 1994 inviting pharmacies to contribute October 1993 and April 1994 data, as well as offering $10 in payment from a dedicated research grant to offset time and paper costs. The data were collected every six months during April and October to minimise any end of year bias associated with the 'safety net' scheme.

It was not a requirement that the same pharmacies participate throughout the course of the study as all data were pooled for each month into two regions. This was stressed in both follow-up letters. Data were pooled into Southern Tasmania (002 telephone area code including Hobart) and Northern Tasmania (003 and 004 area code encompassing Launceston, the North East and the North West of the State).

Not all pharmacies were able to provide comprehensive prescription data, either due to technical difficulties in retrieving data from the computer or for perceived confidentiality reasons (in relation to total dispensing volumes). For calculations involving DDDs/1000 population/day it was necessary that the total prescriptions (dispensing volume) for each pharmacy be provided although it was not necessary that the collection period was a full month (see also Section 4.3.2). Unfortunately some pharmacies did not provide dispensing volumes, therefore their data could not be used for DDDs/1000 population/day calculations.

Table 4.1   *Anti-asthmatic drugs (generic and brand name) shown with their Nordic defined daily dose (DDD) recorded from drug summary lists.*

Generic drug	Brand names	Nordic DDD	Drug type
Salbutamol	Asmol® Respolin® Respax® Ventolin®	10 mg Inhal. solution 0.8 mg Inhal. aerosol/powder 12 mg oral 12 mg parenteral	β-agonist
Terbutaline	Bricanyl®	20 mg Inhal. solution 2 mg Inhal aerosol/powder 15 mg oral 15 mg parenteral	β-agonist
Salmeterol (First listed on the PBS co-payment schedule as an authority item in 1995.)	Serevent®	0.1 mg Inhal. aerosol/powder	β-agonist
Fenoterol (MDI delisted from the PBS co-payment schedule in 1990.)	Berotec®	4 mg Inhal. solution 0.6 mg Inhal aerosol/powder 10 mg oral 10 mg rectal	β-agonist
Orciprenaline	Alupent®	6mg Inhal. aerosol 60 mg oral 60 mg parenteral	β-agonist
Beclomethasone dipropionate	Aldecin® Becloforte® Becotide®	1.5 mg Inhal. solution 0.8 mg Inhal aerosol/powder	inhaled corticosteroid
Budesonide (First listed on the PBS co-payment schedule in 1991.)	Pulmicort®	1.5 mg Inhal. solution 0.8 mg Inhal aerosol/powder	inhaled corticosteroid
Theophylline	Austyn® Elixophylline® Nuelin® Slo-bid® Theodur®	0.4 g oral 0.4 g parenteral 0.4 g rectal	methyl xanthine
Aminophylline	Cardophyllin®	0.6 g oral 0.6 g parenteral 0.6 g rectal	methyl xanthine
Choline theophyllinate	Choledyl®	0.6 g oral 0.6 g parenteral 0.6 g rectal	methyl xanthine
Ipratropium bromide	Atrovent®	0.12 mg Inhal. aerosol/powder 0.3 mg Inhal. solution	anti-cholinergic
Sodium cromoglycate	Intal®	80 mg Inhal. powder/solution 40 mg Inhal. aerosol	anti-allergic (excluding inhaled corticosteroids)

DDDs adapted from Anatomical Therapeutic Chemcial (ATC) classification index, January 1993, WHO Collaborating Centre for Drug Statistics Methodology, Oslo, Norway.

However, these data were used in the analysis of regional differences in prescribing since the relative dispensing ratios of different anti-asthmatic drugs were examined and dispensing volumes were not necessary for this analysis (see also Section 4.3.3).

Those pharmacies that provided dispensing volumes for the entire month were compared with the average Australian dispensing volumes per month. This data was provided by the Pharmacy Guild of Australia from a random sample of 263, 244, 271 and 273 pharmacies throughout Australia for 1991, 1992, 1993 and 1994 respectively. These figures were compared with the Tasmanian dispensing volume per month using two-tailed one sample t-tests (Instat® Ver 2.0, Graphpad Software, San Diego, CA, USA).

4.3.2   Analysis of prescription data: calculation and analysis of Statewide DDDs/1000 population/day

The calculation of Statewide DDDs/1000 population/day required drug summary data from responding pharmacies to be extrapolated to the total Tasmanian population. It was not necessary that the data be complete for the entire month. This calculation of Statewide DDDs/1000 population/day was performed in two steps. Firstly, the drug summary data was converted to a ratio of anti-asthmatic DDDs dispensed per total prescription volume. The second step involved extrapolating this ratio to find the DDDs of anti-asthmatics dispensed to the entire Tasmanian population. These calculations are described in more detail in Sections 4.3.2.1 and 4.3.2.2.

4.3.2.1   Calculation of DDDs dispensed per total prescription volume

The unit quantities of anti-asthmatic drugs and the total prescription volumes (PBS/RPBS, general and private) from which they were dispensed were recorded

from the drug summary lists and entered into a spreadsheet (Microsoft Excel® 4.0, Microsoft Corporation, Redmond, WA, USA). An example of the spreadsheet layout is shown in Figure 4.4. The unit quantities of each drug dispensed were then converted into DDDs dispensed and grouped into one of five classes of anti-asthmatic drugs. Drugs were grouped into β-agonists, inhaled corticosteroids, methyl xanthines, ipratropium bromide and sodium cromoglycate (see Table 4.1). Each of the five groups were then divided by the total prescription volumes from which they were dispensed giving a ratio of DDDs per total prescriptions for each of the five classes. This was repeated for each pharmacy that provided prescription totals with their drug summary data. Simple descriptive statistics including mean, 95% confidence limits of the mean and standard deviations were calculated for DDDs dispensed to total prescription volume ratios for each of the five groups. This procedure was repeated for each month of sampling.

4.3.2.2 Extrapolation of DDDs dispensed to the Tasmanian population and calculation of DDDs/1000 population/day

Unfortunately, the Pharmacy Guild of Australia had only limited information regarding the number of total prescriptions dispensed per year in Tasmania based on samples of only 10, 20 and 13 pharmacies for 1991, 1992 and 1993 respectively. These sample sizes were less than the number of responding pharmacies in this study. While there is no published information of total prescriptions dispensed in Tasmania, there are reliable data on dispensing under the PBS within the State. Because of this, the number of total prescriptions dispensed per year in Tasmania was estimated by multiplying the number of Tasmanian PBS prescriptions per year by the ratio of Australian total prescriptions to Australian PBS prescriptions. Australian total prescriptions were provided by the Pharmacy Guild of Australia (based on random samples of 263, 244, 271 and 273 pharmacies throughout Australia for 12 months trading up until the 30th of June 1991, 1992, 1993 and 1994 respectively). Australian PBS prescriptions (Government derived figures

published in the Pharmacy Guild of Australia Guild Digest) were obtained from the Pharmacy Guild of Australia.

The estimated total Tasmanian prescriptions were then divided by 466.802, 469.685, 471.400 and 475.100 for 1991, 1992, 1993 and 1994 respectively (Tasmanian population divided by 1000; 1994 population estimated at 30th June 1994, source: Australian Bureau of Statistics) and 365 (days in a year) to give total prescriptions/1000 population/day. This number was then multiplied by the mean, 95% confidence interval and standard deviation DDDs dispensed:total prescription volume ratio for each class of anti-asthmatic drug to give the mean, 95% confidence interval and standard deviation for DDDs/1000 population/day for each class of drug for each month. The calculation outline is shown in Figure 4.5 with an example calculation. These Tasmanian DDDs/1000 population/day data for October 1991, 1992 and 1993 were then compared with DUSC national data from 1991 to 1993 using two-tailed one sample t-tests (Instat® Ver 2.0, Graphpad Software, San Diego, CA, USA).

4.3.3   Analysis of prescription data: analysis of regional differences in prescribing

To calculate DDDs/1000 population/day, the amounts of drugs utilised by a given sample population must be divided by a known population size. DDDs/1000 population/day could not be used as a measure of drug utilisation in both South and North regions as population boundaries were unable to be defined from the responding pharmacies and estimates of PBS prescriptions for each region were needed to calculate DDDs/1000 population/day. Regional differences were studied by examining the relative prescribing of each of the five anti-asthmatic drug groups (β-agonists, inhaled steroids, methyl xanthines, ipratropium bromide and sodium cromoglycate). Drug summary data that did not include dispensing volumes (i.e.

Amfac-Chemdata

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- DAILY TRADING SUBTOTALS -    14/5/92  ▬▬▬▬▬. page   1.
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DRUG ID	SALES VALUE	BASE PRICE	PROFIT	GOVT PAYMENT	NO	WHS NO
ATROMID-S CAP 500MG 100	15.00	8.88	6.12		1	112458
ATROPINE-SUL AMP 600MCG 5	1.36	1.36			1	
ATROVENT INHALER AER200 1	20.72	20.72			2	######
ATROVENT RESP SOLN20ML 2	50.50	50.50			5	
AUGMENTIN SYRP 125MG/5M 1	13.55	4.87	8.68		1	
AUGMENTIN TAB 250/125 15	15.86	15.86			2	111013
AUGMENTIN-FT SYRP 250MG 1	15.00	6.96	8.04		1	
BECLOFORTE AERO 250MCG 1	99.00	99.00			4	
BECONASE NAS-SPR AD 1	23.20	9.86	13.34		1	
BECOTIDE INHALER 100MCG 1	103.85	94.16	9.69		8	######
BECOTIDE INHALER 50MCG 1	17.67	17.67			3	######
BETALOC TAB 100MG 60	9.05	9.05				308213
BETNOVATE CRM 0.05%15G 1	5.00					

Tasmanian Pharmacy Computer Users Group

PA PDE	SI PDE	Order	ITEM (minimum 1)	AMOUNTS --->	Dispensed	On hand
068950	112458		ATROMID-S CAPS 500mg --->	2 Rxs =	200	
083534	040592		ATROPT EYE DROPS 1% 15mL ===>	1 Rxs =	1	
062391	318691		ATROVENT INHALER --->	5 Rxs =	5	
071021	240283		ATROVENT NEBUL SOLN 20mL ===>	7 Rxs =	21	
379352	354100		ATROVENT UDV 250mcg (30) --->	3 Rxs =	5	
379360	354092		ATROVENT UDV 500mcg (30) ===>	5 Rxs =	6	
434825	111146		AUGMENTIN FORTE SYR 75mL --->	7 Rxs =	7	
434809	111088		AUGMENTIN FORTE TABS ===>	16 Rxs =	240	
434817	111120		AUGMENTIN SYRUP 75mL --->	12 Rxs =	12	
434795	111013		AUGMENTIN TABS ===>	47 Rxs =	765	
661627	029545		AURORIX TABS 150mg --->	5 Rxs =	300	
015903	178780		AUSTYN CAPS 300mg ===>	2 Rxs =	200	
097004	282087		AVIL TABS 50mg --->	1 Rxs =	50	
073199	096321		AVOMINE TABS 25mg ===>	10 Rxs =	300	
073199	096321		AVOMINE THEOC TABS 25mg --->	1 Rxs =	30	
454672	071862		BACTRIM DS TABS ===>	5 Rxs =	50	
597309	300095		BACTRIM SYRUP 100mL S/F --->	7 Rxs =	2	
534587	080689		BACTROBAN OINT 15g 2% ===>	3 Rxs =	9	
595179	311324		BECLOFORTE INHALER 250 --->	23 Rxs =	23	
485194	193813		BECONASE AQ NASAL SPRAY ===>	13 Rxs =	13	
392464	053330		BECONASE NASAL SPRAY --->	9 Rxs =	9	
502219	304147		BECOTIDE INHALER 100 ===>	24 Rxs =	24	
281212	155713		BECOTIDE INHALER 50 --->			
002	021915		BECOTIDE ROTACAPS ===			
			BENADRYL CAPS 50			

Figure 4.1 *An example of drug summary reports received from both Amfac-Chemdata and TPCUG pharmacy dispensing computer systems.*

Amfac-Chemdata instructions:

F2 - END DAY → Do you want a cancellation report? N - no.

Select End of Day run type. M - manual.

End of Day Procedure Options.
R - reporting.

OK to run reporting? F1 - (FROM ARCHIVE)

* It is important to select the F1 option (From Archive) otherwise it is not possible to nominate a time period.

Summary to Labels printer, Repeats printer, Notes printer, Alternate printer, Screen.
(Please select appropriate printer.)

Which type of paper? W - wide continuous.

Select criteria for detailed printout. N - no script printout.

Drug summary printout required? Y - yes.

Insert Dual History Archive Disk in floppy drive.

Archive Disk mounted.

Dates run from : dd/mm/yy

to : dd/mm/yy

Number of Dual History records on disk : ####

Enter dates for Summary : Start Date
to : End Date

F1 - LONG SEARCH

OK to print trading totals? Y - yes ** → Print ACCNT audit trail? N - no → Q - quit from End Day.

** These are needed to calculate asthma prescriptions as the percentage of total prescriptions. If you are not prepared to divulge the sales figures, could you please forward the first two columns of Trading Totals - Table 1 (i.e. No. Items for G NHS, S NHS, Pens., Entl., Conc., Repat, D Bag, Gvtot, Priv., TOTAL).
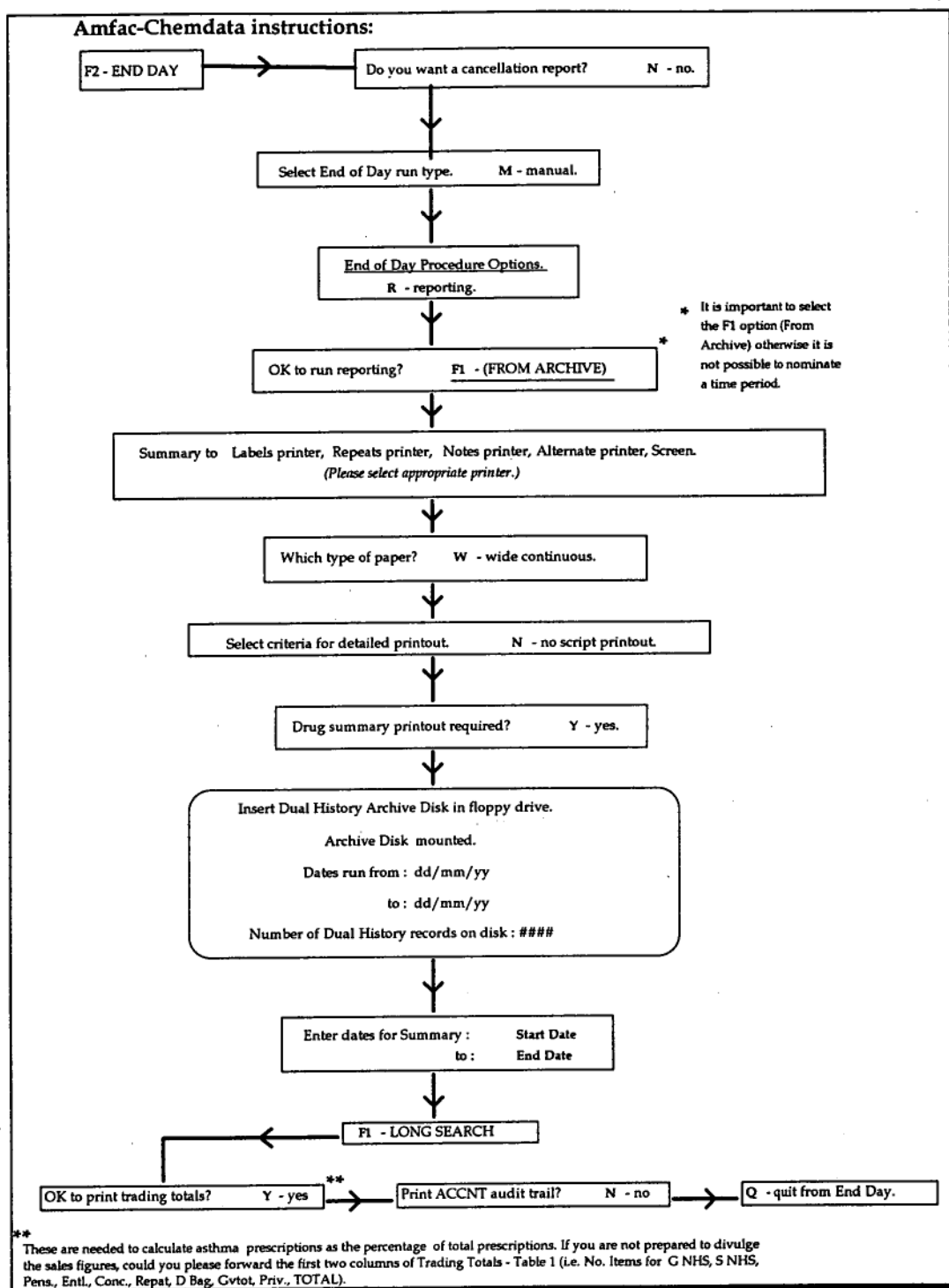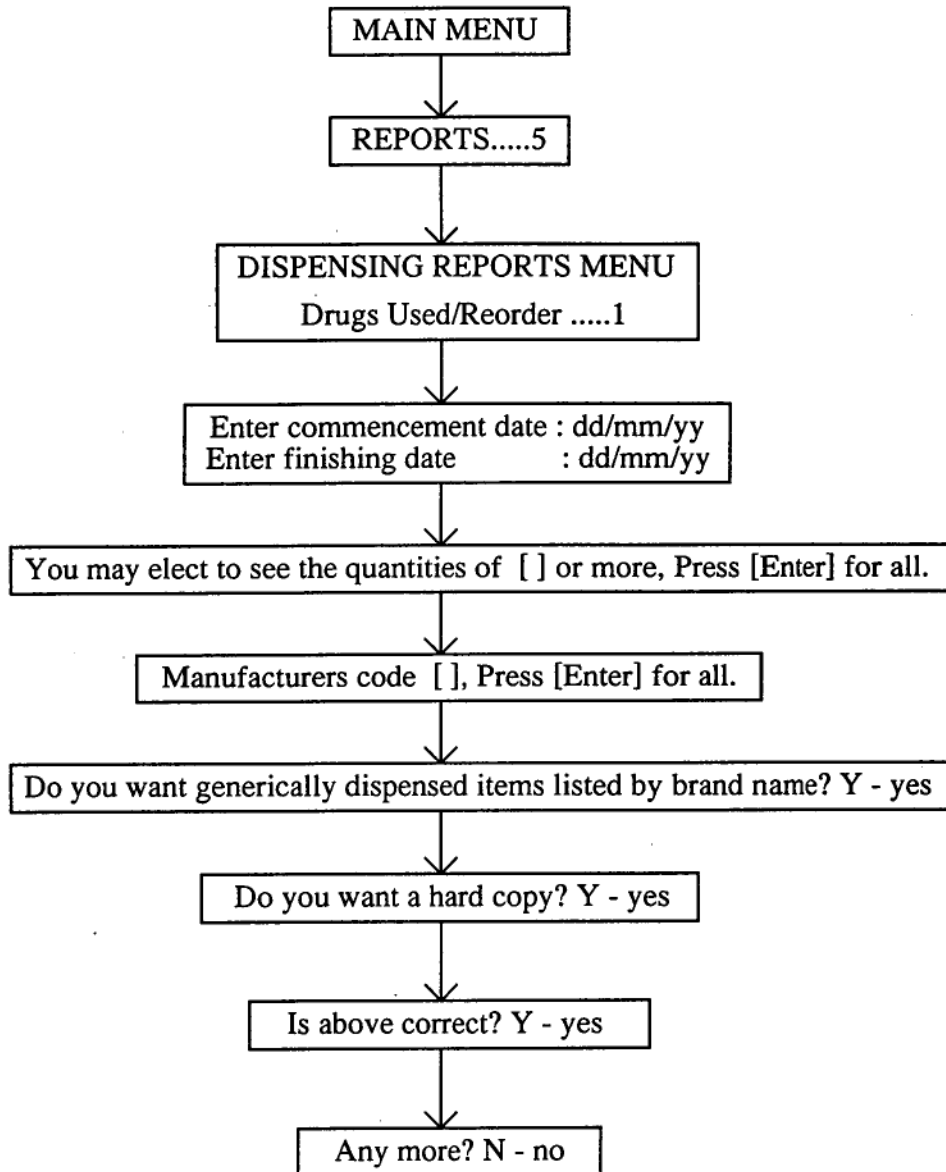
Figure 4.2 *Outline for obtaining drug summary lists from Amfac-Chemdata computer dispensing system.*

Tasmanian Pharmacy Computer Users Group Instructions:

<u>It is essential that data from the entire month is analysed.</u>
i.e. all data for April 1993, October 1993 and April 1994.

```
              ┌──────────────────┐
              │   MAIN MENU      │
              └──────────────────┘
                       ↓
              ┌──────────────────┐
              │  REPORTS.....5   │
              └──────────────────┘
                       ↓
         ┌──────────────────────────────┐
         │ DISPENSING REPORTS MENU       │
         │   Drugs Used/Reorder .....1   │
         └──────────────────────────────┘
                       ↓
       ┌───────────────────────────────────────┐
       │ Enter commencement date : dd/mm/yy     │
       │ Enter finishing date        : dd/mm/yy │
       └───────────────────────────────────────┘
                       ↓
  ┌────────────────────────────────────────────────────────┐
  │ You may elect to see the quantities of [ ] or more,      │
  │ Press [Enter] for all.                                   │
  └────────────────────────────────────────────────────────┘
                       ↓
     ┌────────────────────────────────────────────────┐
     │ Manufacturers code [ ], Press [Enter] for all.   │
     └────────────────────────────────────────────────┘
                       ↓
  ┌────────────────────────────────────────────────────────┐
  │ Do you want generically dispensed items listed by        │
  │ brand name? Y - yes                                      │
  └────────────────────────────────────────────────────────┘
                       ↓
          ┌──────────────────────────────┐
          │ Do you want a hard copy? Y - yes │
          └──────────────────────────────┘
                       ↓
            ┌──────────────────────────┐
            │ Is above correct? Y - yes │
            └──────────────────────────┘
                       ↓
             ┌──────────────────────┐
             │ Any more? N - no     │
             └──────────────────────┘
```

N.B. It is advisable that the above procedure be carried out after closing to avoid tying up the computer.
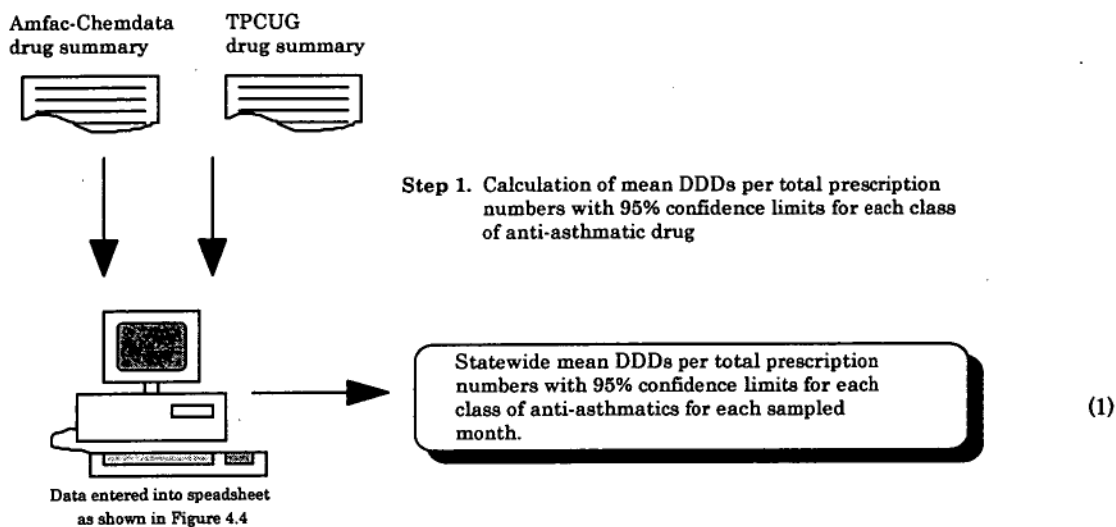
Figure 4.3   *Outline for obtaining drug summary lists from TPCUG computer dispensing system.*
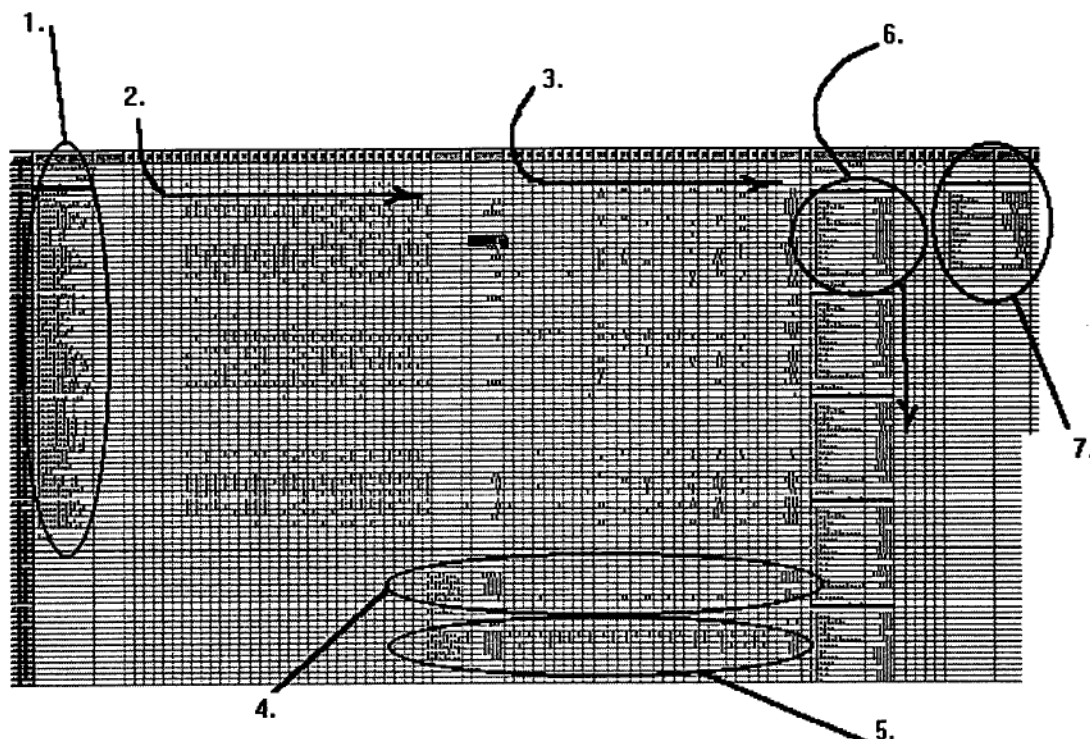
1. List of anti-asthmatic drugs and unit quantities.

2. Quantities of anti-asthmatic drugs dispensed for each individual pharmacy in each column.

3. Anti-asthmatic DDDs dispensed for each individual pharmacy in each column.

4. Anti-asthmatic DDDs dispensed and total prescription numbers for each individual pharmacy in each column grouped into β_2-agonists, inhaled steroids, methyl xanthines, ipratropium bromide and sodium cromoglycate.

5. Grouped anti-asthmatic DDDs dispensed for each individual pharmacy (from 4) divided by the corresponding total prescriptions for that pharmacy.

6. Descriptive statistics performed on each class of anti-asthmatic (from 5).

7. Descriptive statistics performed on total prescription numbers.

Figure 4.4   *An example of Excel® spreadsheet layouts used to calculate the DDDs dispensed per total prescription numbers. This was then used in the calculation of DDDs/1000 population/day for each sample month (shown in Figure 6.3).*

Amfac-Chemdata
drug summary

TPCUG
drug summary

Step 1. Calculation of mean DDDs per total prescription numbers with 95% confidence limits for each class of anti-asthmatic drug

Statewide mean DDDs per total prescription numbers with 95% confidence limits for each class of anti-asthmatics for each sampled month. (1)

Data entered into speadsheet
as shown in Figure 4.4

Step 2. Calculation of Tasmanian DDDs per 1000 people per day

$$\text{Tasmanian PBS prescriptions} \times \left[\frac{\text{Australian total prescriptions per year}}{\text{Australian PBS prescriptions per year}} \right] = \text{Estimated Tasmanian total prescriptions per year} \quad (2)$$

$$\text{Estimated Tasmanian total prescriptions per year} \times \frac{1}{(\text{Tas. population}/1000)} \times \frac{1}{365} = \text{Tasmanian total prescriptions per 1000 population per day} \quad (3)$$

$$\text{Tas. total prescriptions per 1000 population per day} \times \text{Tasmanian mean DDDs for each class of anti-asthmatics per total prescriptions} = \text{DDDs per thousand population per day for each class of anti-asthmatic} \quad (4)$$

$$\text{Tas. total prescriptions per 1000 population per day} \times \text{95\% confidence limits on Tasmanian mean DDDs for each class of anti-asthmatics per total prescriptions} = \text{95\% confidence limits on DDDs per thousand population per day for each class of anti-asthmatic} \quad (5)$$

Example calculation for April 1991 inhaled corticosteroids.

Mean DDDs per total prescription numbers = 0.5307 *(from 1)*
95% confidence limits on mean DDDs per total prescription numbers = 0.074

$$2{,}640{,}507 \times \left[\frac{165{,}217{,}476}{96{,}300{,}259} \right] = 4{,}530{,}184 \quad \text{Estimated Tasmanian total prescriptions per year} \quad \textit{(from 2)}$$

$$4{,}530{,}184 \times \frac{1}{(466{,}802/1000)} \times \frac{1}{365} = 26.59 \quad \text{Tasmanian total prescriptions per 1000 population per day} \quad \textit{(from 3)}$$

Mean: $26.59 \times 0.5307 = 14.11$ DDDs/1000 population/day *(from 4)*

95% confidence: $26.59 \times 0.074 = 1.96$ DDDs/1000 population/day *(from 5)*

Figure 4.5 *Outline of calculation of DDDs per thousand population per day with 95% confidence limits.*

could not be used for calculation of DDDs/1000 population/day) were combined with data used for calculation of DDDs/1000 population/day for analysis of regional differences. The dispensed quantity of each dosage form for each drug was grouped into Southern, Northern and Statewide data and converted to DDDs. Drugs were grouped into the five classes of anti-asthmatics. Statewide prescribing trends for each class of compound were examined over the sampling period using a chi-square test for each region (Statview® 4.01, Abacus Concepts Inc., Berkeley CA, USA).

The regional differences in prescribing between the North and South were examined for each class of compound using a chi-square test for each month. Southern and Northern prescribing trends with time for each class of compound were examined over the sampling period using a chi-square test for each region.

4.3.4 Australian and Tasmanian asthma morbidity and mortality

Tasmanian and Australian asthma mortality data were obtained from the Australian Bureau of Statistics. Tasmanian morbidity data (Statewide and regional; based on hospital admissions with asthma as the primary diagnosis) were obtained from the Tasmanian State Health Department for each month of sampling. Statewide hospital admissions included both public and private hospital admissions. Regional data only included public hospital admissions due to confidentiality agreements with the private hospitals. These Statewide and regional measures of morbidity were plotted with the Statewide and regional ratios of β-agonist: inhaled steroid DDDs dispensed. A Spearman rank correlation test was used to test for any association between Statewide morbidity and the relative prescribing of relieving and preventive medication.

4.4   RESULTS

4.4.1   Analysis of prescription data

The numbers of responding pharmacies for each time period are shown in Table 4.2. Australia-wide average dispensing volumes are shown in Table 4.3. The mean monthly dispensing volumes for responding pharmacies that included prescription totals were significantly lower than the Australian averages for community pharmacies (provided by the Pharmacy Guild of Australia) in 4 out of 7 sampled months. Significant differences were found in monthly pharmacy prescription volumes for April 1991 (t=3.05, df=25, p < 0.01), April 1993 (t=2.05, df=37, p < 0.05), October 1993 (t=2.28, df=41, p < 0.05) and April 1994 (t=3.42, df=40, p < 0.01). It should be noted, however, that the average Tasmanian monthly dispensing volumes from responding pharmacies could only be calculated from those pharmacies that provided complete monthly data with prescription totals. Therefore, these figures should only be seen as a guide to the dispensing volumes of all responding pharmacies in this study. In addition, true dispensing volumes per pharmacy may be lower in Tasmania than the national average as the Tasmanian population:pharmacy ratio is almost 7% lower than the national average (Guild Digest, 1994).

4.4.2   Tasmanian prescribing trends from April 1991 to April 1994

DDDs/1000 population/day for each of the 5 groups of anti-asthmatic medications and totals were calculated as outlined in Section 4.3.2. These are shown in Table 4.4. It was found that the total anti-asthmatic DDDs/1000 population/day dispensed in Tasmania increased slightly over the period of the study with a peak in October 1992 but dropped in April 1994.

Table 4.2   *The number of responding pharmacies, pharmacy dispensing volumes and total prescriptions analysed for anti-asthmatics in the North, South and Statewide over the course of the study. Data contributed by all pharmacies were used to examine regional relative differences in prescribing. Data from those pharmacies that provided prescription totals were also used to calculate DDDs/1000 population/day. Data from pharmacies that included prescription totals and were complete for the entire month were used as a guide to the dispensing volumes of the responding pharmacies.*

	April 91	October 91	April 92	October 92	April 93	October 93	April 94
South contributing pharmacies[a]	15 (20.0%)	21 (28.0%)	23 (30.7%)	22 (29.7%)	21 (28.3%)	24 (32.4%)	23 (31.1%)
South total prescriptions[b]	21,663 (13)	37,719 (18)	44,330 (21)	46,740 (22)	40,467 (21)	50,897 (24)	43,579 (23)
South total prescriptions per month per pharmacy (mean ± SD)[c]	1,666 ± 584 (13)	2,096 ± 808 (18)	2,189 ± 1,075 (20)	2,168 ± 1,088 (21)	1,986 ± 664 (20)	2,121 ± 724 (24)	1,955 ± 743 (22)
North contributing pharmacies[a]	15 (20.5%)	16 (22.9%)	24 (34.2%)	20 (28.6%)	20 (28.6%)	19 (27.1%)	20 (28.6%)
North total prescriptions[b]	28,340 (13)	33,033 (14)	54,286 (22)	47,989 (19)	47,787 (19)	48,022 (19)	46,064 (20)
North total prescriptions per month per pharmacy (mean ± SD)[c]	2,180 ± 844 (13)	2,360 ± 1,056 (14)	2,468 ± 1,130 (22)	2,525 ± 1,244 (19)	2,535 ± 1,173 (18)	2,595 ± 1,275 (18)	2,372 ± 1,209 (19)
State contributing pharmacies[a]	30 (20.2%)	37 (25.5%)	47 (32.4%)	42 (29.2%)	41 (28.5%)	43 (29.9%)	43 (29.9%)
State total prescriptions[b]	50,003 (26)	70,752 (32)	98,616 (43)	94,729 (41)	88,254 (40)	98,919 (43)	89,643 (43)
State total prescriptions per month per pharmacy (mean ± SD)[c]	1,923 ± 758 (26)	2,211 ± 919 (32)	2,335 ± 1,100 (42)	2,338 ± 1,162 (40)	2,246 ± 967 (38)	2,324 ± 1,013 (42)	2,148 ± 996 (41)

[a]   Number of pharmacies contributing data with the response rate shown in parentheses. Used for analysis of regional differences in prescribing where prescription totals not needed.

[b]   From pharmacies contributing data with prescription totals but not necessarily for the entire month. The number of responding pharmacies are shown in parentheses. Data from these respondents were used for DDDs/1000 population/day calculations.

[c]   Dispensing volumes calculated only from those pharmacies that provided complete monthly data with prescription totals. The number of responding pharmacies with complete data are shown in parentheses.

Table 4.3   *Australia-wide average dispensing volumes from community pharmacies provided by the Pharmacy Guild of Australia for the financial year.*

	1990-91	1991-92	1992-93	1993-94
Sample size (number of pharmacies)	263	244	271	273
Average total prescription volume per year (PBS, RPBS, general and private)	30,876	30,448	33,393	34,852
Average total prescription volume per week (PBS, RPBS, general and private)	594	586	642	670
Average total prescription volume per month (PBS, RPBS, general and private)*	2,376	2,344	2,568	2,680

*calculated from prescription volume per week

Drug utilisation trends (in terms of DDDs/1000 population/day) for each of the five groups of anti-asthmatics are shown in Figure 4.6. It was found that the DDDs/1000 population/day of β-agonists dispensed in Tasmania was relatively similar for April and October 1991, fell slightly in April 1992, then increased in October 1992 before decreasing steadily for the remaining months up to and including April 1994. It was found that the DDDs/1000 population/day of inhaled corticosteroids increased steadily to a peak value in October 1992 before levelling out for the remaining months up to and including April 1994. The DDDs/1000 population/day of methyl xanthines decreased steadily by 43% over the period of the study as shown in Figure 4.6. The DDDs/1000 population/day of ipratropium bromide increased steadily to a peak value in October 1993 before levelling out for April 1994 to remain at greater than twice the level of April 1991 prescribing. It was found that the DDDs/1000 population/day of sodium cromoglycate increased steadily to a peak value in October 1993 (approximately a 60% increase over April 1991) before levelling out for April 1994.

Relative prescribing trends of each class of anti-asthmatic were examined for the State over the period of the study. Overall, there were relatively large increases in

inhaled corticosteroid and ipratropium use, an increase in sodium cromoglycate use and a decline in methyl xanthine use during the time period from April 1991 to April 1994 (chi-square=23088, df=24, p < 0.0001). β-Agonist use fell after April 1991 and was fairly stable for the remainder of the study.

4.4.3 Tasmanian prescribing and comparison to Australia-wide DUSC data

DUSC data is obtained from a stratified random sample of pharmacies throughout Australia. The data obtained from October for 1991, 1992 and 1993 was compared with the DUSC data for 1991, 1992 and 1993 (Table 4.5 and Figure 4.7).

As can be seen from Figures 4.6 and 4.7, general similarities were found between the Tasmanian DDDs/1000 population/day and the DUSC data. These similarities included (i) a slight increase in β-agonist DDDs/1000 population/day in 1992 before levelling out with the Tasmanian levels dropping slightly, (ii) large increases in the prescribing of inhaled corticosteroids and ipratropium bromide (iii) a decrease in methyl xanthine prescribing, and (iv) an increase in sodium cromoglycate prescribing. The total anti-asthmatics (both DUSC data and Tasmanian data) remained fairly stable over the period of the study.

It was found that there were statistically significant differences in Tasmanian utilisation of anti-athmatics compared to the data obtained from DUSC. The prescribing of total anti-asthmatics in Tasmania were significantly higher than national DUSC data in 1991 (t=2.62, df=31, p < 0.05) and 1992 (t=2.93, df=40, p < 0.01). These differences are clearly shown in Figure 4.8.

The utilisation of β-agonists in Tasmania was significantly higher than the DUSC national data for 1991 (t=3.35, df=31, p < 0.01), 1992 (t=3.78, df=40, p < 0.001)

and 1993 (t=2.40, df=41, p < 0.05). These differences are clearly shown in Figure 4.9. It should, however, be noted that the DUSC data for β-agonist DDDs/1000 population/day displayed a dip in 1991 compared to the previous and following years, as can be seen in Figure 4.7.

The dispensing of inhaled corticosteroids fluctuated in Tasmania in comparison to national DUSC data, with Tasmanian DDDs/1000 population/day significantly higher than DUSC figures for 1991 (t=2.71, df=31, p=0.01). These differences are shown in Figure 4.10. The prescribing of methyl xanthines was significantly higher than national DUSC data for 1992 (t=2.36, df=40, p < 0.05) as shown in Figure 4.11. There were no significant differences in Tasmanian prescribing of ipratropium bromide in comparison to the national DUSC data for the period of the study (Figure 4.12). The prescribing of sodium cromoglycate fluctuated in comparison to national DUSC data with Tasmanian DDDs/1000 population/day significantly higher than DUSC data for 1992 (t=2.28, df=40, p < 0.05) and 1993 (t=2.18, df=41, p < 0.05). These differences in sodium cromoglycate prescribing are shown in Figure 4.13.

4.4.4 Regional prescribing trends

Regional utilisation trends for each class of anti-asthmatic were examined over the period of the study. Southern Tasmania showed a large increase in inhaled corticosteroid use, a smaller increase in ipratropium bromide and sodium cromoglycate use and a decline in methyl xanthine use over the time period April 1991 to April 1994 (chi-square=10682, df=24, p < 0.0001). β-Agonist use fell after April 1991 and was fairly stable for the remainder of the study.

Similarly, Northern Tasmania showed a large increase in inhaled corticosteroid use, an increase in ipratropium bromide and sodium cromoglycate use and a

decline in methyl xanthine use from April 1991 to April 1994 (chi-square=13394, df=24, p < 0.0001). β-Agonist use fell after April 1991 and was fairly stable for the remainder of the study.

4.4.5   Regional differences in prescribing

Regional differences in prescribing for each of the classes of anti-asthmatics are summarised in Table 4.6. Regional differences in the relative prescribing of anti-asthmatic classes between the South and North of the State were examined using a chi-square test for each month of sampling. It was found that in April 1991 among the anti-asthmatic drug classes there were significantly higher levels of prescribing of β-agonists in the South, and higher levels of methyl xanthine and ipratropium bromide prescribing in the North (chi-square=331, df=4, p < 0.0001). This same pattern continued in October 1991 (chi-square=439, df=4, p < 0.0001), April 1992 (chi-square=1078, df=4, p < 0.0001), October 1992 (chi-square=492, df=4, p < 0.0001) and April 1993 (chi-square=1158, df=4, p < 0.0001). In addition, in April 1993 there were relatively higher levels of sodium cromoglycate prescribed in the South of the State. In October 1993 there were higher levels of inhaled corticosteroids prescribed in the South, higher levels of ipratropium prescribed in the North with little significant difference in the prescribing of β-agonists, methyl xanthines and sodium cromoglycate (chi-square=1340, df=4, p < 0.0001). In April 1994, a similar pattern continued with significantly higher levels of prescribing of β-agonists and sodium cromoglycate in the South, and higher levels of methyl xanthines and ipratropium prescribing in the North (chi-square=1282, df=4, p < 0.0001).

These regional differences are best illustrated in Figures 4.14, 4.15, 4.16, 4.17 and 4.18 for β-agonists, inhaled corticosteroids, methyl xanthines, ipratropium and sodium cromoglycate respectively. These figures show the dispensing of each

class of anti-asthmatic drug, expressed as a percentage of total anti-asthmatic DDDs dispensed, in each region, and Statewide for each sampled month. It can be seen that in general terms there was a greater percentage of β-agonists (Figure 4.14), inhaled corticosteroids (Figure 4.15) and sodium cromoglycate (Figure 4.18) and less methyl xanthines (Figure 4.16) and ipratropium bromide (Figure 4.17) dispensed as a percentage of total anti-asthmatic drugs in Southern Tasmania compared to Northern Tasmania.

4.4.6 Australian and Tasmanian asthma morbidity and mortality trends

Australian and Tasmanian mortality data and Tasmanian morbidity data (based on hospital admissions) are shown in Table 4.7. It can be seen from Table 4.7 that Tasmanian and Australian asthma mortality death rates per 100,000 population (non age-standardised) are similar. A Spearman rank correlation test was used to test for an association between morbidity and the ratio of β-agonist:inhaled corticosteroid DDDs dispensed Statewide (Figure 4.19) but no association was found (Spearman $r=0.04$, $n=7$, $p > 0.05$). There were no significant differences in mortality rates between Tasmanian and Australian data from 1991 to 1993 ($t=0.94$, $df=2$, $p > 0.05$). Statewide and regional trends in morbidity, mortality and anti-asthmatic drug use are illustrated in Figure 4.20.

Table 4.4   *Figures used in the calculation of total prescriptions per 1000 population and DDDs/1000 population/day of anti-asthmatics dispensed from Tasmanian community pharmacies.*

	April 91	October 91	April 92	October 92	April 93	October 93	April 94
Australian average prescription volume per pharmacy per year (PBS, RPBS, general and private)[a]	30,876	30,448	30,448	33,393	33,393	34,852	34,852
Number of Australian community pharmacies[b]	5,351	5,091	5,091	5,018	5,018	4,980	4,980
Estimated Australian total prescriptions per year[c]	165,217,476	155,010,768	155,010,768	167,566,074	167,566,074	173,562,960	173,562,960
Australian PBS prescriptions per year[d]	96,300,259	94,120,245	94,120,245	105,952,602	105,952,602	115,041,099	115,041,099
Tasmanian PBS prescriptions per year[d]	2,640,507	2,603,906	2,603,906	2,963,280	2,963,280	3,199,956	3,199,956
Estimated Tasmanian total prescriptions per year[c]	4,530,184	4,288,487	4,288,487	4,686,484	4,686,484	4,827,786	4,827,786
Tasmanian population[e]	466,802	469,685	469,685	471,400	471,400	475,100	475,100
Tasmanian total prescriptions per 1000 population per day[c]	26.59	25.02	25.02	27.24	27.24	27.84	27.84

Tasmanian DDDs per 1000 population per day   (All numbers in units of DDD/1000 population/day)

	April 91	October 91	April 92	October 92	April 93	October 93	April 94
β-agonists							
Mean	40.70	39.01	34.67	45.37	41.95	42.54	39.36
95% confidence interval of the mean	3.85	3.65	3.26	4.50	4.72	3.99	3.52
Standard deviation	10.02	10.52	10.92	14.71	15.23	13.36	11.78
Inhaled corticosteroids							
Mean	14.11	18.32	18.16	25.41	21.43	25.12	22.75
95% confidence interval of the mean	1.96	2.47	1.88	2.82	2.81	2.89	2.62
Standard deviation	5.10	7.13	6.30	9.23	9.06	9.68	8.77
Methyl xanthines							
Mean	11.61	10.58	8.86	10.34	7.69	7.81	6.58
95% confidence interval of the mean	1.64	1.16	1.39	1.51	1.12	1.02	0.92
Standard deviation	4.27	3.34	4.64	4.93	3.62	3.39	3.08
Ipratropium bromide							
Mean	3.04	4.47	3.55	6.83	6.28	8.05	7.23
95% confidence interval of the mean	0.66	1.11	0.59	1.24	1.20	1.56	1.22
Standard deviation	1.71	3.20	1.99	4.06	3.86	5.22	4.08
Sodium cromoglycate							
Mean	0.97	0.96	0.84	1.50	1.34	1.67	1.47
95% confidence interval of the mean	0.30	0.29	0.18	0.38	0.39	0.34	0.31
Standard deviation	0.78	0.84	0.60	1.24	1.26	1.13	1.05
Total Anti-asthmatics							
Mean	70.43	73.34	66.08	89.45	78.69	85.19	77.40
95% confidence interval of the mean	8.42	8.67	7.31	10.46	10.24	9.79	8.60
Standard deviation	17.63	21.69	19.81	29.24	29.41	29.39	25.70

[a] Source: Personal Communication (Pharmacy Guild of Australia; Canberra).

[b] Source: Personal Communication (Pharmacy Guild of Australia; Canberra).

[c] Estimation calculations outlined in Section 4.3.2 and Figure 4.5.

[d] Source: Personal Communication (Department of Community Services and Health; Canberra).

[e] Source: Australian Bureau of Statistics. October 1993 and April 1994 figures based on Australian Bureau of Statistics estimates.

Table 4.5   *DDD/1000 population/day of anti-asthmatics dispensed from Australian community pharmacies provided by DUSC.*

	1990	1991	1992	1993	1994
β-agonists	39.98	32.78	36.68	37.60	35.14
Inhaled corticosteroids	12.64	14.90	22.63	24.52	22.78
Methyl xanthines	13.70	9.56	8.52	6.99	5.43
Ipratropium bromide	4.66	5.17	7.17	8.99	9.62
Sodium cromoglycate	1.07	0.87	1.06	1.29	1.28
Totals	72.05	63.28	76.01	79.39	74.25

Table 4.6   *Total regional DDDs of anti-asthmatics dispensed from all Tasmanian community pharmacies that contributed data. The number of contributing pharmacies are shown in Table 4.2.*

	β-agonists	inhaled corticosteroids	methyl xanthines	ipratropium bromide	sodium cromoglycate
April 91					
South	37,762	12,973	9,495	2,383	880
North	51,118	18,473	15,328	4,742	1,144
October 91					
South	63,888	29,995	16,786	6,050	1,457
North	68,620	33,145	20,519	8,967	1,933
April 92					
South	63,306	33,196	14,430	4,783	1,760,
North	90,986	45,925	25,599	11,017	2,145
October 92					
South	71,392	39,649	15,295	9,042	2,390
North	83,115	47,784	20,059	14,225	2,890
April 93					
South	55,896	29,684	8,953	6,658	1,840
North	74,356	39,510	16,085	12,083	1,835
October 93					
South	72,226	44,755	12,926	10,800	3,300
North	75,800	42,361	14,533	16,683	2,620
April 94					
South	61,460	34,324	8,675	8,775	2,290
North	68,825	40,248	12,512	14,850	2,540

Table 4.7   *Australian and Tasmanian mortality data and Tasmanian morbidity data based on hospital admissions.*

Australian Asthma Mortality

	Australian population*	Deaths			Deaths per 100,000 population		
		M	F	Total	M	F	Total
1991	17,284,000	335	415	750	1.94	2.40	4.34
1992	17,482,593	332	427	759	1.90	2.44	4.34
1993	17,661,468	326	451	777	1.85	2.55	4.40

Tasmanian Asthma Mortality

	Tasmanian population*	Deaths			Deaths per 100,000 population		
		M	F	Total	M	F	Total
1991	466,802	10	11	21	2.14	2.36	4.50
1992	469,685	9	6	15	1.92	3.19	5.11
1993	471,400	7	6	13	1.48	2.76	4.24

*estimated resident population at 30th June (Source: Australian Bureau of Statistics).

Tasmanian Asthma Morbidity[#]

Month	Year	Northern Tasmania Hospital admissions[a]	Southern Tasmania Hospital admissions[a]	Statewide Total Admissions[b]
April	1991	45	31	82
October	1991	49	31	92
April	1992	59	33	103
October	1992	59	40	114
April	1993	43	54	109
October	1993	42	27	85
April	1994	44	12	62

[#] Source: Personal communication (Department of Community and Health Services; Hobart.)

[a] based on admissions to hospital with ICD primary diagnosis of asthma in public hospitals only

[b] based on admissions to hospital with ICD primary diagnosis of asthma in public and private hospitals
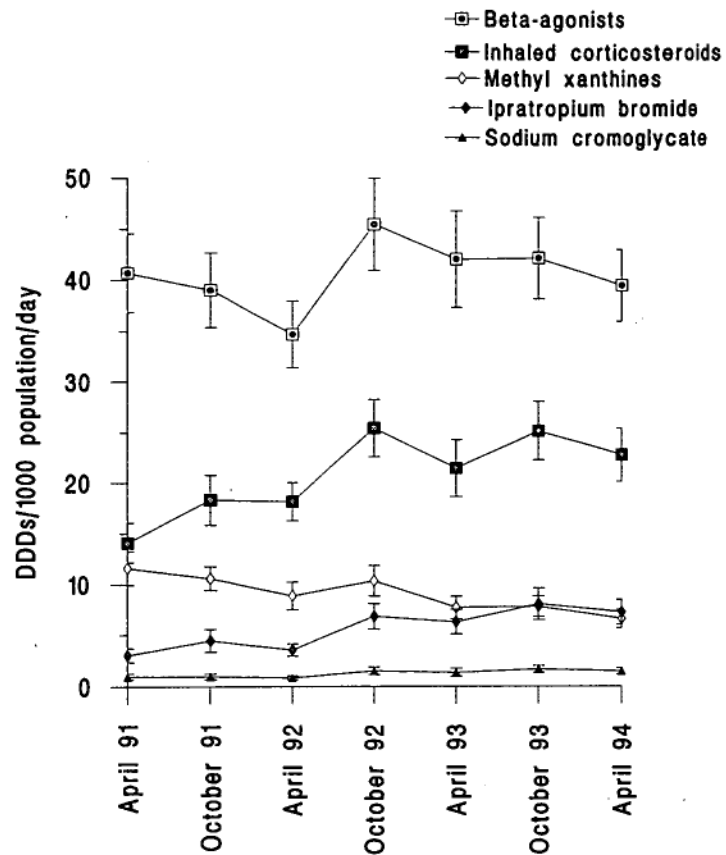
Figure 4.6   *Defined daily dose of anti-asthmatic drugs per thousand population per day dispensed from Tasmanian community pharmacies. Error bars represent ± 95% confidence intervals.*
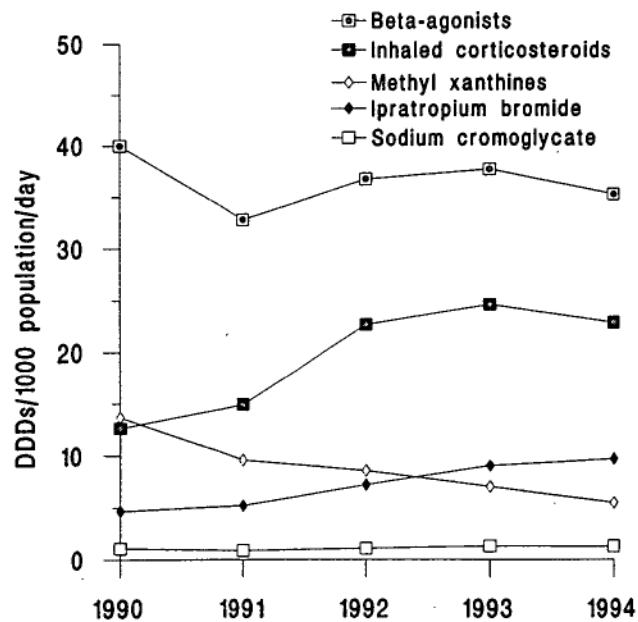

Figure 4.7   *Defined daily dose of anti-asthmatic drugs per thousand population per day from Australia wide DUSC survey data.*
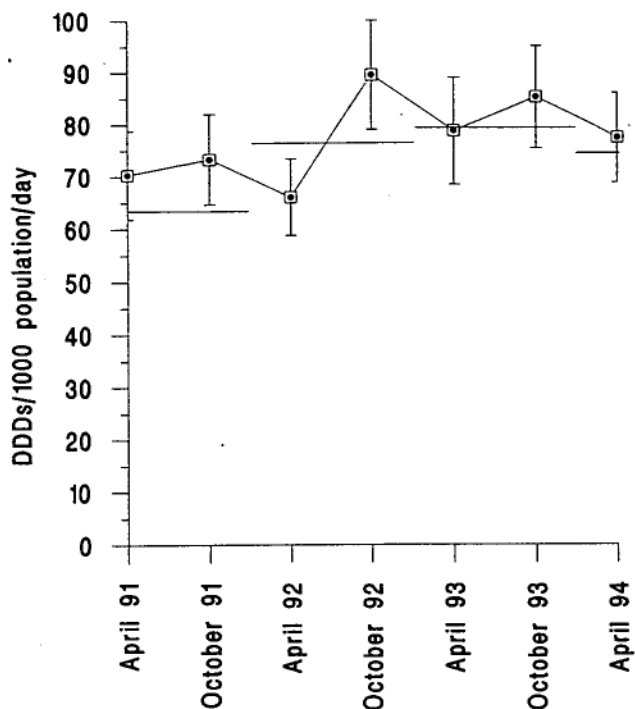
Figure 4.8   *Comparison of defined daily doses of total anti-asthmatics s per thousand population per day dispensed from Tasmanian community pharmacies (shown as a line plot with error bars representing ±95% confidence intervals) with Australia wide DUSC data (broken line).*
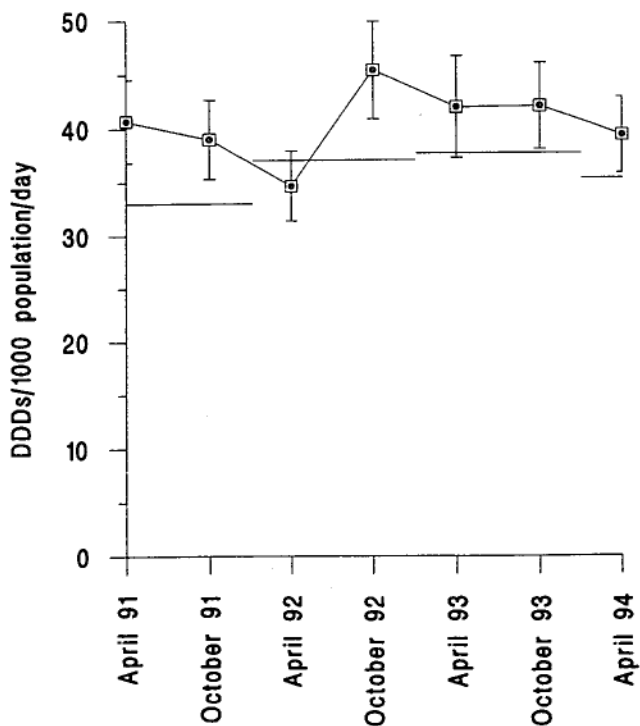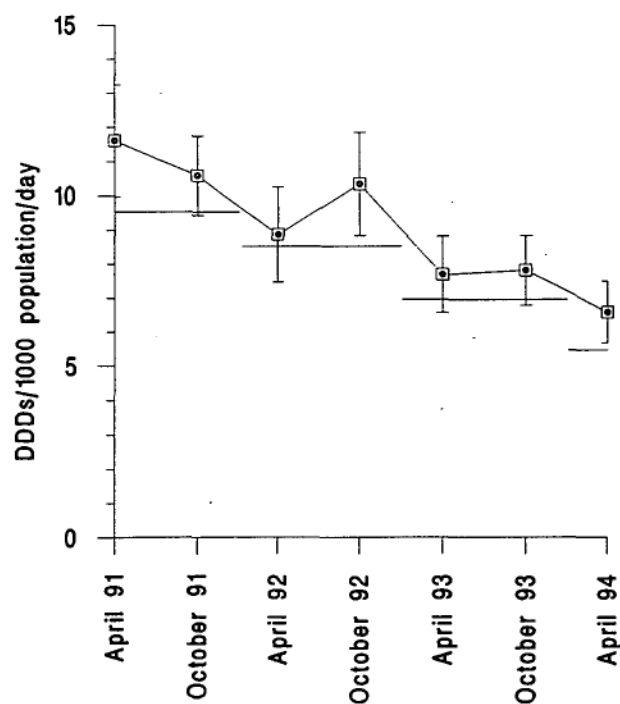

Figure 4.9   *Comparison of defined daily dose of β_2-agonists per thousand population per day dispensed from Tasmanian community pharmacies (shown as a line plot with error bars representing ± 95% confidence intervals) with Australia wide DUSC data (broken line).*
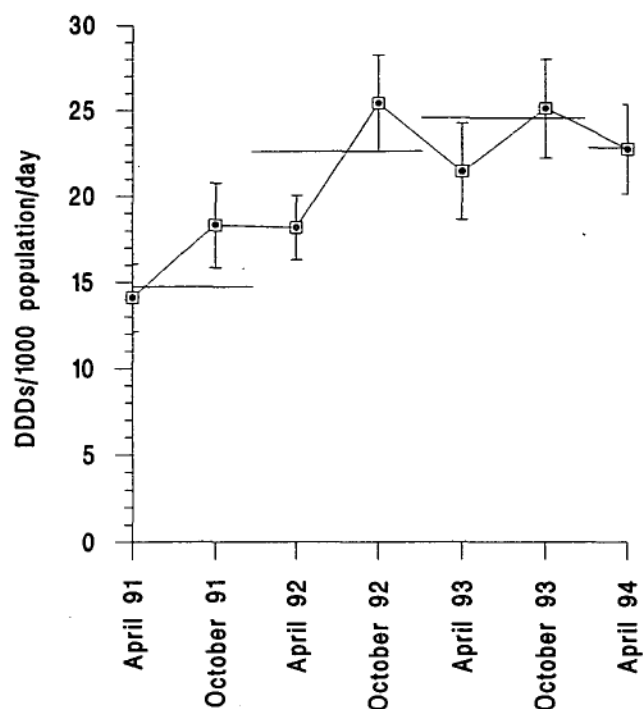
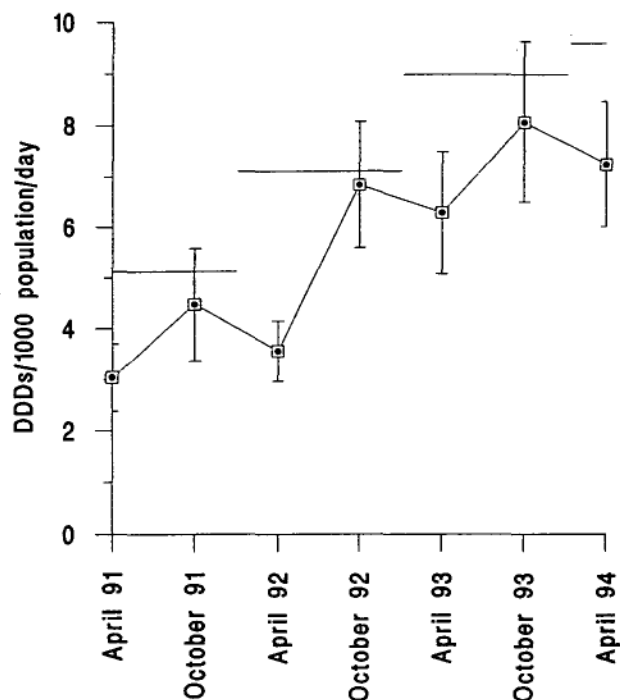**Figure 4.10**   *Comparison of defined daily doses in inhaled corticosteroids per thousand population per day dispensed from Tasmanian community pharmacies (shown as a line plot with error bars representing ± 95% confidence intervals) with Australia wide DUSC data (broken line).*

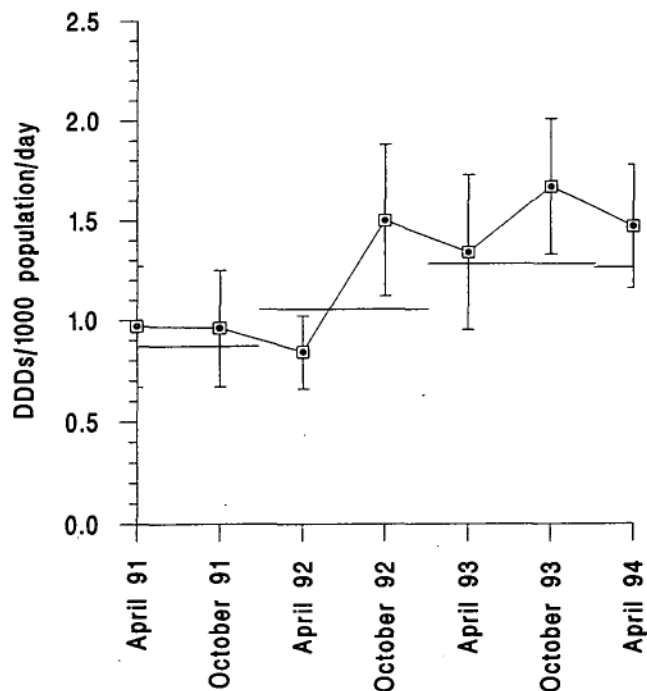**Figure 4.11**   *Comparison of defined daily doses of methyl xanthines per thousand population per day dispensed from Tasmanian community pharmacies (shown as a line plot with error bars representing ± 95% confidence intervals) with Australia wide DUSC data (broken line).*

Figure 4.12   *Comparison of defined daily doses of ipratropium bromide per thousand*
population per day dispensed from Tasmanian community pharmacies
(shown as a line plot with error bars representing ± 95% confidence intervals)
with Australia wide DUSC data (broken line).

Figure 4.13   *Comparison of defined daily doses of sodium cromoglycate per*
thousand population per day dispensed from Tasmanian community
pharmacies (shown as a line plot with error bars representing ± 95%
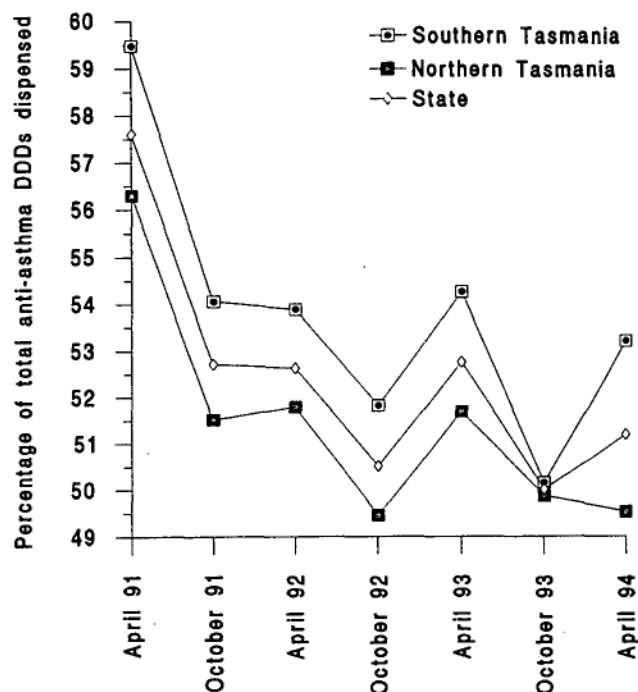*confidence intervals) with Australia wide DUSC data (broken line).*

Figure 4.14   β-*agonist DDDs dispensed as a percentage of total anti-asthmatic DDDs*
dispensed in Southern and Northern Tasmania. The Statewide average is also shown.
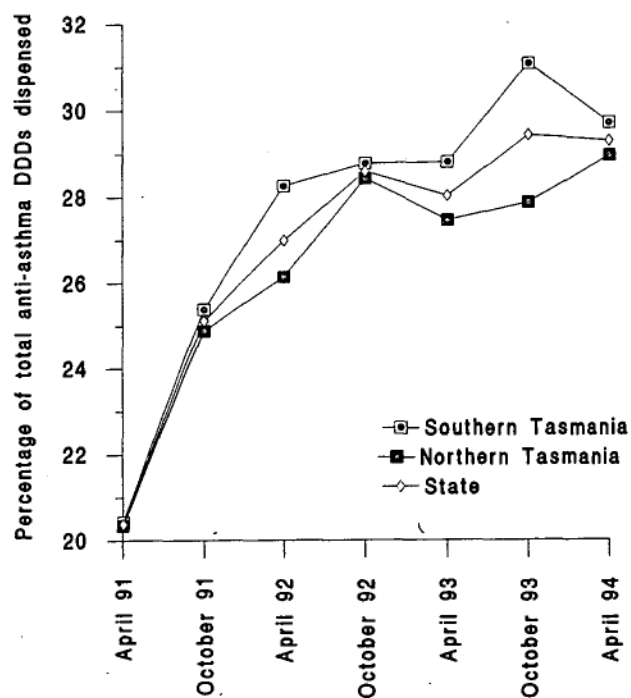

Figure 4.15   *Inhaled corticosteroid DDDs dispensed as a percentage of total anti-asthmatic*
DDDs dispensed in Southern and Northern Tasmania. The Statewide
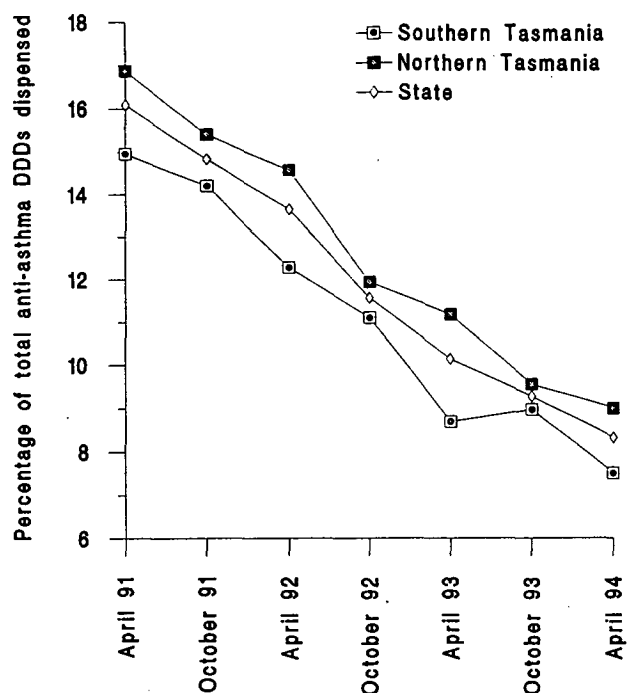*average is also shown.*

Figure 4.16   *Methyl xanthine DDDs dispensed as a percentage of total anti-asthmatic DDDs dispensed in Southern and Northern Tasmania. The Statewide average is also shown.*
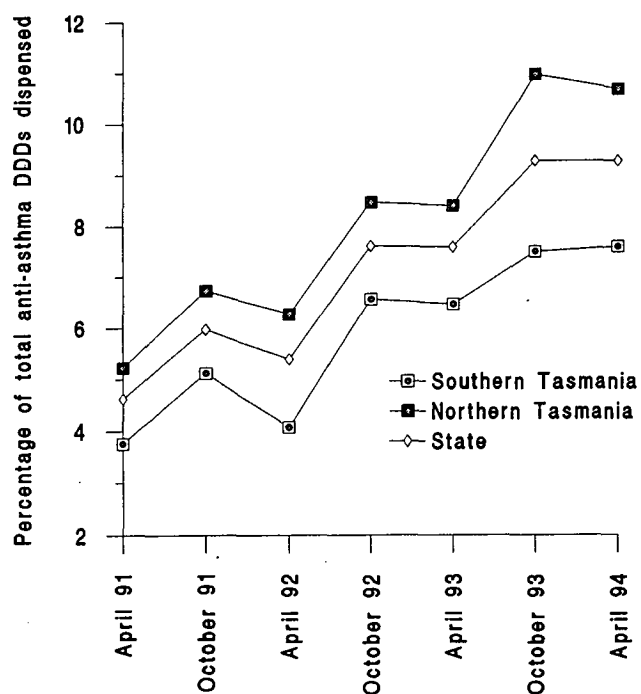

Figure 4.17   *Ipratropium bromide DDDs dispensed as a percentage of total anti-asthmatic DDDs dispensed in Southern and Northern Tasmania. The Statewide average is also shown.*
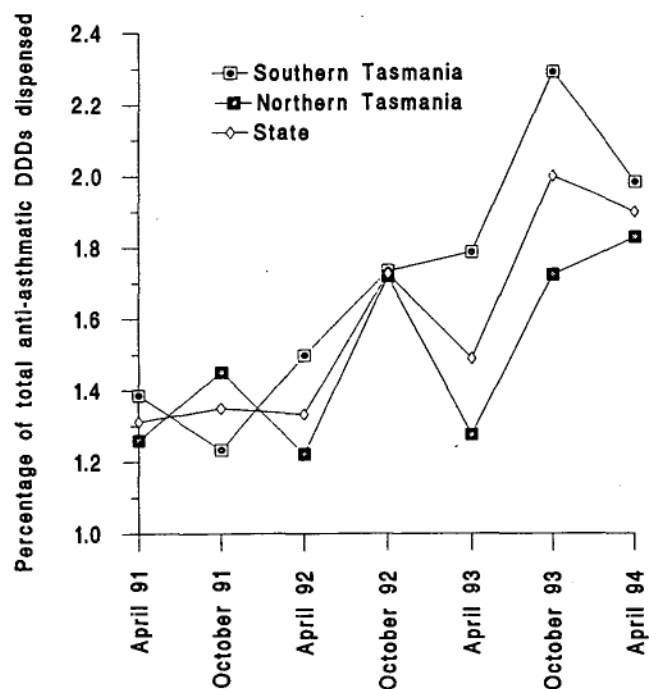
Figure 4.18   *Sodium cromoglycate DDDs dispensed as a percentage of total anti-asthmatic DDDs dispensed in Southern and Northern Tasmania. The Statewide average is also shown.*
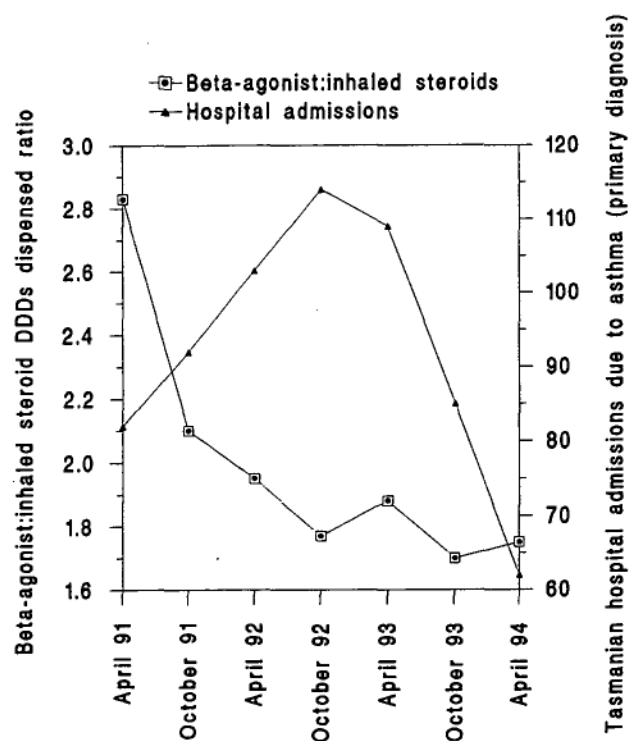

Figure 4.19   *Ratio of β-agonist / inhaled steroids DDDs dispensed from Tasmanian community pharmacies shown together with Tasmanian hospital admissions due to asthma as the primary diagnosis.*
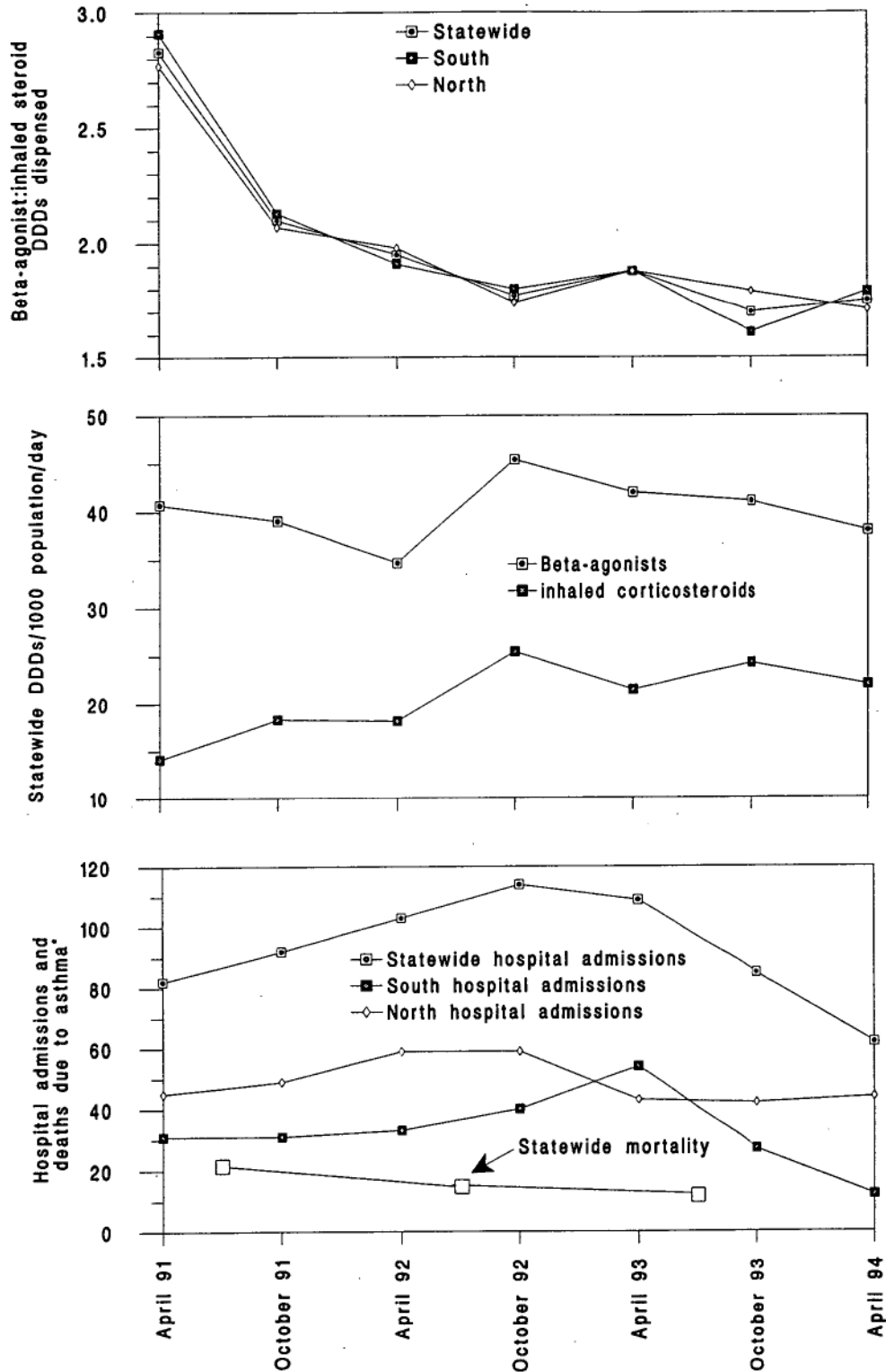
Figure 4.20   *Statewide and regional trends in mortality and morbidity shown with statewide DDDs/1000 population and the ratio of Statewide and regional β-agonist:inhaled steroid DDDs dispensed.*

* See table 4.7

4.5   DISCUSSION

4.5.1   Pharmacoepidemiology, usefulness and limitations in monitoring prescribing trends

Pharmacoepidemiology can be a useful tool to monitor prescribing trends, particularly those brought about by changes in disease understanding, as this current study demonstrates. Recent overseas pharmacoepidemiological studies have focussed on the prescribing of NSAIDs (Rodriguez and Jick 1994; Figueras et al, 1994; Traversa et al, 1995), anti-hypertensives (Manolio et al, 1995), antibiotics (Atanasova and Terziivanov, 1995), anti-arrhythmic drugs (Avanzini et al, 1995) and prescribing within target groups such as the elderly (Lindberg et al, 1994; Davidson et al, 1995). These are several areas that could be targets for future Australian pharmacoepidemiology studies.

Pharmacoepidemiology also provides a means of evaluating educational intervention programs aimed at improving prescribing practices, such as the use of academic detailing to modify the dosage prescribing of allopurinol (Peterson and Sugden, 1995) and the study presented here. In addition, a method of estimating disease prevalence using pharmacoepidemiological drug consumption data comparable to that achieved with conventional cross-sectional studies but at a lower cost has been suggested (Sartor and Walckiers, 1995).

Economic considerations in the prescribing of drugs is another area in which pharmacoepidemiology has an important role, especially as the cost of drugs increase and expensive drugs are brought onto the market. For example, Manolio et al (1995) recently questioned the widespread utilisation of ACE inhibitors and calcium channel blockers in the United States since their introduction in the early 1980s. It was argued that without convincing evidence of the advantages of ACE inhibitors and calcium channel blockers, it was difficult to justify the large decline in the market share of less expensive anti-hypertensive agents, such as diuretics and

β-blockers, and that US$3.1 billion would have been saved had the prescribing practices of 1982 remained in effect in 1992 (Manolio et al, 1995). Although this example may be a little extreme, it does highlight the way in which pharmacoepidemiology can be used to monitor prescribing for maximum cost-effectiveness.

As outlined earlier, the cost of asthma in Australia in 1991 was estimated to be between $585 and $720 million (NAC report, 1992). In 1992, the supply of salbutamol, beclomethasone dipropionate, ipratropium bromide and budesonide cost the Australian government $135 million alone (Anonymous, 1994). Obviously the increases in the prescribing of inhaled corticosteroids will impact on government costs, since inhaled corticosteroids are significantly more expensive than the β-agonists in MDI formulations. Naish et al (1995), after a study examining asthma prescribing habits in East London, suggested that pressure to reduce costs of asthma prescribing may lead to a reduction in prophylactic treatment and lower the quality of care as cost consciousness and good clinical practice are not necessarily linked. This leads to pharmacoeconomic cost-effectiveness issues that are beyond the scope of this present study.

Like PBS data, the data presented in this study lacks patient identifying numbers. This means that only information regarding the number of prescriptions and quantities dispensed are available, and not the number of patients prescribed a drug, individual doses or combination therapy data. A major consideration with patient identifying numbers is patient confidentiality, which could be at risk if identifying numbers were used. Although PBS data and the data used in this study are less comprehensive (since they lack a means for patient identification) it is easier and less controversial to obtain data from these sources.

As discussed earlier, the method of using community based pharmacy computerised dispensing records, as used in this study, is more comprehensive than PBS data alone (Section 3.3). This is particularly so in this study as a major portion of anti-asthmatic therapy, the bronchodilator MDIs, are priced under the maximum patient contribution and for that reason, MDI prescriptions for general patients or S3R sales are not included in the PBS database. DUSC estimates of total prescribing are derived from PBS data supplemented with private prescription survey data obtained from computerised dispensing data from around 250 pharmacies throughout Australia. DUSC data which has been supplemented in this way, like the data collected in this study, are more complete than PBS data alone. DUSC data only includes over the counter MDI sales of salbutamol from those states where recording is required (Victoria, South Australia and Tasmania). For this reason there is a possibility that DUSC data may underestimate total anti-asthmatic and β-agonist utilisation.

The impact of hospital outpatient dispensing on these drug utilisation figures would be expected to be minimal. Hospital dispensing in the past has been estimated at 12% of the Australian pharmaceutical market (Hurley et al, 1988; Henry et al, 1991). However, in recent years hospital out-patient dispensing has undergone considerable down-sizing and would now be expected to be much smaller than this figure.

As explained earlier, the calculation of the Tasmanian DDDs/1000 population/day for all drugs relies on prescription volumes for the corresponding financial year from the Pharmacy Guild of Australia. These estimates of prescription volume were taken from a random sample of between 263 and 273 pharmacies throughout Australia. These estimations also rely on the ratios of PBS to total prescriptions being similar for Tasmania and the whole of Australia.

In addition, the unit of drug utilisation, in this case DDDs/1000 population/day, may not truly represent the true nature of exposure and should be used with caution when drawing conclusions. For instance, an exposure of 12 DDDs/1000 population/day may indicate 6 people in 1000 taking 2 DDDs of drug per day, or 12 people in 1000 taking 1 DDD each per day. DDDs/1000 population/day is a standardised unit of comparison and should only be used for comparisons such as between different studies, populations and drugs (Cooke, 1991). Analogously, an increase in anti-asthmatic medication DDDs/1000 population/day may indicate that the number of people taking anti-asthmatic drugs have increased (an increase in incidence) or prescribed dosages of anti-asthmatic drugs have increased (increasing severity and/or more vigorous treatment) or a combination of both (Paterson and Musk, 1990). Unfortunately, there have been few international anti-asthmatic drug utilisation studies of this nature for comparison.

A study by Klaukka et al (1991) examined the prescribing of anti-asthmatic drugs in Finland. However, asthma prevalence (self reported prevalence of 1.9% of the total population) and total anti-asthmatic utilisation (49 DDDs/1000 population/day) were considerably less than presented here. Unfortunately the study did not describe prescribing trends for each of the anti-asthmatic medication groups. Factors that affect drug prescribing such as drug availability, drug cost, prescriber education/guidelines and disease incidence (diagnosis/recording) limit the usefulness of international comparisons in pharmacoepidemiology.

Regardless of any possible shortcomings in these data due to reliance on various estimations, which is inherent in all drug utilisation studies of this nature, the data presented is of benefit in comparing national and Tasmanian prescribing trends to

observe whether prescribing practices are changing as recommended by recent management guidelines.

As the full potential of pharmacoepidemiology becomes realised it is likely to become an important tool in the pharmacoeconomic and therapeutic evaluation of drug use and policy decision making within Australia.

4.5.2 Comparison of Tasmanian and National Anti-asthmatic prescribing trends

The prescribing of anti-asthmatic drugs has increased considerably since 1986. Jenkins et al (1990) found that the total prescribing of anti-asthmatic medication in Australia was around 34 DDDs/1000 population/day in 1986 compared to current levels of around 70-80 DDDs/1000 population/day. Care should be exercised, however, when comparing β-agonist and total anti-asthmatic prescribing data (of which β-agonist DDDs make up the majority) in this current study with that of Jenkins et al (1990). Given the lack of a comprehensive pharmacoepidemiology database at the time, the total β-agonist community consumption data from Jenkins et al (1990) relied on linear regression estimations from PBS/RPBS and IMS data, as not all community salbutamol inhaler sales were included in the PBS and RPBS databases. This 1986 estimate of 34 DDDs/1000 population/day is far less than current prescribing reported in this study, both in Tasmania (77 DDDs/1000 population/day for April 1994) and nationally (74 DDDs/1000 population/day for 1994). This increase in prescribing would also be expected given the rising incidence of the disease reported in the National Health Survey (1989-90) from 2% in 1977-78 to 8% in 1989-90.

It must be remembered that disease incidence, as reported in the National Health Survey, is self-reported by the public. It is therefore likely that the incidence may be influenced by public awareness campaigns (such as the asthma awareness

campaign in 1988 and the NAC in 1990) so a strict comparison between 1977-78 and 1989-90 asthma incidence may not be entirely valid.

The trends seen in both the national and Tasmanian utilisation were not unexpected given the awareness campaigns and the therapeutic shift toward anti-inflammatory in recent years. Following the fenoterol controversy in which concerns were raised that fenoterol may have been responsible for the New Zealand epidemic of asthma deaths in the late 1970s (Pearce et al 1990; Sears et al, 1990) and the publication of studies suggesting that regular β_2-agonist use may worsen asthma control (Sears et al, 1990; van Schayck et al, 1991; Spitzer et al, 1992) the use of β_2-agonists has been reassessed. Following this concern, fenoterol MDI and nebuliser solution were delisted from the PBS co-payment schedule in Australia in 1990. It is therefore not surprising that the national utilisation of fenoterol, which never had a large market share in Australia, has declined by almost 85% between 1990 and 1994 to 0.22 DDDs/1000 population/day or less than 0.7% of total β-agonist utilisation.

There were concerns that any potential problem with fenoterol may be common to all β_2-agonists (Rubinfield, 1991) and coupled with the developing understanding of asthma as an inflammatory disease, it was suggested that more emphasis should be placed on anti-inflammatory therapy with more prudent use of β_2-agonists (Barnes, 1989; Barnes and Chung, 1992; Lipworth and McDevitt, 1992). Current management guidelines now suggest that additional preventive therapy (inhaled corticosteroids and/or sodium cromoglycate) must be considered in all but the mildest cases of asthma (Anonymous, 1992; Tse and Bridges-Webb, 1993; Thompson and Watkins, 1994; Rees and Price, 1995a).

Jenkins et al (1990) found that the total utilisation of β-agonists (inhaled and systemic) doubled from 10.9 DDDs/1000 population/day in 1980 to 22.6 DDDs/1000 population/day in 1986. This contrasts with a decrease of almost 11% to 35.1 DDDs/1000 population/day for national utilisation of β-agonists from 1990 to 1994 (DUSC data) and a fall of around 3% to 39.4 DDDs/1000 population/day between April 1991 and April 1994 for Tasmanian utilisation of β-agonists. It should be noted, however, that there were considerable fluctuations in both Tasmanian and DUSC data as shown in Figures 4.6 and 4.7 respectively. Although there was a greater fall in the national dispensing of β-agonists, it must be remembered that both national and Tasmanian β-agonist utilisation figures fluctuated by more than 18% and 24% respectively. The smaller decline in the utilisation of β-agonists in Tasmania should not necessarily be interpreted as Tasmanian prescribing being more reliant on β-agonists, but maybe due to fluctuations as outlined above. These data suggest that both national and Tasmanian β-agonist prescribing has passed a peak and is now stable or declining.

As reported earlier, the National Health Survey has reported a rise in the incidence of asthma since 1972 and asthma mortality had been increasing over the last 20 years (Section 2.2) until a recent decline (which may be part of natural fluctuations). Even though β-agonist dispensing did not decline over the period of the study, this does not necessarily suggest that patterns of β-agonist use are similar to those prescribing practices before the change in emphasis to the earlier introduction of anti-inflammatory therapy. Firstly, despite monthly fluctuations, β-agonist use did not increase over the period of this study. Secondly, if the incidence of asthma has been increasing over the period of the study at a rate similar to that between the 1977-78 and 1989-90 National Health Surveys, more people would have been expected to be using bronchodilator therapy with drug

utilisation levels increasing toward the end of the study. Thirdly, public and prescriber awareness due to the NAC would suggest that more people may have recently been diagnosed with asthma. These people may be more likely to have asthma of a milder nature (i.e. disease not previously diagnosed, treated or recognised) and if so, would be more likely to be prescribed bronchodilators than anti-inflammatory therapy. It is therefore surprising and encouraging that β-agonist use, despite changing prescribing practices in asthma, did not increase over the period of the study. The most likely scenario is that individual patient reliance on β-agonist therapy has decreased.

There has been concern surrounding the change in Poisons scheduling for salbutamol MDIs in Australian States. Once an S4 item requiring a prescription from a doctor, salbutamol MDIs have been available over the counter after consultation with a pharmacist in all Australian states since 1985. Salbutamol MDIs are classed as S3Rs in Tasmania (i.e. S3 item that is required to be recorded by law). Concern has been expressed based on the possibility of infrequent medical consultation and under treatment of asthma (Gibson et al, 1993b) and the large increases in over the counter MDI sales observed by Jenkins et al (1990).

Secondary to the drug summary data (that consisted of combined prescription and S3R sales), some drug summary lists also included the number of over the counter S3R items dispensed separately (available on the Amfac-Chemdata system). This enabled the percent of total MDIs dispensed as S3R over the counter sales to be calculated for a small number of pharmacies. Despite the concerns outlined above, it was found that only a small percentage (6.5%) of total salbutamol MDIs were sold over the counter as S3Rs. There are, however, large limitations with these data for two reasons. The estimate relies on the assumption that all S3R prescriptions were recorded by the pharmacist (as legally required), and there were only a small

number of pharmacies (less than 6% of all monthly lists, provided by only 7 different pharmacies over the period of the study) that included this information. Most responding pharmacists removed this S3R information presumably because, unlike the drug summary lists, patient names and addresses were included with the S3R prescription information. Despite these limitations, this figure compares favourably with that of Comino et al (1995) who found that 11% of asthmatic respondents purchased over the counter bronchodilators in Hobart. Over the counter bronchodilator sales were found to be higher in Adelaide (15%), Melbourne (19%), Brisbane (26%), Perth (50%) and Sydney (57%; Comino et al, 1995).

There were only a few private prescriptions of the long acting β_2-agonist salmeterol recorded in this study. Salmeterol (which was released on the PBS as an authority item in early 1995) accounted for less than 0.03% of the total national anti-asthmatic DDDs dispensed (1994 DUSC figures) and 0.12% of the total Tasmanian anti-asthmatic DDDs dispensed for April 1994 (representing only 5 prescriptions). Perhaps in light of some concerns about development of tolerance to the protective effects of long acting β_2-agonists (Cheung et al, 1992; Skorodin, 1993; Grove and Lipworth, 1995), there is not a high level of utilisation of this drug at this early stage. It would be interesting to model the market uptake of salmeterol in a similar approach to that used by Birkett and McManus (1995).

Inhaled corticosteroid utilisation increased by 80% to 22.8 DDDs/1000 population/day between 1991 and 1994 nationally and by 61% between April 1991 and April 1994 in Tasmania to 22.8 DDDs/1000 population/day. National utilisation of beclomethasone dipropionate (Aldecin®, Becotide®, Becloforte®) has fallen by almost 14% from 12.6 to 10.9 DDDs/1000 population/day between 1990 and 1994. Budesonide (Pulmicort®) utilisation has increased since its listing on the

PBS co-payment schedule in 1991 by over 5-fold from 2.4 to 11.9 DDDs/1000 population/day between 1991 to 1994. Budesonide has several advantages over beclomethasone dipropionate which may explain its popularity. Budesonide is available in both a higher dose formulation (400 µg per dose) and a breath activated device. Budesonide also appears to have a higher topical to systemic potency ratio than beclomethasone dipropionate (Brattsand et al, 1982)

Theses trends of increased inhaled corticosteroid usage are consistent with current management guidelines. Inhaled corticosteroids are now advised in all but the mildest forms of adult asthma (McFadden and Gilbert, 1992; Anonymous, 1992; Skorodin, 1993; Tse and Bridges-Webb, 1993; Barnes, 1995; Paterson et al, 1995; Rees and Price, 1995a) and the recommended dosages of inhaled corticosteroids have been increasing. There has been some concern, however, that inhaled corticosteroids are being increasingly used for children with relatively mild asthma, perhaps at the expense of using sodium cromoglycate (Phelan, 1995).

Beclomethasone dipropionate was originally marketed as 50 µg per inhalation in Australia, however, dosages have increased over the years. For example, in the original trials dosage schedules were formulated with a limit of 1200 µg/day. A 250 µg per inhalation MDI (Becloforte®) was listed on the PBS in late 1988 as an authority item (needing special approval to be prescribed) but as dosages have increased, it is now available without authority.

The introduction of high doses of inhaled corticosteroids facilitated by a 250 µg per dose inhaler (beclomethasone dipropionate) was a major therapeutic advance in the management of asthma (Douglass and Bowes, 1990). In addition, budesonide is also now available in a 400 µg per dose MDI. Despite the well documented risk of adrenal suppression with dosages above 1500 µg/day, high dose inhaled

corticosteroids (over 2000 µg/day) are now widely used to control more severe asthma and their use can alleviate or markedly reduce the need for oral glucocorticoid therapy (Kamada, 1994; Barnes, 1995). There is little evidence of glucocorticoid related side effects in doses up to 1500 µg/day (Barnes et al, 1995), however, doses exceeding 1000 µg/day do pose an increased risk of adrenal suppression (Kamada, 1994).

The introduction of the higher dose formulation and spacer has improved the delivery of corticosteroids, allowing them to be prescribed to people who may have been unable or reluctant to take the drug in the past (Douglass and Bowes, 1990). The use of spacer devices has been shown to reduce the incidence of oral candidiasis (Köng, 1985; Salzman et al, 1988), particularly so in patients who have a poor MDI technique. In addition, four times daily regimens of inhaled corticosteroids have been associated with a higher incidence of oral candidiasis than twice daily regimens (Toogood et al, 1984; Smith and Hodson, 1986). Although the current management guidelines place greater emphasis on the earlier introduction of anti-inflammatory therapy, the availability of higher dose MDIs needing fewer daily inhalations, and the introduction of spacer devices, have also helped make inhaled corticosteroids more popular by reducing adverse effects such as oral candidiasis and hoarseness.

This increase in inhaled corticosteroid drug utilisation adds more evidence that prescribing practices have changed in line with management guidelines with the earlier introduction of preventive therapy. The Tasmanian utilisation of inhaled corticosteroids, although not displaying as great an increase, was virtually the same as 1994 national data with an April 1994 Tasmanian figure of 22.9 compared with the national data of 22.8 DDDs/1000 population/day.

It has been shown in England that 15% of patients receiving inhaled corticosteroids had a diagnosis other than asthma (Campbell et al, 1995). In addition β_2-agonists, ipratropium bromide and theophylline are widely used in other diseases such as COPD (Section 2.5). Since the prescription data collected here was not linked to indication, due care should be exercised when interpreting the inhaled corticosteroid data. However, despite this data not being linked to indication, even when taking into account 15% variability, there has been a real increase in the utilisation of inhaled corticosteroids.

Further supporting evidence of less reliance on bronchodilator therapy is that the ratio of β-agonist:inhaled corticosteroid DDDs dispensed has decreased from 7.3 (or 4.1 not including general prescription and over the counter sales estimates) in 1986 (Jenkins et al, 1990) to 1.5 (national data) or 1.7 (Tasmanian data) in 1994. The national ratio has decreased by 51% between 1990 and 1994 while the Tasmanian ratio has decreased by 40% between April 1991 and April 1994. As β-agonist utilisation has remained fairly stable over the period of the study, this increase in inhaled corticosteroid usage has been the main contributor to the decrease in the ratio of β-agonist:inhaled corticosteroid DDDs dispensed. This suggests that prescribing practices have changed, with probably a combination of more people now receiving inhaled corticosteroid therapy and higher doses now being used.

Theophylline and its xanthine derivatives have suffered a large decrease in prescriptions over the last 4 years. Jenkins et al (1990) reported total methyl xanthine utilisation as 6.8 DDDs/1000 population/day in 1986. DUSC data indicates 1994 utilisation as 5.4 DDDs/1000 population/day, down 60% from 1990. Tasmanian utilisation has also fallen by a similar margin to 6.6 DDDs/1000 population/day in April 1994, down 43% from April 1991.

Theophylline is no longer considered a first-line drug in asthma (Alpers, 1991). Current management guidelines now suggest less emphasis on the use of oral theophyllines for chronic asthma (Anonymous, 1992; Tse and Bridges-Webb, 1993). Its popularity may have decreased for several reasons; the development of new drugs such as high dose inhaled corticosteroids and ipratropium bromide (Skorodin, 1993), studies questioning the use of theophylline in the emergency treatment of asthma (Littenberg, 1988) and dosage difficulties associated with its narrow therapeutic range and pharmacokinetic variability (Johnston, 1990; Jenne, 1994).

Despite recent questions regarding the efficacy of theophylline, it remains a potent and effective bronchodilator for asthma, and to a lesser degree, for COPD (Addis, 1990; Skorodin, 1993). Theophylline is useful in the treatment of chronic persistent asthma that is difficult to control with maximum doses of inhaled corticosteroids (Breslin, 1993), nocturnal asthma (Alpers, 1991; Frew and Holgate, 1993; Skorodin, 1993), patients with acute severe asthma who are progressing into respiratory failure (Addis, 1990; Jenne, 1994), and patients with severe COPD who are dependent on bronchodilators and oxygen (Jenne, 1994). In addition, theophylline has an additive or synergistic effect on bronchodilation when combined with β_2-agonists and/or ipratropium (Jenne, 1987; Nishimura et al, 1992; Thomas et al, 1992), may possess a mild anti-inflammatory action (Jenne, 1994; Rees and Price, 1995a) and has positive effects on mucociliary clearance, diaphragmatic function and fatigue (Jenne, 1994).

The negative aspects of theophylline use have been well documented. The variable clearance, narrow therapeutic index and the severity of the toxic reactions of theophyllines necessitate close attention to dosage regimens and subsequent

monitoring of plasma levels (Jenne, 1994) making theophylline a difficult drug to prescribe (Johnston, 1990). In addition adverse effects such as nausea, vomiting, and abdominal discomfort are common but headache, malaise and convulsions can also occur (Rees and Price, 1995a).

Although expensive, immunoassay paper test strips for theophylline are now available providing a theophylline plasma level from a capillary blood sample within 30 minutes (Aronson et al, 1992). With plasma levels of theophylline in community patients now easily measurable, these adverse effects should be minimised with regular monitoring of levels. It is therefore a little surprising as to the magnitude of the fall in theophylline utilisation over the period of this study. Further studies into the relationship between the degree of theophylline plasma monitoring in the community, incidence of adverse effects, drug efficacy and the pharmacoeconomic aspects of prescribing of a relatively inexpensive drug should be examined. After all, theophylline is still of benefit in some asthmatics, has been a mainstay of asthma treatment for several decades and the tools are now available to better utilise this drug.

There was a surprising rise in the use of ipratropium over the period of the study. Utilisation of ipratropium increased by 106% between 1990 and 1994 nationally and 138% between April 1991 and April 1994 in Tasmania. The large increase in ipratropium use may be associated with a combination of increased use in older patients with COPD and the fact that ipratropium may easily be added to an existing anti-asthmatic medication regimen.

Ipratropium is more commonly of benefit in patients with chronic bronchitis or emphysema (Alpers, 1991; Kemp, 1993; Skorodin, 1993; Rees and Price, 1995). Ipratropium use in asthma is limited and is of most benefit to older patients, the

very young (Rees and Price, 1995a), or in patients with acute severe asthma (Bryant, 1985; Rebuck et al, 1987; O'Driscoll et al, 1989; Bendefy, 1991). Additional clinical benefit has been noted when ipratropium is combined with a β_2-agonist in COPD (Massey and Gotz, 1985; Mann et al, 1988). In addition, because the onset of action is slower but the action is more prolonged lasting up to 8 hours, the addition of ipratropium to an anti-asthmatic regimen may provide better therapeutic coverage in some patients but it is not recommended as a single drug therapy in asthma (Alpers, 1991; Breslin, 1993).

Comparison of ipratropium utilisation with that of Jenkins et al (1990) was not possible as the 1986 data in that study did not include ipratropium. Ipratropium was not released on the PBS co-payment schedule in Australia until 1985.

It is unlikely that the rise in ipratropium use has offset or masked a lack of an increase in β-agonist bronchodilator use. Even when grouping ipratropium with β-agonists as bronchodilators, the bronchodilator:inhaled corticosteroid ratio decreased between 1990 to 1994 from 3.5 to 2.0 (down 44%) nationally and from 3.1 to 2.3 (down 27%) in Tasmania between April 1991 and 1994.

One disturbing finding in this study was the relatively minor use of sodium cromoglycate in Australia. Both national and Tasmanian levels of utilisation were around 1 DDD/1000 population/day. This was similar to usage in 1986 (Jenkins et al, 1990). In addition, Jenkins et al (1990) noted a 39% fall in sodium cromoglycate usage between 1975 and 1986.

In Canada, the use of sodium cromoglycate as well as inhaled corticosteroids began to rise in the late 1980s coinciding with the development of Canadian guidelines for asthma treatment (Toogood, 1983). This has not happened in Australia.

Prescribing of sodium cromoglycate in Finland has been reported at around 2 DDDs/1000 population/day and as much as 7 DDDs/1000 population/day in some regions (Kluakka et al, 1991). Given that the Finnish utilisation of all anti-asthmatics totalled 49 DDDs/1000 population/day (with a lower incidence of asthma), this represented over 4% of all anti-asthmatic DDDs. In the current prescribing environment in Australia, this would represent a utilisation of around 3 DDDs/1000 population/day, double that found in this current study.

Given that 12% of people under the age of 25 years reported asthma, accounting for 56% of all persons with asthma (National Health Survey, 1989-90), it seems peculiar that utilisation of a preventive drug with proven efficacy and which is recommended as first-line therapy in younger people (Alpers, 1991; Breslin, 1993; Skorodin, 1993; Rees and Price, 1995b) has remained relatively low. In addition, sodium cromoglycate is relatively free of harmful adverse effects (Skorodin, 1993) has been well promoted in current management guidelines (Anonymous, 1992; Tse and Bridges-Webb, 1993; Rees and Price, 1995a) and is now available in a higher dosage form (Intal Forte®).

Taken regularly, sodium cromoglycate will control symptoms in about 60% of school age children with frequent symptoms (Rees and Price, 1995b). A trial period of at least 4-8 weeks of regular use should be allowed before sodium cromoglycate is dismissed as ineffective (McFadden and Gilbert, 1993; Skorodin, 1993; Price, 1995a). To improve compliance, patients should be warned about this lag time (Skorodin, 1983) and this may be one reason why sodium cromoglycate is not utilised more. Sodium cromolgycate is not effective in all patients and there seem to be no indicators to predict which patients will respond (Barnes, 1989). In addition, sodium cromoglycate is generally not used together with inhaled steroids (Anonymous, 1992; Breslin, 1993).

The release of nedocromil sodium (Tilade®) in Australia in April 1995 will be of interest given the surprisingly low market share of sodium cromolgycate (the only currently available anti-allergic medication) in a prescribing environment which should favour such drugs. Will the introduction of nedocromil sodium, the first non-steroid anti-inflammatory preparation specifically for adults (Brogden and Sorkin, 1993), promote greater interest in this class of drugs? The drug utilisation results found here suggest that more attention should be given to the prescribing of anti-allergic drugs.

There is no doubt that utilisation patterns have changed over the three years of this study despite the fluctuations. The national utilisation of β-agonists was low for 1991 compared with 1990 and 1992 while there was a large increase in utilisation of inhaled corticosteroids from 1991 to 1992. Tasmanian utilisation of β-agonists dropped sharply between October 1991 to April 1992 while there was an increase in inhaled corticosteroid utilisation between April 1991 and 1992. These events occurred after the launch of both the NAC's Medical Practitioners and Pharmacists Asthma Management Plans in 1991. It is difficult to conclude that these utilisation changes are due entirely to the awareness and educational intervention of the NAC, although studies have documented how patient and prescriber education can improve clinical outcomes in patients with asthma (Toelle et al 1993; Pauley et al, 1995). The shift in prescribing emphasis may also have been contributed to by the publicity surrounding suggestions that β-agonists may worsen asthma. There was, however, an increase in 1992 (nationally) and October 1992 (Tasmania) in the utilisation of both inhaled corticosteroids and β-agonists, before β-agonist utilisation levelled out and declined slightly in Tasmania. The cause of this could be associated with an increased awareness of the disease, brought about by the NAC, resulting in more diagnoses, or natural fluctuations in

morbidity. However, there was no marked increase in asthma deaths during this period.

The ratios of both bronchodilators (β-agonist plus ipratropium bromide) and β-agonist to inhaled corticosteroids are slightly higher in Tasmania than the national data which suggests there may either be still some room for improvement in prescribing practices in Tasmania or the higher ratios in Tasmania may reflect a higher disease incidence and severity. However, there were no significant differences in mortality rates between the Tasmanian and Australian data from 1991 to 1993.

The national DUSC figures, like the data presented in this study are merely estimations of drug utilisation. To place the accuracy of the data into perspective, the survey component of the DUSC estimations of prescribing presented here were collected from around 250 pharmacies Australia-wide initially, dropping to around 190 in 1993 (personal communication, Secretary of DUSC). This represented around one pharmacy sample per 69,000 people in 1991 rising to one sample per 93,000 people in 1993. In comparison, the Tasmanian data from October 1991 represented around one pharmacy sample per 13,000 people falling to around one sample per 11,000 people in October 1993 (calculated from 37 and 43 pharmacies respectively). In addition DUSC estimations of prescribing only included over the counter MDI sales from Victoria, South Australia and Tasmania. This would suggest that the Tasmanian data presented in this study should be far more representative of prescribing practices within Tasmania than the DUSC data is representative of national prescribing.

4.5.3 Comparison of Tasmanian regional prescribing trends

There were minor differences in prescribing within Tasmania. As discussed earlier (Section 4.4.5) there was slightly greater relative utilisation of inhaled corticosteroids and β-agonists in the South of the State and greater relative utilisation of ipratropium and methyl xanthines in the North. The ratios of β-agonist:inhaled corticosteroid were very similar, except for October 1993 where the Southern Tasmania ratio was 1.6 compared to Northern Tasmania's 1.8.

Asthma morbidity during the period of the study, as measured by hospital admissions in Tasmania, rose to a peak in October 1992, then fell for the remainder of the study. Utilisation of both β-agonists and inhaled corticosteroids increased at the same time. The cause of the increase in morbidity and drug utilisation is unclear but may have been associated with factors such as an influenza epidemic or a severe winter. However, given a lack of appropriate retrospective data and the limitations of analyses of secular trends, it is difficult to identify the reasons for these increases.

The major contributor to the observed fall was Southern Tasmanian admissions while Northern Tasmanian admissions did not fall to the same extent. It is probably not valid to relate the fall in hospital admissions with the decline in the ratio of β-agonist:inhaled corticosteroid DDDs dispensed for the following reasons. The admissions numbers are small and susceptible to greater relative fluctuations and the degree of correlation between asthma morbidity in the general community and hospital admissions is unclear.

It appears that in general, Southern and Northern Tasmanian prescribing are similar, and have followed a similar trend in line with the national data and current guidelines.

4.5.4   Does this data support either the 'severity' or 'β-agonist' hypothesis?

There also seems to be evidence that there may be now less reliance on β-agonist therapy. Despite an increasing incidence of the disease and greater awareness and diagnosis, β-agonist utilisation has remained relatively stable (both nationally and in Tasmania) and may be declining. This suggests that for a given individual within the population, the dose of β-agonist used has decreased. In addition, a decrease in the dispensed DDDs ratio of β-agonist:inhaled corticosteroid has been observed. There has been a decline in national asthma mortality from the late 1980s. National and Tasmanian asthma mortality has stabilised over the period of the study. Tasmanian hospital admissions, a crude measure of morbidity, also appear to have stabilised over the period of the study and may be declining.

The question arises, does this data support either the 'β-agonist hypothesis' or the 'severity hypothesis'. The 'severity hypothesis' speculates that increasing use of β-agonists is a marker of greater disease severity, which itself is associated with an increased risk of fatal or near fatal asthma (Suissa et al, 1994). The 'β-agonist hypothesis' implies that the over reliance on β-agonists is responsible for the adverse asthma outcomes independent of disease severity (Suissa et al, 1994).

Unfortunately the data presented here have limitations, of which there are two different aspects. Firstly, there are well documented limitations with analysis of secular trends, or ecological association studies as they are sometimes known (Eisdale et al, 1987; Strom, 1994). It is difficult to control confounding variables, they observe groups and so lack data on individuals, and disease trends may not be ideally recorded (Strom, 1994). As stated earlier (Section 3.2.1), to find a true causal association, random, bias and confounding (indirect) errors must be excluded. In addition there must be coherence with existing information (biological

plausibility), consistency and specificity of the association, a time sequence, and a strong association (Strom, 1989).

Secondly, there are limitations with the use of DDDs/1000 population/day to measure drug utilisation as stated earlier, it provides no information on individual dosages. For example, without other supporting evidence, a static level of drug utilisation measured in DDDs/1000 population/day may suggest either (i) levels have remained stable, (ii) more people taking less drug, or (iii) less people taking more drug. Rises and falls in DDDs/1000 population/day utilisation figures further confound this problem. However, when combined with other information, this uncertainty can be minimised. As discussed earlier, it can most likely be inferred from this study that individual reliance on β-agonists has decreased.

Like the study by Jenkins et al (1990), these limitations in using DDDs/1000 population/day make this current study unable to support or disprove either the 'β-agonist hypothesis' or the 'severity hypothesis'. This was noted by both Paterson and Musk (1990) and Jenkins and Bowes (1991), and in the study by Jenkins et al (1990). What this study does show is that there is now much greater utilisation of inhaled steroids and less reliance on β-agonists as sole therapy which is in-line with current management guidelines. At the same time, mortality and morbidity appear to be stabilising or declining from the levels in the late 1980s.

Further studies of this nature are required to monitor prescribing, asthma morbidity/mortality, and to assess the impact of recommended treatment regimens on therapeutic outcomes (Toogood, 1993). It is important not to become complacent and shift to other more topical issues. Asthma knowledge, drug therapy, morbidity and mortality have shown they are dynamic processes with

changes occurring in the past. It is likely there will be changes in the future and observational studies such as analysis of secular trends are an ideal means of monitoring these trends.

4.6   CONCLUSION

In recent years it has been recognised that asthma should not simply be regarded as bronchoconstriction but involves a characteristic inflammatory response. This, together with concerns that β-agonist may worsen asthma, have resulted in current management guidelines placing greater emphasis on the earlier introduction of inhaled corticosteroids and anti-allergy drugs.

Tasmanian prescribing trends of anti-asthmatic medications reflect these new guidelines and are similar to national prescribing trends. There has been a large increase, both nationally and in Tasmania, in inhaled corticosteroid utilisation and β-agonist utilisation appears to have stabilised and may be declining. There was a surprisingly low level of utilisation of sodium cromoglycate, suggesting that greater attention should be given to the prescribing of sodium cromoglycate and the newly introduced nedocromil sodium.

Australian and Tasmanian asthma mortality may have declined from levels in the late 1980s but it is difficult to attribute that this is entirely due to changes in prescribing and/or the NAC, or the changes may simply be part of natural fluctuations due to the limitations of secular trend analyses.

CHAPTER 5

High Performance Liquid Chromatographic Assay for the Simultaneous Determination of Ipratropium Bromide, Fenoterol, Salbutamol and Terbutaline in Nebuliser Solution

5.1   SUMMARY

A reversed-phase ion-pair high performance liquid chromatography assay was developed for the simultaneous determination of ipratropium bromide, fenoterol hydrobromide, salbutamol sulphate and terbutaline sulphate in nebuliser solution. Chromatographic separation was achieved with a Nova-Pak® C_{18} 4 μm 10 cm x 8 mm i.d. Radial-pak® cartridge inside a Waters RCM 8 x 10 compression module using ternary gradient analysis. Detection was performed using UV detection at 220 nm. The standard curves were linear over the following ranges: ipratropium bromide 20.8- 250.0 μg/ml, fenoterol hydrobromide 27.8-500.0 μg/ml, salbutamol sulphate 34.7-2500.0 μg/ml and terbutaline sulphate 69.5-2500 μg/ml. Inter-day and intra-day coefficients of variation for each compound ranged from 4.5-5.2% and 3.5-3.9% respectively. The assay procedure was developed to allow the accurate determination of constituents in various combinations of nebuliser solution, as well as being stability indicating. This provides a convenient means of testing long-term compatibility and stability following the post-manufacture mixing of commonly used nebulised preparations.

5.2   INTRODUCTION

Inhalation of respirator solutions via nebuliser is an integral component of the modern treatment of airways diseases, particularly for patients unable to use metered dose inhalers (Johnson, 1989; Anonymous, 1987; Horsley, 1988). Nebulised drugs commonly used in the treatment of asthma include ipratropium bromide, which is a quaternary derivative of atropine, and the β2-agonists fenoterol, salbutamol, and terbutaline. The structures of these compounds are shown in Figure 5.1.
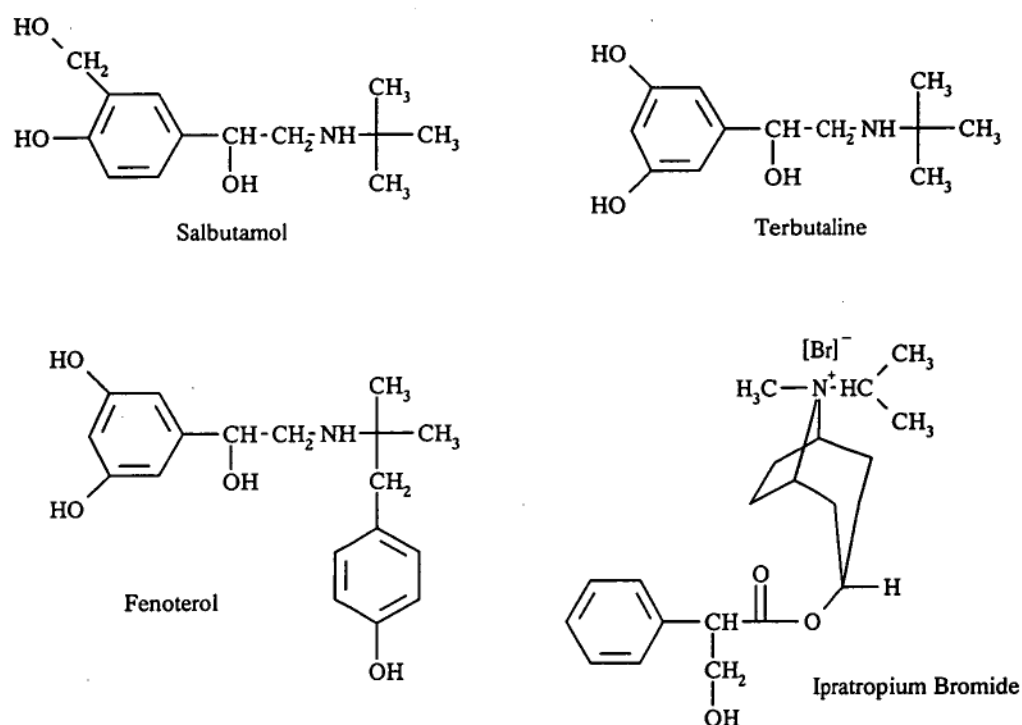
Figure 5.1   *Structures of salbutamol, terbutaline, fenoterol and ipratropium bromide.*

While ipratropium bromide is not a first-line or sole drug for asthma, recent studies have advocated the use of ipratropium bromide in acute severe asthma in combination with β2-agonists (Bryant, 1985; Rebuck et al, 1987; O'Driscoll et al, 1989, Mann et al, 1988). The bronchodilator effect of ipratropium bromide in chronic bronchitis appears to be comparable, and may be superior in some cases, to that of the β2–agonists (Mann et al, 1988). In addition, in chronic obstructive pulmonary disease, various studies have shown additional clinical benefit is gained when ipratropium bromide is combined with other bronchodilating drugs (Barnes, 1989; Massey and Gotz, 1985; Mann et al, 1988).

The mixing of nebuliser solutions is a common practice (Caldwell et al, 1991; O'Driscoll and Cochrane, 1987) which provides a means of shortening the time that patients spend nebulising inhaled drugs. It has been suggested that the convenience and reduced administration time resulting from mixing nebuliser solutions may increase patient compliance (Roberts and Rossi, 1993). The post-

manufacture mixing of these nebuliser solutions, however, gives rise to issues of compatibility and stability. Iacono et al (1987) showed that ipratropium was stable for a period of up to 1 hour after mixing with salbutamol and sodium cromoglycate, while longer term admixture stability and compatibility with fenoterol and terbutaline were not discussed. A need for a single assay that would allow testing of the compatibility and stability of different combinations of these β_2-agonists with ipratropium was evident.

HPLC has many advantages over gas chromatography for the analysis of polar, non-volatile and thermally labile compounds in aqueous matrices (Mehta, 1992). In this study, a reversed-phase ion-pair HPLC assay using Pic® reagent was developed to determine the concentrations of ipratropium, salbutamol, fenoterol and terbutaline present in a nebuliser solution, either alone or in any combination. Ion-pair chromatography was utilised because of the quaternary structure of ipratropium; this allowed retention of the neutral ion-pair on the reversed phase column.

5.3   EXPERIMENTAL

5.3.1   Materials

Ipratropium bromide powder was kindly donated by Boehringer Ingelheim Pty. Ltd. (Ingelheim, Germany) for the development of the assay.

The following were used in the assay procedure: ipratropium bromide (Atrovent® 0.025% nebuliser solution; Boehringer Ingelheim Pty. Ltd., Artarmon, NSW, Australia), fenoterol hydrobromide (Berotec® 0.1% w/v nebuliser solution; Boehringer Ingelheim Pty. Ltd., Artarmon, NSW, Australia), salbutamol sulphate (Ventolin® 0.5% w/v nebuliser solution; Glaxo Australia Pty. Ltd., Boronia, Vic., Australia), terbutaline sulphate (Bricanyl® 1% w/v nebulising solution; Astra Pharmaceuticals Pty. Ltd., North Ryde, NSW, Australia), and mepivacaine

hydrochloride (Carbocaine® 1% w/v sterile injection, Winthrop Laboratories, New York, USA).

Methanol and tetrahydrofuran (liquid chromatography grade) were purchased from Waters Associates, Lane Cove, NSW, Australia. The ion pair reagent (Pic® B-8 Reagent Low UV; containing water, methanol, octane sulphonic acid and calcium acetate in undisclosed quantities) was purchased from Waters Associates, Milford, MA, USA.

5.3.2   Equipment

The HPLC system consisted of a Varian Solvent Delivery System Model 9010 (Varian Chromatography Systems, Walnut Creek, CA, USA) and a Nova-Pak® C_{18} 4 μm 10 cm x 8 mm i.d. Radial-pak® liquid chromatography cartridge (Waters Associates, Milford, MA, USA) inside a Waters RCM 8 x 10 compression module (Waters Associates, Milford, MA, USA). A Rheodyne injector Model 7161 (Rheodyne Inc., Cotati, CA, USA) with a 10 μl external loop was used. Detection was performed using a Varian Variable Wavelength UV-VIS Detector Model 9050 (Varian Chromatography Systems, Walnut Creek, CA, USA) set at 220 nm.

Chromatographic peaks were digitised, integrated and recorded with a Varian GC Star Workstation (Varian Chromatography Systems, Walnut Creek, CA, USA).

5.3.3   Method development

The quaternary nature of the ipratropium molecule resulted in a fast elution with the solvent front in a normal reversed-phase HPLC system. Ipratropium bromide standard was used to confirm the retention time. This situation was easily solved with the addition of an ion-pair reagent to the mobile phase. The ipratropium ion formed an ion-pair with the hydrophobic counter-ion made available by the addition of an octane sulphonic acid ion-pair reagent. The relatively hydrophobic

ion-pair was retained to a greater extent on the non polar C_{18} column and hence, resolved from the solvent front. Hexane, heptane and octane sulphonic acid were investigated as ion-pair reagents. Octane sulphonic acid was finally chosen as the ion-pair reagent as the slightly longer hydrophobic tail resulted in a greater retention factor without the need to increase the ion-pair reagent concentration. The increased retention permitted more range for optimising the organic solvent components in the mobile phase. The addition of an ion-pair reagent also enhanced the resolution of the β_2-agonists and mepivacaine with reduced peak tailing evident. Chromatograms of ipratropium, salbutamol and a blank distilled water sample are shown in Figure 5.2 with and without octane sulphonic acid ion-pair reagent (Pic® B-8 Reagent Low UV) in the mobile phase, with an identical organic component in each mobile phase (25% tetrahydrofuran, 10% methanol in distilled water).

Ternary gradient analysis was used to allow the satisfactory resolution of all five compounds. A solvent with relatively strong elution power such as tetrahydrofuran was required for the satisfactory elution of the hydrophobic ion-pairs. Initially in the assay development, fenoterol was found to elute late in the chromatogram with a poor peak shape under the starting mobile phase conditions. This was corrected by two means. Firstly, the tetrahydrofuran concentration was increased after 7.7 minutes, which subsequently decreased the retention time and improved the peak shape of fenoterol. Secondly, an increasing methanol component after 7.7 minutes was found to further decrease the retention time and improve the peak shape of fenoterol.

5.3.4 Chromatographic conditions

Ternary gradient analysis (mobile phases A, B and C) was used to achieve satisfactory solute elution. Mobile phase A was a solution of tetrahydrofuran - distilled water (40:60, v/v) containing 0.0025 M Pic® B-8 Reagent Low UV. This
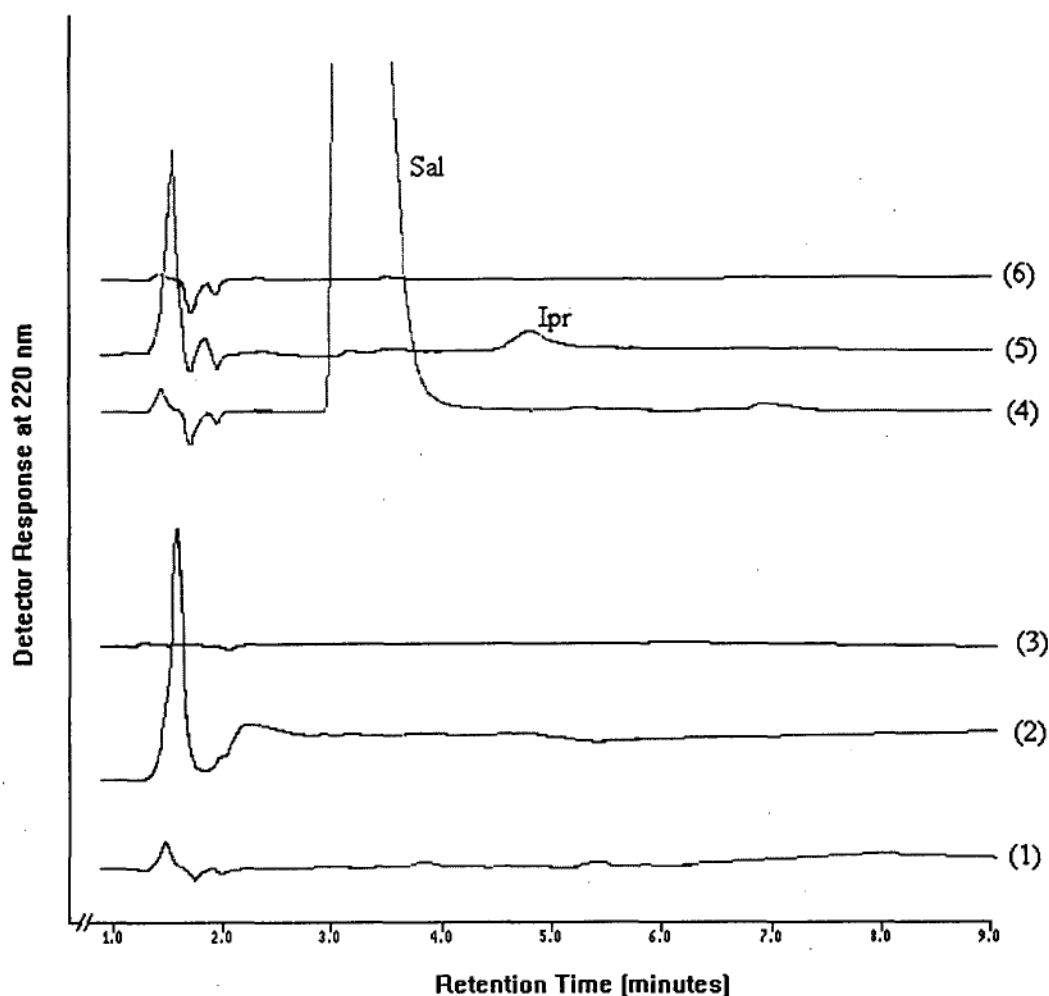
Figure 5.2   *Chromatograms of samples of salbutamol 5 mg/ml (1), ipratropium 250 µg/ml (2),*
distilled water blank (3) without an octane sulphonic acid ion-pair reagent in the
mobile phase; and samples of salbutamol 5 mg/ml (4), ipratropium 250 µg/ml (5),
distilled water blank (6)with an octane sulphonic acid ion-pair reagent in the mobile
phase.

was prepared by diluting the contents of one vial of Pic® B-8 Reagent Low UV in

100 ml of distilled water. Fifty millilitres of this solution was then added to 400 ml

of tetrahydrofuran and made up to 1000 ml with distilled water. This resulted in a

solution containing 0.0025 M Pic® B-8 Reagent Low UV which was half the

recommended strength of 0.005 M (accompanying Pic® instructions). The level of

ion-pair reagent was reduced to minimise the cost of the assay and did not impair

performance under the conditions of this assay. Mobile phase B was distilled water

and mobile phase C was methanol - distilled water (50:50, v/v). All mobile phases

were filtered through a 0.5 μm filter (Lido Manufacturing Corp., Bensenville, IL, USA) using a vacuum flask before use.

Flow was 2.0 ml/min of 50% mobile phase A and 50% mobile phase B up to 7.7 minutes, then changing linearly to 60% mobile phase A, 15% mobile phase B and 25% mobile phase C at 13.0 minutes. Run time was 13.0 minutes with a 5.0 minute equilibration time.

5.3.5   Calibration curve

An internal standard solution of mepivacaine was prepared by adding 2 ml of mepivacaine hydrochloride 1% to 8 ml of distilled water to give a solution of 2.0 mg/ml.

A 2 ml stock solution containing ipratropium, fenoterol, salbutamol and terbutaline nebuliser solutions (3:1:1:1, v/v/v/v) was prepared from the proprietary nebuliser solutions to give a solution containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml. Further dilutions of the stock solution were made; 200 μl aliquots of the stock solution were added to appropriate volumes of distilled water to give concentrations of 66.7%, 33.3%, 16.7%, 8.3% and 4.2% of the stock solution concentrations.

In addition, individual solutions of fenoterol 500 μg/ml, salbutamol 2.5 mg/ml, terbutaline 2.5 mg/ml (prepared from proprietary nebuliser solutions) and ipratropium 250 μg/ml (proprietary nebuliser solution) were used to give maximum points on the calibration curves.

5.3.6   Sample preparation and calibration curve

Three hundred microlitres of sample solution was added to 250 μl of internal standard solution and mixed using a vortex mixer (Super-Mixer, Lab-Line Instruments Inc., Melrose Park, IL, USA). Twenty microlitres of the mixture was injected for analysis and peak area to internal standard area ratios were calculated using the Varian GC Star workstation and a calibration curve for ipratropium, fenoterol, salbutamol and terbutaline constructed by computer using a linear least squares regression method (Cricket Graph® 1.3.2, Cricket Software, Malvern, PA, USA).

5.3.7   Precision

Intra-day variation was calculated by analysing five individually prepared samples of 300 μl of stock solution mixed with 250 μl of internal standard solution at evenly spaced intervals throughout the sampling day. The coefficient of variation was calculated for each drug from peak/ internal standard area ratios.

Inter-day variation was calculated by analysing a daily prepared sample of 300 μl of stock solution containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml, mixed with 250 μl of internal standard solution each day over a five day period. The coefficient of variation was calculated for each drug from peak/ internal standard area ratios.

5.3.8   Stability indicating nature of the assay

Each of the four nebuliser solutions were degraded with four different conditions: heat, hydrogen peroxide, hydrochloric acid and sodium hydroxide.

Heat degradation samples were prepared by adding 1 ml of each of the proprietary nebuliser solutions to separate glass ampoules, which were then sealed and heated at 200°C for 2 hours. After cooling, each heat degraded sample was diluted with an

appropriate volume of distilled water to give a theoretical maximum concentration equivalent to that in the stock solution (either ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml or terbutaline 1.67 mg/ml).

Hydrogen peroxide degradation samples were prepared by adding 100 μl of hydrogen peroxide (35% w/w) to 200 μl of nebuliser solution in a glass vial and heating at 75°C for 20 minutes. After cooling, each nebuliser solution was diluted with an appropriate volume of distilled water to give a theoretical maximum concentration equivalent to that in the stock solution.

Samples were degraded under acidic conditions by adding 100 μl of hydrochloric acid (2 M) to 200 μl of nebuliser solution in a glass vial and heating at 75°C for 20 minutes. After cooling, each nebuliser solution was neutralised with 100 μl of sodium hydroxide (2 M). Fenoterol, salbutamol and terbutaline samples were further diluted with distilled water to give final theoretical maximum concentrations equivalent to that in the stock solution.

Samples were degraded under basic conditions by adding 100 μl of sodium hydroxide (2 M) to 200 μl of nebuliser solution in a glass vial and heating at 75°C for 20 minutes. After cooling, each nebuliser solution was neutralised with 100 μl of hydrochloric acid (2 M). Fenoterol, salbutamol and terbutaline samples were further diluted with distilled water to give final theoretical maximum concentrations equivalent to that in the stock solution.

An aliquot of 300 μl was taken from each degraded nebuliser solution and added to 250 μl of internal standard. All degradation samples were chromatographed under identical conditions to the calibration curve samples.

5.3.9 Elution of preservatives

Possible co-elution of drugs and preservatives contained in the proprietary nebuliser solutions was examined by comparing chromatograms of solutions of benzalkonium chloride 10.5 mg/ml, EDTA (ethylenediamine tetra-acetic acid) 7.0 mg/ml and chlorbutol 6.6 mg/ml in distilled water with a chromatogram of a stock solution nebuliser solution containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml. The levels of preservatives were significantly higher than that typically encountered in proprietary nebuliser solutions.

5.3.10 Chromatographic parameters

Chromatographic parameters of capacity factor (k') and peak symmetry (A_{10}) were measured for all compounds from the calibration curve stock solution containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml (Li Wan Po and Irwin, 1980).

5.4 RESULTS AND DISCUSSION

5.4.1 Chromatographic conditions

The chromatogram of a stock solution sample containing ipratropium, fenoterol, salbutamol and terbutaline with mepivacaine as internal standard is shown in Figure 5.3. Retention times were as follows: salbutamol 3.2 minutes, terbutaline 4.3 minutes, ipratropium 5.9 minutes, mepivacaine 8.2 minutes and fenoterol 12.7 minutes.
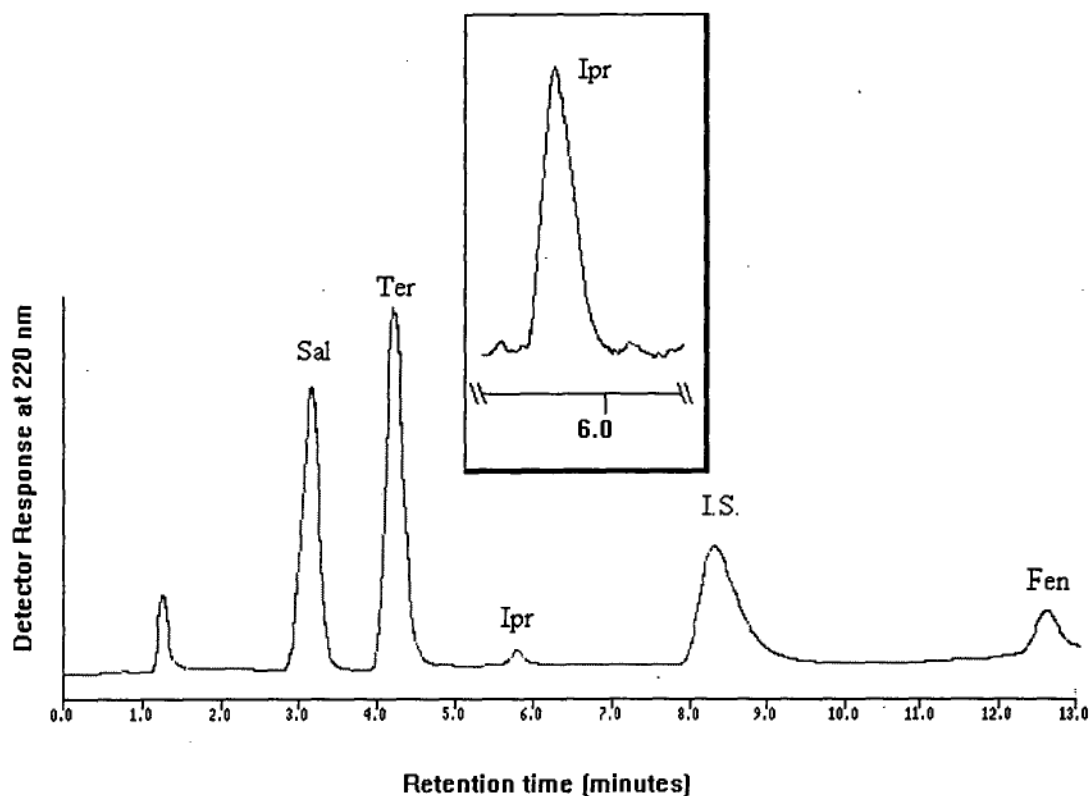
Figure 5.3   *Chromatogram of a stock solution sample containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml with mepivacaine as internal standard (I.S.).*

5.4.2   Calibration curve

Calibration curves for ipratropium, fenoterol, salbutamol and terbutaline are shown in Figures 5.4, 5.5, 5.6 and 5.7 respectively. The calibration curve parameters are shown in Table 5.1. It can be seen that the standard curves were linear over the following ranges: ipratropium 20.8-250.0 μg/ml, fenoterol 27.8-500.0 μg/ml, salbutamol 34.7-2500.0 μg/ml and terbutaline 69.5-2500 μg/ml. Ipratropium, fenoterol, salbutamol and terbutaline were all detectable at levels below the calibration range.

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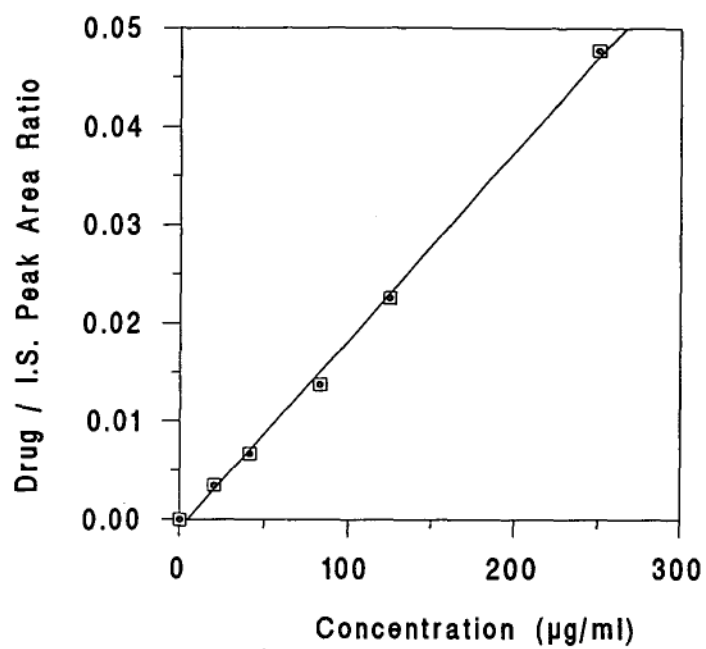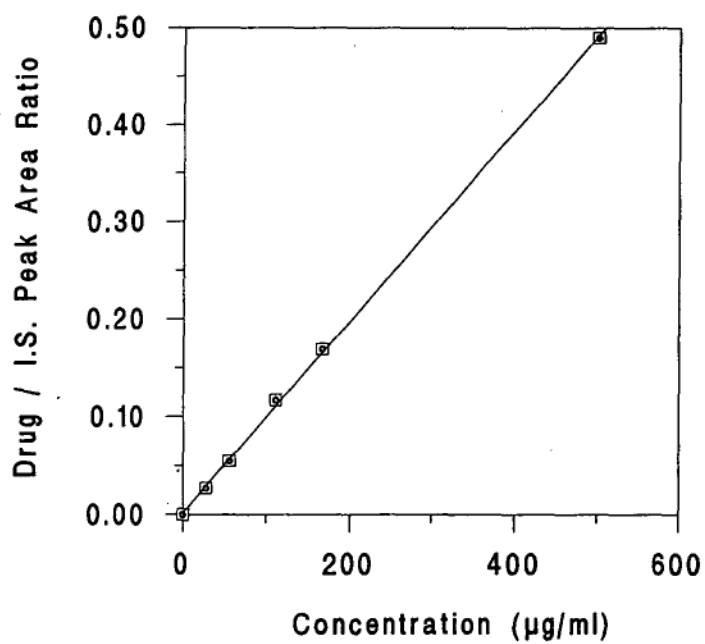**Figure 5.4** *Calibration curve for ipratropium bromide.*

Figure 5.5 *Calibration curve for fenoterol.*
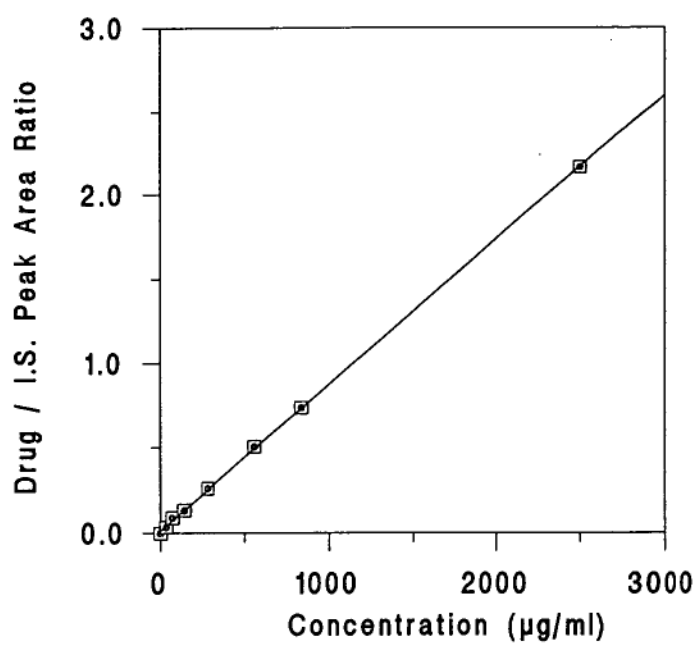
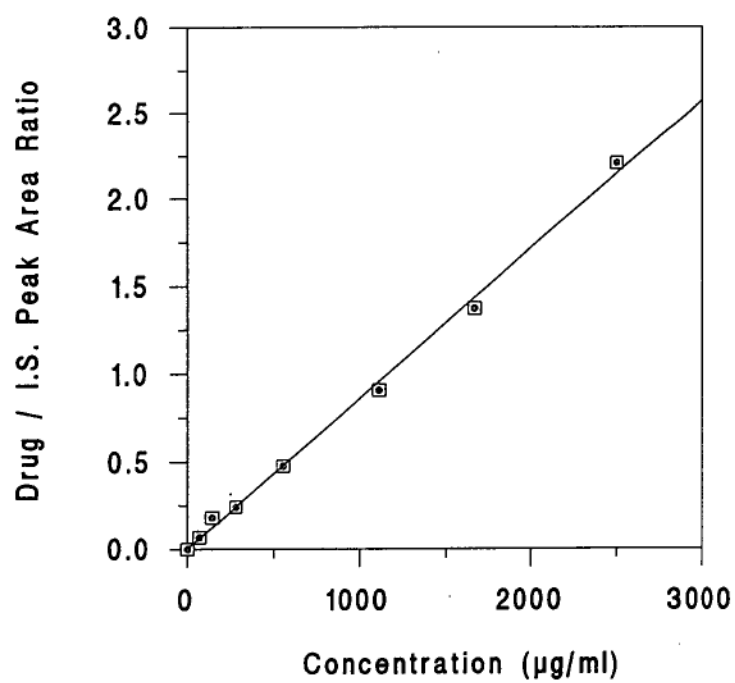**Figure 5.6** *Calibration curve for salbutamol.*

Figure 5.7 *Calibration curve for terbutaline.*

Table 5.1 *Calibration curve parameters of ipratropium, fenoterol, salbutamol and terbutaline.*

	(n)	Linear Range (μg/ml)		Regression Line Equation (y = Peak Area Ratio) (x = Concentration μg/ml)	Correlation Coefficient (r)
Ipratropium	6	20.8 -	250.0	y = -0.000997 + 0.000192 x	1.00
Fenoterol	6	27.8 -	500.0	y = 0.00251 + 0.000979 x	1.00
Salbutamol	8	34.7 -	2500.0	y = 0.0168 + 0.000861 x	1.00
Terbutaline	8	69.5 -	2500.0	y = 0.00392 + 0.000857 x	1.00

5.4.3 Precision

Intra-day variation was determined by calculating the coefficient of variation. The coefficient of variation for each drug was (all n=5): ipratropium 3.5%, fenoterol 3.5%, salbutamol 3.9%, and terbutaline 3.9%. Inter-day variation was (all n=5): ipratropium 4.8%, fenoterol 5.1%, salbutamol 4.5% and terbutaline 5.2%.

5.4.4 Stability indicating nature of the assay

Peak areas of heat degraded samples of salbutamol and terbutaline were reduced to 3% and 54% of the peak areas from the original stock solution respectively. A higher attenuation revealed that the original ipratropium peak at 5.9 minutes had been completely degraded under the heat degradation conditions with only a flat baseline remaining and a small degradation peak at 5.0 minutes. Similarly, the original fenoterol was completely degraded under the heat degradation conditions. A minor degradation product of terbutaline with a retention time of 5.6 minutes shouldered the non-degraded ipratropium peak (retention time 5.9 minutes) but did not affect the accurate integration of this peak. None of the degradation products of ipratropium, fenoterol, salbutamol or excipients in their respective nebuliser solutions produced interference peaks with the stock solution containing

ipratropium 125 µg/ml, fenoterol 167 µg/ml, salbutamol 833 µg/ml and terbutaline 1.67 mg/ml.

Peak areas of samples degraded by hydrogen peroxide were reduced to 36%, 71%, 78% and 64% of the original ipratropium, fenoterol, salbutamol and terbutaline peak areas, respectively, with degradation product peaks in the solvent front and small peaks late in the chromatogram. Peak areas of samples degraded under acidic conditions were reduced to 88%, 79% and 69% of the original fenoterol, salbutamol and terbutaline peak areas, respectively, with small degradation product peaks late in the chromatogram. A lower attenuation revealed that the original ipratropium peak at 5.9 minutes had been completely degraded under acidic conditions with only a flat baseline remaining.

Peak areas of samples degraded under basic conditions were reduced to 82%, 84% and 65% of the fenoterol, salbutamol and terbutaline peak areas, respectively, with small degradation product peaks late in the chromatogram. A lower attenuation again revealed that the original ipratropium peak at 5.9 minutes had been completely degraded under basic conditions with only a flat baseline remaining. Chromatograms of degraded ipratropium, fenoterol, salbutamol and terbutaline samples under each of the degradation conditions are shown in Figures 5.8, 5.9, 5.10 and 5.11 respectively.

It was not feasible to establish mass balance for all of the drugs under all of the degradation conditions because of the many unknown decomposition products present in the samples. Furthermore, peak homogeneity could not be measured as the laboratory did not have a diode array detector. However, the assay was determined to be stability indicating for ipratropium, fenoterol, salbutamol and terbutaline under heat, hydrogen peroxide, acid and base degradation given the

Figure 5.8 *Comparison of chromatograms of non-degraded stock solution (1), and heat (2), hydrogen peroxide (3), acid (4) and base (5) degraded ipratropium nebuliser solution under identical attenuation and identical initial strength (125 μg/ml). See Section 5.3.8.*

significant reduction in peak areas and the absence of interfering peaks from decomposition products.

5.4.5 Elution of preservatives

A chromatogram of possible interfering preservative excipients (benzalkonium chloride, EDTA and chlorbutol) is shown in Figure 5.12. It can clearly be seen that these preservatives, even at significantly higher levels than in proprietary nebuliser solutions, did not interfere with any of the chromatographic peaks of interest.

Figure 5.9   *Comparison of chromatograms of non-degraded stock solution (1), and heat (2), hydrogen peroxide (3), acid (4) and base (5) degraded fenoterol nebuliser solution under identical attenuation and identical initial strength (167 µg/ml). See Section 5.3.8.*
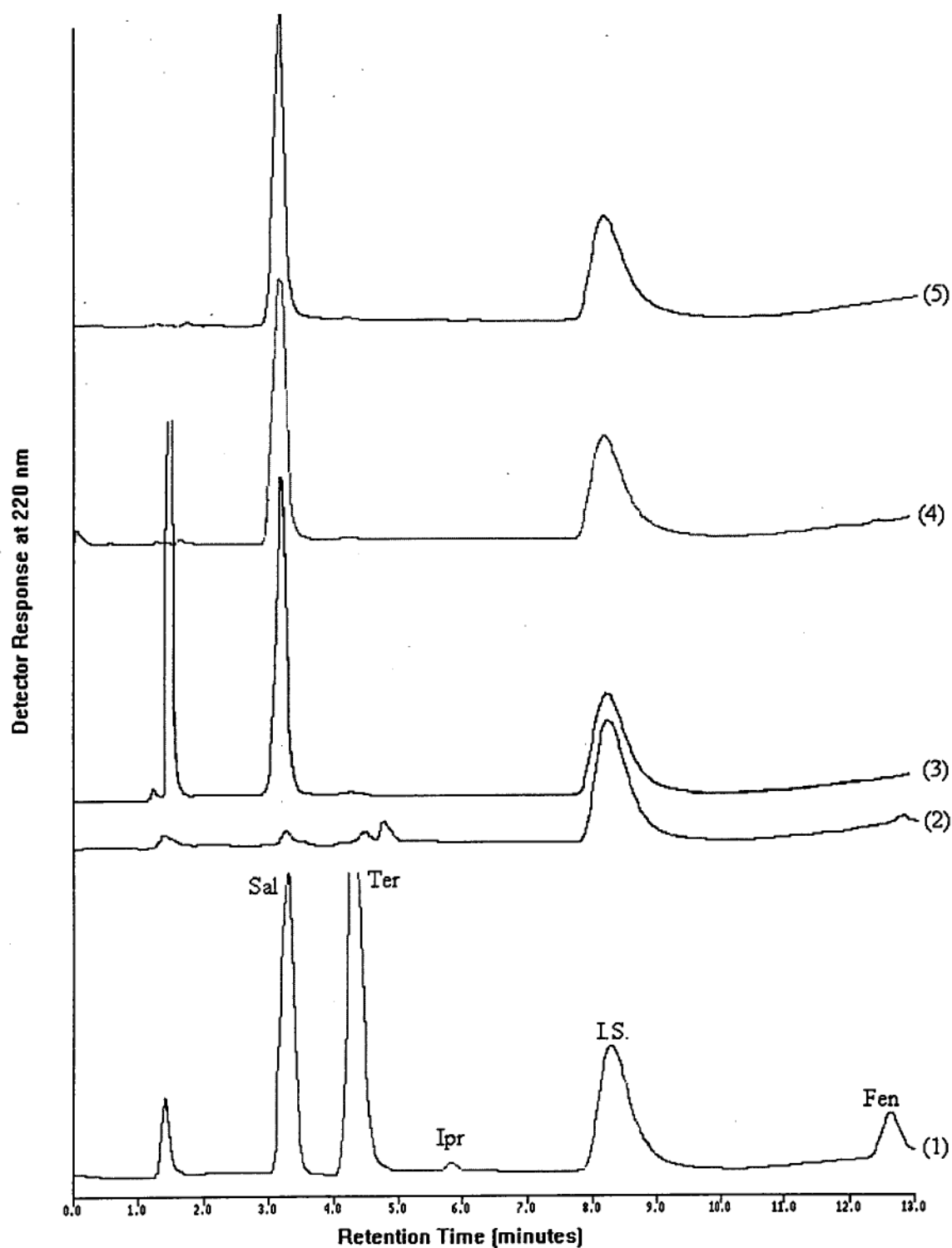
Figure 5.10 *Comparison of chromatograms of non-degraded stock solution (1), and heat (2), hydrogen peroxide (3), acid (4) and base (5) degraded salbutamol nebuliser solution under identical attenuation and identical initial strength (833 μg/ml). See Section 5.3.8.*
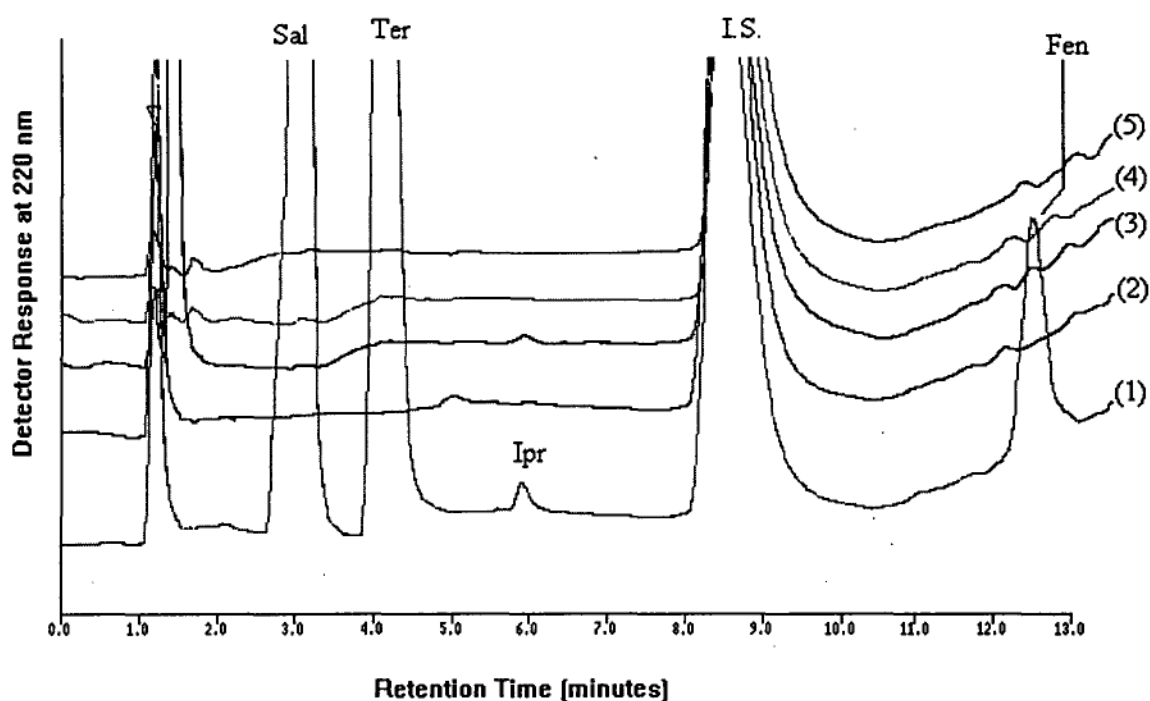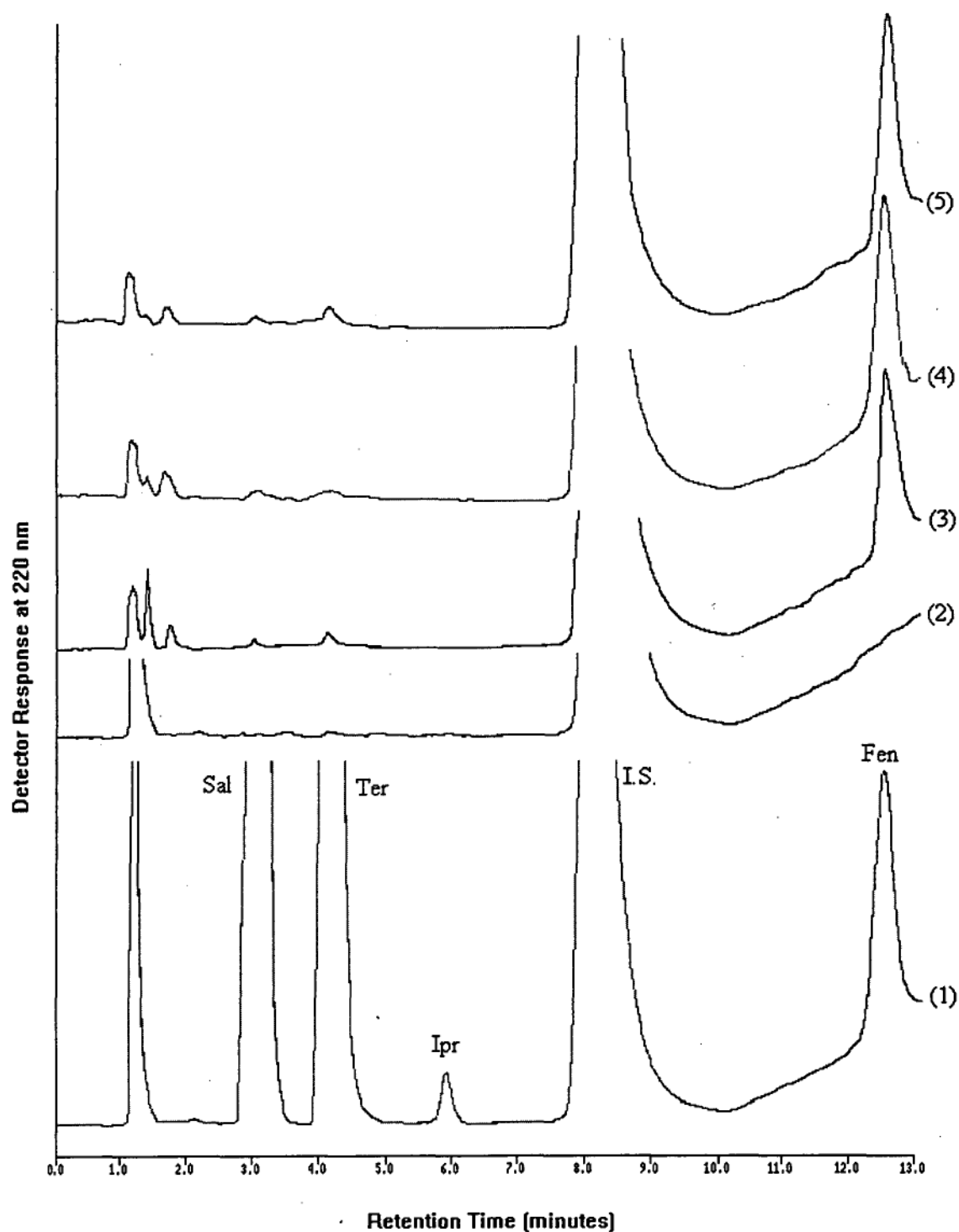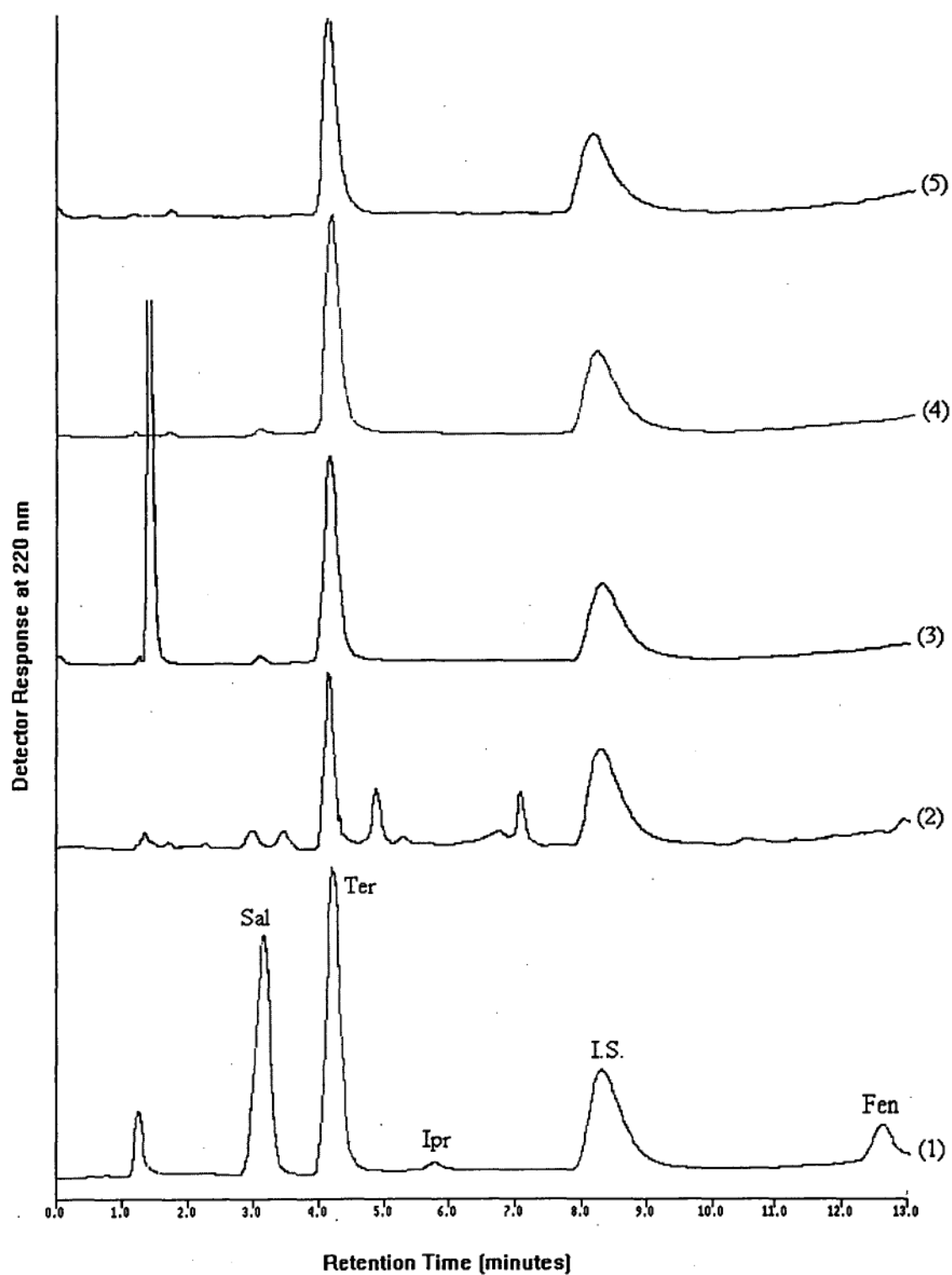
Figure 5.11   *Comparison of chromatograms of non-degraded stock solution (1), and heat (2), hydrogen peroxide (3), acid (4) and base (5) degraded terbutaline nebuliser solution under identical attenuation and identical initial strength (1.67 mg/ml). See Section 5.3.8.*
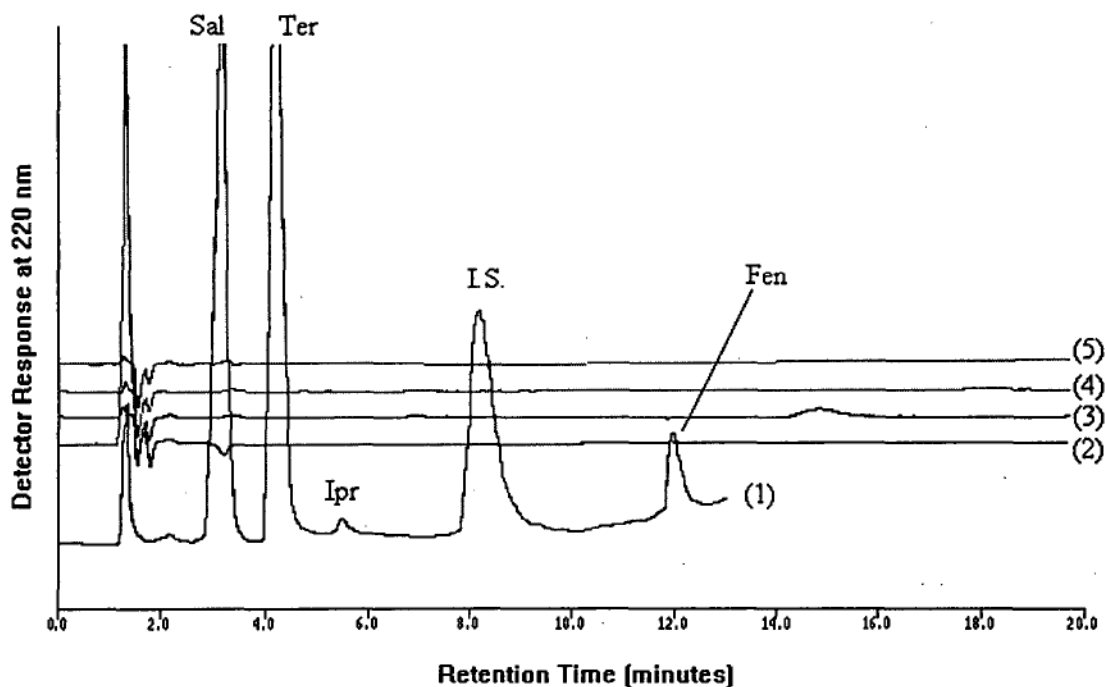
Figure 5.12   *Comparison of chromatograms from a stock solution containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml (1) with samples of distilled water blank (2), benzalkonium chloride 10.5 mg/ml (3), EDTA 7.0 mg/ml (4) and chlorbutol 6.6 mg/ml (5). See Section 5.3.9.*

5.4.6   Chromatographic parameters

The chromatographic parameters are shown in Table 5.2. There was significant peak asymmetry for mepivacaine. Peak symmetry of less than 1.5 is preferred for quantitative analysis; however, it is generally accepted that severe peak tailing of basic drugs in reversed-phase chromatography is caused by interaction of solutes with free silanol groups in the packing (Vervoot et al, 1992). Vervoot et al (1992) also found that the addition of an ion pair reagent (sodium 1-hexanesulphonate) did not decrease peak tailing with some protonated basic drugs, as may be the case with mepivacaine and the ion-pair reagent in this assay. The tailing of the mepivacaine peak was reduced considerably by using a relatively high proportion

of tetrahydrofuran in mobile phase A. The asymmetry of this peak did not affect assay performance.

Table 5.2 *Typical column parameters calculated from a chromatogram of a stock solution containing ipratropium 125 μg/ml, fenoterol 167 μg/ml, salbutamol 833 μg/ml and terbutaline 1.67 mg/ml with mepivacaine as internal standard.*

	Retention time t (min)	Capacity Factor k'	Peak Symmetry (A_{10})
Salbutamol	3.2	1.6	1.13
Terbutaline	4.3	2.5	1.75
Ipratropium	5.9	3.8	2.11
Mepivacaine	8.2	5.6	2.65
Fenoterol	12.7	9.2	1.55

5.5   CONCLUSION

The assay provides a method of determining concentrations of drugs in common nebuliser solution admixtures. The assay is also stability indicating and provides a basis for assays to determine the stability of common nebuliser admixtures containing ipratropium and β_2-agonists.

CHAPTER 6

Stability of Ipratropium Bromide and Salbutamol Nebuliser Admixtures

6.1 SUMMARY

This study aimed to determine the physicochemical stability of an admixture of ipratropium bromide and salbutamol nebuliser solution (ipratropium bromide: salbutamol, 1:1 v/v). Solutions were stored for 5 days in a refrigerator at 4 °C, at 22 °C protected from light and at 22 °C under 24 hour fluorescent lighting. Concentrations of ipratropium and salbutamol were periodically determined using a modification of the HPLC assay developed in Chapter 5. It was found that the nebuliser solution admixtures retained greater than 90% of their original concentrations of ipratropium and salbutamol for the duration of the study. Differences in losses between storage conditions were not statistically significant. An expiry period of 5 days for these admixtures would seem reasonable in practice.

6.2 INTRODUCTION

For patients unable to use metered dose inhalers, inhalation of nebulised respirator solutions is an integral component of the modern treatment of airways diseases (Anonymous 1987; Horsley, 1988; Johnson, 1989; Anonymous, 1993b). Drugs available as respirator solutions for the treatment of acute or chronic obstructive airways diseases in Australia include ipratropium bromide, fenoterol, salbutamol and terbutaline.

The mixing of ipratropium and β_2-agonist nebuliser solutions is a common practice (Iacono et al, 1987; O'Driscoll et al, 1987; Caldwell et al, 1991). The bulk mixing of ipratropium and β_2-agonist nebuliser solution provides a means of shortening the time that patients spend nebulising inhaled drugs, as well as producing savings in costs and nursing time when compared to unit-of-use pre-diluted sterile nebules (Fry and Williamson, 1991). It has also been suggested that the convenience and reduced administration time resulting from mixing nebuliser solutions may increase

patient compliance (Roberts and Rossi, 1993). The post-manufacture mixing of these nebuliser solutions, however, gives rise to issues of compatibility and stability.

Compatibility of ipratropium and salbutamol immediately prior to administration has been studied. Iacono et al (1987) showed that ipratropium in nebuliser solution retained greater than 90% of its initial concentration up to 1 hour after mixing with salbutamol and sodium cromoglycate solutions. However, long term compatibility and stability of admixtures of these solutions is not well documented.

Commencement of this study followed enquiries from pharmacists involved with a district nursing service regarding the stability of ipratropium:salbutamol admixtures. The nurses provide drugs including nebuliser admixtures to out-patients on a regular basis, typically several visits per week. Because of the common practice of mixing bulk solutions of drug, the objective of this study was to answer the clinically relevant question: do pre-mixed nebuliser admixtures of ipratropium and salbutamol retain greater than 90% of their initial concentrations if stored over a typical working week of 5 days?

6.3   METHOD

6.3.1   Materials

Salbutamol sulphate (pure substance) was kindly donated by Glaxo Australia Pty. Ltd. (Boronia, Vic.) and ipratropium bromide (pure substance) was kindly donated by Boehringer Ingelheim Pty. Ltd. (Ingelheim, Germany). The following nebuliser solutions were used in the assay procedure: ipratropium bromide (Atrovent® 0.025% w/v; Boehringer Ingelheim Pty. Ltd., Artarmon, NSW, Austalia), fenoterol hydrobromide (Berotec® 0.1% w/v; Boehringer Ingelheim Pty. Ltd., Artarmon, NSW, Austalia) and salbutamol sulphate (Ventolin® 0.5% w/v; Glaxo Australia

Pty. Ltd., Boronia, Vic., Australia). Methanol and tetrahydrofuran (liquid chromatography grade) were purchased from Waters Chromatography Division (Millipore Australia Pty. Ltd., Sydney). Pic® B-8 Reagent Low UV (containing water, methanol, octane sulfonic acid and calcium acetate in undisclosed quantities) was purchased from Waters Associates (Milford, MA, USA).

6.3.2 Assay procedure

The high performance liquid chromatography (HPLC) system consisted of a Varian Solvent Delivery System Model 9010 (Varian Chromatography Systems, Walnut Creek, CA, USA) with a Rheodyne injector Model 7161 (Rheodyne Inc., Cotati, CA, USA) with a 10 μl external loop connected to a Varian Variable Wavelength UV-VIS Detector Model 9050 (Varian Chromatography Systems, Walnut Creek, CA, USA). Chromatographic peaks were digitised, integrated and recorded with a Varian GC Star Workstation (Varian Chromatography Systems, Walnut Creek, CA, USA). The analysis was performed using a Nova-Pak® C_{18} 4 μm 10 cm x 8 mm i.d. Radial-pak® liquid chromatography cartridge (Waters Associates, Milford, MA, USA) inside a Waters RCM 8 x 10 compression module (Waters Associates, Milford, MA, USA).

Solute elution was satisfactorily performed using isocratic analysis. This assay was adapted from an assay for combinations of ipratropium and β_2-agonist nebuliser solutions using reversed phase ion-pair chromatography with ternary gradient analysis (Chapter 5). Flow was 1.0 ml/min of a mobile phase containing 30% tetrahydrofuran, 1.9 mM octane sulfonic acid (from a dilution of Low UV Pic® B-8 ion-pair reagent) and 5% methanol in distilled water. Run time was 10 minutes. Detector conditions were as follows: wavelength 272 nm at 0.5 AUFS until 4.1 minutes changing to 220 nm at 0.05 AUFS until 10 minutes. Retention times for salbutamol, ipratropium and the internal standard fenoterol were 3.5, 4.4 and 7.6 minutes respectively.

An internal standard solution of fenoterol 100 μg/ml in distilled water was prepared from the proprietary fenoterol nebuliser solution (1 mg/ml). Calibration curves were constructed daily from freshly prepared admixtures of ipratropium bromide nebuliser solution 250 μg/ml and salbutamol sulphate nebuliser solution 5.0 mg/ml over the following range: ipratropium 31-156 μg/ml and salbutamol 0.625-3.13 mg/ml. The concentration of the proprietary admixtures was verified both at the beginning and the end of the study using a calibration curve constructed from pure ipratropium bromide and salbutamol sulphate powder. The same fenoterol internal standard solution and ipratropium and salbutamol proprietary nebuliser solutions were used throughout the course of the study and were stored at 4°C and protected from light.

Fifty microlitres of each sample was added to 25 μl of internal standard solution and mixed using a vortex mixer (Super-Mixer®, Lab-Line Instruments Inc., Melrose Park, IL, USA). Twenty microlitres of each sample was injected for analysis in duplicate and the results averaged. Peak area to internal standard area ratios were calculated using the Varian GC Star workstation and calibration curves for ipratropium and salbutamol constructed by computer using a linear least squares regression method (Cricket Graph® 1.3.2, Cricket Software, Malvern, PA, USA).

Intra-day variation was calculated for each day of sampling by analysing five samples of ipratropium:salbutamol nebuliser solution (1:1 v/v; containing ipratropium bromide 125 μg/ml and salbutamol 2.5 mg/ml) with internal standard at evenly spaced intervals throughout the sampling day. The coefficient of variation was calculated for each drug from peak area/internal standard area ratios.

The stability indicating nature of the assay was validated by heat, acid and base hydrolysis, and oxidation. Heat degradation samples were prepared by placing

100 μl of salbutamol proprietary nebuliser solution, 100 μl of ipratropium bromide proprietary nebuliser solution or 100 μl of ipratropium:salbutamol nebuliser solution (1:1 v/v) into separate sealed glass ampoules. Ipratropium and salbutamol degradation samples were heated at 200°C for 1, 2 or 3 hours in a gas chromatograph oven. The combined ipratropium:salbutamol nebuliser solution (1:1 v/v) was heated for 2 hours at 200°C.

Acid degradation samples were prepared by adding 50 μl of 1 M HCl to 100 μl of ipratropium:salbutamol nebuliser solution (1:1 v/v) in a glass vial. Samples were heated at 75°C for 20 minutes or 1 hour then neutralised by adding 50 μl of 1 M NaOH. Base degradation samples were prepared by adding 50 μl of 1 M NaOH to 100 μl of ipratropium: salbutamol nebuliser solution (1:1 v/v) in a glass vial. Samples were heated at 75°C for 20 minutes or 1 hour then neutralised by adding 50 μl of 1 M HCl. Oxidation degradation samples were prepared by adding 50 μl of 35% w/w hydrogen peroxide to 100 μl of ipratropium:salbutamol nebuliser solution (1:1 v/v) in a glass vial. Samples were heated at 75°C for 20 minutes or 1 hour then diluted with a further 50 μl of distilled water.

All degradation samples were allowed to cool. A 50 μl aliquot of each sample was added to 25 μl of internal standard solution and chromatographed under identical conditions to the calibration curve samples. Peak area ratios from chromatograms of degraded samples were compared with peak areas from freshly prepared standards that had been diluted to the same extent as the degraded samples. Chromatograms were also examined for any decomposition product peaks interfering with the ipratropium, salbutamol or internal standard peaks.

Known excipients in the proprietary nebuliser solutions, benzalkonium chloride and ethylenediamine tetra-acetic acid (EDTA), were tested for possible co-elution with the peaks of interest. Solutions of benzalkonium chloride 10.7 mg/ml in

distilled water and EDTA 7.0 mg/ml in distilled water were chromatographed under identical conditions to the calibration curve samples.

6.3.3 Stability study

A 20 ml admixture of ipratropium bromide and salbutamol sulphate nebuliser solutions (1:1 v/v; containing ipratropium bromide 125 µg/ml and salbutamol 2.5 mg/ml) was mixed thoroughly. Aliquots of 2 ml were taken and placed into nine pre-weighed 6 ml clear glass vials (Miniature Vial®, Canberra-Packard Pty. Ltd., Mount Waverley, Vic., Australia) and then re-weighed. Three vials were then stored under each of the following conditions: (i) refrigerated at 4 ± 0.5°C; (ii) shallow immersion in an orbital shaking water bath (Model OW 1412, Paton Industries Pty. Ltd., Stepney, South Australia) at 22 ± 0.25°C protected from light by aluminium foil; and (iii) shallow immersion in an orbital shaking water bath at 22 ± 0.25°C subject to continuous fluorescent lighting for the duration of the study. Lighting was provided by an 18 W fluorescent light suspended approximately 20 cm above the solutions. Samples exposed to light were tilted at approximately 45° so that the nebuliser solution admixtures were in the direct path of light and not protected by the opaque vial caps. The solutions stored at 22°C in the water bath were gently shaken at 20 oscillations per minute.

Nebuliser solutions were sampled initially, and on days 1, 2 and 5. Samples were analysed in the same manner as the calibration curve samples. The concentrations of drugs remaining in the nebuliser solutions were calculated from the daily calibration curves.

Potential loss of solvent through evaporation was checked by pre-weighing the storage vials, re-weighing the vials after addition of the 2 ml combined nebuliser solution, and weighing again at the completion of the study. The initial weight of solution in the sample vials, after correcting for the removal of a succession of

100 μL aliquots, was compared with the final weight of solution in the sample vials after the completion of the study.

6.3.4 Statistical methods

Differences in the losses of ipratropium and salbutamol between the solutions and sampling periods were statistically evaluated using repeated measures analysis of variance (Statview® 4.01 package, Abacus Concepts Inc., Berkeley, CA, USA, on a Macintosh® computer).

6.4 RESULTS

6.4.1 Assay procedure

The range of intra-day coefficients of variation over the sampling days for salbutamol was found to be 2.5 - 4.2% (all n = 5). The range of intra-day coefficients of variation for ipratropium was found to be 2.6 - 4.9% (all n = 5). All standard curves throughout the study had correlation coefficients (r) greater than 0.996 on all days of sampling. A chromatogram of an ipratropium:salbutamol (1:1 v/v) admixture is shown in Figure 6.1. Calibration curves are shown in Figures 6.2 and 6.3.

The heat decomposed salbutamol nebuliser solution was originally clear which discoloured to a brown solution with precipitate after heating. The ipratropium solution remained clear without any precipitate. The assay was found to be stability indicating as degradation product peaks did not interfere with either the salbutamol, ipratropium or internal standard peaks (shown in Figure 6.4).

The heat decomposed salbutamol retained approximately 16% of the original peak area after 1 hour which decreased to approximately 4% and 1% at 2 and 3 hours respectively. The heat decomposed ipratropium was degraded below the detection

limit after 1 hour with only a flat baseline remaining. No co-eluting peaks from decomposition products emerged at the retention time of the original ipratropium peak after 2 and 3 hours. This suggested that the peaks of interest in the chromatogram were free of any interference from primary or secondary decomposition products.

The combined heat decomposition sample of ipratropium:salbutamol (1:1 v/v) admixture had similar decomposition chromatograms after 2 hours when compared to the individually heat stressed ipratropium and salbutamol nebuliser solutions suggesting no obvious decomposition interaction between the two components.

Acid degradation of an ipratropium:salbutamol (1:1 v/v) admixture reduced salbutamol peak areas to approximately 49% and 45% of original peak areas after 20 minutes and 1 hour respectively. The ipratropium peaks were degraded below the detection limit after 20 minutes with no peaks from primary or secondary decomposition products emerging at the retention time of ipratropium after 1 hour.

Base degradation of an ipratropium:salbutamol (1:1 v/v) admixture reduced salbutamol peak areas to approximately 53% and 38% of original peak areas after 20 minutes and 1 hour respectively. The ipratropium peaks were degraded below the detection limit after 20 minutes with no peaks from primary or secondary decomposition products emerging at the retention time of ipratropium after 1 hour.

Oxidation degradation of an ipratropium: salbutamol (1:1 v/v) admixture reduced salbutamol peak areas to approximately 92% and 87% of original peak areas after 20 minutes and 1 hour respectively. The ipratropium peak areas were reduced to approximately 96% and 95% of original peak areas after 20 minutes and 1 hour
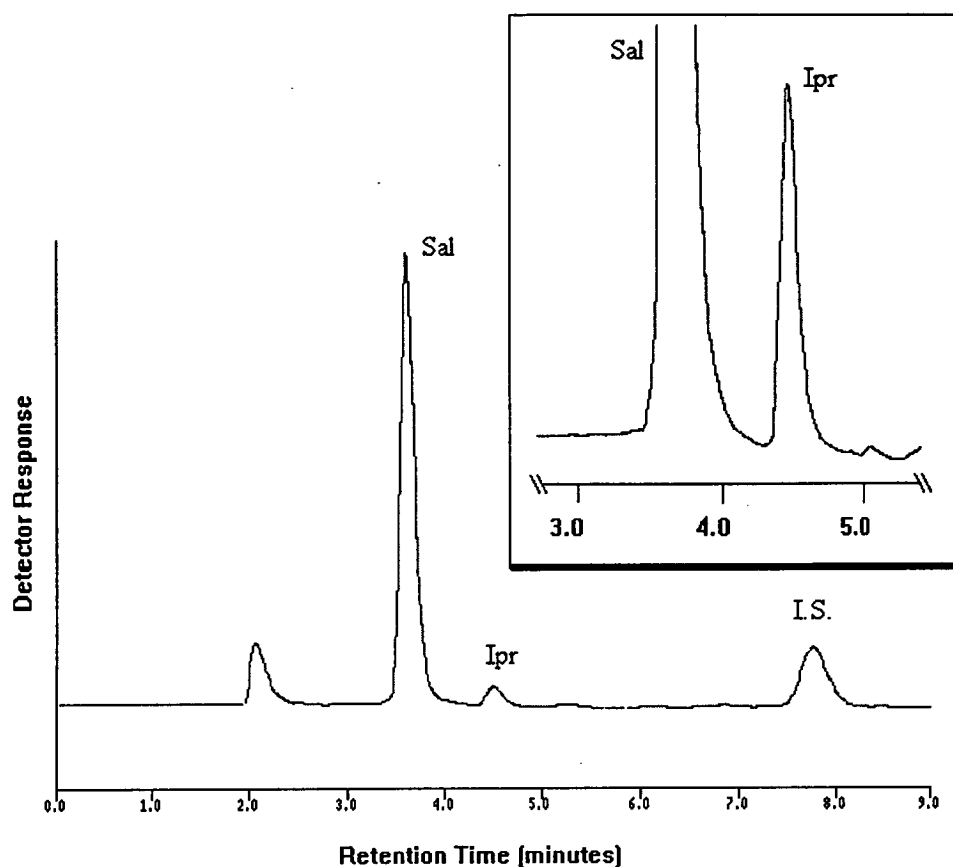
Figure 6.1 *Chromatogram of an ipratropium : salbutamol (1:1 v/v) admixture. Inset shows ipratropium and salbutamol peak separation with a 16-fold decrease in attenuation*

respectively. Salbutamol produced four significant decomposition products at retention times of 4.2, 4.9, 5.7 and 9.4 minutes after heat stressing at 200°C. The largest (at 5.7 minutes) had a peak height approximately 10% of the parent drug and twice as large as the peak due to ipratropium. The peaks at 4.2 and 4.9 minutes were of comparable height to the ipratropium peak.

Ipratropium produced four significant decomposition products at retention times of 4.2, 4.9, 5.9 and 9.3 minutes after heat stressing at 200°C. The peak at 5.9 minutes was comparable in size to that of the parent drug. The peaks at 4.2 and 4.9 minutes were approximately 30% and 15% of the height of the parent drug. All decomposition product peaks had baseline separation from the peaks of interest and did not affect integration. These decomposition peaks were not seen during the
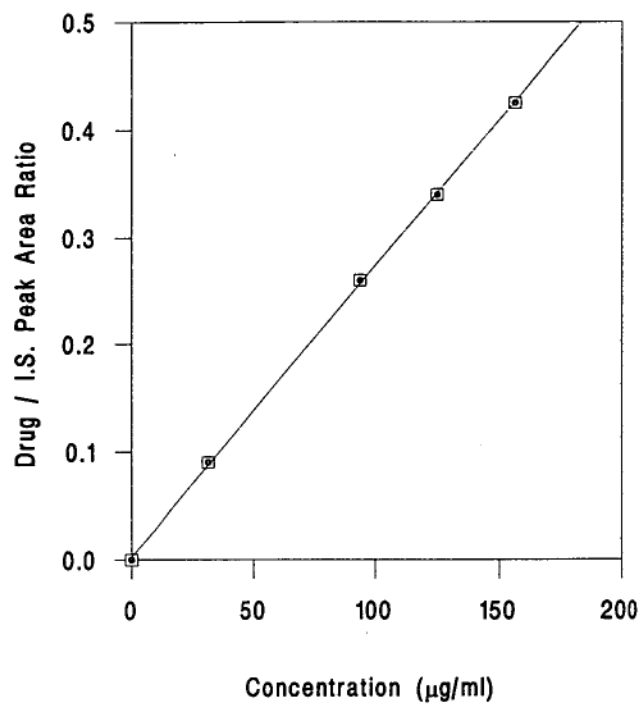
Figure 6.2 *Calibration curve of ipratropium.*
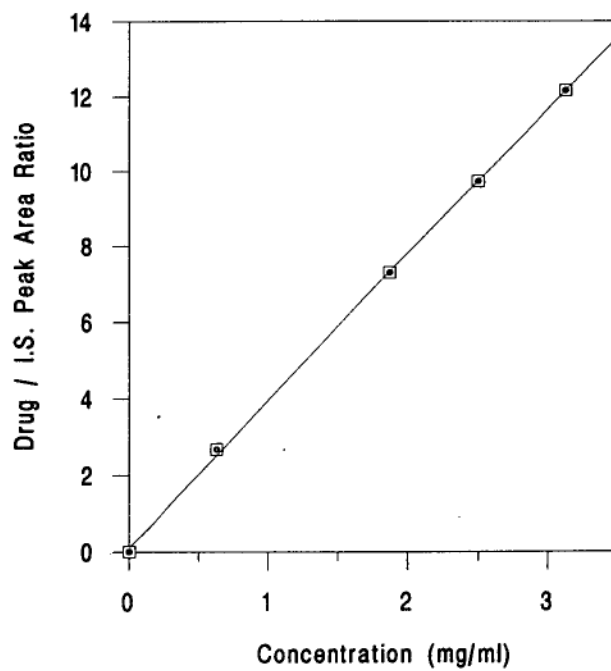
Figure 6.3 *Calibration curve of salbutamol.*
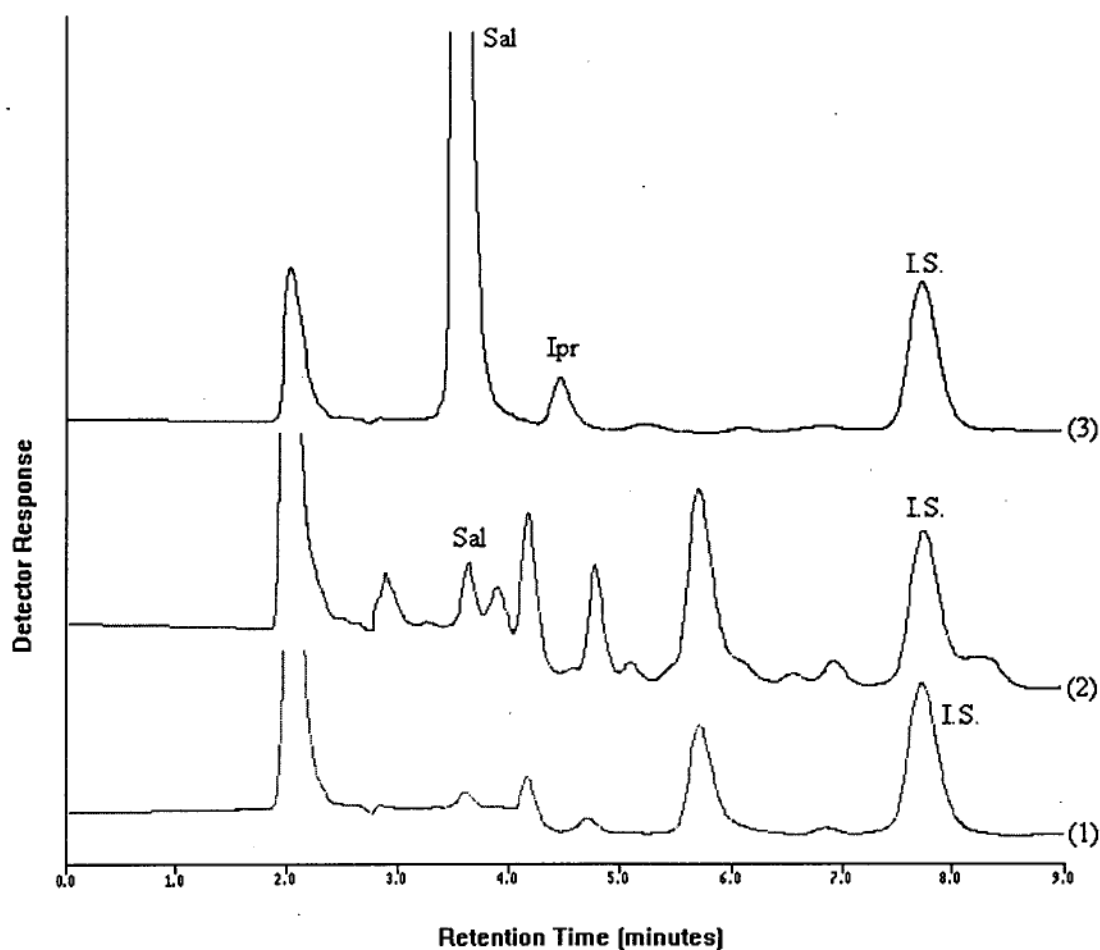
Figure 6.4   *Chromatograms showing degradation of ipratropium (1) and salbutamol (2) at 200°C compared to a non-degraded ipratropium:salbutamol (1:1 v/v) admixture (3).*

course of the study and were only observed under heat stressing, acid and base hydrolysis and oxidation.

Benzalkonium chloride and EDTA did not produce any significant peaks under the chromatographic conditions and hence, did not interfere with the chromatography of any of the peaks of interest.

6.4.2   Stability study

Degradation was expressed as the percentage of the measured initial drug concentration in the combined nebuliser solution immediately after mixing. The percentages of initial concentration of each drug remaining with time are shown in Table 6.1. Degradation profiles are shown in Figures 6.5, 6.6 and 6.7 for

ipratropium: salbutamol 1:1 v/v admixtures stored at 4°C (dark), 22°C (dark) and 22°C (light) respectively. All admixtures clearly retained at least 90% of their initial concentrations for the duration of the study. The nebuliser solution admixtures remained clear with no discolouration, cloudiness or precipitation throughout the course of the study. Evaporation loss was minimal with all solutions retaining at least 97.5% of their expected weight.

Repeated measures analysis of variance for salbutamol revealed no statistically significant difference in loss between the storage conditions (F=0.29, 2 and 6 df, p > 0.50). There was a significant difference in the concentration of salbutamol with the storage periods (F=6.10, 3 and 18 df, p < 0.01), attributable to the initial slight fall between days zero and one. Similarly, repeated measures analysis of variance for ipratropium revealed no statistically significant difference in loss between the storage conditions (F=0.69, 2 and 6 df, p > 0.20), but a significant difference in the concentration of ipratropium with the storage periods (F=4.84, 3 and 18 df, p < 0.05).

Table 6.1   *Degradation of ipratropium and salbutamol (1:1 v/v) nebuliser solution admixtures over a five day period.*

	Storage at 4°C (Dark)		Storage at 22°C (Dark)		Storage at 22°C (Light)	
	Salbutamol	Ipratropium	Salbutamol	Ipratropium	Salbutamol	Ipratropium
	Mean ± SD (% of initial concentration)		Mean ± SD (% of initial concentration)		Mean ± SD (% of initial concentration)	
Initial	2.43 ±0.09 (100.0)	129.2 ±5.4 (100.0)	2.43 ±0.09 (100.0)	129.2 ±5.4 (100.0)	2.43 ±0.09 (100.0)	129.2 ±5.4 (100.0)
Day 1	2.34 ±0.05 (96.3)	127.6 ±2.4 (98.8)	2.34 ±0.07 (96.3)	127.9 ±5.0 (99.0)	2.37 ±0.05 (97.5)	129.7 ±2.4 (100.4)
Day 2	2.35 ±0.03 (96.7)	126.0 ±2.1 (97.5)	2.41 ±0.06 (99.2)	127.8 ±3.1 (98.9)	2.39 ±0.02 (98.4)	127.6 ±1.3 (98.8)
Day 5	2.43 ±0.08 (100.0)	126.2 ±5.5 (97.7)	2.35 ±0.06 (96.7)	123.1 ±5.3 (95.3)	2.41 ±0.12 (99.2)	127.6 ±5.7 (98.8)
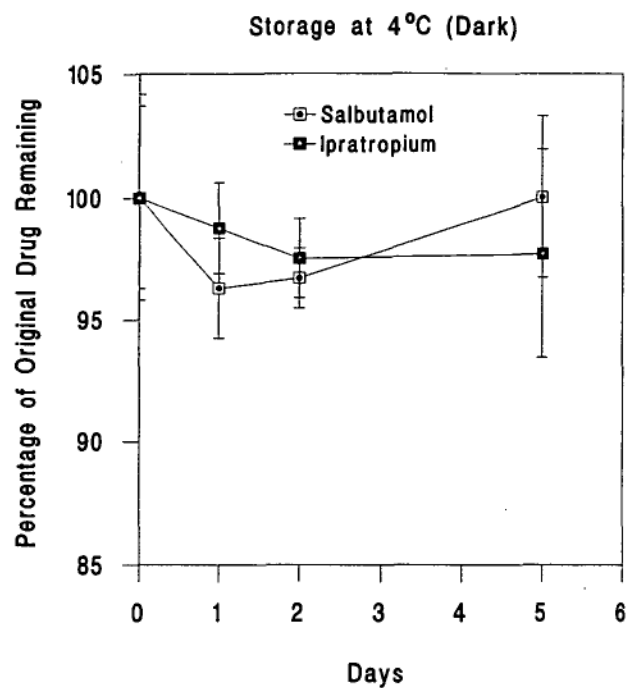
Figure 6.5 *Degradation profile of ipratropium:salbutamol (1:1 v/v) nebuliser admixture at 4°C and protected from light.*
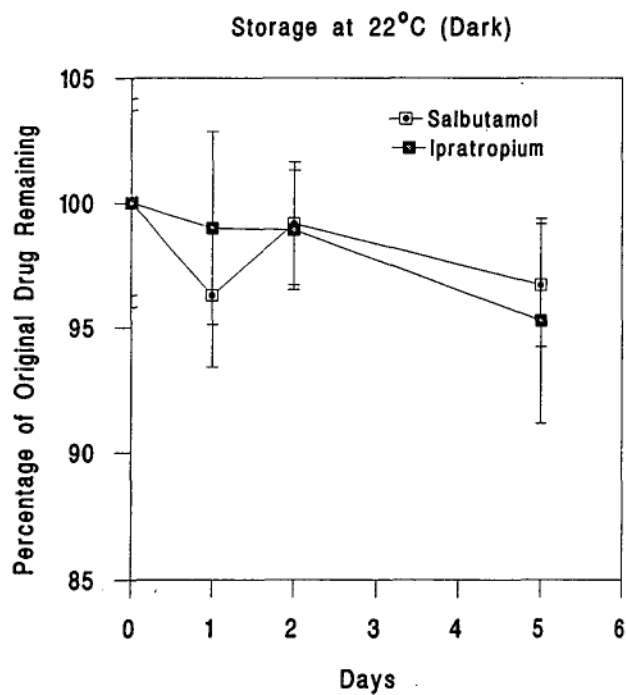
Figure 6.6 *Degradation profile of ipratropium:salbutamol (1:1 v/v) nebuliser admixture at 22°C and protected from light.*

Storage at 22°C (Light)
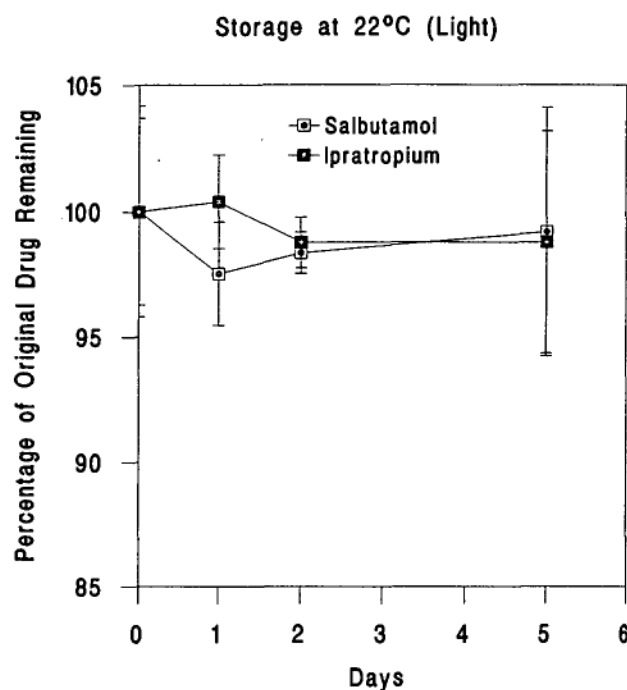
Figure 6.7 *Degradation profile of ipratropium:salbutamol (1:1 v/v) nebuliser admixture at 22°C and exposed to light.*

6.5 DISCUSSION

The results indicate that admixtures of proprietary ipratropium and salbutamol (1:1 v/v) nebuliser solutions retain greater than 90% of their initial concentrations when stored for periods of up to 5 days. Ipratropium was found to be slightly less stable than salbutamol. There were no statistically significant differences in stability between the storage conditions for either salbutamol or ipratropium.

It was not the purpose of this study to determine a precise expiry period but merely to examine the stability over a typical working week of 5 days. These findings suggest that it would seem reasonable in practice to recommend that admixtures of ipratropium bromide (Atrovent®) and salbutamol sulphate (Ventolin®) nebuliser solutions (1:1 v/v) can be stored between 4°C and 22°C for periods up to 5 days. This would allow bulk admixtures to be prepared and stored,

resulting in reduced preparation time and drug costs when compared to unit-of-use pre-diluted nebules (Fry and Williamson, 1991).

This study was based purely on physicochemical stability and did not take into account microbial contamination or clinical efficacy. Benzalkonium chloride is contained in Atrovent® (0.025%) and Ventolin® (0.01%) nebuliser solutions (Roberts and Rossi, 1993). Ipratropium nebuliser solution also contains EDTA (Roberts and Rossi, 1993). After these nebuliser solutions are combined to give a 1:1 v/v admixture the overall concentration of benzalkonium chloride is 0.0175% (less than that present in proprietary ipratropium solution). Roberts and Rossi (1993) noted that the manufacturer was considering lowering the concentration of benzalkonium chloride in Atrovent® so the importance of this dilution of preservative concentration in the ipratropium and salbutamol (1:1 v/v) nebuliser solution admixtures is unclear. Reliance on benzalkonium chloride as a preservative in nebuliser solutions has led to the nosocomial spread of microorganisms (Hamill et al, 1995). It is also worth noting that this storage period of 5 days would not apply to unit-of-use preservative free nebules. It is therefore important that patients are counselled and understand this difference, as well as being counselled about the appropriate storage and hygiene measures when using nebulised drugs (Anonymous, 1993b; Mathew, 1990).

6.6   CONCLUSION

Admixtures of proprietary ipratropium bromide and salbutamol nebuliser solutions (1:1 v/v) retain greater than 90% of their initial concentrations if stored between 4°C and 22°C for periods of up to five days. An expiry period of 5 days would seem reasonable in practice.

CHAPTER 7

Background on Salbutamol Pharmacokinetics

7.1 SUMMARY

The pharmacokinetics of salbutamol are outlined in the following sections. The majority of an inhaled dose is swallowed, is absorbed from the gastrointestinal tract and is subject to extensive first-pass metabolism in the intestinal wall and liver. Only a relatively small fraction of the dose that is inhaled exerts a local bronchodilatory effect in the lungs following systemic absorption. The only known metabolite of salbutamol in humans is the sulphate ester metabolite, salbutamol-4'-O-sulphate which is substantially less pharmacologically active than the parent drug. Salbutamol-4'-O-sulphate is freely filtered from the kidneys. Active tubular secretion is thought to play a major role in the renal excretion of salbutamol. The ratio of unchanged drug to metabolite in urine varies with the amount of dose subject to the first-pass effect according to the route of administration.

7.2 ABSORPTION

Despite their effectiveness and widespread use over more than two decades, there is surprisingly little information available on the pharmacokinetics of β_2-agonists (Morgan, 1990). In the past, this has mainly been due to difficulty in measuring low serum or plasma concentrations, particularly with regard to inhalation therapy. This is because the inhalation route of administration delivers the drug directly to the site of action requiring smaller doses; in addition, the volumes of distribution of the β_2-agonists are large (Chrysten, 1994). The combination of these two factors means plasma levels following inhalation in therapeutic doses are relatively low.

In clinical practice, salbutamol is administered by inhalation, orally and by intramuscular and intravenous injection (Morgan, 1990). Of these routes of administration, it is the inhalation route that is most effective (Alpers, 1991), by either MDI, DPI (such as the Ventolin Rotahaler®) or nebulisation

(Taburet and Schmit, 1994). Many factors may effect the bioavailability from inhalation and are discussed later.

The bioavailability of salbutamol depends on the route of administration. With oral administration, the systemic availability of salbutamol has been found to be a relatively low 50% (Morgan et al, 1986). Because of the low systemic availability, the oral doses of β-agonists are generally 5 to 10 times greater than the parenteral doses (Morgan, 1990). Salbutamol is usually administered as a racemic mixture, however, there is evidence to suggest that the systemic availability of oral (+)-salbutamol is greater than that of (-)-salbutamol (Tan and Soldin, 1987; Walle et al, 1993a; Walle et al, 1993b).

The size of aerosolised particles is critical in the bioavailability from inhalation. Aerosol particles may be solids or liquids, and tend to settle (sediment), adhere to each other (coagulate) and adhere to structures (deposit) such as tubing and mucosa and are generally heterodispersed with particles covering a range of sizes (Taburet and Schmit, 1994). Aerosol particles larger than 10 μm in diameter are mostly deposited in the oropharynx and swallowed while particles smaller than 0.5 μm are likely to reach the alveoli or be exhaled (Stahlhofen et al, 1980; Taburet and Schmit, 1994). Particles with a diameter of between 1 and 5 μm are most likely to be deposited in the lower respiratory tract and therefore be effective in the treatment of asthma (Newman, 1985).

Surprisingly, the amount of drug reaching the lungs from inhalation is small and variable but typically less than 15% of the inhaled dose (Clay and Clarke, 1987). The majority of the inhaled dose is deposited on the pharynx upon inhalation and then swallowed (Morgan, 1990). Studies using γ-scintigraphy have shown pulmonary delivery at around 10% from a pressurised MDI and around 20% from

a Turbuhaler® (Borgstrom and Nilsson, 1990; Borgstrom et al, 1992) although there is some confusion about these measurements (Melchor et al, 1990). A summary of lung deposition between different inhalation devices is shown in Table 7.1. Factors such as definition of lung deposition (total lung versus lower lung), mucociliary clearance, oral absorption and reliability of inhalation techniques all influence these estimates, which should be treated with caution. Despite these variations in quoted pulmonary deposition, the point to be made is that the majority of an inhaled dose never reaches the lungs.

Table 7.1 *Characteristics of inhalation delivery systems including pulmonary deposition. These figures should only be seen as a guide.*

Delivery System	Pulmonary Deposition (% dose)	Reference
MDI	9-15	Thorsson et al, 1993. Borgstrom and Nilsson, 1990.
MDI + spacer	14-20	Vidgren et al, 1987.
DPI	10-30	Thorsson et al, 1993. Borgstrom et al, 1992. Hindle et al, 1995.
Nebuliser	2-19*	Hardy et al, 1993.

* percent of dose initially loaded into in the nebuliser.

Small differences in inhalation techniques (between patients and inhalation devices) have also been shown to greatly alter the degree of deposition and the dose delivered (Newman et al, 1982; Vidgren et al, 1987, Taburet and Schmit, 1994). Variation in the degree of lung deposition can also be responsible for differences in therapeutic response of the drug between patients with inappropriate MDI inhalation techniques responsible for many failed clinical outcomes (Taburet and Schmit, 1994).

The pharmacokinetics of inhaled salbutamol can essentially be described by a combination of two separate mechanisms. Inhaled salbutamol consists of drug that reaches the lungs (approximately 10%) and drug that is swallowed (approximately 90%). The swallowed fraction of dose is absorbed and metabolised in the same way as an oral formulation (high first-pass effect with a greater percentage of drug recovered in urine as metabolite) while the dose that reaches the lungs is absorbed into the blood stream and metabolised in the same way as an intravenous dose with a lower percentage of recovered drug excreted as metabolite (Jenne and Ahrens, 1987).

A high ratio of metabolite to unchanged drug is seen in urine following inhalation and is similar to that after oral administration, consistent with a significant first-pass effect from oral absorption of the majority of the dose (Morgan, 1990). This ratio is much lower following direct bronchial instillation of drug and resembles the ratio following IV administration, indicating avoidance of the first-pass effect (Laros et al, 1977).

In the past bronchodilation by wet nebulisation has been considered the treatment of choice for in-hospital asthma therapy (Choo-Kang and Grant, 1975). The metabolite/unchanged drug ratio concentration in plasma and urine following nebuliser is less than that with MDI and oral administration indicating a larger proportion of the dose is absorbed via the lungs (Morgan, 1990). However, recent studies have found that administration of salbutamol from an MDI with a spacer (or holding chamber) can provide similar bronchodilation to that achieved by traditional wet nebulisation (Colacone et al, 1993; Gibson et al, 1995; Idris et al, 1993).

In summary, the pulmonary bioavailability of salbutamol with inhalation therapy is low (around 10%) and highly variable with extensive first-pass metabolism of the swallowed dose.

7.3   DISTRIBUTION

Following bolus IV administration, the plasma concentration-time curve of most of the β-agonists including salbutamol is characterised by 2 or 3 exponential phases, indicative of substantial tissue distribution (Goldstein et al, 1987). In keeping with this, salbutamol has a relatively large volume of distribution of around 156 litres (Morgan et al, 1986) and a low plasma protein binding of around 7-8% (Morgan et al, 1986).

The concentration of salbutamol found in plasma varies according to the means of administration. Peak plasma levels of 10-17 ng/ml were found 1-4 hours after a dose of salbutamol in subjects taking 4 mg of oral salbutamol three times a day (Morgan et al, 1986). The reported 'therapeutic range' of salbutamol is 10-20 ng/ml (Glaxo Ltd.; cited by Lewis et al, 1990).

Plasma levels after inhalation are usually much lower. A study by Lewis et al (1990) measured salbutamol plasma levels in patients presenting to hospital with acute asthma. It was found that patients who had been taking only inhaled therapy prior to admission had low plasma levels of salbutamol at less than 3 ng/ml (the lowest detectable limit) except for one patient who had taken 5 mg via nebuliser 2 hours prior to admission and had a level of around 5 ng/ml. In comparison, patients who had been taking oral therapy had higher levels between 6 and 34 ng/ml.

The pharmacological effect of β_2-agonists correlates reasonably well with plasma concentration of drug, however, there is large intersubject variation in the relationship between plasma concentration and response (Morgan, 1990; Taburet and Schmit, 1994). Therapeutic response and onset of adverse effects are the main determinants in dosage optimisation (Morgan, 1990). Plasma concentrations of β_2-agonists are not regarded as a useful parameter for dosage adjustment (Morgan, 1990).

7.4   METABOLISM

Salbutamol undergoes phenolic conjugation with sulphate by phenol sulphotransferase (PST) to the sulphate ester metabolite, salbutamol 4'-O-sulphate, identified by Lin et al (1977; Figure 7.1). Extensive literature searches have shown that the sulphate ester metabolite of salbutamol is the only metabolite that has been detected in humans. Even with relatively large IV and oral doses, no glucuronide conjugates were found in urine or plasma by Morgan et al (1986). The sulphate ester metabolite does not appear to possess significant pharmacological activity (Evans et al, 1973).

Salbutamol 4'-O-Sulphate
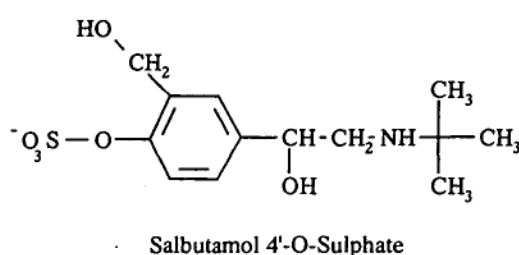
Figure 7.1   *Salbutamol 4'-O-sulphate, the only identified metabolite of salbutamol in man.*

Orally administered salbutamol undergoes extensive first-pass metabolism, attributed entirely to sulphate conjugation (Morgan et al, 1986). There is evidence to suggest that most of the first-pass metabolism may take place in the wall of the

small intestine (Illett et al, 1980; Koster et al, 1985) but this has not been demonstrated unequivocally (Morgan, 1990). PST is widespread in the body and platelet, hepatic and placental PST have also been shown to metabolise β-agonists (Sodha and Schneider, 1984; Walle et al, 1993b).

As mentioned earlier, salbutamol is administered clinically as a racemic mixture. As for most of the β-agonists, the (-)-isomer is mainly responsible for the pharmacological activity of a racemic mixture of salbutamol (Brittain et al, 1973; Waldeck and Widmark, 1985). Differences in the pharmacokinetics of the salbutamol isomers have also been described (Tan and Soldin, 1987).

It has been suggested (Tan and Soldin, 1987; Walle et al, 1993a) that the systemic availability of oral (+)-salbutamol is greater than that of (-)-salbutamol due to preferential first-pass metabolism. This was confirmed by Walle et al (1993b) who found that sulphation efficiency of the pharmacologically more active (-)-salbutamol was approximately 10 times greater than (+)-salbutamol with hepatic, jejunal mucosa and platelet derived PST. PST enzymes, present in humans as the M form (monoamine substrates) and the P form (simple phenol substrates) were also examined in the same study. It was found that only the M form demonstrated sulphation of the salbutamol substrate (Walle et al, 1993b).

7.5   EXCRETION

Salbutamol is both excreted from the kidneys unchanged and as the metabolite with elimination dependent on the route of administration. After intravenous administration most of the drug is excreted as unchanged drug whereas after oral administration, a greater proportion of the drug is excreted as the metabolite (Morgan et al, 1986), reflecting the first-pass effect.

β_2-Agonists are polar and basic and have a high renal clearance unaffected by pH (Chrysten, 1994). The protein binding corrected renal clearance of salbutamol is greater than the glomerular filtration rate suggesting active tubular secretion (Morgan, 1990). The sulphate ester does not appear to be actively secreted (Morgan et al, 1986). In addition, the active tubular secretion of salbutamol may be stereoselective as is the case with terbutaline (Borgstrom et al, 1989).

Typically, urine levels of drugs excreted by the kidneys tend to be higher than plasma levels as the collection of urine in the body effectively acts as a reservoir for excreted drug. Urine levels of salbutamol have been reported at around 100 ng/ml following a 200 μg dose (Horn et al, 1989) and between 160-580 ng/ml after a 400 μg dose of inhaled salbutamol from MDI (Hindle et al, 1993). Urinary salbutamol excretion has been used as a measure of relative bioavailability to the lung (Hindle et al, 1993).

There is little biliary excretion of β_2-agonists in humans (Nilsson et al, 1973) and only 1-12% of the dose has been recovered from faeces following administration of both oral and inhaled tritiated salbutamol (Walker et al, 1972).

Common pharmacokinetic parameters of salbutamol are summarised in Table 7.2. and the fate of an inhaled dose of salbutamol is outlined in Figure 7.2.

1. The majority of drug is deposited in the oropharynx region and swallowed (90%).

2. About 10% of the inhaled dose reaches the lungs and exerts a local pharmacological effect.

3. Swallowed drug is absorbed systemically but is subject to extensive stereoselective first-pass metabolism in the intestinal wall and liver by PST to the pharmacologically less active salbutamol 4'-O-sulphate. Salbutamol is also metabolised in the platelets by PST.

4. Plasma levels are highly variable, dependent on means of inhalation, and are typically less than 5 ng/ml.

5. Systemic salbutamol is metabolised to the inactive sulphate metabolite in the liver.

6. Salbutamol has a high renal clearance and is actively secreted from the kidneys. Salbutamol 4'-O- sulphate is freely filtered.

7. Less than 12% of the dose is recovered in faeces.

Figure 7.2 *Fate of inhaled salbutamol. Inhaled salbutamol basically follows two routes. The majority of drug (approximately 90%) is swallowed with metabolism in a similar manner to oral administration with substantial first-pass metabolism. The drug that is deposited in the lungs is metabolised in a similar manner to intravenous administration with less first-pass metabolism.*

Table 7.2 *Common pharmacokinetic parameters of (±)-salbutamol. Adapted from Morgan, 1990.*

Plasma protein binding (%)	Cl (L/h/kg)	Cl_R (L/h/kg)	V_Z (L/kg)	t1/2 (h)	D_f (%)	Unchanged drug in plasma[a] (%) IV	PO	Excreted unchanged in urine[b] IV	PO	Reference
8	0.41, 0.38, 0.46	0.28	1.9, 2.2, 2.5	3.2, 3.9, 4.0, 5.0, 6.0	4	> 50	25, 25	64, 65	20, 32	Evans et al (1973), Goldstein et al (1987), Martin et al (1971), Morgan et al (1986), Powell et al (1985, 1986), Walker et al (1972), Vaisman et al (1987).

[a] Percentage of total drug and metabolites.
[b] Percentage of absorbed dose.
Cl = total plasma clearance.
Cl_R = renal clearance.

V_Z = apparent volume of distribution during the terminal phase.
t1/2 = elimination half-life.
D_f = intravenous dose excreted in faeces.
PO = oral.

CHAPTER 8

Assay Development for the Determination of Salbutamol in Urine

8.1 SUMMARY

An assay was developed for the determination of salbutamol in the urine of asthmatic patients. Several solid-phase extraction (SPE) sorbents were tested and compared with liquid-liquid extractions. The SPE was then further refined by studying retention behaviour of salbutamol on the sorbent under different solvent compositions and modifications in SPE procedures. Analysis was performed using reversed-phase HPLC with UV detection. The recovery of salbutamol from urine was 48 ± 5.0% with a calibration range of 47.3 ng/ml to 1760 ng/ml. The detection limit was estimated at 15 ng/ml (SN ratio of 3 dB). Intra-day and inter-day coefficients of variation were 8.6% and 11.1% (at 473 ng/ml) respectively. Enzymatic hydrolysis was performed for 2 hours to determine total salbutamol levels (unchanged + metabolite) enabling indirect determination of salbutamol metabolite levels.

8.2 INTRODUCTION

8.2.1 Background

Extensive literature searches indicate that until recently, there has been little research investigating the monitoring of plasma or urinary levels of the inhaled β_2-agonist bronchodilators in asthmatic patients, primarily because of the difficulties in measuring the low concentrations generally seen in clinical practice with inhaled therapy (Horn et al, 1989; Horn et al 1990). Preparations of salbutamol available include tablets, syrup and injection, but it is inhaled salbutamol that is most commonly administered (Jenkins et al, 1990). Inhaled salbutamol is available as a 100 μg metered dose inhaler (MDI), 200 μg Rotacaps® (a dry powder inhaler; DPI) and a respirator solution and unit-of-use pre-diluted nebules (2.5 and 5.0 mg/ml) which are delivered by nebuliser.

Administration by MDI results in direct stimulation of β_2-receptors in the bronchial smooth muscle. Because approximately 10% of the drug is delivered

directly to the site of action after inhalation (the remainder is subject to extensive first-pass metabolism as discussed in Chapter 7), a smaller dose of drug is usually required (usually 100 μg per inhalation) which results in fewer systemic side-effects than if the drug is given by the oral route. The smaller dose of drug delivered by MDI, however, poses problems for studies involving the determination of drug levels in biological matrices.

Because of its hydrophilicity and the low concentrations found in plasma at therapeutic dosages, extraction of the drug free from interfering compounds is relatively difficult (Ong et al, 1989). Typically, urine levels tend to be higher than plasma levels as the collection of urine in the body effectively acts as a reservoir for salbutamol and its metabolite before being eliminated from the body. This makes measuring drug levels easier although the scope of pharmacokinetic parameters obtainable may be limited.

The aim of this study was to develop an assay capable of determining salbutamol levels in urine following inhalation therapy. It was anticipated that to be clinically useful, this assay would need to measure salbutamol to a lower calibration range of around 20 ng/ml in urine. Horn et al (1989) found that in patients who had taken 200 μg salbutamol from a MDI, samples taken 1 hour after administration contained 101 $\pm$ 77 ng/ml (n=20) of salbutamol.

8.2.2 Previous methods of detection

The isolation and measurement of organic compounds, in particular drugs at low concentrations in a biological matrix, is a significant analytical challenge (McDowall, 1989). Chromatography and immunoassays are the two principal techniques used for the measurement of drug concentrations in biological matrices (Mehta, 1992). In general, immunoassay techniques for drug analysis are expensive,

time consuming to develop, and can be used only for those drugs for which reagent kits are available, usually only when a need for TDM has been clearly indicated (Mehta, 1992). For these reasons, an immunoassay technique for measuring salbutamol in this current study was not feasible, leaving chromatographic techniques as the most practical means of measuring salbutamol at therapeutic concentrations. Given the relatively polar nature of salbutamol, reversed-phase HPLC was chosen as the method of analysis, although high performance thin layer chromatography (HPTLC), gas chromatography (GC) and GC-MS have been used to measure therapeutic levels of salbutamol in the past (Horn et al, 1989). Advantages of reversed-phase HPLC compared to gas chromatography are shown in Table 8.1.

Table 8.1   *Advantages of reversed-phase HPLC compared to GC in relation to drug analysis, as adapted from Mehta (1992).*

1. HPLC is faster than GC and can be used for polar, non-volatile and thermally labile compounds. This permits the determination of a wide variety of drugs including salbutamol.

2. Unlike GC, derivatization is not necessary in most cases.

3. HPLC does not destroy the sample allowing it to be recovered for additional studies.

4. A wide variety of silica based stationary phases with different functionalities are available. In addition, new stationary phases have been developed that allow the direct separation of chiral drugs.

5. Aqueous mobile phases are compatible with clinical samples.

Several recent studies involving measurement of therapeutic levels of salbutamol are summarised in Table 8.2. It should be noted that most of these studies used HPLC for measuring therapeutic levels of salbutamol, however, the method of detection typically used amperometric or fluorescent detectors, not UV detection as used here. In general, UV detection offers the advantages of being a robust and

relatively inexpensive means of detection but lacks the specificity and sensitivity of amperometric and fluorescence detection (Mehta, 1992).

8.2.3   Solid-phase extraction background

To measure a drug in a biological matrix usually requires a clean up step to remove interfering endogenous compounds and in many cases, other drugs. This clean up step has been traditionally performed using liquid-liquid extractions whereby the drug is unionised by changes in pH in the aqueous phase allowing it to partition into a lipophilic organic phase. The partitioning of compounds from the biological fluid can be modified by changes in pH and organic phase to be more selective toward the drug of interest leaving possible interfering endogenous compounds behind in the aqueous phase.

These liquid-liquid extractions can be very effective for the extraction of lipophilic drugs from biological matrices, however, as stated earlier, the extraction of relatively non-polar molecules such as salbutamol from plasma or urine, free from interfering compounds at therapeutic concentrations, is relatively difficult (Ong et al, 1989).

An alternative method of isolating an analyte from a biological matrix is solid-phase extraction (SPE). Solid-phase extraction separates compounds by utilising the principles of liquid chromatography which involves the analytes being separated according to the degree of which each component is partitioned or adsorbed by the stationary or solid-phase (McDowall, 1989). As the sample solution passes through the sorbent bed (usually under vacuum), the isolate is adsorbed onto the solid-phase while other sample components pass through the solid-phase bed leaving a concentrated isolate (Van Horne, 1985). The isolate can then be selectively removed (or eluted) from the sorbent bed by using a minimum volume of a solvent

Table 8.2   *Previous studies measuring therapeutic levels of salbutamol and other β_2-agonists.*

Title	Extraction procedure	Detection	Detection range	Author(s)
Reversed-phase HPLC determination of salbutamol in rabbit plasma.	Extracted onto a solid phase Sep-Pak® C_{18} cartridge using ion-pair reagent (heptane sulphonic acid). Recovery was 89.9 ± 2.8% (mean ± SD, n=3).	Fluorescence detection.	10 - 300 ng/ml.	Kurosawa et al, 1983.
Determination of salbutamol in human serum by reversed-phase HPLC with amperometric detection.	Solid phase Sep-Pak® C_{18} cartridge then extracted into di(ethylhexyl) phosphate (DEHP) as an ion-pair solution (heptane sulphonic acid). Recovery was 79 ± 8.6% (mean ± SD, n=12).	Ampero-metric detection.	0.4 - 20 ng/ml.	Tan and Soldin, 1984.
Determination of salbutamol in human plasma and urine by high performance thin-layer chromatography.	Solid phase extraction using Sep-Pak® C_{18} cartridge (plasma) and Prep-I type W XAD-2 cartridges (urine) and conversion to an indoaniline dye by reaction with dimethyl-*p*-phenylenediamine.	Absorption microdensit-ometry at 650 nm.	20 - 700 ng/ml.	Colthup et al, 1985.
Simultaneous determination of salbutamol and terbutaline at overdose levels in human plasma by HPLC with electrochemical detection.	Pre-column sample purification and enrichment.	Electro-chemical detection	1 - 100 ng/ml. Calibration curve over the range 20 - 100 ng/ml.	Sagar et al, 1993.
Measurement of terbutaline and salbutamol in plasma by HPLC with fluorescence detection.	Solid-phase extraction on alkali-treated Bond-Elut.	Fluorescence detection.	Detection limit 0.5 ng/ml. Calibration curve over the range 25 - 125 ng/ml.	McCarthy et al, 1993.
Plasma assay of salbutamol by means of HPLC with amperometric determination using a loop column for injection of plasma extracts. Application to the evaluation of subcutaneous administration of salbutamol.	Solid-phase extraction on Bond-Elut® C_{18} cartridge . Recovery was 93 ±10.6% (mean ± SD, n=9).	Ampero-metric detection.	0.5- 15 ng/ml. Calibration curve over the range 2.5 - 15 ng/ml.	Tamisier-Karolak et al, 1992.

Table 8.2 *continued.*

An HPLC method for the determination of albuterol (salbutamol) enantiomers in human serum using solid phase extraction and chemical derivatisation.	Solid-phase extraction and derivatisation of racemic albuterol to a diastereomeric thiourea.	Fluorescence detection.	1.0- 20.0 ng/ml. Calibration curve over the range 2.0 - 20.0 ng/ml.	He and Stewart, 1992.
Residue screening for the β-agonists clenbuterol, salbutamol and cimaterol in urine using enzyme immunoassay and HPLC.	Combined solid-phase extraction with C_{18} silica gel and enzyme immunoassay.	-	1 ppb	Meyer et al, 1991.
Analysis of albuterol in plasma based on immunoaffinity chromatographic clean-up combined with HPLC with fluorescence detection.	Immunoaffinity clean-up recovery was 96.1 ±1.1% (mean ± SD, n=8). Overall recovery was 80.1 ± 5.3 % (mean ± SD, n=8).	Fluorescence detection.	0.5 ng/ml. Calibration curve over the range 0.8 - 6.4 ng/ml.	Ong et al, 1989.
Determination of salbutamol enantiomers in human plasma and urine by chiral high-performance liquid chromatography.	Solid-phase extraction with acetonitrile wash and methanol elution.	Fluorescence detection.	0.25-500 ng/ml. Calibration curve over the range 1.25-500 ng/ml.	Boulton and Fawcett, 1995.

for which the isolate has a strong affinity. The properties of the solid-phase sorbent bed (usually consisting of a functional group covalently bonded to a silica substrate) determine the selectivity for the isolate (Van Horne, 1985).

A list of common sorbents and their properties are shown in Table 8.3. Most of the recent research studies on measuring therapeutic levels of the β_2-agonists have utilised SPE (as can be seen in Table 8.2). It was therefore decided to investigate SPE as a possible clean up step for separating salbutamol from an aqueous urinary matrix containing interfering endogenous compounds and other drugs.

8.2.4   Outline of the rationale for assay development

SPE was initially compared with liquid-liquid extraction. Different sorbents were tested in Section 8.3.3. An analytical HPLC column was then used in Section 8.3.4

Table 8.3   *Commercially available solid-phase extraction sorbents, functional groups and their properties (adapted from Van Horne, 1985).*

Abbreviation	Functional Group	Polarity	Properties
C18	Octadecyl	Non-polar	Most non-polar sorbent available and is the most retentive of all sorbents for isolates being retained by non-polar mechanisms. C18 does not retain polar molecules well. C18 sorbents are not very selective which is often a benefit when the isolates vary widely in structure but final extracts may not be as pure as when more selective absorbents are employed.
C8	Octyl	Non-polar	Similar properties to the C18 phase but not quite as retentive for non-polar isolates and a slightly higher potential for polar interactions than C18 due to its shorter chain length.
C2	Ethyl	Non-polar/polar	C2 is a fairly polar sorbent due to is short chain length. C2's polarity is slightly greater than CN for polar interactions.
CH	Cyclohexyl	Non-polar	CH has approximately the same polarity as a C2 sorbent. CH is a medium polarity sorbent that exhibits unique selectivities for certain isolates.
PH	Phenyl	Non-polar	PH has approximately the same polarity as a C8 sorbent. Like CH, PH exhibits a slightly different selectivity from other non-polar sorbents due to the electron density of the aromatic ring.
CN	Cyanopropyl	Non-polar/polar	CN is a medium polarity sorbent with many uses. The cyano group gives CN a unique selectivity.

to examine the retention characteristics of salbutamol under different conditions of solvent and pH with the solid-phase chosen from Section 8.3.3. The SPE technique was optimised using this information in Section 8.3.5. Finally the chromatography conditions from Chapters 5 and 6 were optimised for salbutamol urine extracts in Section 8.3.6. Internal standards were examined in Section 8.3.7. Calibration curves for salbutamol were examined in Section 8.3.8 and hydrolysis conditions in Section 8.3.9.

8.3 METHOD

8.3.1 Materials

Salbutamol pure base was kindly donated by Glaxo Australia Pty. Ltd. (Boronia, Vic., Australia). Methanol and tetrahydrofuran (liquid chromatography grade) were purchased from Waters Associates (Lane Cove, NSW, Australia). The ion pair reagent Pic® B-8 Reagent Low UV (containing water, methanol, octane sulphonic acid and calcium acetate in undisclosed quantities) was purchased from Waters Associates (Milford, MA, USA). Decane sulphonic acid was purchased from the Sigma Chemical Co. (St Louis, MO, USA). β-Glucuronidase/arylsulphatase (derived from *helix pomatia*) was obtained from Boehringer Mannheim, Germany. All other chemicals used were laboratory grade reagents.

8.3.2 Equipment

The HPLC system consisted of a Varian Solvent Delivery System Model 9010 (Varian Chromatography Systems, Walnut Creek, CA, USA) and a Rheodyne injector Model 7161 (Rheodyne Inc., Cotati, CA, USA) with a 10 μl external loop connected to a Varian Variable Wavelength UV-VIS Detector Model 9050 (Varian Chromatography Systems, Walnut Creek, CA, USA). Detection was performed at 220 nm. Chromatographic peaks were digitised, integrated and recorded with a Varian GC Star Workstation (Varian Chromatography Systems, Walnut Creek, CA, USA).

The analysis of salbutamol extraction procedures was performed on a Nova-Pak® C_{18} 4 μm 10 cm x 8 mm i.d. Radial-pak® liquid chromatography cartridge (Waters Associates, Milford, MA, USA) inside a Waters RCM 8 x 10 compression module (Waters Associates, Milford, MA, USA). The retention behaviour of salbutamol in phenyl solid phases (Section 8.3.4) was examined using a μBondapak Phenyl 10 μm

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300 mm x 3.9 mm i.d. steel chromatography column (Waters Associates, Milford, MA, USA).

The following SPE sorbents were used in Section 8.3.3: C_{18}, C_8, C_2 and phenyl 1 ml volume disposable extraction columns from a Bond Elut® Applications Development Kit (Analytichem International, Harbour City, CA, USA). Phenyl 1 ml volume Baker Bond spe® disposable extraction columns (JT. Baker Inc., Phillipsburg, NJ, USA) were used in Sections 8.3.5 to 8.3.9 and in Chapter 9.

8.3.3 Initial investigation of the solid-phase recovery of salbutamol

The solid-phase recovery and clean-up of salbutamol from both water and urine was investigated using several potentially suitable sorbents chosen from the characteristics outlined in Table 8.3. The SPE technique was compared with a liquid-liquid extraction and a SPE technique using an ion-pair reagent to aid retention.

Analysis of samples was performed by injecting into a 10 μl fixed loop for HPLC analysis on a Nova-Pak C_{18} 4 μm 10 cm x 8 mm i.d. Radial-pak liquid chromatography cartridge with a mobile phase consisting of 30% tetrahydrofuran, 1.9 mM octane sulphonic acid (from a dilution of Low UV Pic® B-8 ion-pair reagent) and 5% methanol in distilled water. Flow rate was 1.5 ml/min with UV detection performed at 220 nm.

8.3.3.1 Preparation of salbutamol standards and their use in the assessment of SPE technique

A stock solution of salbutamol 500 ng/ml in distilled water was prepared from salbutamol base to spike water and urine samples in Sections 8.3.3.2, 8.3.3.3 and 8.3.3.4 and was also used to estimate recovery in Section 8.3.8. The 100 μl aliquots of this 500 ng/ml stock solution that were used to spike urine blanks contained 50 ng of salbutamol. Assuming 100% recovery, this amount would be present in

the final sample extract and after reconstitution with 100 μl of distilled water, would be present in a concentration of 500 ng/ml, equivalent to the stock solution. The chromatograms obtained from Sections 8.3.3.2, 8.3.3.3, 8.3.3.4 and the recovery estimation in Section 8.3.8 were compared against a chromatogram of the 500 ng/ml stock solution representing maximum theoretical recovery. These comparisons enabled an assessment of the different SPE techniques against a standard reference (Sections 8.3.3.2, 8.3.3.3, 8.3.3.4) and an estimate of salbutamol recovery (Section 8.3.8).

8.3.3.2 Comparisons of SPE, SPE using ion-pair, and liquid-liquid extractions

Spiked samples were prepared by adding 100 μl of a 500 ng/ml salbutamol stock solution to 1 ml of distilled water and mixing thoroughly using a vortex mixer (Super-Mixer, Lab-Line Instruments Inc., Melrose Park, IL, USA). Liquid-liquid extractions were performed by adding 250 μl of 1 M HCl, mixing, and discarding the organic phase (either ethyl acetate or hexane), adding 500 μl of 1 M NaOH and either 1 ml of ethyl acetate or hexane, mixing, collecting the organic layer, evaporating under nitrogen at 45°C and adding 100 μl of distilled water.

A simple SPE technique was performed using 1 ml volume C_{18} Bond Elut disposable extraction columns. The technique consisted of (i) sorbent conditioning, (ii) introduction of sample to the column, (iii) sorbent wash and (iv) solute elution steps. This procedure is illustrated in Figure 8.1.

Sorbent conditioning was performed by allowing 1 ml of methanol to pass through the disposable extraction column followed by 1 ml of pH 6.7 phosphate buffer. After the buffer had passed through the column, the spiked sample was introduced while the column sorbent was still wet and allowed to pass through the column. The sorbent was then washed with 2 x 500 μl aliquots of pH 6.7

phosphate buffer. Once the washings had been removed the solute was eluted with 2 x 500 μl aliquots of methanol, evaporated under nitrogen at 45°C and 100 μl of distilled water was added. All of the steps involving solvents passing through the disposable extraction columns were performed under slight vacuum. The column sorbent was not allowed to air-dry between sorbent conditioning and introduction of sample onto the column.

SPE was also attempted using an ion-pair reagent (octane sulphonic acid) to aid retention on the column. The procedure was identical to the SPE technique above except 1 ml of octane sulphonic acid (0.005 M) was added to the spiked sample and used in the wash steps instead of pH 6.7 phosphate buffer. All samples were then analysed by HPLC under the conditions outlined in the beginning of Section 8.3.2 and compared with the 100% theoretical recovery chromatogram.

It was found that the SPE technique using C_{18} sorbent produced satisfactory recovery with good peak heights of a similar magnitude to the stock solution (theoretical 100% recovery; Section 8.3.3.1). Both of the liquid/liquid extractions recovered far less salbutamol than the C_{18} sorbent as would be expected given the relatively polar nature of salbutamol. The ion-pair SPE method also produced worse results than the C_{18} sorbent without ion-pair. Overlayed chromatograms are shown in Figure 8.2.

8.3.3.3 Comparisons of the retentive properties of C_{18}, C_8, C_2 and phenyl solid-phase sorbents in an aqueous matrix

The retentive properties of C_{18}, C_8, C_2 and phenyl solid-phases were examined using 1 ml volume Bond Elut disposable extraction columns. Spiked water samples were prepared by adding 100 μl of a 500 ng/ml salbutamol stock solution to 1 ml of distilled water then mixing thoroughly using a vortex mixer. A simple SPE technique was performed identical to that outlined above in Section 8.3.3.2. A
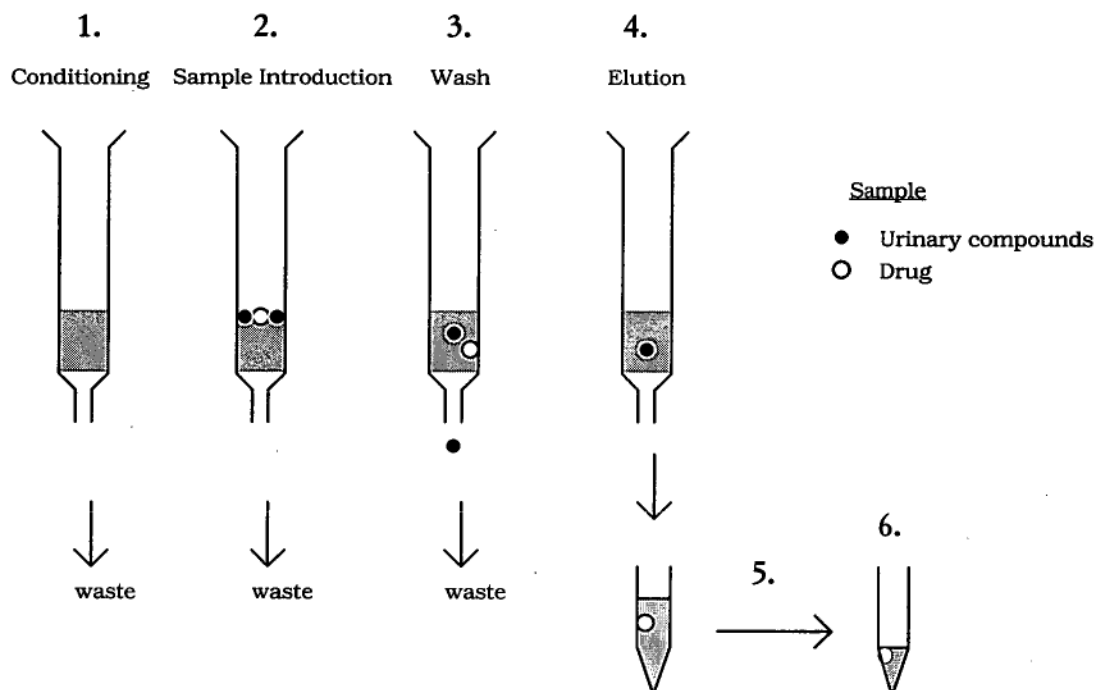
Figure 8.1   *Solid-phase extraction (SPE) technique employed for comparisons of SPE, SPE using ion-pair, and liquid-liquid extractions (Section 8.3.3.2). 1. Sorbent conditioning with 1 x 1 ml of methanol and 1 x 1 ml pH 6.7 phosphate buffer. 2. Sample introduction to sorbent (1 ml of urine added to sorbent). 3. Sorbent wash with 2 x 500 μl of pH 6.7 phosphate buffer. 4. Solute elution with 2 x 500 μl of methanol. 5. Eluent evaporation under nitrogen with 45°C. 6. Sample reconstitution with 100 μl of distilled water ready for HPLC analysis.*

strong additional elution consisting of 2 x 250 μl aliquots of tetrahydrofuran was used in an attempt to ensure that all the salbutamol was removed from the column. Extractions were repeated five times to examine reproducibility. All samples were then analysed by HPLC under the conditions outlined in the beginning of Section 8.3.3.

Recoveries were examined by comparing the relative salbutamol peak areas. It was found that the secondary tetrahydrofuran elution removed a considerable amount of salbutamol in all cases. The mean relative recovery was greatest with the phenyl SPE column, of which very little salbutamol was removed by the first methanol elution. The mean relative recoveries and reproducibility are shown in Figure 8.3.
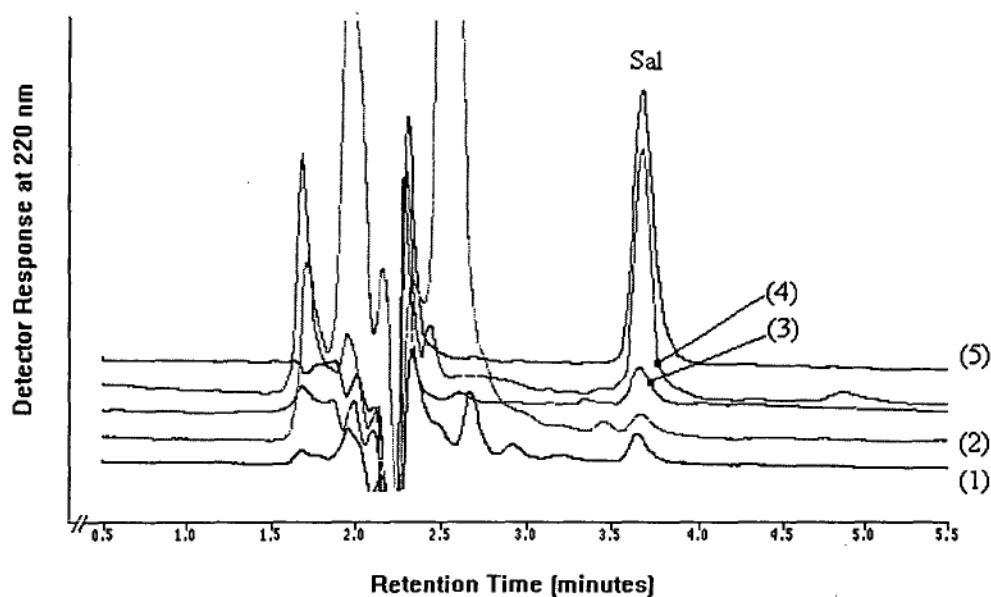
Figure 8.2   *Comparison of SPE, SPE with ion-pair and liquid/liquid extractions are shown.*
(1) Liquid/liquid extraction with hexane. (2) Liquid/liquid extraction with ethyl
acetate. (3) SPE with ion-pair reagent (octane sulphonic acid) used in extraction. (4) SPE
without ion-pair reagent. (5) 500 ng/ml stock solution equivalent to 100% recovery (see
Section 8.3.3.1).

8.3.3.4 Comparison of the retentive properties of C_{18}, C_8, C_2 and phenyl solid-phase sorbents in a urinary matrix

Although the extraction properties of the various disposable extraction columns were investigated in Section 8.3.3.3 in extracting salbutamol from water, it was necessary to investigate the various extraction properties obtained from the same batch of spiked urine. This would enable the selectivity and clean-up properties of the various solid-phases with respect to salbutamol to be investigated. For example, a column that recovered 90% of the solute of interest but also retained 90% of interfering endogenous solutes in the chromatography may not be ideal, whereas a column recovering less of the solute of interest but without interfering compounds may be more suitable. This is discussed in more detail in Section 8.2.3.

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The retentive and selective properties of C_{18}, C_8, C_2 and phenyl solid-phases were examined using 1 ml volume Bond Elut disposable extraction columns. Spiked urine samples were prepared by adding 100 μl of a 500 ng/ml salbutamol stock solution to 1 ml of blank urine and mixing thoroughly using a vortex mixer. A simple SPE technique was performed identical to that outlined above in Section 8.3.3.2 with the exception that the elution consisted of 1 x 250 μl aliquot of tetrahydrofuran. It was found that elution with 2 x 250 μl aliquots of tetrahydrofuran resulted in a significant amount of interfering urinary solute.
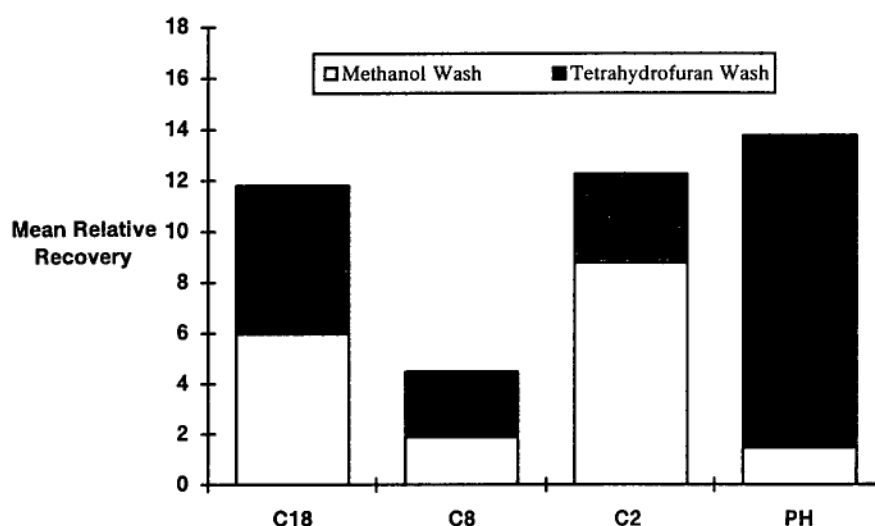
Figure 8.3   *Comparison of mean salbutamol recovery from C18, C8, C2 and PH solid phases after methanol elution and an additional tetrahydrofuran elution.(Section 8.3.3.3). Coefficients of variation (all n = 5) for initial methanol wash were as follows: 18%, 80%, 25% and 18% for C18, C8, C2 and PH solid phases respectively. Coefficients of variation (all n = 5) for additional tetrahydrofuran wash were as follows: 25%, 67%, 55% and 15% for C18, C8, C2 and PH solid phases respectively.*

All samples were then analysed by HPLC under the conditions outlined in the beginning of Section 8.3.3 and compared with the 100% theoretical recovery chromatogram.

The retentive properties of C_{18}, C_8, C_2 and phenyl solid-phase sorbents were found to vary mostly in their salbutamol selectivity. Extraction was as expected given the characteristics outlined in Table 8.3. The C_{18} sorbent retained a large number of different compounds with a relatively messy chromatogram and lower recovery of salbutamol which is not unexpected given the relatively polar nature of salbutamol. The C_8 and C_2 sorbents provided a progressively cleaner chromatogram with decreasing hydrocarbon chain length, each with similar salbutamol recovery. The phenyl sorbent provided both good salbutamol recovery and a high relative selectivity and was selected as the choice for the SPE procedure. Chromatograms of the different sorbents are shown in Figure 8.4.

8.3.4 Behaviour of salbutamol in phenyl solid-phases

Based on the results in Section 8.4.1 it was decided to perform the SPE of urinary salbutamol with disposable Bond Elut® phenyl solid phase extraction columns. In this section the behaviour of salbutamol on an analytical phenyl HPLC column was examined. It was hoped that the chromatographic column parameters measured for salbutamol on the analytical HPLC column could be utilised on the phenyl SPE columns to optimise the extractions. Capacity factors (k') were measured with varying methanol and tetrahydrofuran components in the mobile phase. The capacity factor is defined as the number of column volumes of solvent required to elute the solute. This is shown below.

$$k' = \frac{t - t_0}{t_0}$$

k' = capacity factor

t = solute elution time

t_0 = elution time without retention i.e. solvent complex elution time

The influence of pH on solute retention was also examined. It was hoped that this would enable a prediction of the behaviour of salbutamol in phenyl disposable extraction columns. This prediction would then allow tailoring of the organic components in the SPE wash and elution steps for optimum extraction using phenyl disposable extraction columns.
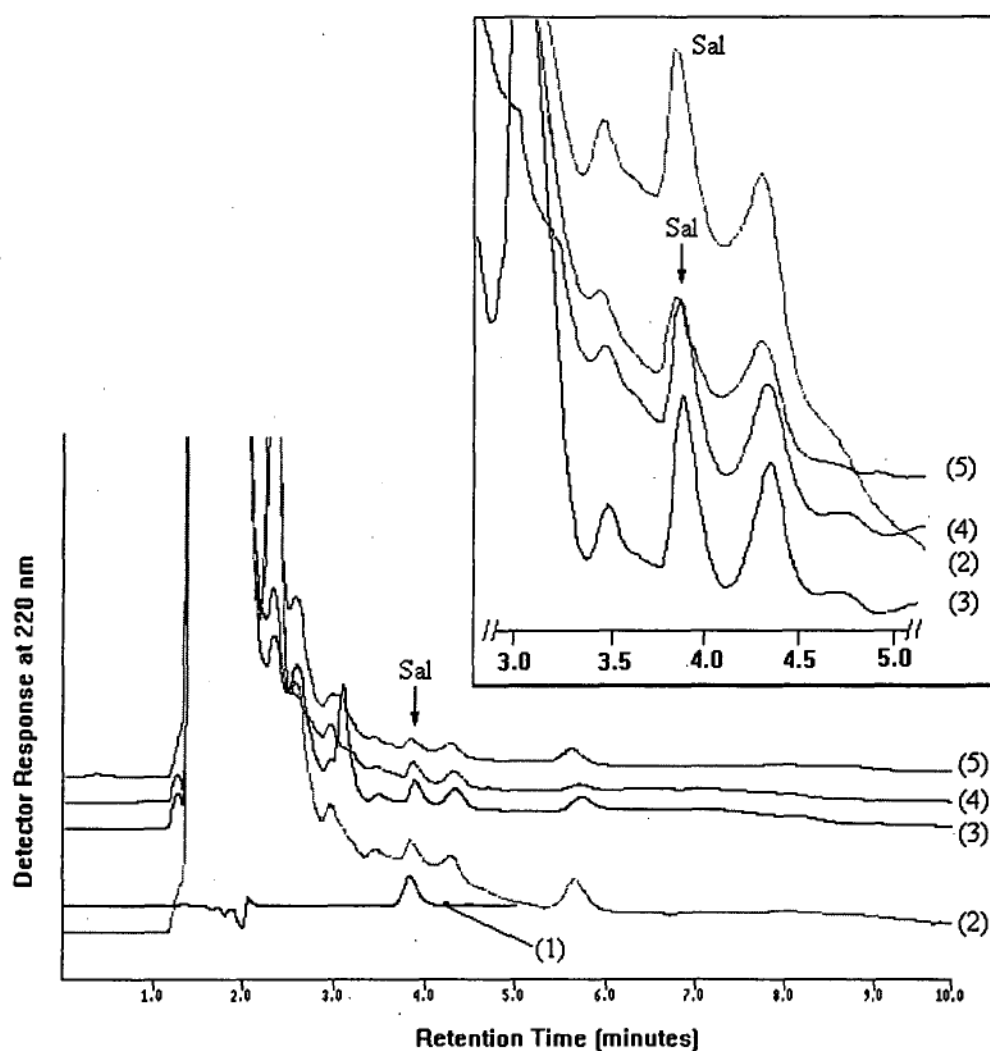
Figure 8.4 *Comparison of the retentive properties of (2) C_8, (3) phenyl, (4) C_2, and (5) C_{18} solid-phase sorbents in a urinary matrix with a baseline offset to aid display shown with (1) salbutamol standard (equivalent to theoretical 100% extraction). Inset, although confused, shows the salbutamol peaks for the same chromatograms under a lower attenuation. The salbutamol peak is shown (Sal).*

A solution of salbutamol 0.5 mg/ml in distilled water was prepared from salbutamol base. Twenty microlitres of this solution were injected into the HPLC connected to a phenyl column with UV detection performed at 220 nm (Section 8.3.2). The organic component and pH of the mobile phase were then varied as described below and the respective t and t_0 parameters recorded.

The effect of methanol on the retention behaviour of salbutamol on a phenyl solid phase was examined using eight different compositions of mobile phase consisting of 25, 40, 50, 60, 65, 75, 80 and 90% methanol in distilled water at a flow rate of 1.0 ml/min. Similarly, the effect of tetrahydrofuran on the retention behaviour of salbutamol on a phenyl solid phase was examined using mobile phases consisting of 3, 5, 10, 15, 20, 30, 40 and 60% tetrahydrofuran in distilled water at a flow rate of 1.0 ml/min. The capacity factor parameters for salbutamol were recorded under each of these conditions.

The effect of pH on retention behaviour was examined using six different mobile phases consisting of 10% methanol in Walpole's acetate buffer APF (pH 3.9 and 5.0), phosphate buffer (pH 6.1 and 8.1) and borate buffer (pH 8.1). The capacity factor parameters for salbutamol were recorded under each of these pH values with a flow rate of 1.0 ml/min.

The effect of methanol on the elution of salbutamol on a phenyl column is shown in Figure 8.5. The fastest salbutamol elution occurred with methanol percentages of between 40% and 70% in distilled water. Similarly the effect of tetrahydrofuran on the elution of salbutamol is shown in Figure 8.6 with fastest elution occurring with tetrahydrofuran percentages of between 10% and 30%. In terms of relative elution strength of salbutamol on a phenyl column, tetrahydrofuran was around 15 times more powerful than methanol.

The effect of pH on elution of salbutamol on a phenyl column is shown in Figure 8.7. The capacity values obtained should only be seen as a guide because different buffers were used which could also greatly affect the elution parameters. Faster elution was found with the lower pH values of the acetate buffer. Better retention of salbutamol was found at higher pH values with the phosphate and borate buffers.
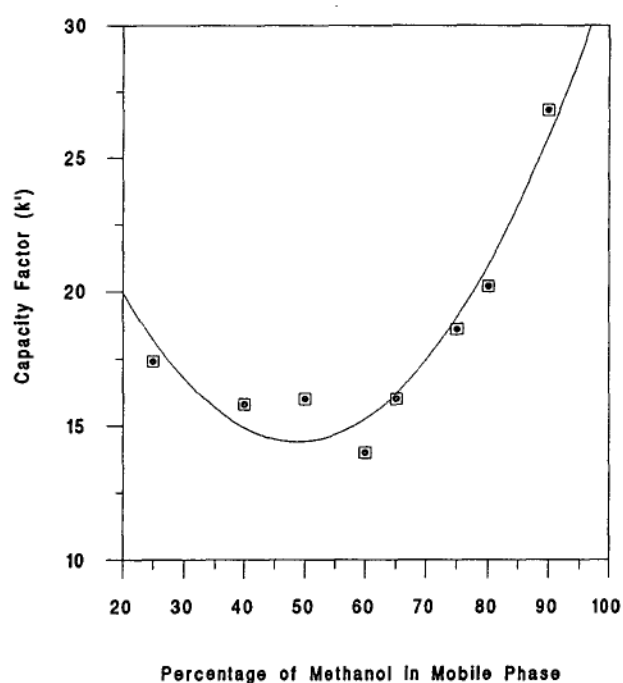
Figure 8.5   *Effect of methanol on the elution of salbutamol from a phenyl analytical column.*
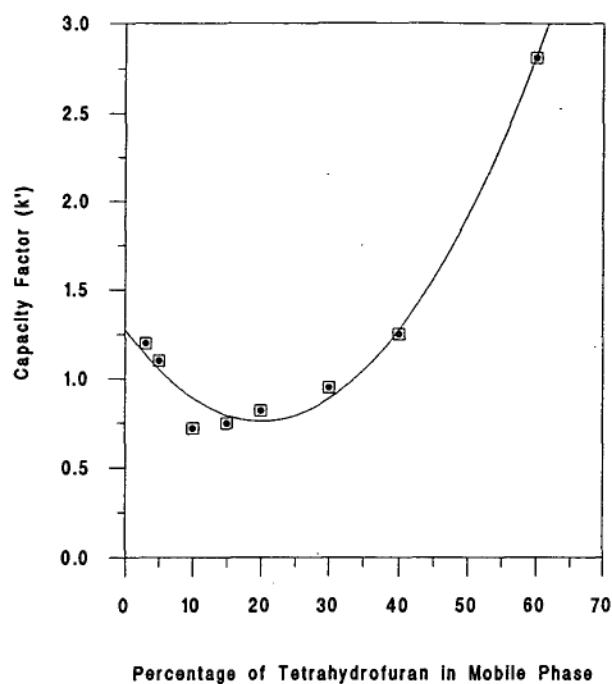
Figure 8.6 *Effect of tetrahydrofuran on the elution of salbutamol from a phenyl analytical column.*
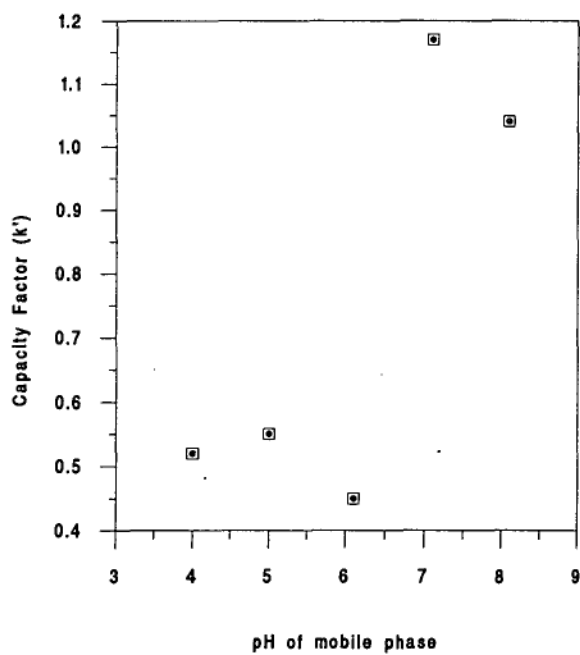
Figure 8.7 *Effect of pH on the elution of salbutamol from a phenyl analytical column.*

8.3.5 Optimisation of extraction

Various parameters in the extraction procedure outlined in Section 8.3.3 were changed to optimise the extraction procedure. Based on observations in Sections 8.3.3 and 8.3.4, changes were made in the introduction of sample on to the disposable extraction column, sorbent wash and elution steps. Solutions containing 10% tetrahydrofuran in pH 8.1 and 4.0 buffers were tested in sample A and F. It was hoped that this would provide a fast elution of salbutamol (given the low capacity factors illustrated in Figures 8.6 and 8.7) while leaving other endogenous products on the column. Samples B, C and D were given two washes of methanol in buffer and water (washing solutions with high capacity factors from Figure 8.5) in the hope of washing endogenous compounds off the column while leaving salbutamol behind. Sample E was given pre-treatment with a buffer before introduction onto the SPE column. The different SPE procedures are listed in Table 8.4.

It was found that the SPE of sabutamol was improved by pre-mixing pH 8.1 phosphate buffer with the urine before introduction of the sample onto the SPE column (sample E). It was also found that improvements were made over the control SPE method when the wash step was performed with 1x250 µl of 10% methanol in pH 8.1 buffer followed by 1x250 µl of 80% methanol in distilled water (sample B). Both these improvements were incorporated into the final SPE method used for sample analysis in Section 8.3.8. No improvements over the simple control SPE were made in the elution step. These results are shown in Figure 8.8.

8.3.6 Optimisation of chromatography

The chromatography of a salbutamol stock solution (500 ng/ml in distilled water) was tested under different flow rates, ion-pair reagents and UV detection wavelengths to increase resolution, peak shape and detection response. The

Table 8.4 *Solid-phase extraction steps tested to optimise the SPE of salbutamol in Section 8.3.5 based on observations obtained from Sections 8.3.3 and 8.3.8.*

Sample	Sorbent Conditioning	Sample Introduction	Sorbent Wash	Elution
Control	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine added to SPE column	2 x 250 µl pH 8.1 buffer	1 x 250 µl tetrahydrofuran
A	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine added to SPE column	2 x 250 µl pH 8.1 buffer	1 x 100 µl 10% tetrahydrofuran in pH 8.1 buffer
B	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine added to SPE column	1 x 250 µl 10% methanol in pH 8.1 buffer followed by 1 x 250 µl 80% methanol in water	1 x 250 µl tetrahydrofuran
C	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine added to SPE column	2 x 250 µl 10% methanol in pH 8.1 buffer	1 x 250 µl tetrahydrofuran
D	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine added to SPE column	2 x 250 µl 80% methanol in water	1 x 250 µl tetrahydrofuran
E	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine + 500µl pH 8.1 buffer mixed and added to SPE column	2 x 250 µl pH 8.1 buffer	1 x 250 µl tetrahydrofuran
F	2 x 250 µl methanol 2 x 250 µl pH 8.1 buffer	1 ml urine added to SPE column	2 x 250 µl pH 8.1 buffer	1 x 100 µl 10% tetrahydrofuran in pH 8.0 buffer
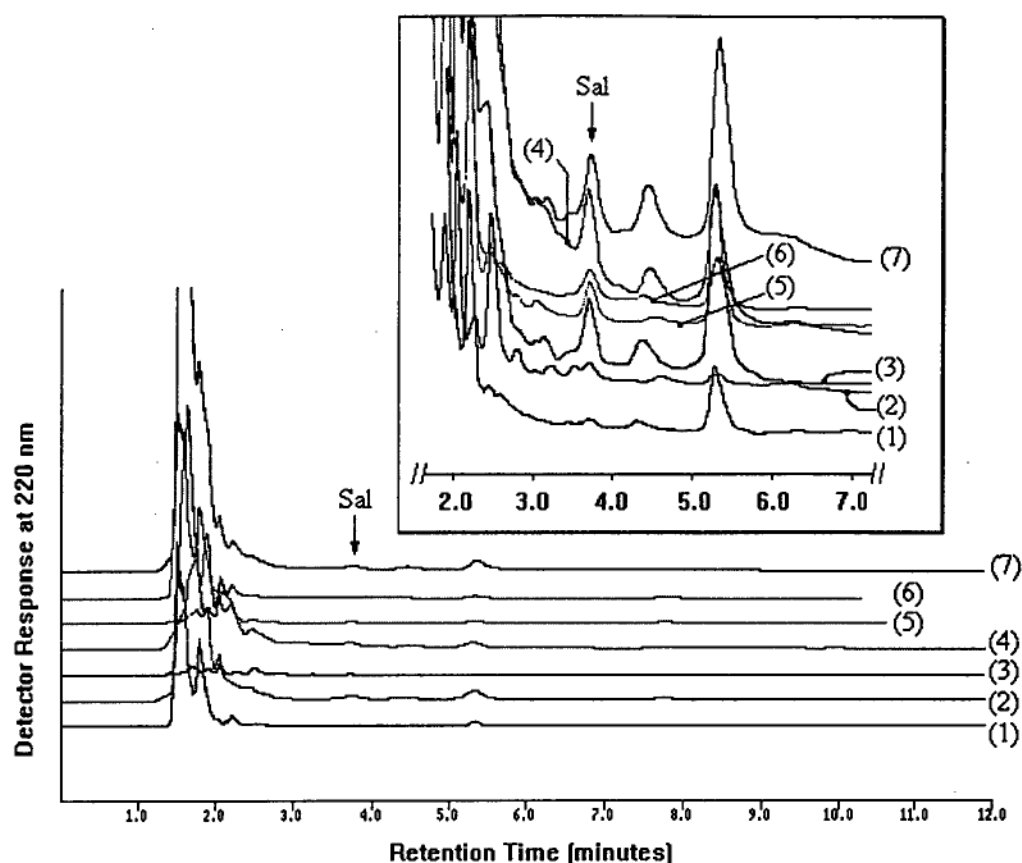
Figure 8.8 *Optimisation of solid phase extraction technique from samples outlined in Table 8.4 summarised as follows. Improvements were found with Sample B and Sample E.*

(1) Control: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine added to SPE column, 2 x 250 µl washes of pH 8.1 buffer and elution with a 1 x 250 µl wash of tetrahydrofuran.

(2) Sample A: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine added to SPE column, 2 x 250 µl washes of pH 8.1 buffer, and elution with a 1 x 100 µl wash of 10% tetrahydrofuran in pH 8.1 buffer.

(3) Sample B: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine added to SPE column, 1 x 250 µl wash of 10% methanol in pH 8.1 buffer followed by 1 x 250 µl 80% methanol in water wash and elution with a 1 x 250 µl wash of tetrahydrofuran.

(4) Sample C: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine added to SPE column, 2 x 250 µl washes of 10% methanol in pH 8.1 buffer and elution with a 1 x 250 µl wash of tetrahydrofuran

(5) Sample D: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine added to SPE column, 2 x 250 µl washes of 80% methanol in water and elution with a 1 x 250 µl wash of tetrahydrofuran.

(6) Sample E: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine and 500 µl pH 8.1 buffer mixed and added to SPE column, 2 x 250 µl washes of pH 8.1 buffer and elution with a 1 x 250 µl wash of tetrahydrofuran.

(7) Sample F: Sorbent conditioning with 2 x 250 µl of methanol and 2 x 250 µl washes of pH 8.1 buffer, 1 ml urine added to SPE column, 2 x 250 µl washes of pH 8.1 buffer and elution with a 1 x 100 µl wash of 10% tetrahydrofuran in pH 8.0 buffer.

commercial Low UV Pic® B-8 ion-pair reagent was compared with a 0.0025 M decane sulphonic acid ion-pair reagent.

The following mobile phases were used:

(i)   20% tetrahydrofuran, 1.9 mM octane sulphonic acid (from a dilution of Low UV Pic® B-8 ion-pair reagent) and 5% methanol in distilled water with a flow rate of 1.5 ml/min.

(ii)   20% tetrahydrofuran and 2.0 mM decane sulphonic acid in distilled water with a flow rate of 1.5 ml/min.

(iii)   18% tetrahydrofuran, 18% methanol and 1.6 mM decane sulphonic acid in distilled water with a flow rate of 1.5 ml/min.

(iv)   Mobile phase identical to (ii) with a flow rate of 2.5 ml/min.

Different wavelengths were also examined by injecting a 500 ng/ml solution of salbutamol into the HPLC fixed loop injection port and comparing the detection response by measuring peak heights at 220, 225, 230, 235 and 240 nm.

Salbutamol (500 ng/ml in distilled water) was best chromatographed using a mobile phase consisting of 20% tetrahydrofuran, 1.9 mM octane sulphonic acid (from a dilution of commercial Low UV Pic® B-8 ion-pair reagent) and 5% methanol in distilled water with a flow rate of 1.5 ml/min, developed from Chapter 5. A comparison with the other mobile phases tested is shown in Figure 8.9. It was also found that increasing wavelength dramatically reduced the relative absorbance of salbutamol as shown in Table 8.5.
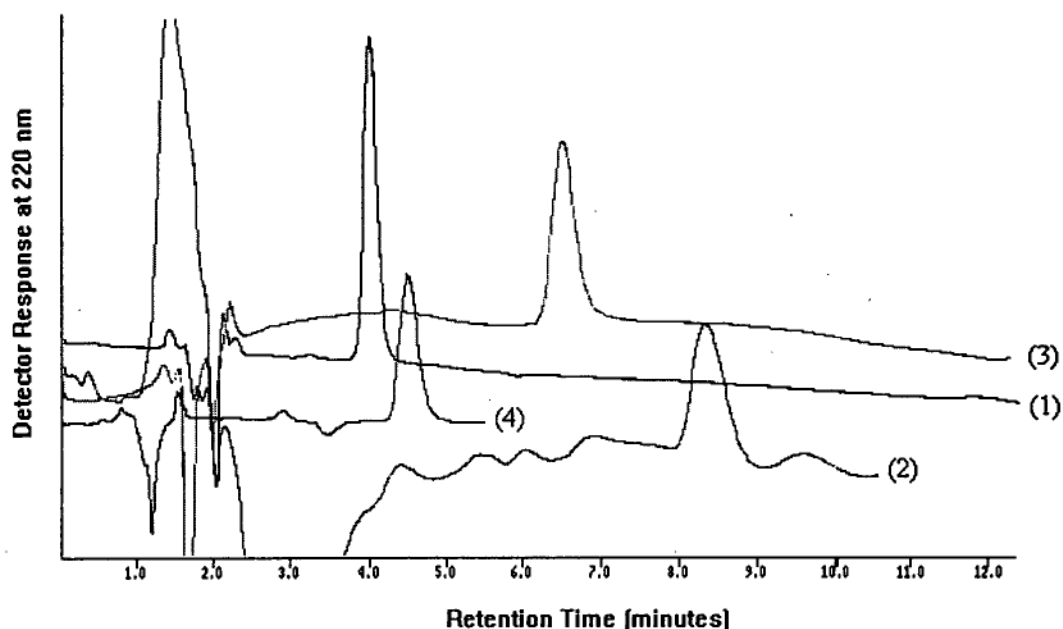
Figure 8.9   *Optimisation of the chromatography of salbutamol as described in Section 8.3.6. Mobile phases and flow rates were as follows: (1) 20% tetrahydrofuran, 1.9 mM octane sulphonic acid (from a dilution of Low UV Pic® B-8 ion-pair reagent) and 5% methanol in distilled water with a flow rate of 1.5 ml/min. Salbutamol peak shown at 4 minutes. (2) 20% tetrahydrofuran and 2.0 mM decane sulphonic acid in distilled water with a flow rate of 1.5 ml/min. Salbutamol peak shown at 8.4 minutes. (3) 18% tetrahydrofuran, 18% methanol and 1.6 mM decane sulphonic acid in distilled water with a flow rate 1.5 ml/min. Salbutamol peak shown at 6.5 minutes. (4) Mobile phase identical to (2) with a flow rate of 2.5 ml/min. Salbutamol peak shown at 4.5 minutes.*

Table 8.5   *Relative absorbance of salbutamol with increasing frequency represented as the percentage of maximum absorbance.*

wavelength (nm)	Relative absorbance (%)
220	97
225	100
230	71
235	32

8.3.7 Internal standard

Several compounds were tested for suitability as an internal standard in the urine assay for salbutamol. It was preferred that other β_2-agonists were not used as potential internal standards as some asthmatic patients could be using more than one generic type of β_2-agonist bronchodilator. The ideal internal standard would be recovered well from the SPE technique and elute late in the chromatogram with little interference from endogenous compounds and salbutamol.

Drugs of a similar nature to salbutamol were tested for their suitability as an internal standard, that is relatively polar, basic drugs, with relatively low molecular weights. Several β-blockers (which are contraindicated in asthmatic patients and would therefore not be expected to be administered to these patients), sympathomimetics (including the β-agonists fenoterol, isoprenaline and terbutaline), local anaesthetics and ketamine were tested. These compounds were tested for retention and peak shape under the chromatographic conditions developed for salbutamol (identical to that used below in Section 8.3.8). These drugs are shown in Table 8.6.

Table 8.6   *Potential internal standards tested.*

β-blockers	miscellaneous	β-agonists
atenolol	mepivacaine	fenoterol
metoprolol	ephedrine*	isoprenaline
oxprenolol	ketamine	terbutaline
pindolol		
propranolol		
timolol		

* $\alpha + \beta$ agonist

All of the potential internal standards tested except fenoterol were found to be unsatisfactory under the given chromatographic conditions with a long retention time and peak tailing a major problem.

8.3.8 Standard curve: method conditions, parameters and reproducibility

A 100 ml stock solution of salbutamol in distilled water (194 µg/ml) was prepared from pure salbutamol base. Two aliquots of 25 µl of this solution were then diluted 1:10 and 1:100 with distilled water to give stock solutions of 19.4 µg/ml and 1.94 µg/ml. Aliquots of 25, 50 and 100 µl were taken from both of these solutions and each added to 1 ml of blank urine from a healthy subject with no prior use of any β-agonist. These spiked urine samples with concentrations of salbutamol between 47.3 ng/ml and 1.76 µg/ml were used to construct a calibration curve. An internal standard stock solution of fenoterol (100 µg/ml) was prepared from a proprietary fenoterol nebuliser solution (Berotec®). Fifty microlitres of internal standard was added to each sample and mixed using a vortex mixer. Calibration curves were constructed on each day of sampling and stock solutions were refrigerated at 4°C.

The spiked calibration curve samples were then extracted using the following SPE method using 1 ml Baker Bond spe® disposable extraction columns. Five hundred microlitres of pH 8.1 phosphate buffer were added to 1 ml of spiked urine sample and mixed using a vortex mixer. The solid-phase sorbent was conditioned with 2 washes of methanol (250 µl per wash) and 2 washes of pH 8.1 phosphate buffer under a slight vacuum to aid passage through the SPE column. The buffered samples were then introduced onto the columns and washed with 250 µl of a 10% methanol in pH 8.1 phosphate buffer solution and 250 µl of 80% methanol in distilled water. The columns were not allowed to dry in between steps and were

not re-used. Sample elution was performed with a 250 μl wash of tetrahydrofuran and the eluent concentrated to approximately half volume under nitrogen (unlike samples in Section 8.3.3 and 8.3.5 that were evaporated to near dryness). The concentrated samples were then centrifuged for 2 minutes at 15,000 rpm in a microcentrifuge (Heltich Mikrolitre, Type 2020).

The chromatography was performed with the equipment outlined in Section 8.3.2. A mobile phase consisting of 20% tetrahydrofuran, 19 mM Low UV Pic B-8® (from a dilution of Low UV Pic B-8® ion-pair reagent) and 5% methanol in distilled water was used with a flow rate of 1.5 ml/min and detection performed at 225 nm. Calibration curves were calculated from salbutamol:internal standard peak height ratios (obtained from the Varian GC Star Workstation®).

Standard curves were constructed from spiked urine samples on each day of sampling. Intra-day coefficient of variation was calculated from five salbutamol spiked urine samples of 47.3 ng/ml (lowest level of the calibration curve) and 473 ng/ml. The 473 ng/ml solutions were also used to examine the recovery of salbutamol by comparing peak heights with a stock solution of salbutamol equivalent to 100% recovery (Section 8.3.3.1). Inter-day coefficient of variation was calculated from a 10 ml urine sample containing salbutamol (473 ng/ml) with 1 ml aliquots extracted on five separate days for analysis. The detection limit, based on a SN ratio of 3 dB, was also examined.

An overlayed chromatogram showing a blank and spiked urine sample used for the calibration curve is shown in Figure 8.10. An example of the calibration curve is shown in Figure 8.11 together with daily calibration curve parameters used in Chapter 9. The calibration curve range was 47.3 ng/ml to 1760 ng/ml. Intra-day coefficients of variation were found to be 9.5% (n = 5; 47.3 ng/ml) and 8.6% (n = 5;

473 ng/ml). The inter-day coefficient of variation was 11.1% (n=5; 473 ng/ml). Recovery of salbutamol from urine was estimated at a disappointing 48 ± 5.0% (mean ± SD, n=5). This was much lower than early indications of around 70%. This decrease in recovery may have been due to variations in many minor factors in the SPE technique such as flow rates, temperature, pH, solvent compositions and handling errors. This highlights the delicate nature of SPE techniques. The detection limit was found to be 15 ng/ml (SN ratio of 3 dB) but inadequate clean-up prevented calibration to that level.
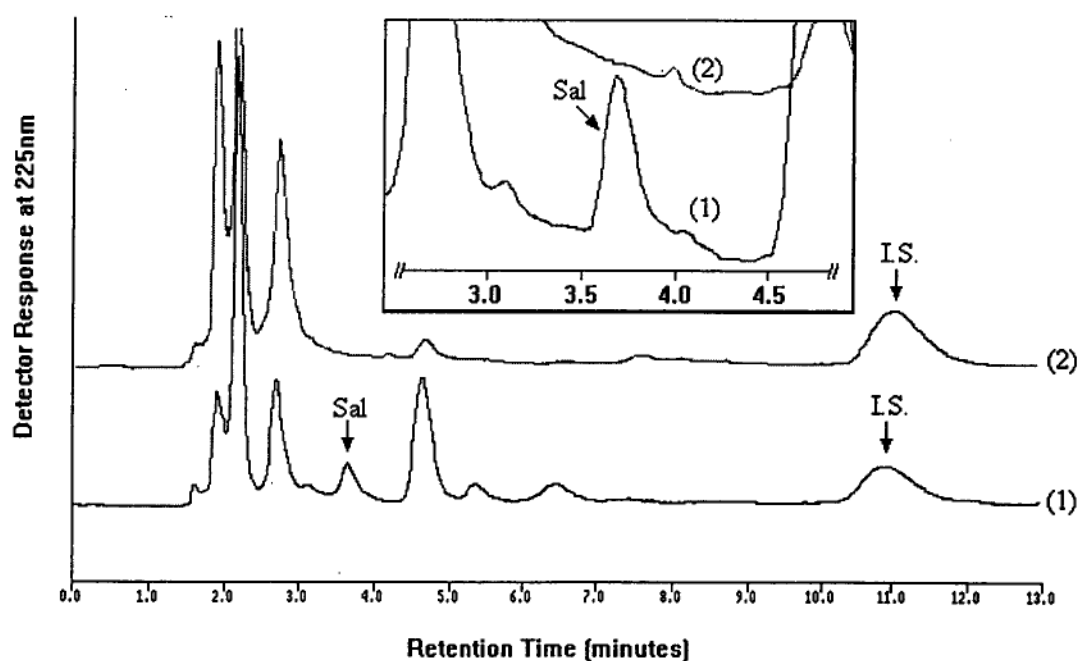
Figure 8.10 *Overlayed chromatogram showing a spiked urine sample (1) with 473 ng/ml of salbutamol used for the calibration curve compared to a urine blank (2). Inset shows the salbutamol peak at a lower attenuation.*

y = 0.0402 + 0.0011x   r^2 = 0.9983
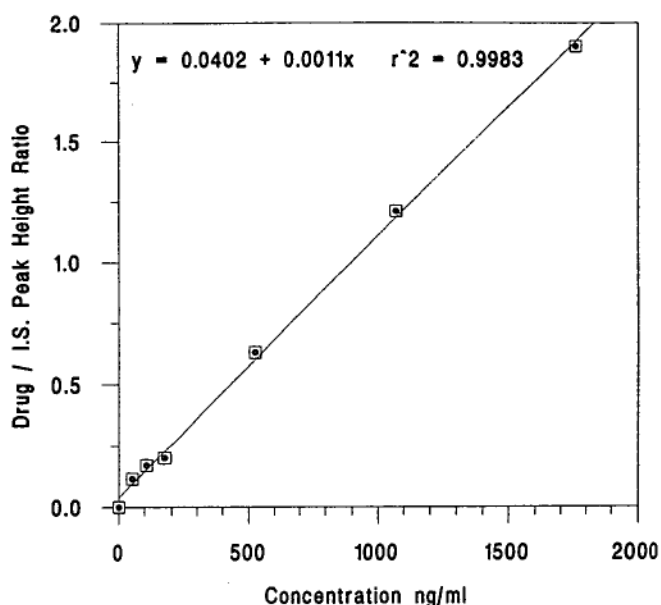
Regression line equations for calibration curves used in the analysis of samples.
(x = concentration ng/ml, y = peak height ratio)

y = 0.0402 + 0.0011x   r=0.999

y = 0.0407 + 0.0013x   r=1.000

y = -0.0300 + 0.0013x   r=0.986

y = 0.0000 + 0.0014x   r=1.000

y = 0.0805 + 0.0011x   r=0.972

y = 0.0000 + 0.0011x   r=1.000

Figure 8.11   *Calibration curve of salbutamol in urine. Daily regression line equations and correlation coefficients from all curves used for analysis of samples are also shown.*

8.3.9   Hydrolysis of salbutamol and determination of the major metabolite of salbutamol

The major metabolite of salbutamol, salbutamol-4'-O-sulphate, was measured indirectly by subtracting the level of unchanged salbutamol from the total hydrolysed sample. Hydrolysis was performed enzymatically by incubating samples at 37°C with a mixture of β-glucuronidase (5.5 U/ml at 38°C; phenolphthalein-β-glucuronide as substrate) and arylsulphatase (2.6 U/ml at 38°C; phenolphthalein disulphate as substrate) derived from *helix pomatia*. The hydrolysis procedure was similar to that used by Hildebrandt et al (1994) in the

analysis of fenoterol conjugates which was analogous to a method used for the analysis of ritodrine conjugates (Brashear et al, 1988).

The time period necessary for hydrolysis was examined. The extraction procedure outlined in Section 8.3.8 was used to examine the salbutamol urine concentration in spot samples from both a community and a hospital patient. The community patient was a 13 year-old mild asthmatic with a reported salbutamol dosage of 400 µg (via MDI) in the preceding 24 hours. The hospital patient was a 59 year-old severe asthmatic with a reported dosage of 20 mg (via nebuliser) in the preceding 24 hours. Four 1 ml aliquots were taken from each sample and added to 500 µl of pH 8.1 phosphate buffer and 50 µl of internal standard solution. These samples were hydrolysed by addition of 50 µl of β-glucuronidase/arylsulphatase mixture and heated at 37°C in a water bath. Samples were removed from the water bath and extracted and analysed using the procedure outlined in Section 8.3.8, initally before hydrolysis and after 30 minutes, 1, 2 and 12 hours of incubation. Salbutamol:internal standard peak height ratios were compared with the non-hydrolysed samples to determine an adequate hydrolysis time.

A chromatogram of a urine sample from a community and hospital patient before and after 2 hours of hydrolysis is shown in Figure 8.12. The plot of measured free salbutamol (hydrolysed from salbutamol sulphate ester) versus hydrolysis time is shown in Figure 8.13 for both a community and hospital patient. This plot was used to determine an adequate time for hydrolysis. The levels of salbutamol in samples that were hydrolysed for 12 hours were unable to be measured because of
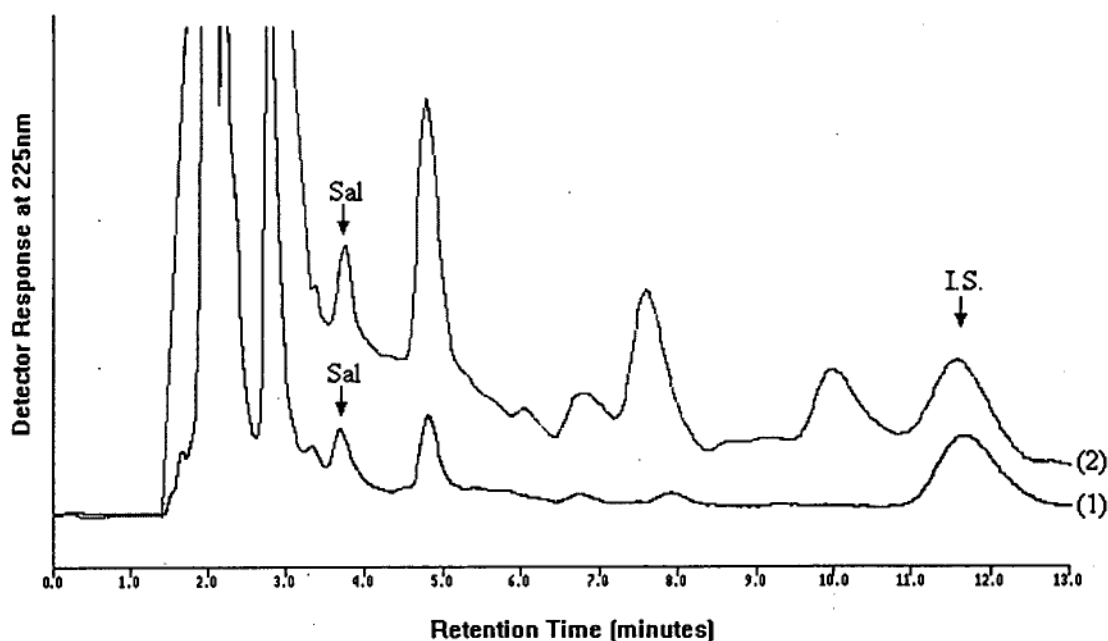
Figure 8.12   *Overlayed chromatogram showing a community patient salbutamol sample before hydrolysis (1) and after 2 hours of hydrolysis (2) with a 90% increase in salbutamol levels.*
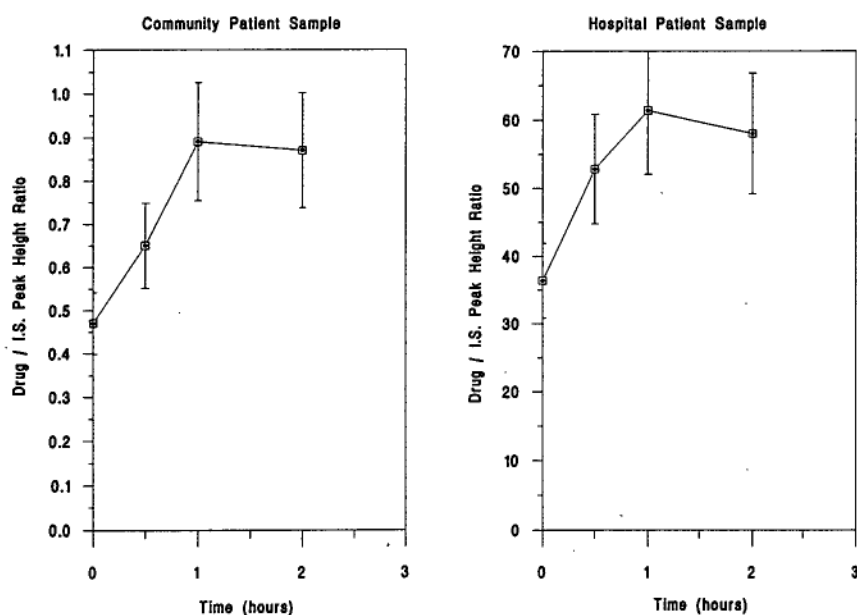
Figure 8.13   *The effect of hydrolysis time on the amount of salbutamol measured in urine (shown here as drug / i.s. peak height ratio) from unchanged salbutamol eliminated by the kidneys as well as salbutamol hydrolysed from the sulphate ester metabolite. Error bars represent the intra-day coefficient of variation for hydrolysed samples (15.2%).*

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large co-eluting peaks, presumably from hydrolysis products from other urine constituents.

A hydrolysis time of 2 hours was decided on for the analysis of the samples. More extensive hydrolysis appeared to produce interfering poorly chromatographed peaks and the hydrolysis appeared to be complete after 1 hour based on Figure 8.13. The amount of hydrolytic enzyme added to the samples seemed adequate given that both the community and hospital sample (with a final salbutamol concentration approximately 70 times greater than the community sample) reached their peak at 1 hour.

Hydrolysed spiked urine was examined to see if hydrolysis affected the calibration curve parameters. Spiked urine samples (92.4, 473 and 924 ng/ml) identical to those used for the calibration curve (Section 8.3.8) were added to 500 μl of pH 8.1 phosphate buffer, 50 μl of internal standard solution and 50 μl of β-glucuronidase/arylsulphatase mixture. Samples were heated at 37°C in a water bath for 2 hours then extracted and analysed in an identical manner to that outlined in Section 8.3.8. These hydrolysed samples were compared with their non-hydrolysed counterparts (used for the calibration curve in Section 8.3.8 and analysed on the same day) for possible interference from either the β-glucuronidase/arylsulphatase mixture or hydrolysis of endogenous compounds. The intra-day coefficient of variation for the hydrolysed samples was examined by hydrolysing five separate 473 ng/ml spiked urine samples.

As mentioned above, the chromatography of the hydrolysed samples were less than ideal. As expected, this impacted on the intra-day coefficient of variation with an intra-day coefficient of variation of 15.2% (473 ng/ml), however, the hydrolysed spiked calibration samples were similar to their non-hydrolysed

counterparts (t=0.079, df=2, p=0.942). An overlayed chromatogram showing a hydrolysed blank and spiked urine sample (473 ng/ml) is shown in Figure 8.14.
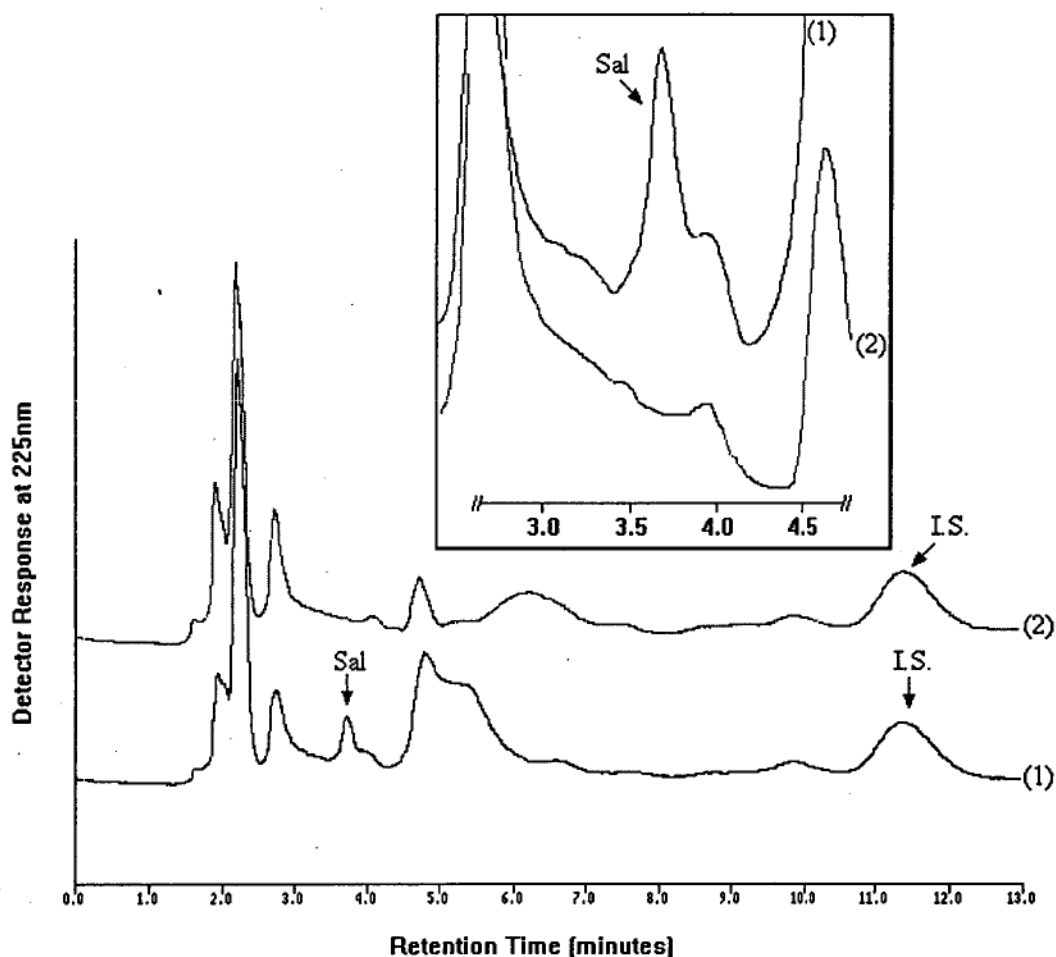
Figure 8.14   *Overlayed chromatogram showing a hydrolysed spiked urine sample (1) with 473 ng/ml of salbutamol compared to a hydrolysed urine blank (2). Inset shows the salbutamol peak at a lower attenuation.*

8.4   DISCUSSION

It was found that SPE can provide reasonable recoveries of salbutamol, a drug that is relatively difficult to extract from biological matrices given its relatively polar nature. The solid-phase sorbents provided greater recovery than liquid-liquid extractions from urine with a phenyl solid-phase sorbent giving the best clean-up and recovery for this application. The examination of drug retention on an

analytical column was found to be a useful tool in developing the overall SPE technique with regard to solvent compositions.

Despite initial indications of levels of recovery around 70%, the final recovery was around a disappointing 50%, although this recovery was quite reproducible. The studies listed in Table 8.2 reported higher recoveries than in this current study, however, these studies were performed in plasma and recoveries of β-agonists can vary greatly between plasma and aqueous solutions (McCarthy et al, 1993).

There was a major problem with interference in the chromatograms from poorly resolved co-elution peaks. This was particularly a problem with the hydrolysed samples. These interfering peaks were probably due to hydrolysed endogenous urinary compounds, both sulphate and glucuronide conjugates. This problem may have been reduced if arylsulphatase enzyme was used alone and not an arylsulphatase/β-glucuronidase mixture. Given that urine acts as a reservoir not only for drugs of interest but interfering compounds, a SPE technique procedure with more thorough sample clean-up would be preferable for further work in this area.

8.5   CONCLUSION

Further research would be well served by using a more sensitive means of detection such as fluorescence detection which was unavailable in this laboratory at the time of this study. In addition, sample clean-up with the SPE technique requires further refinement if measurement of hydrolysed salbutamol is a requirement. Despite the shortcomings presented above, the calibration curve parameters presented here were satisfactory for the type of study described in the next chapter.

CHAPTER 9

Investigation of Urinary Levels of Salbutamol in Asthmatic Patients

9.1 SUMMARY

Some studies have indicated that over-reliance on inhaled β_2-agonist bronchodilator therapy may worsen asthma control and increase morbidity. Urine levels of salbutamol were examined as a potential indicator of over-use in asthmatic patients. Salbutamol levels (unhydrolysed and hydrolysed) were measured in 'spot' urine samples in 102 asthmatic patients (64 community patients and 38 hospital in-patients) using the assay developed in the previous chapter. The correlation between levels and dosage in the preceding 24 hours was examined. Urinary levels were also corrected for dilution by expressing results per milligram of excreted creatinine.

Median levels of unchanged and total drug were found to be 378 ng/ml (range 0-34.4 μg/ml) and 2.55 μg/ml (range 0-49.8 μg/ml) for community and hospital groups respectively. When community and hospital groups were combined, modest correlations were found between salbutamol levels and dosage (Spearman r=0.67, p< 0.005 and Spearman r=0.54, p< 0.005) for unchanged and total drug respectively. Correlations were only slightly improved when correcting for urinary dilution. The inter-patient differences were large. Even when controlling for dosage in the preceding 24 hours, there was a 380 and 810-fold variation in levels for unchanged and total drug respectively. In addition there was evidence to suggest saturation of salbutamol metabolism at relatively low levels. Even when conceding the limitations of 'spot' urine analysis, the enormous inter-patient variability, which may be largely due to differences in the pharmacokinetics and/or administration technique, may have clinical implications. Further work specifically aimed at these findings is required to expand the work presented here.

9.2 INTRODUCTION

As discussed in Chapter 2, despite advances in drug therapy and delivery systems, asthma incidence and anti-asthmatic drug sales have increased in most industrialised countries over the last two decades (Barnes, 1988; Beasley et al, 1990; Hay and Higenbottam, 1987; Kluakka et al, 1991). Several studies have suggested a link between β-agonist therapy and worsening of asthma symptoms and control (Sears et al, 1990; van Schayck et al, 1991; Spitzer et al, 1992). In addition, β-agonists have been implicated in the asthma death epidemics in the United

Kingdom (isoprenaline) and fenoterol in New Zealand (Speizer et al, 1968; Pearce et al, 1990).

Although there is no doubt that selective β_2-agonists remain the most effective bronchodilators in acute asthma (Kemp, 1993) there have been concerns raised that this class of compounds may be over-used. It has been suggested in current management guidelines that there should be a greater emphasis on the earlier introduction of inhaled corticosteroids and less reliance on β_2-agonists (Anonymous, 1992; Tse and Bridges-Webb, 1993). These issues were discussed in more detail in Section 2.5.

Urine monitoring of salbutamol was investigated as a possible means of easily identifying over-use of β_2-agonists in asthmatic patients. It has been noted previously that asthmatic patients appear to use more therapy than they admit to (Chryssidis et al, 1981; Horn et al, 1989). This study aimed to assess the inter-patient variation of urinary salbutamol levels in a relatively large group of asthmatic patients. The correlation between reported dosage of inhaled drug and urine levels of salbutamol was examined to see whether monitoring urine levels would be of benefit in identifying those patients who may be over-using their medication. A single 'spot' urine sample was used to assess the urinary salbutamol level as multiple samples or measuring urine volumes would prove impractical in an out-patient community setting.

Because the half-life of salbutamol is around 3-6 hours, it was decided that the dosage in the 24 hours preceding the urine sample would give a reasonable variable with which to correlate the urine data. Horn et al (1989) found that intervening micturition had little effect on measured salbutamol levels. Urinary levels were also found to be relatively constant for at least 4 hours after a single inhaled dose of salbutamol (Horn et al, 1989). Urinary levels of drug must not, however, be

considered equivalent to plasma levels with regard to the scope of obtainable pharmacokinetic parameters. The limitations of urinary data are discussed in Section 9.5.

Urine levels of salbutamol would of course be subject to urinary dilution with the level determined by the degree of hydration and the urine volume. Biological monitoring of industrial solvents is widely used in the industrial health area to assess exposure of workers to solvents, usually from pulmonary absorption. Because obtaining plasma samples for analysis of xenobiotics can be time consuming and levels can be influenced by peak exposures, 'spot' urine samples are often used to assess exposure (Bernard and Lauwerys, 1986). Results are typically corrected for urine dilution by expression of the solvent metabolite found in urine per gram of creatinine, an endogenous waste product excreted in urine (Apostoli et al, 1982; Bernard and Lauwerys, 1986). The concentration of creatinine, like the metabolite of interest, varies with the degree of urine dilution. Regardless of dilution, the proportion of the metabolite of interest to creatinine remains unchanged. Urinary creatinine turnover is relatively constant over a wide range of renal damage and does not depend on urine flow rate or dietary protein intake (Koushanpour and Kriz, 1976). Urinary creatinine excretion does, however, vary with age and gender. Males aged 70-79 years excrete around 60% of the urinary creatinine of men aged 20-29 years and females excrete around 85% of the urinary creatinine of males (Kampmann et al, 1974).

9.3   METHOD

9.3.1   Patient recruitment and profiles

Community patients were recruited by two means. Three government owned community health centres, each having several general practitioners, were contacted by telephone. A meeting was arranged to explain the project to the general practitioners working at the centres. A meeting was also organised at the

local university health centre. General practitioners were asked to briefly explain the project to asthmatic patients, obtain informed consent from the patient if they were interested in participating and fill in the brief questionnaire. A $5 reimbursement was offered to each practice for each patient sample obtained. Questionnaire and informed consent forms were supplied to the practices as well as a supply 70 ml plastic sample bottles.

Patients who agreed to participate (informed consent form shown in Appendix 3) were asked to provide details necessary for the questionnaire (Appendix 4) and a 'spot' urine sample. It was requested that samples be provided by the patient at or as close to the time of the questionnaire interview as possible. To be eligible for recruitment into the project, patients must have used salbutamol by any means of administration within the previous 24 hours and be able to estimate their dosage. It was not feasible for patients to measure urine volume (allowing the amount of drug excreted to be calculated from volume and concentration), so only single 'spot' samples were taken.

Questionnaire forms included an assessment of disease severity based on bronchodilator use, patient history, peak expiratory flow rate and variability from current guidelines for asthma management (Tse and Bridges-Webb, 1993). The criteria for groupings are shown on the questionnaire form (Appendix 4). Most patients were grouped according to their patient history and bronchodilator use.

Community patients were also recruited through advertisements in two local newspapers (Appendix 5) and posters placed on noticeboards around the University of Tasmania's Hobart campus (Appendix 6). Patients were asked to telephone the School of Pharmacy if interested in participating in the project. Appointments were made at either the School of Pharmacy or at the patient's home. Informed consent, questionnaire details and a fresh urine sample at the time

of interview were obtained from these patients. These patients were offered $5 reimbursement for their time. Urine samples were frozen at -14°C until analysis.

Hospital patients were recruited at the Royal Hobart Hospital by ward pharmacists identifying asthmatic inpatients prescribed salbutamol. In-patients identified as current salbutamol users were then approached and the project explained to each patient. If informed consent was given, patient details were obtained from the drug chart and the urine samples collected by the nursing staff, noting the time of sampling. Information sheets about the project were left on wards for the benefit of the nursing staff (Appendix 7). Drug charts were then examined retrospectively to obtain the dosage of salbutamol in the 24 hours preceding the sample. Four hospital patients who had been receiving large doses of salbutamol immediately prior to the 24 hours preceding the sample time were included in the study. Urine samples were frozen at -14°C until analysis. This project was approved by the Royal Hobart Hospital Research and Ethics Committees.

9.3.2   Determination of salbutamol levels

Salbutamol levels were determined by the assay developed in Chapter 8 and is summarised in Figure 9.1. Samples were gently thawed at room temperature ready for analysis. Samples were analysed in three batches on consecutive days with both salbutamol (unchanged) and total salbutamol (after enzymatic hydrolysis) being measured. Samples from patients using nebulised salbutamol were diluted 1:10 with distilled water to assure the salbutamol level was within the calibration range. Other samples above the calibration range were appropriately diluted and re-analysed. The urinary levels of the salbutamol metabolite were expressed as equivalent concentrations of salbutamol due to the nature of determination (unchanged salbutamol subtracted from total hydrolysed salbutamol) as discussed in Section 8.3.9.
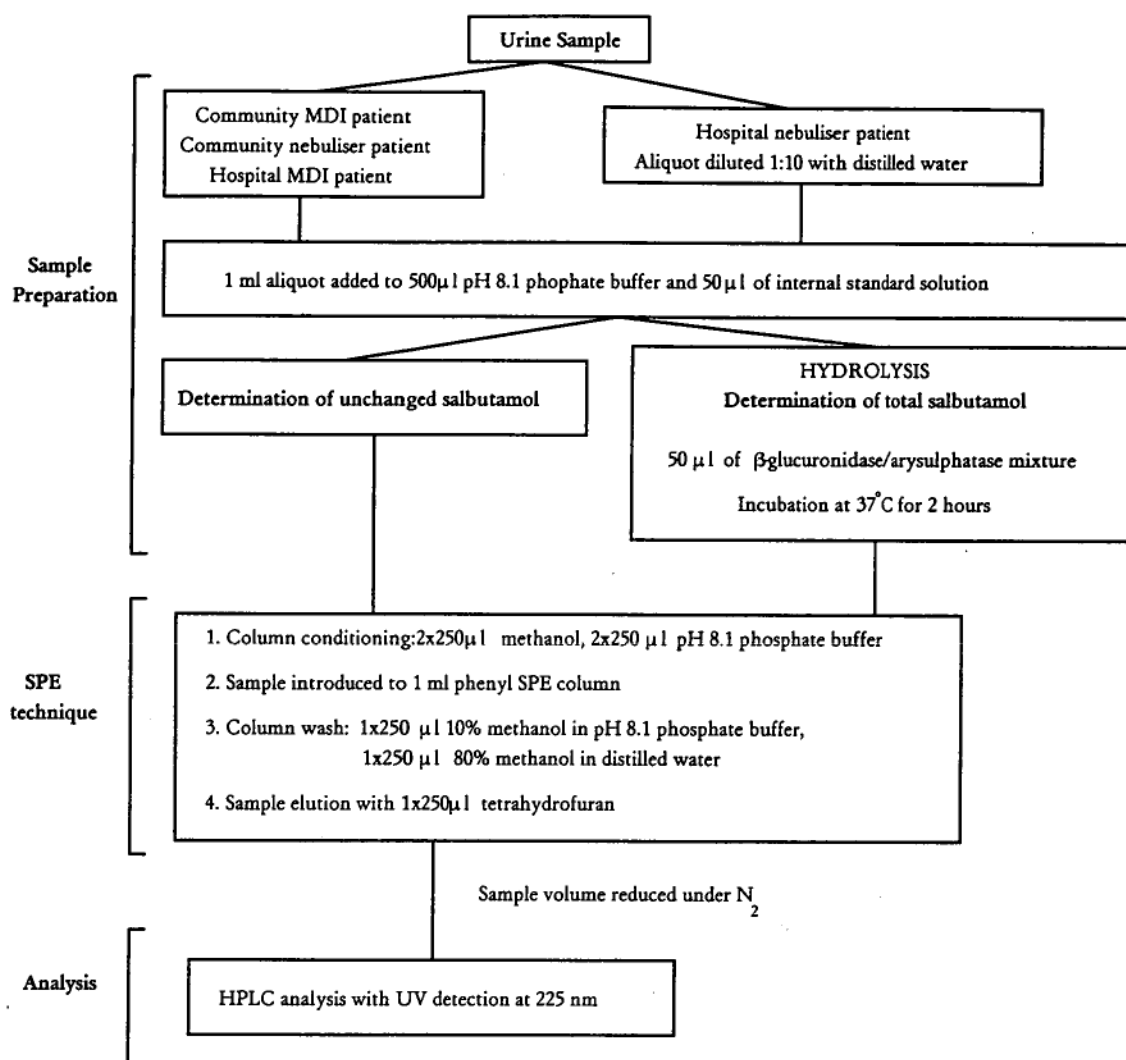
Figure 9.1 *Summary of the method of determination of urinary levels of salbutamol developed in Chapter 8 and used in Chapter 9.*

9.3.3 Determination of urinary creatinine

The Jaffé reaction (alkaline picrate) was the basis for measurement of urinary creatinine. Measurement was performed using a technique developed from Clarke and Thompson (1949) and Supaluknari et al (1987). A picric acid solution of 13.60 g/l in distilled water and a creatinine stock solution of 2.05 mg/ml in 0.1 N HCl were prepared. The creatinine solution was then diluted 1:50 with distilled water to give a solution of 0.041 mg/ml.

Picric acid was obtained from May and Baker Ltd., Dagenham, UK. Creatinine was obtained from Sigma Chemical Co., St. Louis, MO, USA.

This 1:50 dilution was then used to prepare calibration curve samples by taking aliquots of 10, 25, 50, 100, 250, 500 and 1000 µl and making up to 2.01 ml with distilled water (giving creatinine concentrations between 0.204 and 20.4 µg/ml). An absorbance blank consisting of 2.01 ml of distilled water was also prepared. One millilitre of picric acid solution (13.60 g/l) and 500 µl of 1.4 N NaOH were added and the sample mixed with a vortex mixer. The sample was allowed to stand at room temperature for 15 minutes before the absorbance was read at 500 nm using a spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, NY, USA) against the absorbance blank. A calibration curve was then constructed and an intra-day coefficient of variation measured using samples containing 10.2 µg/ml creatinine.

Urine samples were diluted by adding 10 µl of urine to 2 ml of distilled water. Analysis was then performed as for the calibration curve samples by adding 1 ml of picric acid solution (13.60 g/l) and 500 µl of 1.4 N NaOH and the sample mixed with a vortex mixer. The sample was allowed to stand at room temperature for 15 minutes before the absorbance was read at 500 nm using a spectrophotometer (Spectronic 20, Bausch and Lomb) against the absorbance blank.

9.3.4 Statistical analysis

The statistical analyses were performed using Statview® 4.01 (Abacus Concepts Inc., Berkeley, CA, USA) for the Macintosh®. Non-parametric tests were used to examine both patient profiles and the relationships between levels of drug and metabolite with and without correction for urine dilution and reported dosage. The relationship between level and time since last dose was also examined in community patients. Urinary levels of salbutamol below the calibration limit of 47.3 ng/ml were treated as 0 ng/ml for statistical purposes.

The relationship between the unchanged salbutamol level in urine corrected for dose and creatinine clearance was examined to see if there was a link between renal function and urinary excretion of salbutamol. Serum creatinine levels were available close to the time of sampling for some hospital patients. Creatinine clearance was estimated using the Cockroft-Gault equation (Cockcroft and Gault, 1976).

9.4 RESULTS

9.4.1 Patient recruitment and profiles

One hundred and two patients were recruited, consisting of 64 community patients and 38 hospital in-patients. There were 31 males and 33 females in the community group and 19 males and 19 females in the hospital group. There were no significant differences in either the disease severity or salbutamol dosage between genders.

As would be expected, the hospital group were generally older (Table 9.1), had greater disease severity (chi-square=36.4, df=2, $p < 0.005$), received more salbutamol (Table 9.2) and were more likely to be receiving prophylactic therapy (chi-square=10.4, df=1, $p < 0.005$). The differences in dosage are also shown in Figure 9.2. The community group consisted of 24 (38%) mild asthmatics, 29 (45%) moderate asthmatics and 11 (17%) severe asthmatics. The hospital group consisted of 10 (26%) moderate asthmatics and 28 (74%) severe asthmatics. Thirty-eight (59%) of the community patients and 34 (89%) of the hospital patients were receiving prophylactic medication.

The patients receiving prophylactic therapy were more likely to have greater disease severity (chi-square=27.5, df=2, $p < 0.005$). Of the 30 patients not receiving prophylactic therapy, 17 (57%) were mild asthmatics, 9 (30%) moderate asthmatics and 4 (13%) severe asthmatics. Of the 72 patients receiving prophylactic therapy, 7

(10%) were mild asthmatics, 30 (42%) moderate asthmatics and 35 (49%) severe asthmatics. Because of the hospital patient characteristics (older patients with greater disease severity), it is not surprising that the patients receiving prophylactic therapy were more likely to be older (Table 9.3) and be taking higher doses of salbutamol (Table 9.4).
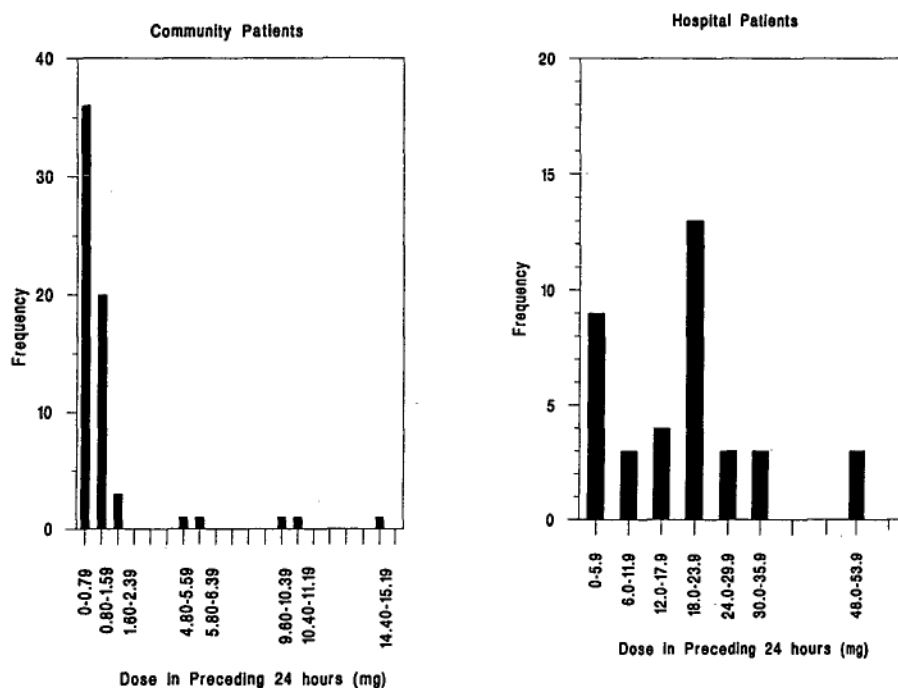
Figure 9.2   *Distribution of salbutamol dosages for community and hospital patients.*

The patients using a nebuliser were more likely to be from the hospital group (chi-square=56.8, df=1, p < 0.005) with greater disease severity (chi-square=49.5, df=2, p < 0.005). In addition, patients with more severe disease were more likely to be receiving higher dosages of salbutamol (Table 9.5) and were more likely to have had previous admissions to hospital with acute asthma in the past year (Table 9.6)

Table 9.1   *Differences in age between community and hospital groups.*

	n	Median (Range) years	Mann-Whitney U	z	p
Community	64	25.5 (11-77)	362	-5.92	< 0.005
Hospital	38	64 (18-83)			

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Table 9.2   *Differences in salbutamol dosage (over the 24 hours prior to sample) between community and hospital groups.*

	n	Median (Range) µg	Mann-Whitney U	z	p
Community	64	400 (100-15,000)	218.5	-6.74	< 0.005
Hospital	36	20,000 (0-50,000)			

Table 9.3   *Differences in age between patients receiving or not receiving prophylactic therapy.*

Prophylaxis	n	Median (Range) years	Mann-Whitney U	z	p
No	30	21 (18-74)	560	-3.82	< 0.005
Yes	72	61 (11-83)			

Table 9.4   *Differences in salbutamol dosage (over the 24 hours prior to sample) between patients receiving or not receiving prophylactic therapy.*

Prophylaxis	n	Median (Range) µg	Mann-Whitney U	z	p
No	30	300 (0-20,000)	479	-4.32	< 0.005
Yes	70	1,500 (0-50,000)			

Table 9.5   *Differences in salbutamol dosage (over the 24 hours prior to sample) with disease severity.*

Severity	n	Median (Range) µg	Kruskal-Wallis H	p
Mild	24	200 (100-1,000)	59.4	< 0.005
Moderate	38	800 (0-20,000)		
Severe	38	20,000 (400-50,000)		

Table 9.6   *Differences in hospital admissions due to asthma in the last year with disease severity.*

Severity	n	Median (Range) number of admissions	Kruskal-Wallis H	p
Mild	24	0 (0-0)	41.0	< 0.005
Moderate	39	0 (0-1)		
Severe	39	1 (0-5)		

9.4.2   Salbutamol levels

The chromatograms for salbutamol in urine and hydrolysed salbutamol in urine are shown in Figures 9.3 and 9.4 respectively. The calibration curve and reproducibility parameters are outlined in Section 8.3.8. As stated in Section 8.3.8, the assay was not ideal with low sensitivity and interfering peaks a major problem, especially with the hydrolysed samples. The salbutamol peaks were unresolvable for 17 unhydrolysed samples (3 community, 14 hospital) and 52 hydrolysed

samples (35 community, 17 hospital). Individual levels of salbutamol and metabolite together with patient profiles are shown in Appendix 8.

The calibration curve for the measurement of creatinine in urine is shown in Figure 9.5. The intra-day coefficient of variation was found to be 2.1%. The range of the calibration curve was 204 ng/ml to 20.4 µg/ml.
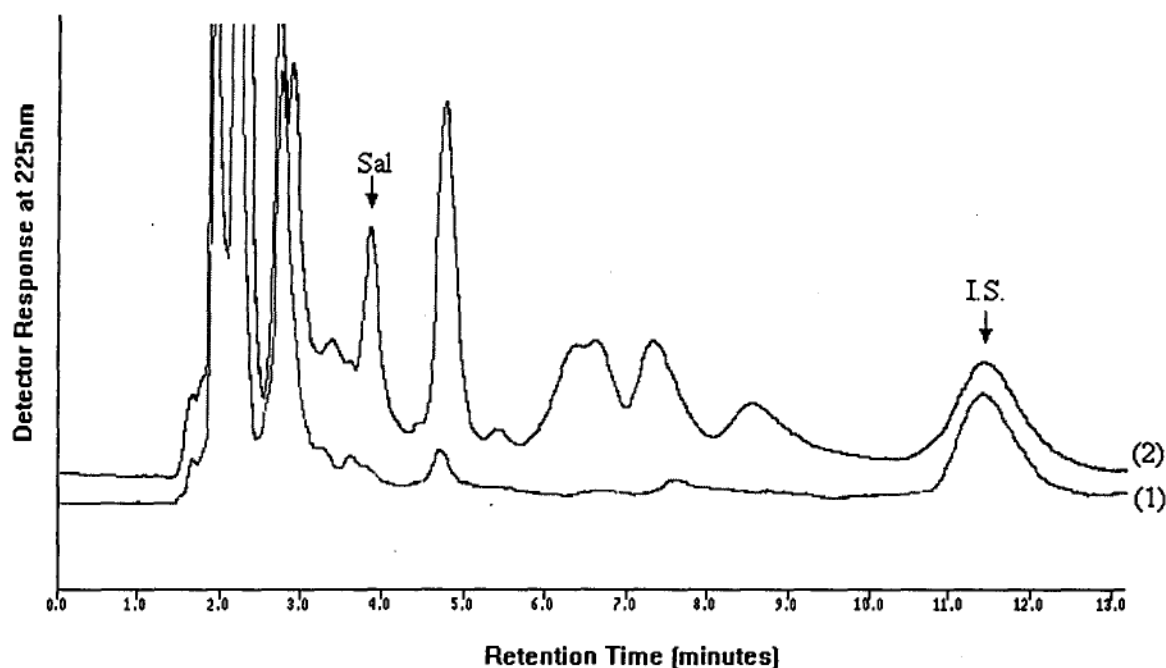
Figure 9.3   *Chromatogram of a urine sample from a community patient (2) showing salbutamol (1261 ng/ml) and fenoterol internal standard (I.S.) compared with a blank sample spiked with I.S. (1).*

The median level of unchanged salbutamol in urine was 378 ng/ml (range 0-34.4 µg/ml) and the median level of total drug (consisting of unchanged drug and metabolite) was 2552.4 ng/ml (range 0-49.8 µg/ml). The community group had lower levels of unchanged drug (median 246.7 ng/ml; range 0-4713.8 ng/ml) than the hospital group (median 3.061 µg/ml; range 0.471-34.4 µg/ml), as would be expected from the differences in dosage (Table 9.2).

Time since last dose (within 24 hours preceding the sample) was examined in community patients to see if this variable significantly affected the levels reported in the urine. There were no significant correlations between time since last dose and either salbutamol, metabolite or total levels corrected for dose for either the community patients or those community patients using MDIs only. Similarly there were no correlations between time since last dose and either salbutamol, metabolite or total levels corrected for both urinary dilution and dose for either the community patients or those community patients using MDIs only. All Spearman r values were less than ±0.14. Time since last dose was not examined in hospital patients as dosing was more frequent.
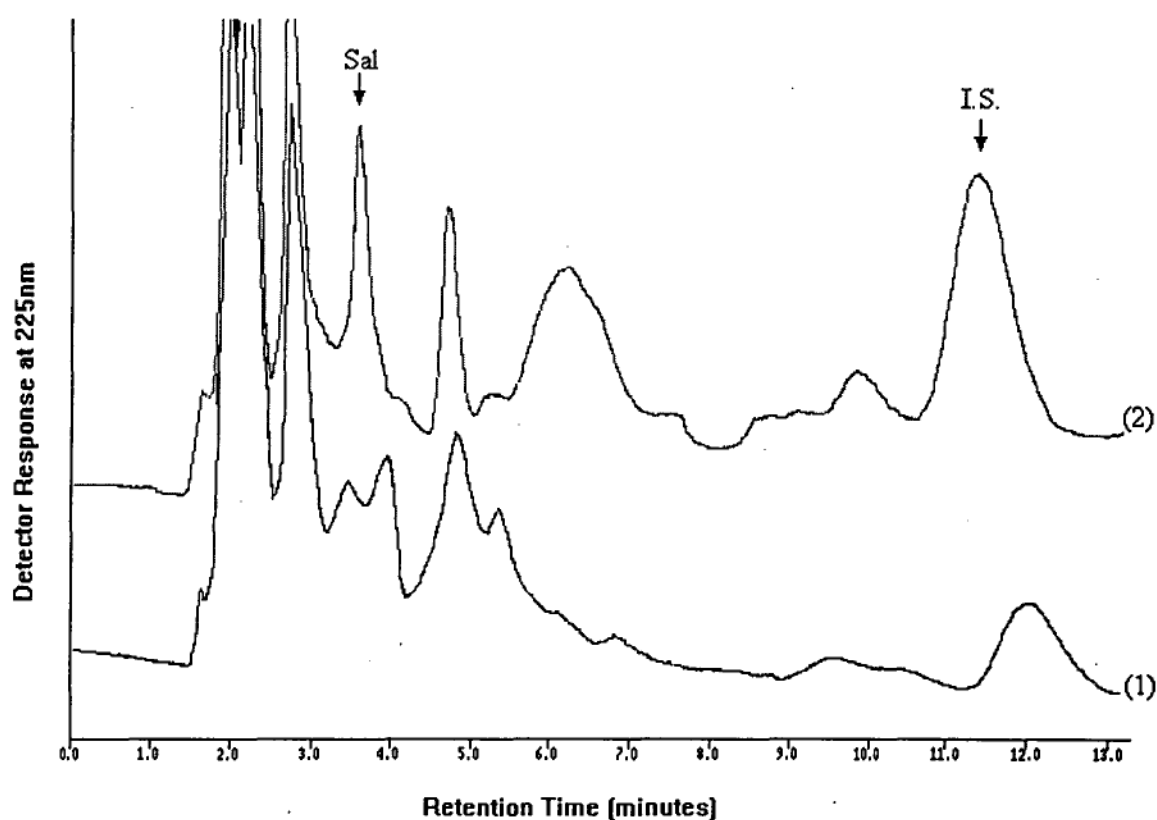
Figure 9.4   *Chromatogram of a urine sample from a community patient after hydrolysis (2) showing salbutamol (767 ng/ml) and fenoterol internal standard (I.S.) compared with a blank sample spiked with I.S. (1).*

$$y = 0.00438 + 0.05174x$$
$$r^2 = 0.99940$$
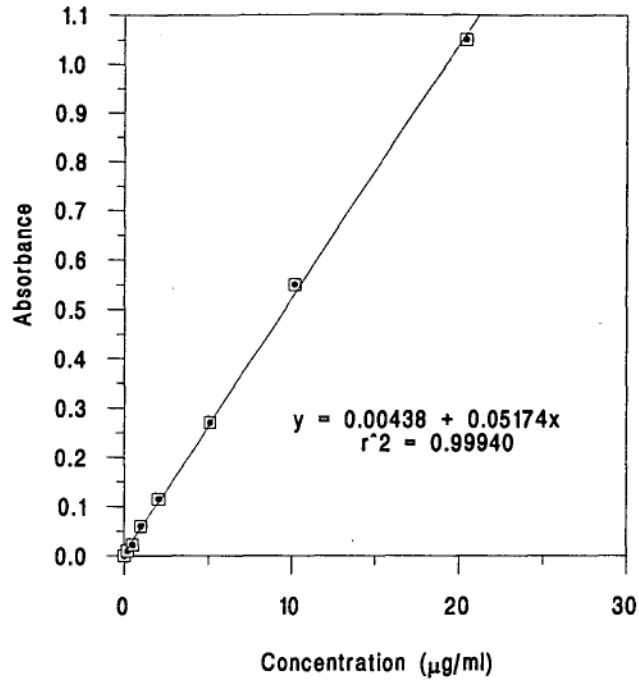
Figure 9.5 *Calibration curve for measurement of creatinine in urine.*

Urinary creatinine levels were also examined. It was found that creatinine levels in the community group were higher than the hospital group (Table 9.8). There was a negative correlation between age and creatinine level in urine (Spearman $r=-0.38$, $n=102$, $p < 0.005$). There were no significant differences in creatinine levels between males and females.

Table 9.7 *Differences in unchanged salbutamol levels between community and hospital patients.*

	n	Median (Range) ng/ml	Mann-Whitney U	z	p
Community	61	246.7 (0-4,713.8)	79	-6.38	< 0.005
Hospital	24	3061 (471-34,400)			

Table 9.8 *Differences in urinary creatinine levels between community and hospital patients.*

	n	Median (Range) ng/ml	Mann-Whitney U	z	p
Community	64	1.44 (0.216-5.23)	736.5	-3.32	< 0.005
Hospital	38	0.701 (0.25-3.52)			

There was a weak correlation between salbutamol dosage in the preceding 24 hours and unchanged salbutamol urine level for the community (Spearman r=0.42, n=61, p < 0.005) group but no significant correlation within the hospital group (Spearman r=0.33, n=24, p=0.11). This is shown in Figure 9.6. The correlations between salbutamol dosage in the preceding 24 hours and unchanged salbutamol urine level were not improved for the community patients using MDI only (Spearman r=0.33, n=56, p=0.01) or hospital patients using nebuliser only (Spearman r=0.30, n=20, p=0.19).

There were no positive correlations between the urinary level of the salbutamol metabolite (calculated as the equivalent weight of salbutamol) and dosage in the preceding 24 hours for either the community (Spearman r=-0.09, n=29, p=0.64) or hospital groups (Spearman r=-0.57, n=21, p=0.01) as shown in Figure 9.7. Again the correlations were not improved for the community patients using MDI only (Spearman r=-0.05, n=27, p=0.78) or hospital patients using nebuliser only (Spearman r=-0.44, n=16, p=0.09).

No significant correlations were found between total salbutamol (salbutamol and metabolite) and dose for either the community (Spearman r=0.03, n=29, p=0.88) or hospital (Spearman r=-0.37, n=20, p=0.11) groups as shown in Figure 9.8. There were no significant correlations between salbutamol dosage in the preceding 24 hours and total salbutamol urine level for the community patients using MDI only (Spearman r=0.01, n=27, p=0.98) or hospital patients using nebuliser only (Spearman r=-0.18, n=16, p=0.49).

After correction for urinary dilution by expression of results per gram of creatinine, the correlations were still poor with large variation between dose and urine levels. There was a modest improvement in the correlation between salbutamol dosage in the preceding 24 hours and unchanged salbutamol per

milligram of creatinine in urine for the community group (Spearman $r=0.50$, $n=61$, $p<0.005$) but not the hospital group (Spearman $r=0.23$, $n=24$, $p=0.27$). This is shown in Figure 9.9. The correlations between salbutamol dosage in the preceding 24 hours and unchanged salbutamol urine level (corrected for urinary dilution) were not improved for the community patients using MDI only (Spearman $r=0.41$, $n=56$, $p<0.005$) but were improved slightly for the hospital patients using nebuliser only (Spearman $r=0.26$, $n=20$, $p=0.27$).

There were no positive correlations between the urinary level of the salbutamol metabolite (calculated as the equivalent weight of salbutamol) per milligram of creatinine and dosage in the preceding 24 hours for either the community (Spearman $r=-0.02$, $n=29$, $p=0.92$) or hospital patients (Spearman $r=-0.47$, $n=21$, $p=0.04$), as shown in Figure 9.10. There were no correlations between salbutamol dosage in the preceding 24 hours and salbutamol metabolite in urine (corrected for urinary dilution) for the community patients using MDI only (Spearman $r=-0.02$, $n=27$, $p=0.93$) or hospital patients using nebuliser only (Spearman $r=-0.34$, $n=16$, $p=0.18$).

No significant correlations were found between total salbutamol (salbutamol and metabolite) per milligram of creatinine for either the community (Spearman $r=0.11$, $n=29$, $p=0.57$) or hospital groups (Spearman $r=-0.28$, $n=21$, $p=0.23$) as shown in Figure 9.11. Similarly there were no significant correlations between salbutamol dosage in the preceding 24 hours and total salbutamol in urine (corrected for urinary dilution) for the community patients using MDI only (Spearman $r=0.05$, $n=29$, $p=0.81$) or hospital patients using nebuliser only (Spearman $r=-0.21$, $n=16$, $p=0.41$). The fact that there were several negative correlations highlights the large scatter associated with these comparisons.

When the hospital and community data were pooled as one group, giving a wider range of dosages and levels, the correlations between dosages and levels did improve. There were significant correlations between dosage in the preceding 24 hours and salbutamol level (Spearman r=0.67, n=85, p < 0.005), salbutamol metabolite level (Spearman r=0.35, n=49, p=0.01) and total (salbutamol and metabolite) recovered drug (Spearman r=0.54, n=49, p < 0.005). After correction for urine dilution, there were correlations between dosage in the preceding 24 hours and salbutamol level (Spearman r=0.69, n=85, p < 0.005), salbutamol metabolite level (Spearman r=0.41, n=49, p < 0.005) and total (salbutamol and metabolite) recovered drug (Spearman r=0.57, n=49, p < 0.005). The correlations were slightly stronger after correction for dilution, however, there was still a relatively large scatter.

The percent of total recovered drug excreted as the metabolite was also examined. While the median percentage of total recovered drug as the metabolite was lower in the group that had used a nebuliser in the previous 24 hours compared to those who had not used a nebuliser the difference was not significant (Table 9.9). There was a weak negative correlation between total dose in the preceding 24 hours and the percent of total recovered drug excreted as metabolite for hospital and community groups combined (Spearman r=-0.29, n=46, p=0.05) and the community patients using MDIs only (Spearman r=-0.44, n=24, p=0.04), but not quite significant for hospital patients using nebuliser only (Spearman r=-0.49, n=16, p=0.06). This is shown in Figure 9.12.

The relationship between amount of unchanged salbutamol divided by dose and estimated creatinine clearance was also examined (shown in Figure 9.13). There was no correlation with renal function and urinary excretion of salbutamol (Spearman r=-0.10, n=11, p=0.77). There were no significant

community/hospital differences in salbutamol urine level corrected for dose as shown in Table 9.10.

Table 9.9   *Differences in percentage of total recovered drug as metabolite between patients who had used a nebuliser in the previous 24 hours and those who had not.*

Nebuliser use in previous 24 hours?	n	Median (Range) % of total recovered drug as metabolite	Mann-Whitney U	z	p
Yes	18	70.3 (0-95)	225.5	-0.78	0.44
No	29	84.0 (0-100)			

Table 9.10   *Differences in salbutamol urine level corrected for dosage between hospital and community patients.*

	n	Median (Range) salbutamol level (ng/ml) divided by dosage (µg)	Mann-Whitney U	z	p
Community	61	0.42 (0-4.71)	559	-1.16	0.25
Hospital	22	0.25 (0.16-6.04)			

The inter-patient variation between urine level of unchanged drug, metabolite and total level corrected for dosage in preceding 24 hours was examined. It was found that even when controlling for stated dosage in the preceding 24 hours there were large variations up to around 400-fold in salbutamol levels, 1200-fold in metabolite levels and 800-fold in total levels (Table 9.11). Inter-patient variability between patients using MDI only and those using nebulised drug only was also examined. Variability, although reduced to some extent, remained relatively large (over 40-fold variation in salbutamol level corrected for dosage in patients using MDI only and over 100-fold for patients using nebulised drug only), as shown in Table 9.11.

Table 9.11   *Variation in urine levels, urine levels corrected for dosage in preceding 24 hours and urine levels corrected for dilution. Variation calculated as maximum level divided by lowest detected level above the detection limit. Also shown is community inter-patient variation with patients taking MDI only and hospital inter-patient variation with those patients using nebulised salbutamol only.*

Variation in salbutamol urine levels

	Salbutamol	Metabolite	Total (salbutamol + metabolite)
Community	93-fold	85-fold	15-fold
Hospital	73-fold	19-fold	12-fold
Community (MDI)	92-fold	85-fold	91-fold
Hospital (Neb)	73-fold	18-fold	10-fold

Variation in salbutamol urine levels corrected for dosage

	Salbutamol	Metabolite	Total (salbutamol + metabolite)
Community	262-fold	1028-fold	810-fold
Hospital	378-fold	1170-fold	451-fold
Community (MDI)	40-fold	241-fold	177-fold
Hospital (Neb)	107-fold	69-fold	43-fold

Variation in salbutamol urine levels corrected for urine dilution

	Salbutamol	Metabolite	Total (salbutamol + metabolite)
Community	319-fold	63-fold	100-fold
Hospital	205-fold	36-fold	25-fold
Community (MDI)	319-fold	63-fold	100-fold
Hospital (Neb)	205-fold	24-fold	25-fold

Variation in urine levels corrected for urine dilution and dosage

	Salbutamol	Metabolite	Total (salbutamol + metabolite)
Community	976-fold	549-fold	400-fold
Hospital	747-fold	2049-fold	955-fold
Community (MDI)	174-fold	146-fold	96-fold
Hospital (Neb)	305-fold	146-fold	69-fold
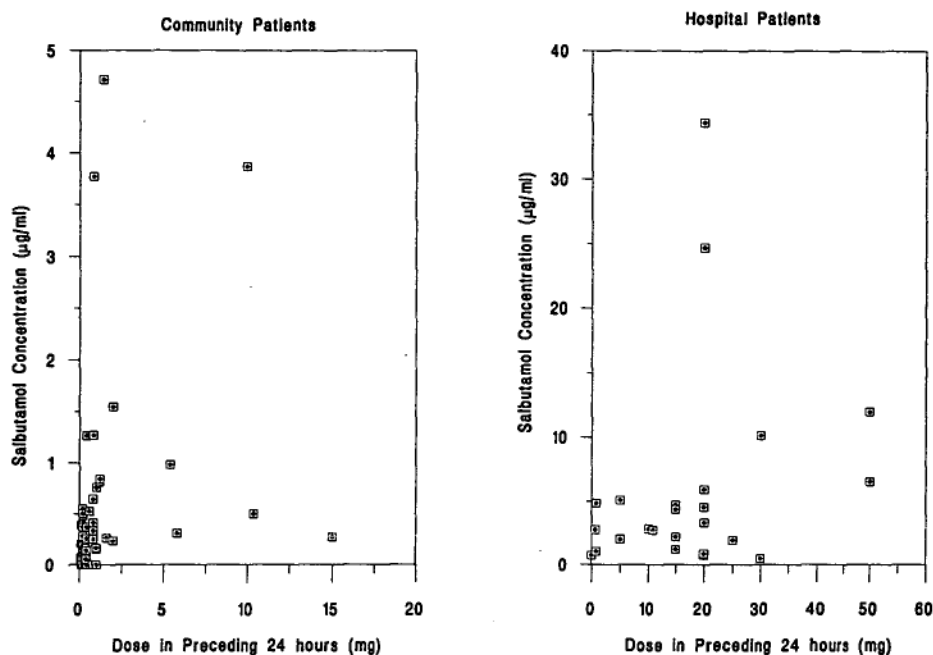
Figure 9.6   *Relationship between salbutamol concentration in urine and dose in the preceding 24 hours for both community (Spearman r=0.42, p< 0.005) and hospital (Spearman r=0.33, p=0.11) patients.*
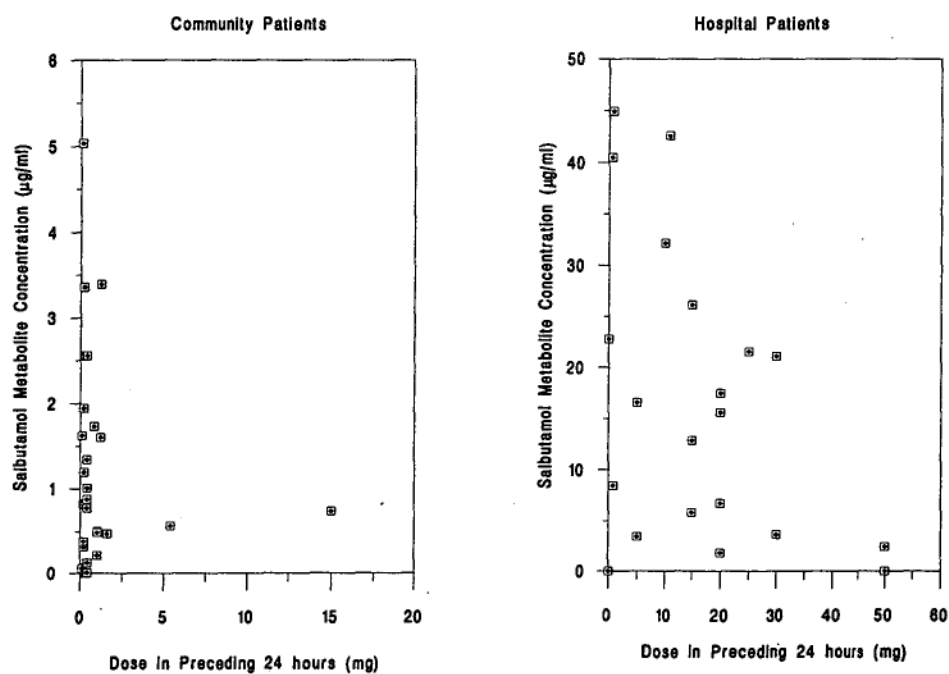
Figure 9.7   *Relationship between salbutamol metabolite concentration in urine and dose in the preceding 24 hours for both community (Spearman r=-0.09, p=0.64) and hospital (Spearman r=-0.57, p=0.01) patients. Salbutamol metabolite expressed as μg of equivalent salbutamol.*
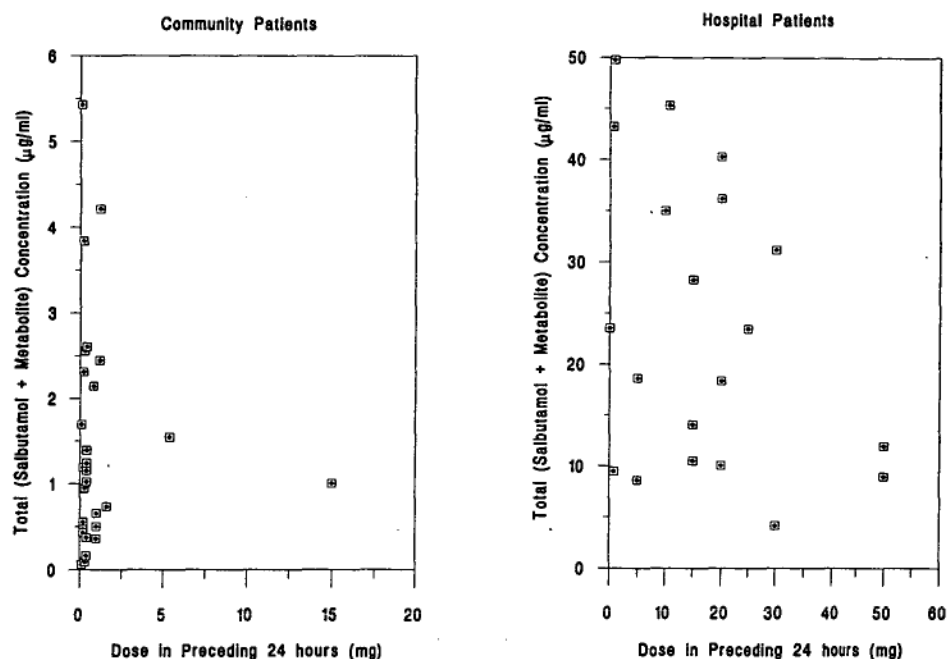
Figure 9.8   *Relationship between the concentration of total salbutamol (salbutamol + metabolite) recovered in urine and dose in the preceding 24 hours for both community (Spearman r=0.03, p=0.88) and hospital (Spearman r=-0.37, p=0.11) patients.*
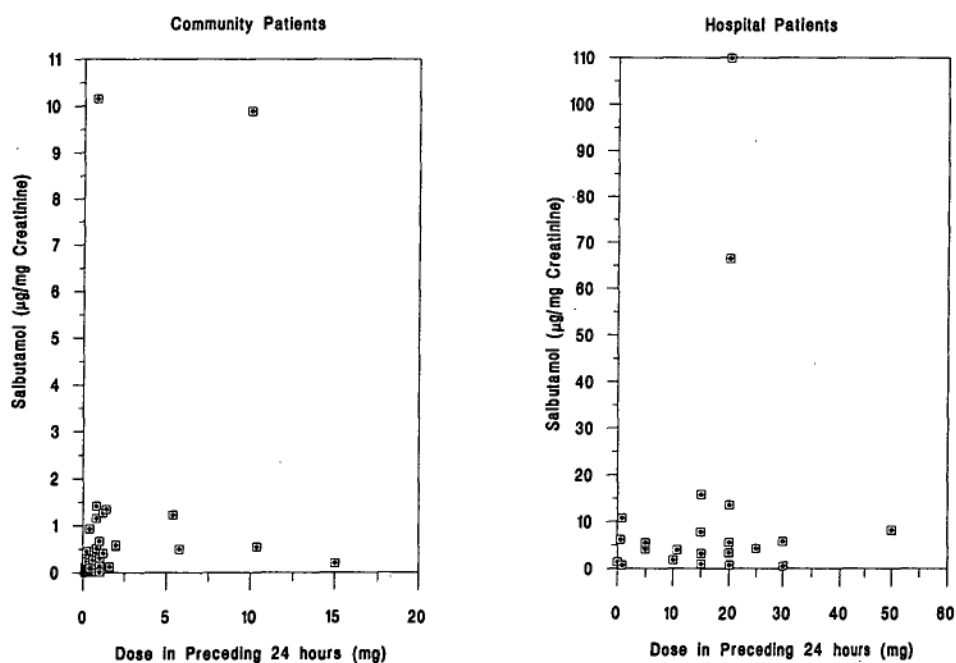
Figure 9.9   *Relationship between salbutamol in urine corrected for dilution (per mg of creatinine) and dose in the preceding 24 hours for both community (Spearman r=0.50, p< 0.005) and hospital (Spearman r=0.23, p=0.27) patients.*
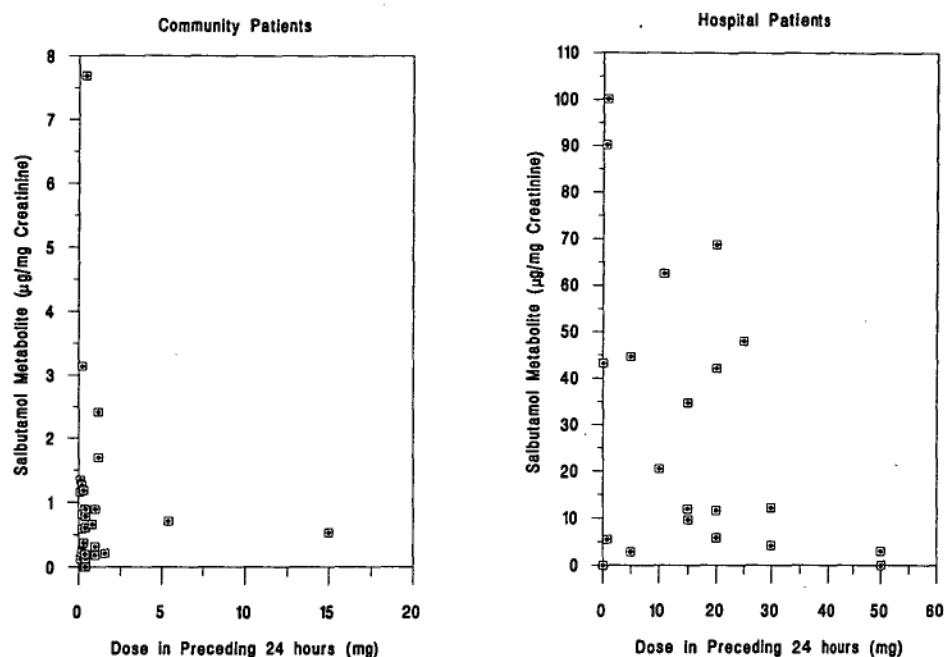
Figure 9.10 *Relationship between salbutamol metabolite in urine corrected for dilution (per mg of creatinine) and dose in the preceding 24 hours for both community (Spearman r = -0.02, p = 0.92) and hospital (Spearman r = -0.47, p = 0.04) patients. Salbutamol metabolite expressed as μg of equivalent salbutamol.*
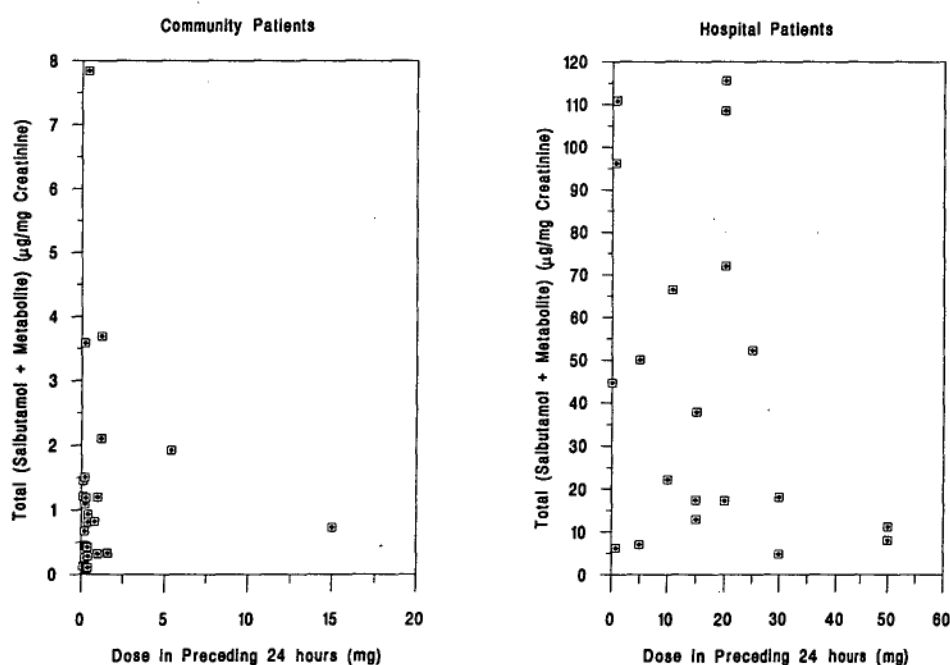
Figure 9.11 *Relationship between total salbutamol (salbutamol + metabolite) in urine corrected for dilution (per mg of creatinine) and dose in the preceding 24 hours for both community (Spearman r = 0.11, p = 0.57) and hospital (Spearman r = -0.28, p = 0.23) patients.*
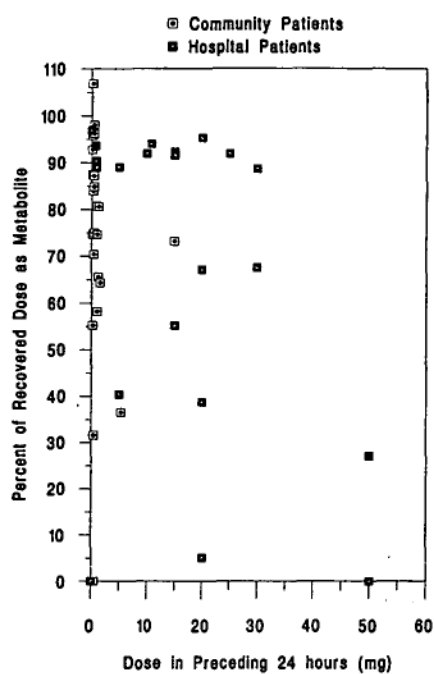
Figure 9.12 . *Relationship between percent of recovered drug as metabolite and dose in the preceding 24 hours.*
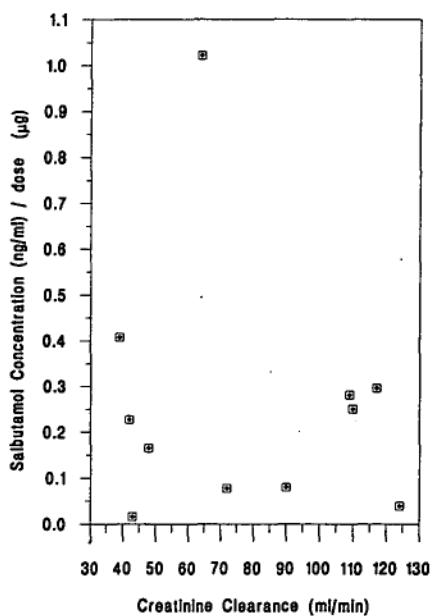
Figure 9.13 . *Relationship between renal function and urinary salbutamol level corrected for dose in preceding 24 hours.*

9.5   DISCUSSION

The recruitment of patients, especially community patients, was surprisingly difficult given the widespread knowledge and incidence of the disease. Recruitment of community patients initially relied on general practitioners to obtain patient consent, questionnaire details and a urine sample. Despite initial assurances of large recruitment numbers and many telephone calls to remind general practitioners, this avenue proved unsatisfactory. The time involvement appeared to be the main reason general practitioners failed to recruit sufficient numbers of patients, with the $5 reimbursement proving an inadequate incentive. Around 50% of community patients were recruited from the advertisement placed in the local newspapers. This avenue proved to be the most effective means of patient recruitment for this study.

The methods of recruiting community patients may have lead to this sample being somewhat unrepresentative of the community as a whole. The usage of salbutamol by community patients in this study was compared with a study by Westley-Wise et al (1993) which examined 319 pharmacy clients receiving β_2-agonists in NSW. It was found that 44% of patients were prescribed less than or equal to 200 μg/day, 30% prescribed greater than 200 μg/day and less than or equal to 600 μg/day and 26% prescribed greater than 600 μg/day. Almost 60% of patients had been prescribed prophylactic therapy in the previous year.

In this current study, the 28% of the community patients reported taking less than or equal to 200 μg/day of salbutamol, 28% received greater than 200 μg/day and less than or equal to 600 μg/day and 44% received greater than 600 μg/day. Of the 64 community patients, 59% were currently taking prophylactic therapy. The community patients in this current study appear to have reported taking higher dosages of salbutamol than pharmacy clients in the study by Westley-Wise et al (1993). Previous studies have suggested that patients use more β_2-agonists than they

admit or are prescribed (Chryssidis et al, 1981; Horn et al, 1989). This may account for the higher reported dosage seen in this study compared to the prescribed dosage reported by Westley-Wise et al (1993).

The majority of patients in this study were either using salbutamol from MDI or nebuliser. One hospital patient (patient 69; Appendix 8) was using MDI in conjunction with a volumetric spacer but had not used it in the previous 24 hours. No patients reported using DPIs.

As expected, the community patient group was quite different to the hospital patient group. It has been shown in Section 9.4 that hospital patients tended to be older, have more severe disease, were more likely to be receiving prophylactic medication, were receiving higher dosages of salbutamol and had lower creatinine excretion rates than the community group. For this reason, the community and hospital groups were treated as two distinct groups with regard to correlation data and inter-patient variability which is discussed later.

Obviously the large numbers of unresolvable salbutamol peaks in the urine sample chromatograms was disturbing and reflected a major limitation of the assay. As discussed in Section 8.5, sample recovery, clean-up and detector sensitivity were relatively poor. Ideally, a more sensitive detection method such as fluorescence detection should have been used, however, this equipment was unavailable in this laboratory. Hydrolysis of urine samples also posed difficulties with large co-eluting peaks, presumably from endogenous urine products, that may not have been present to the same extent in plasma. This study did not examine the (+) and (-) salbutamol isomers but the racemic mixture. Pharmacokinetic differences between the isomers are discussed in Chapter 7.

The median free salbutamol levels from the community group were of a similar magnitude to that found by Horn et al (1989) and Horn et al (1990). The median salbutamol level of the community group in this current study was 246 ng/ml with a median dose of 400 μg over the preceding 24 hours. Horn et al (1989) found that previously healthy subjects with no recent drug exposure who had taken 200 μg of salbutamol had levels of around 100 ng/ml in urine for up to 4 hours after dosing. Horn et al (1989) also found that asthmatic patients who had reported taking 200 μg of salbutamol on the same day prior to their sample had urine levels around 200 ng/ml. Horn et al (1990) found that patients taking 1600 μg a day had median levels around 500 ng/ml. The median salbutamol level for patients in the hospital group in this current study was 3.1 μg/ml with a median nebulised dosage of 20 mg over the preceding 24 hours. These values were also similar to Horn et al (1990) who found that in a group of patients taking 8 mg of salbutamol per day (via specially prepared Rotacaps®), median urine levels of salbutamol were 962 ng/ml.

In this study urine volume determination, viewed as time consuming and messy, was not performed for two reasons. Firstly there was a relatively large number of patients, various settings, and sample collection relied heavily on help from people not directly involved in the study such as nurses and general practitioners. Secondly, the study aimed to examine if a single 'spot' urine sample was of benefit in identifying possible over-use of salbutamol. It was anticipated that this should be a quick test in an out-patient setting for maximum compliance.

Unfortunately this meant that the determination of the actual amount of drug and metabolite excreted (calculated from concentration in a given volume) was not possible. A single concentration of drug in urine is obviously dependent on urine volume. Typical daily volumes of urine are around 1.2 litres but may vary between 0.3 to 2.7 litres per day for adult males and females (Liappis, 1973). This variation

would be expected to affect the concentration of the metabolite or drug by a similar amount.

The correlations between reported total dose in the preceding 24 hours and urinary salbutamol, metabolite and total levels were disappointing although most were statistically significant. This was not totally unexpected given the possible inter-patient variability in urine volumes, administration technique and pharmacokinetics. There was no indication of significant correlation between time since last dose (within 24 hours prior to the sample being taken) and either salbutamol level corrected for total dosage in the preceding 24 hours or salbutamol level corrected for total dosage in the preceding 24 hours and urinary dilution. From this it appears that total dosage within a 24 hour period may be a valid measure of 'average' exposure to salbutamol.

As mentioned earlier, when monitoring levels of solvent metabolites in exposed workers, single 'spot' urine samples are often taken for convenience without measuring total urine volume (Bernard and Lauwerys, 1986). In an attempt to control for the effect of urine dilution, levels are usually expressed per gram of creatinine (Apostoli et al, 1982; Bernard and Lauwerys, 1986). Creatinine is an endogenous product excreted from the kidneys in relatively constant amounts (Koushanpour and Kriz, 1976). Because the creatinine is diluted in urine to the same extent as the metabolite or drug, the effect of urine dilution is reduced.

Creatinine excretion does decline with age (Kampmann et al, 1974). This effect is not so important with workers who are typically less than 60-65 years but becomes more significant in this study where older patients, in particular the hospital patient group, were compared with younger patients. Another limitation with the creatinine method used is this study it that the Jaffé reaction (alkaline picrate) is not entirely specific for creatinine. Glucose, aceto-acetic acid, acetone,

ascorbic acid and pyruvate can all potentially interfere with this colourimetric assay (Lentner, 1984).

There was only a modest improvement in correlation between salbutamol in urine expressed per gram of creatinine and dose in the preceding 24 hours for community patients compared to the samples not corrected for urinary dilution. When community and hospital patients were combined as one group, the correlation between salbutamol level corrected for urinary dilution and dose in preceding 24 hours was slightly improved for unchanged salbutamol, salbutamol metabolite and total salbutamol. The community and hospital groups were distinctively different with regard to dosages, levels and creatinine excretion so these improvements in correlation when the two groups are combined together should be viewed with caution.

Dosages in the preceding 24 hours were as reported by the community patients and may not have been accurate estimations. In the past it has been frequently noted that patients are unreliable with reporting dosages accurately (Sackett and Snow, 1979). Despite relying on community patients accurately reporting their dose of drug in the preceding 24 hours, it is unlikely that this was a major factor in the poor correlation. There were poor correlations between measured salbutamol, metabolite and total levels in urine and reported dose in the preceding 24 hours for both the community and hospital groups. The dose for the hospital patients was taken retrospectively from drug charts and did not rely on patients accurately reporting their dosage like the community groups, yet there was still large variation. Therefore it was most likely that the poor correlations were real and due to variations in the measured levels rather than errors in the reported dosages.

It is suggested that despite these limitations, correction for urinary dilution by expressing the level of drug per gram of creatinine may have a role in 'spot' sample testing of compliance.

There was a surprisingly large inter-patient variation between levels especially after correction for dosage (Table 9.10). The variation was large for both community and hospital patients which suggests that this variation is not due to inaccurate reporting of dosage.

Urine volume is increased with high water and salt intake and a protein rich diet; low water intake, a diet rich in carbohydrates and profuse sweating can lower urine volume (Lentner, 1981). As mentioned earlier urine volume can vary from around 0.3 to 2.7 litres per day for adult males and females (Liappis, 1973). This is a variation of around 10-fold which would reflect a variation in concentration of salbutamol of around 10-fold. Even when accounting for this 10-fold variation, the inter-patient variation still remains large. Inter-patient variation in urinary dilution or time since last dose (within the preceding 24 hours) could not account for the large variations alone.

Differences in dose related to the route of administration may be responsible for some of the variability observed since a typical nebulised dose is around 10 times higher than MDI (Newman, 1985). However, even when comparing the variability within community patients using MDI only and hospital patients using nebuliser only, there is still a large variation. The inter-patient variation in level divided by dose in community patients reportedly taking MDI only was 40-fold and variation in hospital patients using nebuliser only was 108-fold. Even more surprising is that fact that these variations do not take into account those patients with levels below the detection limit. It would be expected that the variation would be even greater

if the detection limit was lower as several community patients had levels below the detection limit.

This large inter-patient variation is most likely due to a combination of differences in pharmacokinetics, administration technique and urinary dilution. It has been noted previously that there are large inter-patient variations in plasma concentrations of salbutamol required to produce similar responses (Morgan, 1990; Penna et al, 1993). Even in the same individual, responsiveness can decrease with time during continuous therapy (Morgan, 1990). Therapeutic response and onset of side effects is therefore often a better indicator of required dose than plasma levels (Morgan, 1990).

The median percent of recovered dose excreted as metabolite in patients using nebuliser was 70% compared to 84% for those using MDI. This compares with Morgan et al, (1986) who found approximately 60% of recovered dose excreted as metabolite in patients taking oral salbutamol. The median levels in this study are generally higher and are likely to be due to limitations of the assay of hydrolysed samples including the reduced sample size due to unresolvable salbutamol peaks.

Patients using nebuliser (with higher doses than MDI) had a lower median percentage of total recovered drug excreted as metabolite compared to patients using MDI, however, the difference was not significant (Table 9.9). It has been previously reported that the metabolite to unchanged drug ratio in plasma and urine following nebuliser use is less than with oral or MDI administration (Morgan, 1990). It has been suggested that this is due to a greater proportion of the dose being absorbed by the lungs and not swallowed via nebulised administration compared to MDI administration (Shenfield et al, 1974; Morgan, 1990). With less dose absorbed orally, the fraction of drug that undergoes first-pass metabolism is reduced, hence the metabolite to unchanged drug ratio is lower. There is some

evidence in this current study to suggest that the lower metabolite to unchanged drug ratio seen with nebulised therapy may also be caused by dose related metabolism effects.

There were indications that the percent of dose recovered as metabolite decreased with increasing dosage, not just in comparison between MDI and nebulised drug users but also in community patients using MDI alone. In addition, there was almost a significant relationship in hospital patients using nebuliser only. A low fraction of the dose recovered as the metabolite in urine implies a low fraction as the metabolite in the body. This evidence would seem to indicate possible saturable metabolism of salbutamol. If saturation is in fact occurring, observed higher proportions of unchanged drug in plasma or urine following nebulised administration may not indicate more efficient pulmonary delivery via nebuliser but could be due to saturated sulphation of salbutamol.

Extensive literature searches have shown that there has been little study into possible saturation of sulphation pathways with salbutamol. Saturation of sulphation pathways has been well documented in animals with drugs such as salicylamide and paracetamol, both in vivo and in vitro (Kane et al, 1991; Pang et al, 1994). It has also been shown that there are differences in saturation between the gut wall and liver (Goon and Klaassen, 1990). The possible saturation seen here with salbutamol is at a much lower concentration than would normally be associated with saturation of the sulphation pathway observed in the studies shown above. More work in this area is required to confirm that there is in fact saturation at low doses and if so, the therapeutic consequences.

As discussed in Chapter 7, there are large variations in reported pulmonary deposition of MDI, DPI and nebulised salbutamol. It is well documented that variation is affected by factors such as technique, mode of delivery and disease

severity. It seems unlikely that these variations in pulmonary deposition of drug combined with variability in the assay, urine volumes and patient reporting of dosage could alone account for all of the total inter-patient variability in urinary levels of salbutamol seen in this study.

This study suggests that inter-patient pharmacokinetic differences (for example saturable metabolism) and differences in administration technique may play a major part in the overall variability. Does this have any bearings on studies showing worsening of asthma symptoms with regular use of inhaled β_2-agonists? As Morgan (1990) pointed out, plasma levels are not an important parameter with which to adjust dosage as there is very large inter-subject variation in levels required to produce the same response. This study has also clearly shown large inter-patient variations in urinary levels corrected for dosages. This suggests that a dose that may be satisfactory for one patient may be unsatisfactory for another (due to differences in administration technique and/or pharmacokinetics) and has more widespread implications.

It has been shown that regular use of salbutamol can worsen asthma control (Sears et al, 1990; van Schayck et al, 1991). In the study by van Schayck et al (1991), a patient group taking continuous salbutamol with a strict regimen of salbutamol 400 µg four times daily from DPI was compared with patients taking treatment on demand. A large number of patients taking continuous therapy withdrew from the study because of insufficient bronchodilator treatment. In addition, lung function was worse in the continuous treatment group compared to the treatment on demand group. Given the large inter-patient variation seen here (due to differences in administration technique and/or pharmacokinetics), it is possible that the 1600 µg of salbutamol per day did not provide plasma levels adequate to control symptoms in some patients in the study by van Schayck et al (1991). Inadequate asthma control may have been caused, not by drug related increases in disease

severity as suggested by van Schayck et al (1991), but due to an inadequate amount of drug exerting a pharmacological effect.

In the study by Sears et al (1990), patients were taking either continuous fenoterol 400 μg four times daily from DPI or placebo with extra bronchodilator therapy if needed, then after 24 weeks treatment groups were crossed-over. It was found that asthma control was better in the period of taking placebo with extra bronchodilator therapy as required. As in the study by van Schayck et al (1991) inadequate asthma control with continuous therapy in some patients may have been caused, not by drug related increases in disease severity but due to an inadequate amount of drug exerting a pharmacological effect due to inter-patient differences in pharmacokinetics.

Sears et al (1990) did find the average daily use of supplementary bronchodilators during the placebo period did not exceed the combined use of supplementary bronchodilator and fenoterol therapy during the continuous treatment phase for any patient. However, this result may have been confounded as patients taking placebo plus supplementary bronchodilators may have been reluctant to use supplementary bronchodilation for fear of 'over-using' their medication because they believed the placebo DPI to be active drug.

Both of these studies mentioned above should be re-examined in light of the large inter-patient differences that have been observed here. Future studies examining regular versus as needed bronchodilator therapy should be designed to reduce any error brought about by differences in pharmacokinetics and/or administration technique and the variation in drug levels that these bring. Ideally, plasma levels of β_2-agonists should be measured in these studies.

As stated earlier, there are severe limitations with the reliability of 'spot' urine testing. What this study has done is identify two areas, possible metabolism saturation and a large inter-patient variation, that have the potential to be clinically significant with regard to control of asthma symptoms and require further investigation.

9.6   CONCLUSION

Keeping in mind the vast limitations presented by 'spot' urine sampling, this study has shown that there is a large inter-patient variability in excretion of drug in proportion to dose, most likely accounted for by differences in administration technique and/or pharmacokinetics. In addition it was shown that there was possible saturable metabolism of salbutamol at relatively low levels. It is suggested that some studies reporting worsening of asthma control with regular fixed dosages of β_2-agonists are limited due to inter-patient differences in administration technique and/or pharmacokinetics. There are strong indications that more work is urgently required in this area to answer the specific issues raised in this study.

CHAPTER 10

General Discussion

Despite advances in drug treatment over the last twenty years asthma morbidity and mortality in the community remains high. The annual cost of asthma in Australia has been estimated at up to $720 million (NAC report, 1992). With other chronic diseases, in addition to drug treatment, quality of life and prognosis can often be improved by other variables such as life style changes (for example diet and exercise) or treatment such as surgical intervention. This is of course important from a public health aspect. By educating the community with regard to life style changes, morbidity, mortality and health care costs can be reduced. Unfortunately this is not really the case with asthma.

The aetiology of asthma remains unclear and in the majority of cases drug treatment is the only mainstay for improving quality of life for patients with the disease. Therefore the pharmacotherapeutic aspects of asthma are relatively more important than for many other chronic diseases. This is especially so given suggestions that over-reliance on certain types of drug therapy may increase morbidity and mortality. This thesis has examined several current pharmacotherapeutic aspects of asthma and airways diseases in general.

The utilisation of anti-asthmatic medication both nationally and within Tasmania was found to be changing in line with current prescribing guidelines. There was evidence to suggest that reliance on β_2-agonists has diminished with greater emphasis now on inhaled corticosteroid utilisation. There were some regional differences between Northern and Southern Tasmania but drug consumption in both regions appeared to be changing in line with current management guidelines.

It was found that utilisation of sodium cromoglycate was lower than anticipated and it is suggested that this drug may be under-utilised at present.

The practice of mixing nebuliser solutions in bulk has been noted to improve patient compliance but questions have been raised concerning the physicochemical stability of these solutions being mixed in bulk and stored for several days. The evidence presented in this thesis suggests that these solutions are stable for at least a period of up to 5 days if stored at or below 22°C. There is some doubt, however, over adequate preservative strength with the diluted admixture. Microbial contamination was not studied but a storage period of 5 days with refrigeration and protection from light was recommended.

This thesis has also identified large inter-patient variation with respect to urinary salbutamol levels corrected for dose in patients receiving inhaled therapy. Even conceding the limitations of 'spot' urine analysis, this suggests large inter-patient variation in administration technique and/or pharmacokinetics between patients. Inter-patient differences in administration techniques affecting bioavailability are indirectly supported by the large variation seen in pulmonary deposition studies (Table 7.1). In addition, there was evidence to support a hypothesis of saturation of salbutamol metabolism at relatively low levels. Confirmation of both of these findings would have important implications.

Firstly, the fact there are large inter patient variations in levels corrected for dose (with or without correction for dilution) suggests that there is also large inter-patient variation in therapeutic effect corrected for dose. A dose satisfactory for relief of symptoms in one patient may not provide sufficient drug levels in another patient for adequate relief of symptoms. In other words, inter-patient differences in the bioavailability (due to variations in pharmacokinetics and/or administration

technique) may determine the dosage of salbutamol required for control of symptoms rather than disease severity. Studies that have shown worsening of asthma control with regular versus as needed therapy should take into account these large inter-patient differences.

Secondly there is little literature available on salbutamol metabolism or possible low level sulphate metabolism saturation. The clinical importance of this saturation and whether it is linked to the inter-patient differences is not clear at present. More research is urgently required into both of these areas to gain a better understanding of β-agonist pharmacotherapy.

Essentially aspects of the pharmacotherapy of asthma have been examined from a macro to a micro scale in this thesis. The research material presented here has (i) confirmed that anti-asthmatic drug utilisation in the community (both locally and nationally) is improving, (ii) shown bulk mixing of salbutamol and ipratropium nebuliser solutions is reasonable on the grounds of physicochemical stability and, (iii) found large inter-patient variability in β-agonist administration techniques and/or pharmacokinetics, including possible saturable metabolism, that could have significant clinical relevance.

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Dear Mr ▇

The School of Pharmacy is currently conducting a Statewide research study into asthma medication prescribing trends. Given the recent questions concerning the safety of long-term ß₂-agonist use, available literature on inhaled corticosteroids and the perception of asthma as an inflammatory disease rather than simply bronchoconstriction, more consideration should now be applied to the prescribing of inhaled corticosteroids (as recommended by the National Asthma Campaign). This study aims to assess whether these prescribing trends in antiasthma medication are occurring in different regions of the State. This project will use similar methods of data collection as a project completed during 1990 examining psychoactive drug prescribing in Tasmania, the results of which have been accepted for publication by the *Medical Journal of Australia*.

In order to collect reliable data, the study requires the participation of as many Tasmanian community pharmacists as possible; participation will require little time and effort and your co-operation will be greatly appreciated. Prescription data will be collected by utilising dispensing computer systems, either Amfac-Chemdata III or IV, or Tasmanian Pharmacy Computer User's Group. These three systems are used by the School of Pharmacy.

Those pharmacists who participate will be asked to provide data relating to the number of drugs dispensed over a one month period. The method of obtaining these data will utilise the 'reporting' options, available on all three systems, to provide an alphabetical drug summary hard copy of drugs and quantities used during the nominated period. The hard copy can then be sent free of charge by reply paid postage to the School of Pharmacy. The 'reporting' procedure is simple to perform and involves a minimum amount of time and effort on the part of the pharmacist. Details of the data collection procedure are fully explained on the attachments.

It is intended that data will be analysed for prescribing rates of various antiasthma medications including ß₂-agonists, inhaled corticosteroids and theophylline over a one month period, every six months, until 1994. The data collection will be backdated and start from the months of April 1991 and October 1991. Amfac-Chemdata users will require Dual History Archive disks for these months, TPCUG users should be able to access the data from hard disk. It is anticipated that any trends in prescribing will be observed; e.g. a decline in the prescribing ratio of ß₂-agonists to inhaled corticosteroid medication.

All the information will be treated in strict confidence. All data will be pooled, data relating to individual pharmacies and patients will not be examined.

If you have any queries, please phone either of the numbers shown below. Thank you for your anticipated cooperation.

Yours sincerely,

Glenn Jacobson,   Dr. Gregory Peterson,   Dr William Friesen,
Ph.D. Student.   Lecturer.   Senior Lecturer.
ph. (002) 20 2202   ph. (002) 20 2197

Tasmanian Pharmacy Computer Users Group

Also serving members on our big north island

48 Begonia Street

LINDISFARNE, Tas. 7015

Tony Wise   Tel (002) 43 8247   FAX (002) 43 7640
Chung Liauw   Tel (004) 25 8444   FAX (004) 25 5013

PROGRAM & DRUG FILES UPDATE - AUGUST 1st 1992

Get to your dispensing Main Menu, type 9 to exit, put this update disk in, type UPDATE and press the [Enter] key. (Don't do the update before finishing dispensing for this month).

Some new NHS fees apply from August 1. To alter these now, type MENU then press 2 to go to Utilities Menu then press 3 to go to DISPENSING FEES and enter the following altered fees ...

     Maximum NHS Patient payment .. $15.90
     NHS Ready prep. disp. fee $1.57
     NHS Extemp dispensing fee $5.15

New PBS items : Lipex and Zocor 5mg, Gaviscon P, Penslg 250mgl plus Levlen, Logynon, Monofeme and Trifeme "pills". Brand premiums on Microgynon 30, Nordette, Triphasil, Triquilar, Prothiaden and Zyloprim.

Dispensing Program change :- Repat patients obtaining an Entitlement Number remain Category R (don't change to E). All Repat prescriptions go in the Repat bundle whether $2.60 or free. That part you know. To facilitate checking that the Entitlement Number has been put on the relevant Repat 'scripts the serial number will be prefixed with Re to indicate that it is a Repat 'script with an Entitlement Number.

"Special Prices" have been used in lieu of the actual cost prices for Imigran tabs and Prohibit vaccine to produce a dispensed prices that is about the suggested Guild price so that Prohibit is about $40 and the quantity for Imigran tabs is shown as 1 so that 2x Imigran tabs are about $60. Another exception is Tarentin Doxpen as the Guild price list has a (non-existent) 30 pack which seems to be the accepted basis for pricing rather than the 100 pack with broken factor. If necessary enter your price for a prescription anywhere on the comments line e.g. $22.55 (the $ sign is required) and this price will over-ride the computer's price. To alter the COST PRICE of any private item go to the DRUG EDITOR then select Edit COST PRICE & pack 3. However, also advise me A.S.A.P. (by fax if you have one) of any price changes required in the Master File that is distributed in Updates. If you have a comment or question on pricing please pass on to Kevin Morgan on (002) 23 5203 (South Hobart Pharmacy, 360 Macquarie Street, South Hobart)

> The School of Pharmacy is currently conducting a pharmacoepidemiology study into anti-asthma medication prescribing trends across the State. The study is similar to a study published in the *Medical Journal of Australia* which examined the prescribing rates of psychoactive drugs and was feature in *The Australian*, *The Examiner* and *The Mercury* on Monday July 6th 1992. The current study still requires the help of as many community pharmacies as possible (especially in the north of the State) to collect prescription data. Participation requires little time or effort and illustrates to government bodies the important role that pharmacies can play in monitoring drug use in the community. If you are willing to provide prescription data and do not understand this procedure or have misplaced the original letter *(but you have! T.W.)* from the School of Pharmacy, please contact Glenn Jacobson on (002) 20 2203 or Dr Greg Peterson on (002) 20 2197.

N.B. I ask you to participate if you haven't yet done so and you won't have to spell *pharmacoepidemiology!*
                                      Tony Wise

NEW MEMBERS Welcome to Geoff Hill of Cygnet (Geoff took over from Kevin Corby who is at Evandale), Adrian Ryan of Latrobe and Varsha Horekicho of Youngtown. Adrian's and Varsha's were Foundation systems. Locum available : Jacqui Marshall, a regular user of our dispensary system, telephone (002) 29 1499.

P.S. I will be on holidays from August 5 until September 19. For assistance contact Phoenix Computers on Tel (004) 25 6444 or FAX (004) 25 5013, Judy Liauw on Tel (004) 25 6666 or FAX (004) 25 5013 or Kevin Morgan on (002) 23 5203. Have you noted Kevin's new telephone number? Also order printer ribbons, disks or paper from Kevin. T.W.

T
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TPCUG

> READ, CIRCULATE and RETAIN these program notes for reference.

The University of Tasmania at Hobart

Statement of Informed Consent for Research and Teaching Purposes

INVESTIGATION OF URINARY LEVELS OF SALBUTAMOL IN ASTHMATIC PATIENTS RECEIVING INHALED THERAPY

The aim of this study is to investigate the range of levels in urine of the drug salbutamol (Ventolin or Respolin) in a large group of asthmatic patients using the drug. This information may prove useful in improving the treatment of asthma.

As a subject, you will be asked to provide a urine specimen and answer some questions relating to your use of salbutamol (Ventolin or Respolin). Also, we will take some limited information (age and relevant medical history) from your medical record. All this information will be kept strictly confidential. If you agree to participate in this study, please sign this form, indicating your consent, on the understanding that:-

1. I have read the information above and any questions I have asked have been answered to my satisfaction. I agree to participate in the investigation and complete the relevant questionaries provided.

2. I understand that I can refuse to take part in this research, or withdraw from it at any time without affecting my medical care or relationship with the Community Health Centre and my doctors.

3. I agree that research data gathered in this investigation may be published, provided that I cannot be identified as a subject.

After considering all of these points, I accept to participate in this investigation.

Signature : ....   Date : ....

Witness : ....   Date : ....

Further information can be obtained from Dr.Greg Peterson or Mr.Glenn Jacobson at the University of Tasmania (ph 20 2190)

Statement By the Investigator

I have explained this study and the implications of participation to the subject and believe that the participant understands it, and that this consent is based on adequate information.

Signature of Investigator : ....   Date : ....

APPENDIX 4: Questionnaire of salbutamol use (Chapter 9)

INVESTIGATION OF URINARY LEVELS OF SALBUTAMOL
IN ASTHMATIC PATIENTS RECEIVING INHALED THERAPY

Name/I.D No._____   Age :..........   Sex: M/F   Date: ....

1. Current drug therapy <u>prescribed by a doctor</u> for the 24 hours prior to visit
    (drugs and dosages- please indicate where applicable)

Ventolin® Inhaler   ....   Ventolin® Nebules 5.0mg   ....
Ventolin® Rotacaps   ....   Ventolin® Tablets 4mg   ....
Ventolin® Rotahaler   ....   Ventolin® Respirator Solution.......................
Ventolin® Syrup   ....   Ventolin® Injection 500µg   ....
Ventolin® Nebules 2.5mg ....

2. Other drug therapy prescribed by a doctor for the 24 hours prior to visit
    (drugs and dosages)
    ....
    ....
    ....

3. Severity of asthma (according to the 1989 Asthma management plan guidelines below)
          [ ] Severe   [ ] Moderate   [ ] Mild

Grade	History	Bronchodilator use	Variability in PEF	Best PEF *(% of predicted)
Severe	Wakes at night with wheeze, cough, chest tightness on waking in morning; hospital admission in the last year; previous life-threatening attack.	Needed more than four times a day.	>30%	<70%
Moderate	Symptoms on most days.	Needed on most days.	20%-30%	70%-100%
Mild	Mild occasional symptoms, eg only with exercise or infections	Needed occasionally	10%-20%	100%
				*PEF (peak expiratory flow)

4. Number of hospital admissions due to acute asthma in the past 12 months?   ....

5. On a typical day, how many times does this patient <u>actually use</u> a Ventolin metered
    dose inhaler?   (i) Number of times?...........   (ii) Number of puffs each time? ....

6. How many times has this patient <u>actually used</u> a Ventolin metered dose inhaler in the
    last 24 hours?
                    (i) Number of times?...........   (ii) Number of puffs each time? ....
                    (iii) How many hours since the patient last used their Ventolin?..........

7. Has this patient used a Volumatic Spacer in conjunction with a metered dose inhaler
    in the last 24 hours? Y/N

8. In a typical week, does this patient use Ventolin by nebuliser as well? ....
        (i) - If yes, number of times usually per day?..........   (ii) Number of ml. each time? ....

9. In the last 24 hours, has this patient used Ventolin by nebuliser? ....
        (i)- If yes, number of times?..........   (ii) Number of ml. each time? ....

10. Would you expect this patient to have normal renal function? Y/N

ASTHMA
SUFFERERS

Subjects are required for a study into asthma medication usage. The research project is being conducted by the School of Pharmacy, at the University of Tasmania, and is approved by the University Ethics Committee. Participation requires little time or effort. If you are currently using Salbutamol (brand name Ventolin, Respolin, Asmol or Respax) and would like to participate, phone (002) 20 2202 or 20 2190 during business hours for further details. Subjects will be reimbursed $5 for their time.

ASTHMA RESEARCH PROJECT

The School of Pharmacy at the University of Tasmania is conducting a research project, approved by the University Ethics Committee, into asthma medication usage and requires volunteers.

We need asthmatic patients who....

- are currently using Ventolin® medication,
- are willing to provide one urine sample (containers provided),
- are willing to answer a short questionnaire about medication usage.

All patient identifying information is confidential and names are not recorded.

If you are interested in volunteering, please call either Mr. G. Jacobson (ph. 20 2202) or Dr. G. Peterson (ph. 20 2197) at the University of Tasmania.

The University of Tasmania at Hobart

INVESTIGATION OF URINARY LEVELS OF SALBUTAMOL
IN ASTHMATIC PATIENTS RECEIVING INHALED THERAPY

The aim of this study is to investigate the range of levels in urine of the drug salbutamol (Ventolin or Respolin) in a large group of asthmatic patients using the drug. This information may prove useful in improving the treatment of asthma.

Patients using salbutamol that give informed consent are asked to provide a single spot urine specimen and answer some questions relating to their use of salbutamol (Ventolin or Respolin). Also, we will take some limited information (age and relevant medical history) from their medical record. All this information will be kept strictly confidential.

Sample bottles are delivered to the ward and nursing staff are asked to collect a single spot urine specimen (approximately 10 to 50ml) from the patients that give consent. It is important that the time is noted on the sample bottle. The sample bottle can be labelled with the patients UR label. The sample should be refrigerated.

Further information can be obtained from Mr.George Taylor (RHH Pharmacy) or Dr.Greg Peterson or Mr.Glenn Jacobson at the University of Tasmania (ph 20 2190)

APPENDIX 8: Patient profiles of salbutamol users (Chapter 9)

* metabolite level expressed in terms of equivalent salbutamol
** total level expressed in terms of equivalent salbutamol

C = community patient
H = hospital patient

Patient number	Age	Sex	Patient type	Severity	Concurrent prophylactic therapy	Hospital admissions in previous year	Dose in preceding 24 hours (mg)	Route of administration	Urine salbutamol level (ng/ml)	Urine metabolite level (ng/ml)*	Urine total salbutamol level (ng/ml)**	% of recovered drug as metabolite	Creatinine (mg/ml)
1	57	M	C	Severe	Y	0	400	MDI	1262	•	•	•	1.34
2	23	F	C	Moderate	Y	0	400	MDI	163	0	163	0	1.58
3	21	M	C	Mild	N	0	200	MDI	247	305	551	55	1.25
4	32	M	C	Moderate	Y	0	1000	MDI	150	209	359	58	1.15
5	20	M	C	Mild	N	0	100	MDI	0	60	60	•	0.49
6	11	M	C	Moderate	Y	0	1000	MDI	759	•	•	•	1.15
7	38	M	C	Mild	N	0	300	MDI	92	0	92	0	1.17
8	20	F	C	Mild	N	0	400	MDI	255	117	372	32	0.90
9	33	M	C	Severe	Y	0	1000	MDI	0	501	501	•	1.58
10	19	M	C	Moderate	Y	0	1600	MDI	261	471	732	64	2.24
11	29	M	C	Mild	Y	0	1000	MDI	166	487	653	75	0.55
12	26	F	C	Moderate	N	0	200	MDI	548	•	•	•	3.29
13	20	F	C	Mild	N	0	200	MDI	119	349	468	75	0.43
14	21	F	C	Moderate	Y	0	200	MDI	248	•	•	•	1.77
15	18	M	C	Severe	Y	0	2000	MDI	238	•	•	•	0.43
16	20	F	C	Mild	N	0	200	MDI	185	•	•	•	1.48
17	19	F	C	Moderate	Y	0	400	MDI	148	1006	1154	87	1.28
18	18	F	C	Mild	N	0	200	MDI	478	3359	3838	88	1.07
19	69	F	C	Moderate	Y	0	800	MDI	221	•	•	•	1.69
20	66	F	C	Mild	Y	0	100	MDI	390	5035	5425	93	3.75
21	25	M	C	Mild	N	0	200	MDI	127	•	•	•	1.46
22	32	F	C	Mild	Y	0	400	MDI	0	•	•	•	2.00
23	54	M	C	Moderate	Y	0	800	MDI	•	•	•	•	1.25
24	20	M	C	Mild	N	0	800	MDI	0	•	•	•	2.39
25	17	F	C	Moderate	Y	0	400	MDI	0	•	•	•	3.87
26	48	F	C	Severe	Y	2	10400	MDI+neb	500	•	•	•	0.92
27	26	M	C	Moderate	N	0	600	MDI	523	•	•	•	1.96
28	19	F	C	Moderate	Y	0	400	MDI	0	•	•	•	3.13
29	18	M	C	Mild	N	0	300	MDI	143	806	949	85	2.16
30	18	F	C	Severe	Y	2	1400	MDI	4714	•	•	•	3.52
31	24	M	C	Mild	Y	0	200	MDI	0	1190	1190	107	5.23
32	40	M	C	Mild	N	0	400	MDI	53	1338	1391	96	1.50
33	19	F	C	Mild	N	0	200	MDI	281	•	•	•	1.42

Patient number	Age	Sex	Patient type	Severity	Concurrent prophylactic therapy	Hospital admissions in previous year	Dose in preceding 24 hours (mg)	Route of administration	Urine salbutamol level (ng/ml)	Urine metabolite level (ng/ml)*	Urine total salbutamol level (ng/ml)**	% of recovered drug as metabolite	Creatinine (mg/ml)
34	20	M	C	Mild	Y	0	200	MDI	54	374	428	87	1.69
35	25	M	C	Mild	N	0	300	MDI	0	2552	2552	•	2.16
36	19	M	C	Moderate	N	0	1200	MDI	816	3393	4209	81	2.00
37	21	F	C	Mild	N	0	100	MDI	71	1621	1692	96	1.40
38	29	F	C	Mild	N	0	200	MDI	0	•	•	•	0.76
39	18	F	C	Mild	N	0	400	MDI	122	•	•	•	1.23
40	50	M	C	Moderate	N	0	800	MDI	0	•	•	•	0.24
41	61	F	C	Severe	Y	0	800	MDI	3771	•	•	•	0.37
42	20	M	C	Moderate	N	0	200	MDI	498	•	•	•	1.65
43	19	F	C	Moderate	N	0	800	MDI	378	•	•	•	1.09
44	77	M	C	Moderate	Y	0	5400	MDI+neb	980	565	1544	37	0.80
45	26	F	C	Severe	N	0	800	MDI	249	•	•	•	0.22
46	43	F	C	Moderate	Y	0	400	MDI	256	767	1023	75	1.26
47	20	M	C	Moderate	N	0	800	MDI	412	1731	2142	81	2.62
48	39	F	C	Moderate	Y	0	800	MDI	643	•	•	•	1.50
49	21	F	C	Mild	N	0	200	MDI	118	818	936	87	1.40
50	18	F	C	Moderate	Y	0	400	MDI	160	•	•	•	2.39
51	72	M	C	Severe	Y	1	800	MDI	•	•	•	•	0.55
52	62	F	C	Mild	Y	0	800	MDI	1268	•	•	•	2.51
53	33	M	C	Moderate	Y	0	1200	MDI	841	1602	2442	66	0.66
54	28	M	C	Moderate	Y	0	200	MDI	371	1941	2312	84	1.54
55	60	M	C	Moderate	Y	0	2000	MDI	1547	•	•	•	2.66
56	63	F	C	Moderate	Y	0	15000	neb	270	735	1005	73	1.38
57	63	F	C	Mild	Y	0	100	MDI	204	•	•	•	1.85
58	61	M	C	Severe	Y	0	1200	MDI	•	•	•	•	1.46
59	38	F	C	Severe	N	0	5800	MDI+neb	308	•	•	•	0.62
60	66	F	C	Moderate	Y	0	800	MDI	333	•	•	•	0.24
61	66	F	C	Moderate	Y	0	400	MDI	51	2556	2607	98	0.33
62	69	F	C	Severe	Y	1	10000	neb	3866	•	•	•	0.39
63	11	M	C	Moderate	Y	0	400	MDI	141	•	•	•	2.00
64	13	M	C	Moderate	Y	0	400	MDI	367	874	1241	70	4.45
65	74	M	H	Severe	Y	0	25000	neb	•	•	•	•	0.49
66	72	F	H	Severe	Y	0	30000	neb	•	•	•	•	0.25
67	73	F	H	Severe	N	1	20000	neb	•	•	•	•	0.43
68	41	M	H	Moderate	Y	0	800	MDI	1044	8428	9473	89	1.54

Patient number	Age	Sex	Patient type	Severity	Concurrent prophylactic therapy	Hospital admissions in previous year	Dose in preceding 24 hours (mg)	Route of administration	Urine salbutamol level (ng/ml)	Urine metabolite level (ng/ml)*	Urine total salbutamol level (ng/ml)**	% of recovered drug as metabolite	Creatinine (mg/ml)
69	76	M	H	Severe	Y	4	0	•	•	0	•	0	1.50
70	19	F	H	Severe	Y	3	50000	neb	•	•	•	•	1.11
71	68	M	H	Severe	Y	0	30000	neb	470	3664	4134	89	0.88
72	72	F	H	Moderate	Y	0	20000	neb	•	•	•	•	1.61
73	18	F	H	Severe	Y	0	50000	neb	11962	0	11962	0	1.48
74	76	M	H	Severe	Y	5	20000	neb	•	•	•	•	0.64
75	73	F	H	Severe	Y	0	15000	neb	2175	26104	28279	92	2.20
76	73	M	H	Severe	Y	1	20000	neb	•	•	•	•	1.67
77	56	F	H	Moderate	N	1	0	•	•	•	•	•	1.32
78	57	F	H	Moderate	Y	0	600	MDI	2749	40466	43215	94	0.45
79	63	M	H	Severe	Y	2	25000	neb	1913	21544	23457	92	0.45
80	64	M	H	Severe	Y	3	30000	neb	10126	21056	31182	68	1.73
81	38	M	H	Severe	Y	0	50000	neb	6536	2421	8957	27	0.80
82	79	F	H	Moderate	Y	0	20000	neb	733	•	•	•	1.09
83	64	M	H	Severe	Y	1	10000	neb	•	•	•	•	0.90
84	49	F	H	Severe	Y	1	10000	neb	2815	32174	34989	92	1.58
85	83	M	H	Severe	Y	1	20000	neb	3307	6747	10053	67	0.59
86	61	F	H	Moderate	Y	0	15000	neb	1190	12863	14053	92	0.37
87	73	M	H	Moderate	Y	0	0	•	726	22779	23505	97	0.53
88	62	M	H	Moderate	Y	0	800	MDI	4831	44932	49763	90	0.45
89	64	M	H	Moderate	Y	0	0	•	•	•	•	•	3.52
90	63	M	H	Severe	Y	2	20000	neb	•	•	•	•	1.38
91	45	M	H	Moderate	N	0	10800	MDI+neb	2700	42611	45311	94	0.68
92	36	F	H	Severe	Y	0	15000	neb	4700	5789	10489	55	0.60
93	76	M	H	Severe	Y	1	20000	neb	•	•	•	•	0.72
94	63	F	H	Severe	Y	0	15000	neb	4339	•	•	•	0.27
95	79	F	H	Severe	Y	1	5000	neb	5110	3460	8570	40	1.23
96	49	F	H	Severe	Y	1	20000	neb	34389	1810	36199	5	0.31
97	73	F	H	Severe	Y	1	5000	neb	2044	16558	18602	89	0.37
98	64	F	H	Severe	Y	1	20000	neb	864	17496	18360	95	0.25
99	74	F	H	Severe	N	0	20000	neb	4536	•	•	•	0.33
100	64	M	H	Severe	Y	0	20000	neb	5930	•	•	•	1.73
101	65	F	H	Severe	Y	3	25000	neb	•	•	•	•	0.62
102	59	M	H	Severe	Y	1	20000	neb	24682	15611	40293	39	0.37

ABBREVIATIONS

°C	degrees Celsius
cAMP	cyclic 3',5'-guanosine monophophate
COPD	chronic obstructive pulmonary disease
dB	decibel
df	degrees of freedom
DDD	defined daily dose
DPI	dry powder inhaler
DUSC	Drug Utilisation Subcommittee
EDTA	ethylenediamine tetra-acetic acid
i.d.	internal diameter
i.s.	internal standard
M	molar
MDI	metered dose inhaler
μg	microgram
μl	microlitre
mg	milligram
ml	millilitre
mM	millimolar
N	normal
NAC	National Asthma Campaign
ng	nanogram
NSAID	non-steroidal anti-inflammatory drug
p	probability
PBS	Pharmaceutical Benefits Scheme
PMS	post marketing surveillance
RPBS	Repatriation Pharmaceutical Benefits Scheme
S3R	recordable S3 drugs (poisons scheduling)
SN	signal-noise
SPE	solid-phase extraction
UV	ultraviolet

LIST OF PUBLICATIONS

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