Ion Chromatographic Determination of Haloacetates in Environmental Samples

By

Fang Wang

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DECLARATION

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

Fang Wang

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Fang Wang

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LIST OF ABBREVIATIONS

CE	Capillary electrophoresis
DFA	Difluoroacetate
DL	Detection limit
DCA	Dichloroacetate
DBA	Dibromoacetate
FIA	Flow injection analysis
GC	Gas chromatography
G.L.C.	Gss-liquid chromatography
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
MFA	Monofluoroacetate
MCA	Monochloroacetate
MDTFA	Fluoroacetates
MDTCA	Chloroacetates
MDTBA	Bromoacetates
Min	Minutes
MBA	Monobromoacetate
TFA	Trifluoroacetate
TCA	Trichloroacetate
TBA	Tribromo
RSD	Relative standard deviation

LIST OF PUBLICATIONS

Type of Publication	Number	Reference
Papers in referred journals	2	1-2
Posters at international meetings	2	3-4

1. Fang Wang, Greg W. Dicinoski, Yan Zhu and Paul R. Haddad
   Simultaneous determination of monofluoroacetate, difluoroacetate and trifluoroacetate in environmental samples by ion chromatography (Chapter 3). Journal of Chromatography A, 1032 (2004) 31-35.

2. Fang Wang, Greg W. Dicinoski, Yan Zhu and Paul R. Haddad
   Simultaneous determination of fluoroacetates, chloroacetates and bromoacetates in soil samples by ion chromatography (Chapter 5) Aust. J. Chem., in press.

3. Fang Wang, Greg W. Dicinoski, Yan Zhu and Paul R. Haddad
   Simultaneous determination of monofluoroacetate, difluoroacetate and trifluoroacetate in environmental samples by ion chromatography, International Ion Chromatography Baltimore, USA, 2002.

4. Fang Wang, Greg W. Dicinoski, Yan Zhu and Paul R. Haddad
Simultaneous determination of fluoroacetates, chloroacetates
and bromoacetates in soil samples by ion chromatography.
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Abstract

A method is reported for the sensitive, simultaneous determination of mono-, di-, and tri-fluoroacetates by ion chromatography. These species were separated using a Dionex AS17 anion-exchange column employed with a potassium hydroxide gradient (via a Dionex EG40 Eluent Generator) and suppressed conductivity detection. The fluoroacetates were successfully separated from a range of inorganic and organic species likely to be present in environmental samples, in a total analysis time of 35 min (including re-equilibration of the column). Detection limits for mono-, di- and trifluoroacetate were 21, 38 and 36 μg/L, respectively, determined using a signal to noise ratio of 3, and were obtained using a sample injection volume of 50 μL. Precision was less than 0.83% relative standard deviation for replicate injections performed over a period of 30 days. The method was applied to the determination of MFA, DFA and TFA in river water, carrot baits, urine samples and different kinds of plant material including *Bilobum, Calvcinum and Spinosum*, and found the concentration range of mono-(MFA), di-(DFA), tri-fluoroacetate (TFA) to be 11-788, 3.5-17 and 0.22-1.2 ppm in wet weight plant samples respectively.

Further research was carried out using the Dionex equipment specified above.

A comparative study was made of the chromatographic behaviour of nine haloacetic acids mono-(MFA), di-(DFA), tri-fluoroacetate (TFA); mono-(MCA), di-(DCA), tri-chloroacetate (TCA) and mono-(MBA), di-(DBA), tri-bromoacetate (TBA). The

techniques included anion-exchange chromatography with suppressed conductivity and UV detection. All nine haloacetic acids were completely separated under the optimised gradient elution conditions. Using selective detection methods or by pretreating samples with a OnGuard II Ag cartridge, eliminated some interference. The procedure for the simultaneous determination of the nine haloacetic acids was simple and fast. The method detection limits for MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA, and TBA were 21, 40, 40, 48, 28, 86, 67, 55, and 160 ppb respectively, determined using a signal to noise ratio of 3. Applications of these methods for the determination of haloacetic acids in real soil samples are shown.

Table of Contents

Chapter 1

Introduction and Literature Review

Haloacetic acids are a family of organic acids which have halogen atoms substituted on the β carbon of acetic acid, and are generally referred to as fluoroacetic acids, chloroacetic acids, or bromoacetic acids. The significance of analysing them is due to their extensive existence in many components of the aquatic ecosystem, which include precipitation samples [1], snow [2], reservoir water [3], drinking water [4], humic substances [5], fog water and rainwater [6], and in their impact on ecology.

1.1 General physical chemistry properties of haloacetates

The fluoroacetic acids include monofluoroacetic acid (MFA), difluoroacetic acid (DFA) and trifluoroacetic acid (TFA) and are collectively referred to as MDTFA. The outstanding property of the MDTFA is their stability and inertness. The fluorine atom has a van der Waals radius of 1.47Å, a size more comparable to that of oxygen (1.52 Å) than that of the other halogens, for example chlorine (1.8 Å), bromine (1.95 Å) and iodine (2.15Å) [7]. Also, fluorine is extremely electronegative having an electronegativity of 4.0 as compared to an electronegativity of 3.0 for chlorine and 2.8 for bromine [8]. This high electronegativity confers a strong polarity to the carbon-fluorine bond. The carbon-fluorine bond also has one of the largest bond energies in nature. The strength of the carbon-fluorine bond helps contribute to the stability of MDTFA. A dramatic illustration of the strength and the stability of the C-F bond is MFA, which can withstand boiling with 100% sulfuric acid

without any defluorination [9]. The stability makes it difficult for MDTFA to be biologically degraded and results in the persistence and accumulation of MDTFA in the biosphere.

Chloroacetic acids include monochloroacetic acid (MCA), dichloroacetic acid (DCA) and trichloroacetic acid (TCA), and are collectively referred to as MDTCA. Compared with MDTFA, chloroacetic acids are more active in their chemical properties although they have some other properties similar to the fluoroacetic acids. Both the carboxylic acid group and the α–chlorine are very reactive. They readily form esters and amides, and can undergo a variety of α–chlorine substitutions. Electron withdrawing effects of the α–chlorine give chloroacetic acid a higher dissociation constant than that of acetic acid [10].

Bromoacetic acids include monobromoacetic acid (MBA), dibromoacetic acid (DBA) and tribromoacetic acid (TBA), and are collectively referred to as MDTBA. Monobromoacetic acid must be protected from contact with air and moisture, since it is readily hydrolyzed to glycolic acid [10]. The simple derivatives, such as the acid chloride, amides, and esters are well known. Esters of bromoacetic acid are the reagents of choice in the Reformatsly reaction which is used to prepare β-hydroxy acids or α, β-unsaturated acids. Similar reactions with chloroacetate esters proceed slowly or not at all [10]. Bromoacetic acid undergoes many of the same reactions as chloroacetic acid under milder conditions, however is not often used due to its greater cost. Some physical chemical properties of MDTFA, MDTCA and MDTBA are summarised in Table 1.1.

Table 1.1. Physical properties of haloacetic acids [11][12]

Haloacetic acid	Molecular formula and molecular weight	Appearance	b.p. (^{0}C)	m.p. (^{0}C)	D^{20} (g/mL)	pK_a	Toxic Level (mg/kg)
MFA	CH_2FCOOH Mw=78.04	Colorless crystal	167-168	33	1.369	2.59	0.7-2.1 (Human)
DFA	CHF_2COOH Mw=96.03	Colorless liquid	132-134	-1	1.526	1.33	None
TFA	CF_3COOH Mw=114.03	Colorless liquid	71- 72	N*	1.535	0.50	0.2-0.6 (In rats)
MCA	$CH_2ClCOOH$ Mw=94.50	Colorless Solid	189.1	52.5	1.404 (25 ^{0}C)	2.87	76 In rats
DCA	$CHCl_2COOH$ Mw=128.94	Colorless Liquid	194	13.9	1.563	1.26	4500 (In rats)
TCA	CCl_3COOH Mw=163.39	Colorless Crystals	197.5	59	1.622 (64 ^{0}C)	0.52	5000 (In rats)
MBA	$CH_2BrCOOH$ Mw=138.96	Colorless Crystals	208	49	1.934 (30 ^{0}C)	2.90	None
DBA	CBr_2COOH Mw=217.8	Colorless Crystals	232	48	-	1.39	None
TBA	CBr_3COOH Mw=296.74	Colorless Crystals	245	135	-	- 0.147	None

* Not available

1.2 Toxicity

MFA is a highly toxic substance, with a lethal dose (LD_{50}) of 0.7-2.1mg/kg in humans [13]. As early as 1943, Marais found MFA to be the principal toxic component of the South African poisonous plant, *Dichapetalum cymosum*, known locally as *Gifblaar* [14]. The

fresh leaves of the plant are so poisonous that 20 g are sufficient to kill a sheep [15]. Its toxicity is due to 'lethal synthesis' of fluorocitrate, which inhibits the aconitase enzyme of the citric acid cycle [16].

The LD_{50} of TFA is reported to be between 200 and 400 mg/kg (oral exposure to rats), while sodium TFA is only slightly toxic when administered intraperitoneally to mice with no deaths at doses up to 5000mg/kg [17]. Bott and Standley reported that TFA did not significantly affect the metabolism of acetate by microbial communities at environmentally expected concentrations [18], but Visscher reported inhibitory effects on methanogenic activity at TFA concentration ≥ 1 μM [19]. TFA appears to be non-mutagenic in bacteria [20]. DFA is a corrosive, combustible liquid, as well as bring a lachrymator. However, no reports were available concerning its toxicity.

MCA is extremely corrosive and can cause serious chemical burns (LD_{50}=76mg/kg in rats [21]). It is also readily absorbed through the skin where toxic symptoms are often delayed for several hours. MCA is 30-40 times more toxic than DCA (4.5g/kg, Oral LD_{50}), or TCA (5.0g/kg, Oral LD_{50}) [22]. Reports of oral LD_{50} were not located for bromoacetic acid, while MCA, DCA, TCA, MBA, DBA and TBA were reported to be carcinogenic at low concentrations [23] and therefore federal regulation for their monitoring is being considered.

1.3 Sources in the biosphere

Fluorine is the most abundant halogen in the earth's crust and ranks 13th in abundance among all elements [24]. It is no surprise that natural productions of organofluorines exist widely in nature. MFA is the best known of these natural organofluorine compounds. MFA

is produced by plants of the genus *Dichapetalum* as well as *Palicourea marcgravii, Acacia georginae, Gastrolobium grandifluorum,* and *Oxylobium* species [25]. *S. cattleya* is also capable of producing monofluoroacetate [26]. These plants are distributed mainly over the Transvaal region of South Africa [14], Western and Northern Australia [27] and New Zealand [28]. Sodium monofluoroacetate (NaMFA), commonly known as Compound 1080, has been used as a vertebrate pesticide for more than 50 years. Its use has been widespread throughout North America, Australia and New Zealand with peak usage in the 1960s [29]. In Tasmania Australia, the state government administers the use of 1080 poison, which is used to kill brush-tail possums. The kill, left in the environment, becomes another source of MFA in the biosphere.

The role of chlorofluorocarbons (CFC) in the depletion of stratospheric ozone [30, 31] has led to an international agreement to discontinue their production [32]. Partially fluorinated ethanes with relatively short atmospheric residence times, namely CH_2FCF_3 (HFC-134a), $CHCl_2CF_3$(HCFC-123) and $CHClFCF_3$ (HCFC-124), are now being introduced as coolants for refrigeration and air conditioning as an alternative. In the troposphere, they are oxidized by hydroxyl radicals, yielding HF, CO_2, HCl and TFA [33,34]. For HFC-134a, an annual production of 20,000 metric tons was expected for the year 2010 [35], 30-40% of which will be atmospherically oxidized to TFA [33]. The pKa and high water solubility of TFA indicated that wet deposition (i.e. rain, snow, and fog) will be a primary atmospheric removal process [36]. Upon partitioning into water, TFA dissociates to form the trifluoroacetate anion, which remains in the aqueous phase due to its enhanced water solubility. The affinity of TFA for the aqueous phase, coupled with its high stability and virtual resistance to chemical and biological degradation, creates the potential for accumulation in surface water systems, such as vernal pools, playa type lakes, saline lakes,

and other systems that are characterized by high evaporation and little or no out-flow [37]. Based on the assumption that there is at present no environmental TFA, levels of 5-20 pg m^{-3} in air and of 100-160 ng L^{-1} in precipitation have been forecast for 2010 [33]. However, air and water samples collected in Germany, Switzerland and Israel in 1995 already contain levels of TFA in the range predicted for 2010. Since TFA turned out to occur at such high levels in the global environment other sources must also be taken into consideration [38].

The degradation of trifluoromethyl – substituted aromatics, such as the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), is another source of TFA. From 1958 the chemical TFM has been used to control the sea lamprey (Petomyzon marinus) in four of the Great Lakes (Superior, Michigan, Huron, and Ontario). By 1988, more than 1 million kg of TFM had been applied to these lakes, and usage since has been approximately 50000 kg/year [39]. TFM has also been introduced in order to control tadpole infestations in warm water ornamental fish ponds [40]. TFM was confirmed to undergo photohydrotic degration, at 365 nm and under actinic radiation, to produce TFA [41].

Several volatile compounds containing a trifluoromethyl group have been used or are currently used as anaesthetics. Examples include fluroxene, halothane, sevoflurane, desflurane, and isoflurane. TFA is a metabolite of nearly all of the trifluoromethyl-substituted anesthetics. Fluroxene ($CF_3CH_2OCHCH_2$) is metabolized to trifluoroethanol, which is further oxidized to TFA [42]. Oxidative metabolism of halothane yields TFA [43]. Desflurane ($CF_3CHFOCHF_2$) is also oxidized to TFA with release of fluoride [44].

Due to the strength of the C-F bond, the stability of MDTFA is unique. Few reports of biodegradation of MDTFA are available. Visscher et al [45] reported instances of reductive defluorination of TFA under methanogenic and sulfate-reducing conditions. TFA was sequentially defluorinated to DFA, MFA, and acetic acid, which was cleaved to yield methane. Under aerobic conditions, the production of fluoroform has also been reported. This report also illustrates the possibility of co-existence of MFA, DFA and TFA in the environment.

Chloroacetate can be formed via atmospheric breakdown of the airborne C_2-chlorocarbons, trichloroethene, tetrachloroethene, and 1,2,2-trichloroethane, that are used as solvents for degreasing and dry-cleaning [46]. However, industrial production of 1,1,1-trichloroethane has been discontinued because of its ozone-depletion potential. The annual production volumes of tri- and tetrachloroethene have progressively decreased over the past decade. Other possible sources of chloroacetic acid are waste incineration [47] or water chlorination. For TCA, biological processes have also been suggested to contribute [48] however the strengths of these sources are difficult to assess. Major industrial uses for chloroacetic acid are in the manufacture of cellulose ethers (mainly carboxymethylcellulose, CMC), herbicides, and thioglycolic acid. Other industrial uses include the manufacture of glycine, amphoteric surfactants, and cyanoacetic acid.

Bromoacetates are suggested to be formed by the atmospheric degradation of brominated hydrocarbons released to the atmosphere by marine organisms [49].

1.4 Determination Methods

Haloacetate compounds are being increasingly used, and as those substances confer both chemical and biological inactivity, they have become ubiquitous environmental contaminants. Appropriate analysis methods for haloacetates are necessary for environmental protection, the study of haloacetic producing organisms, and the evaluation of the toxicological effects of these compounds. Because of the complexity of the matrix of real samples, the determination of haloacetates generally involves separation procedures. The chromatographic methods, gas chromatography (GC), high performance liquid chromatography (HPLC), ion chromatography (IC) and so on, are frequently used. The following will review the literature up to April 2003.

1.4.1 GC

Prior to IC and HPLC, GC was the dominant separation technique. Haloacetates in various environmental or biological samples were determined using GC. Examples were MFA in animal tissues [50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61], plants [62, 63, 64], water [65, 66, 67] and soils [68, 69], and TFA in water and air [70, 71, 72, 73], urine and serum from patients anaethised with halothane [74, 75, 76], Ceronapril bulk [77], rat milk [78] and TCA in blood [79]. Simultaneous determination was successfully performed on the three compounds (TFA, MCA, DCA) [80], the nine compounds (MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA, TBA) [81], the five compounds (MCA, DCA, TCA, MBA, DBA) [82] and the five compounds (MCA, TCA, MBA, DBA, TBA) [83] in water, and MCA, DCA, MBA, TCA, TFA in sterols [84]. Since fluorine, chlorine and bromine are extremely electronegative, the widely used detector was electron-capture (ECD) [54, 57, 59, 62, 66, 68, 69, 70, 73, 75, 79, 84]. Due to the matrix complexity, GC-MS in the selected ion

monitoring mode was often used to confirm the analytes existence [50, 52, 54, 55, 56, 58, 63, 65, 67, 69, 73,76]. The high polarity of haloacetic acids is not suitable for direct GC analysis, and appropriate derivatizations prior to instrumental analysis are required. Conversion of MFA or TFA to alkyl esters such as methyl [51, 52, 60, 73, 74], ethyl [51, 75] and mainly to pentafluorobenzyl esters [53, 54, 58, 59, 63, 68, 71, 72] was performed. However, haloacetic acids are small volatile molecules with high water solubility and polarity. This makes them difficult to separate from water, which interferes with the later esterification derivatisation reaction. This is often the cause of low recoveries, and time-consuming extraction or drying steps are needed. In order to overcome these problems, Ozawa developed a derivatisation method for the determination of MFA in water samples by GC, where MFA in an aqueous solution acidified with hydrochloric acid was converted to the dichloroanilide derivative by reaction with 2, 4 - dichloroaniline and N, N'- dicyclohexylcarbodiimide [66, 69]. Although the drying steps used by other esterification procedures have been omitted, the derivatisation procedures, although tedious, were retained [62].

Headspace GC (HSGC) is useful for the determination of volatile substances, however by the addition of esterifying reagents to the HS reaction mixture, even non-volatile organic acids can be analysed. The method is based on the derivatisation of organic acids with dimethyl sulfate in concentrated sulfuric acid contained in sealed headspace vials, where the gas phase methyl ester is formed and subjected to GC analyses. MFA in water and air samples [70, 73] and a bulk substance (ceronapri) [78] were determined by this method. Kimball [67] developed a procedure using 1 M HCl as the sample solvent, which minimizes adsorption problems commonly encountered with the chromatographic determination of free acids in aqueous solutions. Commercially available polyethylene glycol capillary

columns are compatible with injections of highly acidic aqueous solutions. The use of the columns does not require a derivatisation procedure and offers advantages over previously described chromatographic methods for the determination of MFA.

Sarrion developed a method involving acidic methanol esterification (AME) procedure for the determination of haloacetic acids (MCA, DCA, TCA, MBA, DBA) in drinking water, which was adopted as the EPA Method 552.2 with some modifications [82, 85]. Briefly, the method employs 11 µL/L of methyl - tert - butyl - ether (MTBE) solution of 2, 3-dibromopropionic acid 22 µg/mL, as surrogate standard, 3 mL of concentrated sulfuric acid (to obtain pH<0.5), 12 g of anhydrous sodium sulfate, 3 g of copper (II) sulfate pentahydrate and 2 mL of MTBE added to 30 mL of water and placed in a 40 mL vial. The vials were sealed with PTFE-faced septa, shaken for 15 min in a mechanical shaker, placed upright and allowed to stand for 5 min. In order to derivatize the haloacetic acids, 1 mL of the MTBE extract and 2 mL of methanol-sulfuric acid (9:1, v/v) were transferred to a 10mL vial, which was placed in a thermo slatted water bath at 50 ^{0}C for 1 h. After cooling to 4 ^{0}C, 5 mL of a $CuSO_4$-Na_2SO_4 solution was added and the mixture was shaken by hand for 2 min. A 300 µL aliquot of the MTBE extract was transferred to a 2 mL vial along with 3 µL of a MTBE solution of 10 mg/L 1,2-dibromopropane of (as internal standard). Finally, 1µL of the MTBE extract was injected into the gas chromatograph. Water samples were analysed using a calibration curve obtained by spiking 30 mL of Milli-Q water with haloacetic acids at seven concentration levels between 0.25 and 20 µg/L. Although many efforts were made to simplify the derivatisation procedures, in general, these are complex and time-consuming. The references relating to GC determination of haloacetic acids are summarised in Table 2.

Table 1.2. Determination of haloacetic acids by GC

Analyte	Sample	Separation condition	Detection	Detection limit	Ref
MFA	Rat tissues	Two-dimensional GC.	MS	10 ng / g (S/N 40)	[50]
MFA	Animal and human tissues	Column: Porapak Q glass column (5ft × 4 mm i.d.). Oven temperature: 200 ^{o}C. Carrier gas: He at 30 ml/min.	MS	Animal: ~ 0.7μg/g Human: 0.1μg/g	[51]
MFA	Biological samples	Column: A Resoflex glass tube (6 ft × 3 mm i.d.). Oven temperature: 75 ^{o}C. Carrier gas: N_2 at 50 ml/min.	MS	0.1 mg	[52]
MFA	Plant tissue and organs of animals	Column: 10% polyethylene glycol 6000 packed column or 10% Reoplex 400 on Chromosorb W solid phase column (2 m ×4 mm i.d.). Oven temperature: 65- 205^{o}C at 3 ^{o}C /min Carrier gas: He	FID	Not reported	[53]
MFA	Baits and avian tissues	Column:7% OV-210 + 3.5%OV-101 on Chromosorb Q, glass column (2 m × 3 mm i.d.). Oven Temperature: 130^{o}C. Carrier gas: N_2 at 20 ml/min.	ECD, MS	Not reported	[54]
MFA	Biological samples	Column: SE 54 fused silica capillary column (20 m × 0.3 mm i.d.). Oven temperature: 140 ^{o}C to 230 ^{o}C at 6^{0}C/min. Carrier gas: H_2 58 kPa.	FID, MS	Not reported	[55]
MFA	Tissue of dogs	Not reported	MS	10 ng/g	[56]

Table 1.2 (Cont.)

MFA	Tissues of coyotes and magpies	Column: QF-1, QF-1/DC-200 on Chromosorb W and OV-210/OV-17 on Ultra Bond 20 M. Oven temperature: 165 ^{o}C. Carrier gas: methane-argon	ECD	None	[57]
MFA	Animal tissues	Column: OV-101 capillary column. Oven temperature: 100 ^{o}C for 0.1 min, to 210 ^{o}C at 10 ^{o}C / min, for 1 min. Carrier gas: He at 45 cm/s.	MS	10 µg/L	[58]
MFA	Animal tissue	Column: 5 % QF-1 on Chromosorb W DMCS glass column (1.8 m × 2 mm i.d.). Oven temperature: 90 ^{o}C. Carrier gas: 10 % methane in argon at 35 ml/min.	ECD	0.03~ 0.05 µg/mL	[59]
MFA	Biol. tissue, food, and bait samples.	Column: Resoflex packed glass tube (6' × 3 mm o.d.). Oven temperature: 75 ^{o}C. Carrier gas: N_2 at 50 ml /min.	ECD	0.1 µg/g	[60]
MFA	Stomach tissue or stomach content	Column: Soft aluminum with Johns-Manvill Chromasorb 102 (100/120 mesh) (3' × 1/8"). Oven temperature: 180 ^{o}C. Carrier: N_2 at 30-35 ml/min.	FID	0.2 µg/mL	[61]
MFA	Toxic plant and its environmental water and soil	Not reported	ECD	0.1 µg /L	[62]

Table 1.2 (Cont.)

MFA	Plant samples	Column: OV-225 glass capillary column (40 m × 0.3 mm i.d.); Durawax 3 and 4 silica capillary column (30m × 0.32 mm i.d.); SE-54 silica capillary (30m × 0.25 mm i.d.).Oven temperature: 50 ^{o}C for 1 min, 39 ^{o}C / min to 100 ^{o}C, 8 ^{o}C to 220 ^{o}C Carrier gas: He	MS	~ 0.005 µg/g	[63]
MFA	Leaves of D. cymo-sum	Column: NEGS on Supelcoport (1.5 m × 4 mm i.d). Oven temperature: 85 ^{o}C Carrier gas: N_2 at 25 ml/min.	FID	No report	[64]
MFA	Water	Column: CBP 10 5 % phenyl-methylpolysiloxane fused-silica capillary column (25 m×0.33 mm i.d.) Oven temperature; 50 ^{o}C. Carrier gas; He at 3 ml/ min.	FID, MS	0.5 µg (S/N 14)	[65]
MFA	Water	Column: DEGS-H_3PO_4(5+1%) on Chromosorb W60-80 mesh and Apiezone grease L-H_3PO_4 (%+2%) on Chromosorb W60-80 mesh glass packed column. Oven temperature: 175^{0}C. Carrier gas: N_2 at 20 ml/min.	ECD	0.6 µg/L	[66]
MFA	Aqueous solvent	Column: 0.25 µm bonded phases of acidified polyethylene glycol capillary column (15 m×0.25 mm i.d.). Oven temperature: programming temperature. Carrier gas: He at 47 cm /s.	MS	200 pg	[67]

Table 1.2 (Cont.)

MFA	Soils	Column: DB-5 capillary column (30 m × 0.53 mm i.d.). Oven temperature: 60°C to 120^{0}C at 4°C / min. 290 ^{o}C for 10 min. Carrier gas; He at 5 ml / min.	ECD	0.2 µg/g	[68]
MFA	Soil Biological samples	Column: 5% DEGS-1% H_3PO_4 on Chromosorb W60-80 mesh and 5% Apiezone grease L-2%H_3PO_4 on Chromosorb W60-80 mesh glass packed column (2.1m × 3 mm i.d.). Oven temperature: 175°C. Carrier gas: N_2 at 20 ml/min.	ECD, MS	Soil: 0.0015 µg/g Bio-sample: 0.003 µg/g	[69]
TFA	Water Air	Column: Pora PLOT Q (Chrompack) fused capillary column (27.5 m × 0.32 mm i.d.) with 2.5 m guard column. Oven temperature: 110 ^{o}C for 9 min, by 20 ^{o}C /min to 200 ^{o}C for 2 min. Carrier gas: He, at 3.0 ~3.3 ml/min.	ECD, MS	Water: 0.025 ~ 10 ng/ml Air: 1 ng/ml	[70]
TFA	Air	Column: CP-Sil 5CB fused capillary column (25 m × 0.25 mm i.d.). Oven temperature: 40 °C for 1 min, by 10 ^{o}C to 800 ^{o}C , 8°C /min to 160 ^{o}C and 20°C /min to 250 °C. Carrier gas: He at 45 cm /s.	MS	80 fg	[71]
TFA	Rainwater	Column: methyl-phenyl-(5%)-silicone fused capillary (25m × 0.25mm i.d.). Oven temperature: 65 °C for 1min, 25°C /min to 159 °C, 1 ^{0}C/min to 161°C, 25°C /min to 240 °C, isothermal for 2 min. Carrier gas; He at 80 kPa.	MS	Not reported	[72]

Table 1.2 (Cont.)

TFA	Rain water	Column: Poraplot Q fused capillary (25 m × 0.32 mm i.d.). Oven temperature: 110°C for 10 min, 20°C /min to 210°C, isothermal for 2 min. Carrier gas: N_2 at 3.2 ml/min	ECD, MS	Not reported	[73]
TFA	Metabolism of halothane	Column: Porapak Q glass column (1 m × 2 mm i.d.). Oven temperature: 115°C for 1 min, 4°C/min. Carrier gas: He at 20 ml /min.	MS	1 μM	[74]
TFA	Urine and serum from patients narcotized with halothane	Column: 4% OV-17 on Chromosorb W HP,100-12 mesh, glass packed column (2m × 2 mm i.d.). Oven temperature: 80°C. Carrier gas: N_2 at 20 ml /min.	ECD	1 μg/mL	[75]
TFA	Microsomal suspension	Column: Porapak Q glass column (2 m × 3 mm i.d.) Oven temperature: 110°C. Carrier gas: N_2.	MS	Not reported	[76]
TFA	Ceronapril bulk	Column: A PoraPLOT Q fused capillary column (10 m × 0.32 mm i.d.). Oven: 140°C for 5 min, by 70°C /min to 200°C, for 8 min. Carrier gas: He at 97 kPa.	FID	40 μg/mL	[77]
TFA	Rat milk	Column: DB-FFAP fused silica column (30 m × 0.32 mm i.d.). Oven temperature: 40 to 90°C at 3°C /min, to 250°C at 30°C, for 10 min. Carrier gas: He.	ECD	0.5 μg/mL	[78]

Table 1.2 (Cont.)

TCA	Blood, urine	Column: Glass 1.8m×3mm I.D. OV-17 Oven temperature: 125^{o}C. Carrier gas: N_2 60mL/min.	ECD	0.1 µg /mL	[79]
TFA MCA DCA	River, tree	Column: silanized fused-silica capillary 80cm×0.10mm Oven temperature: 40^{o}C-160^{o}C at 8^{o} C/min, 160^{o} C-250^{o} C at 20 ^{o}C /min Carrier gas: He 45cm/s	MS	0.01-80fg	[80]
MFA DFA MFA MCA DCA TCA MBA DBA TBA	Aqueous samples	Column: DB-17 Capillary. Oven temperature: 50 ^{o}C to 250^{o}C, 6^{o}C/min Carrier gas: He	MS	0.02-8.0 µg/L	[81]
MCA DCA TCA MBA DBA	Tap water	Column: Fused-silica capillary 30m×0.25mm i.d. Oven temperature: 40-60^{o}C 20^{o}C/min to 120^{o}C for 3min, 280^{o}C for 10min Carrier gas: He 33cm/s.	MS	10-200 ng /L	[82]
MCA TCA MBA DBA TBA	Chlorinated drinking water	Column: DB-5 capillary 20m×0.18mm i.d. Oven temperature: 40-180^{o}C at 3^{o}C/min. Carrier gas: He	MS	Not reported	[83]

Table 1.2 (Cont.)

MCA DCA MBA TCA TFA	Sterols	Column: 20%by wt. B-cyanoethymerl methl silicone polymer 80-90mesh Anakrom ABS maintained at 220^{o}C Oven temperature: 200^{o}C. Carrier gas: N_2 at 60mL/min.	ECD	1×10^{-15} mol /min	[84]
MBA DBA TBA	Standard	Column: OV-351, 25m × 032mm; SE-30 WCOT, 25m×0.30mm i.d. Oven temperature: 50 ^{o}C. Carrier gas: -	FID	Not reported	[86]
DCA	Human plasma	Column: HP-Was 30m × 0.25 mm i.d. Oven temperature: 40^{o}C for 4 min raised to 100^{o}C, 5^{o}C/min, raised to 240 ^{o}C Carrier gas: He at 1.21ml/min.	MS	0.3 -1.5 μM	[87]
MCA DCA TCA MBA DBA	Snow	Column: HP-5, 30m × 0.25mm. Oven temperature: 30^{o}C. (5min) 5^{o}C/min up to 105^{o}C Carrier gas: He, 39cm/s.	MS	12-6 ng /L	[88]
MCA DCA TCA	Human Plasma	Column: 30m × 0.25mmI.D. HP-Wax . Oven temperature: 40^{o}C, 2 min to 100 ^{o}C at 50 ^{o}C /min, then raised to 240 ^{o}C at 50 ^{o}C / min. Carrier gas: He.	MS	0.12-7.83 μM	[89]

Table 1.2 (Cont.)

TCA TCE	Urine	Column: SPB-5, 30m x 0.53mm i.d. Oven temperature: - Carrier gas: -	ICA	0.1 mg/L	[90]
	Herbicides	Column: 15% Apiezon L on Chromaton N-AW-DMCS. Oven temperature: 275 ^{o}C Carrier gas: He 1.21mL/min	ECD	0.1 µg	[91]
TCA	Urine	Column: 10% Carbowax 20M on Chromosorb Waw. 2.5m × 3mm i.d. Oven temperature: 130 ^{o}C Carrier gas: N_2 at 500mL/min .	FID	2.0 mg/L	[92]
TCA	Human serum	Column: 3%OV17, 1.8m×4mm i.d. Oven temperature: 80 ^{o}C Carrier gas: N_2 35 mL/min	ECD	10 mg/L	[93]
TCA	Water, soil, sugar	Column: SE-30 glass column 1.9 m×3.5mm Oven temperature: 90 ^{o}C. Carrier gas: -	FID	0.013, 0.2 mg/kg	[94]
TCA, MCA	Serum, urine	Column: Chromosorb WAW coated with reoplex400 Oven temperature: - Carrier gas: -	FID	13 mmol/kg	[95]
TCA	Blood, urine	Column: OV-17 glass 1.8m×3mm i.d. Oven temperature: 125^{o}C. Carrier gas: N_2 at 60mL/min.	ECD	0.1 µg/mL	[96]

1.4.2 HPLC

Following or paralleling GC analysis, HPLC methods were developed for the determination of fluoroacetates. MFA (compound 1080) is an intensely poisonous rodenticide and predacide, and has been widely used for controlling various vertebrate pests. As a result of the field use of such a highly toxic compound, it was necessary to develop a fast, sensitive and accurate method to analyse baits and tissues in an attempt to monitor the exposure of operators, agricultural stock and protected wildlife species. However, the determination of low level MFA in biological samples was somewhat hindered by its high solubility in water and correspondingly low solubility in organic solvents, its instability when heated in alkaline solution, and the lack of any specific group in the molecule that would facilitate identification. Most of the GC methods described above involved cumbersome extraction and derivatisation procedures, low recoveries and interferences from the matrix. GC-MS methods are more sensitive and selective, but the instrumentation is expensive. The development of HPLC, more or less overcame these shortcomings.

Ray, *et al.* [97] first reported a HPLC method for the determination of MFA in canine gastric content. The procedure involved extraction of MFA with water, methyl ethyl ketone, and diluted base, followed by sample cleanup using octadecylsilane bonded phase cartridges and derivatisation in ethyl acetate solution with O-p-nitrobenzyl-N,N'-diisopropylisourea. The derivative was chromatographed on a 10 μm silica column with UV detection at 254 and 280 nm. Although the method was specific for MFA with the acceptable sensitivity (0.075ppm) and recovery (75-90%), it suffered from lengthy sample preparation. It was necessary to extract MFA from aqueous acid-buffered solution into an organic solvent since the derivatisation reaction was not possible in

aqueous solution. The derivatisation was difficult to drive to completion due to the presence of fluorine hindering the esterification, along with an incubation time of 8 to 10 h. In order to shorten the analysis time and enhance the detection sensitivity, Collins, *et al*. [98 , 99] described a simple and efficient method employing fluorescent derivatisation and separation by HPLC for recovering MFA from poison bait. MFA was derivatised with the fluorescent agent, 4-bromomethyl-7-methoxycoumarin (Br-Mmc) by catalysis with 18-crown-6. The derivative was simple to prepare and the derivatisation time could be reduced to about 30 minutes. The detection sensitivity was lowered to 2 ng/mL (0.002 ppm). However, since the derivatisation only occurred in non-aqueous solution, the extraction and drying procedures prior to derivatisation were still needed. The use of anhydrous conditions and time-consuming solvent extraction resulted in a method incapable of handling numerous samples at one time.

These facts were the most important considerations in the development of a new method for the determination of 1080 in baits. Kramer [100] reported the HPLC analysis of the compound 1080 in meat baits and formulations, whose simplicity was in the fact that the esterification was performed in the presence of water. Filtered aqueous extracts of the bait samples were extracted with methyl ethyl ketone, and then with KOH, with the alkaline phase being diluted with acetonitrile. Aqueous formulations were diluted with acetonitrile. The esterification was performed with the esterifying agent, 4-bromophenacyl bromide (4-BPB) and 18-crown-6 dissolved in acetonitrile. The resulting solution was analysed by HPLC. The crown ether-catalyzed esterification proceeded in the presence of up to 10% water with negligible hydrolysis of the 4-BPB. The derivatisation was rapidly driven to completion by the crown ether which strongly solvated the potassium ion and enhanced the reactivity of the fluoroacetic ion. The

derivative under these conditions was stable for at least 2 days at room temperature. The relatively high detection limit (1.5 ppm) in bait for MFA was a problem.

Dichapetalum Cymosum was the first plant in which an organic fluorine compound was found when Marais [14] discovered that its toxic component was MFA. MFA has been discovered in several other plant species since [101]. Vickery *et al*. [102] considered the possibility that DFA and TFA might also be present in these plants, and attempted to separate them with paper chromatography. They only obtained one spot of MFA with a low detection sensitivity of 1 mg/mL of MFA, DFA or TFA in 20 μL solution, despite expecting a much more sensitive detection. The natural MFA containing in plants has been confirmed. The other earlier methods for determining fluorine either colorimetrically [103] or with a fluoride-selective electrode [104] after the sample extract was ashed, were usually non-specific although sufficiently sensitive for most purposes. GC methods as described above needed a tedious derivatisation to enhance the analyte volatility, while high sensitivity could be obtained [62, 64], especially when coupled with MS detection [64]. Meyer, *et al.* [105] developed a fast HPLC method for the determination of MFA in *Dichapetalum Cymosum*. The plant samples were extracted with 0.1 mM NaOH in a water bath at 80^0C for two hours. The suspension was acidified with 9 M H_2SO_4 and the MFA extracted with diethyl ether. The ether extract was evaporated to dryness, and the residue dissolved in and directly analyzed by HPLC on an Aminex Ion Exclusion, HPX-87H, column. Although the analysis was fast due to no derivatization, the detection sensitivity was far lower than for GC-MS.

In order to decrease the laboratory error to a minimum, sample clean up procedures should be as simple as possible. Due to volatility of the MDTFA, the analyte may be lost

during the evaporation process at high temperatures (>90 ^{0}C) or be degraded to glycolic acid in solution at high pH. Therefore, a procedure that requires extraction with organic solvents and then evaporation of these solvents to concentrate the analytes should be avoided. Unfortunately, most of the reported sample preparation procedures involve time-consuming and error-producing organic solvent extraction, clean up, evaporation and derivatisation, due to a lack of alternative methods. Recently, Minnaar, *et al.* [106] described a simple isocratic HPLC method for the quantitative analysis of MFA in plant and liver samples, which overcame these problems. Sample preparation involved homogenising the sample at room temperature, centrifuging and filtering the sample through a syringe filter. A C-610 organic acid analysis column at ambient temperature with 0.001M H_3PO_4 as an eluent and UV detection at 210 nm offered a optimum separation, sensitivity and accuracy. The average recovery was 94.8%, with a detection limit of 12 μg/L, which was relatively low as far as HPLC methods go, although not compatible with GC-MS detection.

TFA, as one of the metabolites of the important volatile anaesthetic, Halothane (2-bromo-2-chloro-1,1,1- trifluoroethane), has been determined by GC as described above. However, Imbenotte, *et al*. [107] attempted several GC methods, including direct measurement by HSGC and GC analyses after various derivatisation process, but without success. So, they developed a HPLC method to perform this measurement. TFA in urine and plasma was extracted by addition of 18-crown-6 ester, and after acidification of the sample, the 4-bromomethyl-7-methoxycoumarin derivative of TFA was prepared, and then subjected to HPLC on a RP18 column with a 60: 40 mixture of methanol and water as mobile phase. A detection limit of 0.1 μg /mL was obtained with UV detection at 320 nm.

Ogata *et al*. developed a method for the determination of TCA, DCA and TCA in urine. The HPLC method involved the use of a stainless steel column, 500mm×8mm, packed with Hitachi gel 2618 (H form), with detection at 220nm [108]. The detection limit was found to be 5.0 μg /mL. Husain *et al*. [109] reported a method for determination DCA and TCA in standard mixtures, with UV detector was set at 0.001 a.u.f.s. The limit of detection for TCA was found to be 10 ug/L. Recently, a HPLC method coupled with negative ionisation electrospray mass spectrometry has been proposed by Hashimoto and Otsuki [108]. This method, when applied to the nine haloacetic acids containing bromine and chlorine, requires extraction of the analytes with methyl *tert*-butyl ether, followed by concentration under a controlled stream of dry nitrogen. Although the good limits of detection could be reached (0.003-0.070 μg /L), few details on chromatographic separation of mixture of analytes were provided. Moreover, co-elution among the fluoroacetates and chloroacetates were not resolved.

Analysis of bromoacetic acids by HPLC have not been reported. The references regarding the HPLC determination of MDTFA and MDTCA are summarised in Table 1.3.

Table 1.3. Determination of MDTFA by HPLC

Analyte	Sample	Samples Preparation	Separation condition	Detection	DL	Ref
MFA	Canine gastric content	Extracted with water, Me Et ketone, and dil. base, followed by sample cleanup using octadecylsilane bonded phase cartridges and derived in Et acetate soln. with O-p-nitrobenzyl-N,N'-diisopropylisourea.	Column: 4 mm × 30 cm μPorasil (10 μm silica). Mobile phase: 5% methyl acetate in 2,2,4-trimethylpentane	UV (254, 280 nm)	0.075 μg/mL	[97]
MFA	Poison bait	Sample was homogenised; the suspension was acidified and distilled under vacuum; the distillate was evaporated at neutral pH and the distillate was derivatised with 4-bromomethyl-7-methoxycoumarin.	Column: 25 cm RP-8 reverse phase column. Mobile phase: acetonitrile : ethyl acetate:water (9:2:22)	Fluorescence	2 ng/mL	[98]
MFA	Poison bait	Homogenised in water, shaken and left overnight. The suspension was acidified with H_3PO_4 and distil under vacuum. The distillate was derivatised with 4-bromomethyl-7-methoxycoumarin.	Column: 25 cm RP-8 reverse phase column. Mobile phase: acetonitrile :ethyl acetate:water (9:2:22)	Fluorescence	0.002 ng/mL	[99]

Table 1.3 (Cont.)

MFA	Meat baits and formulations	Extracted with ethyl methyl ketone, the organic phase was shaken with 0.5M-KOH, and the alkaline phase was diluted with acetonitrile. Aqueous formulations were diluted with acetonitrile	Column: 25 cm x 4.6 mm reverse phase RP-18 (10 um); 25 cm x 4 mm silica LiChrosorb (10 um). Mobile phase: aq. 37% tetrahydrofuran.	UV (260nm)	2 μg/mL	[100]
MFA	Dichapetalum cymosum	Sample was blended, the suspension was filtered; the filtrate was concentrated and acidified; organic acids were extracted.	Column: Aminex Ion Exclusion HPX-87H Mobile phase: 3.5 mM H_2SO_4	UV (206 nm)	0.1 μg/g	[105]
MFA	Plant material, bovine rumen and liver	Sample was homogenized; the suspension was centrifuged and filtered.	Column: 300mm × 7.8 mm C-610 org. acid anal. Mobile phase: 0.02M H_3PO_4.	UV (210 nm)	12 μg/L	[106]
TFA	Plasma and urine from anesthetized patients	Extracted with 18-crown-6 ether. Derived with 4-bromomethyl-7-methoxycoumarin after acidification of the sample	Column: 300 mm x 6 mm RP 18 (10 um) Mobile phase: methanol :water (60:40)	UV (320 nm)	0.1μg/L	[107]

Table 1.3 (Cont.)

MCA DCA TCA	Urine	Direct analysis	Column: stainless steel 500mmx 8mm internal diameter packed with Hitachi gel 2618 (H-form). Mobile phase: 1% phosphoric acid	UV (220nm) UV	5.0 μg/mL	[108]
DCA TCA	Standard mixture	Direct analysis	Not reported		10 μg/L	[107]
MFA DFA TFA MCA DCA TCA MBA DBA TBA	Waste, river And seawater	Not reported	Column: crosslinked polystrene resin Mobile phase: 3%acetic acid, acetonitrile,water.	ESI-MS	0.003-0.070 μg/L	[110]

1.4.3 Ion chromatography

Since haloacetic acid determinations are quite a new environmental concern with respect to applications to environmental samples, few references are available dealing with the development of ion chromatographic analytical methods for their analysis. Ion chromatography (IC) is one of the fastest growing analytical techniques for the determination of ionic species. It offers a simple, reliable, and relatively fast means for the simultaneous separation and determination of ionic species.

In its earliest embodiments, IC was focused primarily on the analysis of inorganic ions. Today IC has a much wider scope. It now has an important role in the analysis of organic, as well as inorganic, ions. Fluoroacetates have been analysed by IC in various matrices, such as soils [111], peptides [112, 113, 114, 115], natural water [116, 117], rabbit bile [118], and human plasma and urine [119]. Simultaneous determination was successfully performed on TCA and other halogen ions in human samples [120], two haloacetic acids (DCA, TCA) [121], and (DCA, Acetate) [122], five haloacetic acids (MBA, DCA, DBA, TCA, MCA) [123] and six haloacetic acids (MCA, DCA, TCA, MBA, DBA, TBA) mixtures [124, 125] in water samples, and DCA in blood samples [126]. Although a variety of other detection methods are currently used, suppressed conductivity detection is still the most useful tool for haloacetic acid determination. In the early studies, two chromatographic methods have been described by Nair et al. [127] for MCA, DCA, TCA, MBA and DBA based on anion-exchange and ion-exclusion methodologies. The first, coupled with a suppressed conductometric detection, gave detection limits ranging from 8-80ug/L and was applied to the determination of DCA, DBA and TCA in a drinking water sample. Ion-exclusion separation, coupled with UV detection at 210 nm, provided detection limits ranging between 5 and 90 μg/L when applied to the determination of DCA and TCA

in herbicide samples. Sarzanini *et al.* [123] have studied the optimisation of chromatographic procedures (based on ion-interaction and anion-exchange mechanisms) for the separation of MBA, DBA, TBA, DCA and TCA acids in the analysis of drinking waters. Ion-interaction chromatographic (IIC) procedures, coupled with UV detection, have been optimised by studying the effect of mobile phase composition in the presence of different ion pairing reagents (etrabutylammonium and cetyl-trimethylammonium). The anion exchange separations have been carried out on various stationary phases (IonPac AS9, AS10 and AS11) and coupled with different detection systems (spectrophotometric and conductimetric). According to the detection system used, different mobile phase compositions were used and their effect on the separation of haloacetic acids has been studied.

A method was reported by Roehl, *et al.* [124], relating to the application of IC-ESI-MS to drinking water samples, for the confirmation and quantitation of environmentally significant contaminants, i.e. compounds with adverse health effects which are either regulated or being considered for regulation, such as bromate, perchlorate, haloacetic acids (MCA, MBA, DCA, DBA, TCA, TBA). Preliminary work for this report using on–line sample preconcentration prior to an anion-exchange separation with gradient elution and ESI-MS detection has shown that some of the haloacetic acids can be separated and detected selectively at μg/L levels. References relating to IC determination of fluoracetates, chloroacetates and bromoacetes are summarised in Table 1.4.

Table 1.4. Determination of MDTFA by IC

Analyte	Sample	Column	Suppressor	Eluent	Detection	Detect limit	Ref
MFA	Soil	Dionex ion-exchange OmniPac PAX-100, PAX100G	AMMS-II micromembrane suppressor, gradient eluent	0.2mM NaOH-ACN 70% (v/v)	CD	0.1μg/g	[111]
TFA	Peptide, Antoseptoc, Herbicide	Anion ion-exchange 300 column, anion exclusion column (300 mm × 7.8 mm)	335 model suppressor	IEC: 1% phosphoric acid IE: 2.2mM Na_2CO_3; 2.8 mM $NaHCO_3$	UV (210nm) CD	IE: 12μg/L IEC: 65 μg/ L	[112]
TFA	Peptide	Dionex Ion-exchange AS4A and AG4A	Anion micromembrane suppressor	1.5mM Na_2CO_3-2.5mM $NaHCO_3$ –4 %(v/v) acetonitrile	CD	0.3μg/mL	[113]
TFA	Water	AS14 and AG14	ASRS-1 Auto-Suppressor	3.5mM Na_2CO_3 0.8mM $NaHCO_3$	CD	300ng/ml	[114]
TFA	Peptide samples	Dionex HPIC-AS4 (25 cm × 4 mm) with a pre-column (5 cm x 4 mm) of the same packing	None	4mM-$NaHCO_3$	CD	0.1 μg/ml	[115]

Table 1.4 (Cont.)

MFA.	Water	Waters ion-exclusion column (300 mm × 7.8mm) and Guard column. Ion-exchange Pak anion column (50 mm × 4.6mm)	None	IE: 1mM Octanesulfonic acid (pH3). IEC: 3mM octanesulfonate (pH6)	CD	0.015 mg/mL	[116]
TFA	Fresh water surface sediments	HPICE-ASI	Anion micromembrane suppressor	1 mM HCl	CD	20 μg/L	[117]
TFA	Rabbit bile	Anion exchange column (250 × 4 mm)	None	2 mM Na_2CO_3 + 4 mM $NaHCO_3$	CD	0.5 μM	[118]
TFA	Human plasma and urine	Dionex Inopac AS 11 analytical column (4 × 250 mm) and AG 11 guard column (4 × 50 mm)	ASRS-1 Auto-Suppressor	NaOH gradient profile	CD	10 μM	[119]

Table 1.4 (Cont.)

TCA	Human serum	Low-capacity anion-exchange resin columns	Dionex AMMS micro-membrane	35mM NaoH 20mM P-hydroxy benzonitrile and 2% V/V.MeCN (pH12.3)	CD	0.08 mg/L	[120]
DCA TCA	Raw water	IonPac AS11 IonPac GS11	Dionex ASRS	5.0 mM NaOH	CD	0.45-1.5 μg/L	[121]
DCA, Acetate	Water	Dionex IonPacAS11	Doinex ASRS	6.25mM NaOH	CD	2-6 μg/L	[122]
MBA DCA DBA TCA MCA	Tap water	Dionex IonPac AS9, AS10, AS11	Dionex AMMS-II	50% CH_3OH, (pH 5.0)	CD	3.0 mg/L 1.5 mg/L 2.0 mg/L 3.0 mg/L 1.5 mg/L	[123]
MCA DCA TCA MBA DBA TBA	Water	Dionex IonPac AS9-HC and AS16 analytical columns	Dionex ASRS-ULTRA	5-70 mM NaOH, 0.25mL/min.	MS	3 μg/L, 3 μg/L, 1 μg/L, 2 μg/L, 1 μg/L, 1 μg/L respectively	[124]

Table 1.4 (Cont.)

MCA DCA TCA MBA DBA TBA	Water	Dionex AG-11, AS-11; Conc. Column, 4mm idx35mm	2mm anion self-regenerating suppressor	Gradient eluent A: 0.1M NaOH B: 0.001M NaOH	CD	μg/L level	[125]
DCA	Blood, Urine	Dionex AS11 (250x4mm)	ASRS 4mm	Gradient eluent: 0.01mM NaOH + 40% methanol	CD	0.05 μg/mL	[126]
MCA DCA TCA MBA DBA	Peptide sample, Antiseptic solution, Herbicise	Alltech Universal Anion 300 Column,(150mmx4.6)Anion Exclusion Column (30087.8)	Model 335	2.2 mM Na_2CO_3-2.8mM $NaHCO_3$	CD UV-VIS		[127]
DCA TCA	Raw water	Dionex IonPac AS11	Dionex ASRS	5.0mM NaOH and Gradient eluent	CD	0.45 μg/L 1.50 μg/L	[128]

1.4.4 Other methods

In addition to the methods already mentioned, some others are also reported occasionally. These are summarised in Table 1.5.

Among them is capillary zone electrophoresis (CZE), which is worthy of mention. Recently CZE has been developed as a powerful analytical technique for the separation of charged analytes [129, 130]. In the determination of haloacetic compounds it has been used as an alternative to chromatographic methods, since it does not require a tedious derivatisation step, while the analysis times can be reduced [131]. In order to enhance detection sensitivity, liquid-liquid extraction (LLE) has been the most frequently used technique for the enrichment of haloacetic acids [132, 133, 134]. In this case, methyl *tert*-butyl ether (MTBE) is used as the organic phase, with acidification to extract the undissociated acidic compounds of the sample. Nowadays, solid-phase extraction (SPE) is becoming the most frequently used extraction technique for environmental samples, and overcomes some of the problems of LLE, such as the large amounts of generally toxic and inflammable organic solvents or the greater cost and duration of the concentration step [135]. Furthermore, the selectivity, affinity and/or capacity of the SPE process can be adjusted as new materials become available [136]. However, before an sorbent is selected for SPE, some physico-chemical considerations such as the functional groups of the analytes, the nature of the bonded phase and the interactions between the sorbent and the components of the sample matrix must be taken into account [137]. Using an SPE technique followed by CZE with indirect ultraviolet detection, Martinez *et al*. [131] developed a method to determine haloacetic acids from tap water. Four different sorbents, a quaternary ammonium anion exchanger (LC-SAX), a highly cross-linked polymer of styrene-divinybenzene (LiChrolut

EN), a graphitised carbon black (Envi-Carb) and a macroporous poly (divinylbenzene-co-N-vinylpyrrolidone) copolymer (Oasis HLB), were compared as SPE adsorbents. Strong anion exchangers (SAX) were chosen because of their previous use for the extraction of anionic species in tap water and river water [138]. LiChrolut EN, Envi-Carb and Oasis HLB have been chosen because they have been used to determine highly polar species from aqueous samples [137, 138, 139, 140]. Finally, the proposed method was applied to the analysis of these compounds before and after the chlorination step in a water treatment plant, and at different points in the mains water supply in order to study their evolution. The results were compared with those obtained by the LLE-GC-MS method [141] where a previous derivatisation step was necessary.

Table 1.5. Other methods

Analyte	Sample	Method	Detection Limit	Ref
MCA, DCA, TCA, MBA, DBA	Tap water	CE-DAD	5.0-40 μg/L	[131]
MFA	Rodenticide baits	CZE-UV	0.4 μg/mL	[139]
MFA	Biological samples	NMR	1 ppm	[140]
MFA and its sodium salt	Plant leaves	^{19}F NMR	Not report	[141]
MFA	Contaminated water and grass	TLC, OPLC	0.1 μg	[142]
MFA	Plants	Paper chromatography	0.02 mg	[143]

1.5 Aims of the project

Trace amounts of haloacetic acids are widely dispersed throughout the environment, from manufacturing process and several biodegradation processes. Development of a method for the simultaneous determination of haloacetic acids is therefore considered to be important from an environmental standpoint. From the above literature review, the main method for determining haloacetic acids is by GC, but these methods are complicated and time consuming. Compared with other techniques, IC is considered to be a relatively convenient and sensitive technique for the quantitative determination of ionic species, and easily lends itself as an automated technique for multi-species analysis. However, no ion chromatographic methods have been described for the simultaneous determination of the nine haloacetic acids in environmental samples. Therefore, in this project the following aspects will be performed:

- Development of an ion chromatographic method for the simultaneous determination of mono-, di-, and tri-fluoroacetates (MDTFA), with specific emphasis on the determination of MDTFA in toxic plants.
- Development of an ion chromatographic method for the simultaneous determination of nine haloacetic acids, focusing on the optimisation of the separation parameters, while enhancing the detection sensitivity.
- Application of the developed method to determine haloacetic acids in various environmental samples.

1.6 References

[1] G. A. Cowmen. P. C. Singer, *Environ. Sci. Technol*. 1996, 30, 16 -24

[2] A. Grimvall, H. Boren, I.V. Sydow, K. Laniewski. S. Jonsson, Department of Water and Environmental Studies, Linkoping.University, Linkoping, Sweden.

[3] P. C. Uden. W. J. Miller, *Water Works Assoc*. 1983, 75, 524 -527.

[4] G. A. Cowmen, P. C. Singer, *Environ. Sci. Technol*. 30, 16 - 24.

[5] B. D. Quimby, M. F. Delaney, *Anal. Chem.* 1980, 52, 259 - 263.

[6] A. Rompp, O. Klemm, W. Fricke, *Emviron. Sci. Technol*. 2001, 35, 1294 -1298

[7] B. E. Smat, Organoflorine Chemistry: Principles and Commercial Applications, Banks, R. E. Smart, B. E. Tatlow, J. C. Eds, plenum Press: New York, 1994 pp57 - 88.

[8] L. Pauling, The nature of the chemical bond, 3rd ed; Cornell University Press: Ithaca, NY, 1960.

[9] B. C. Saunders, G. J. J. Stacey, *Chem. Soc*. 1948, 1773.

[10] E. E. Royals, Organic Chemistry, Prentice-Hall, Englewood cliffs, N. J. 1954, p.706.

[11] D.R. Lide, CRC handbook of chemistry and physics, CRC Press, New York (1999).

[12] Chemwatch, Chemwatvh Newsletter, Quarterly CD Release 2003/2

[13] S. P. Atzert, A review of sodium monofluoroacetate (compound 1080) its properties, toxicology, and use in predator and rodent control; Special Science Report on wildlife 146; U. S. fish and wildlife Service: Washington, DC, 1971.

[14] J. S. C. Marais, J. Onderstepoort, *Vet. Sci.* 1944, 20, 67.

[15] S. R. Peters, *Endeavour*, 1954, 13, 147.

[16] R. Peters, Carbon-fluorine compounds: Chemistry, biochemistry, and biological activities (A Ciba Foundation Symposium) associated scientific Publishers: Amsterdam, 1972; pp 56 -77.

[17] D. A. Blake, H. F. Cascorbi, R. S. Rozman, F. J. Meyer, *Tocicol. Appl. Pharmacol*.

1969, 15, 83.

[18] T. L Bott, L. J. Standey, proceedings of the 1994 AFEAS Workshop on the environmental fate of trifluoroacetic acid; AFEAS Administrative Organization, SPA-AFEAS, Inc. Miami Beach, FL, 1994.

[19] P. T. Visscher, C. W. Culbertson and R. S. Oremland, *Nature*, 1994, 369, 729.

[20] D. A. Blake, M. C. DiBlasi, G. B. Gorden, *Fundam. Appl. Tocicol*. 1981, 1, 415.

[21] G. Weiss, ed., hazardous Chemicals Data Book, Noyes Data Corp. 1980, pp. 245, 424, 628.

[22] G. Woodward, J. I. Hyg, *Toxicol.* 1941, 23, 78.

[23] J. J. Rook, *Water Treatment Exam.* 1974, 23, 234.

[24] E. A. Paul, P. M. Huang, *Handbook of Environmental Chemistry, Springer Verlag; Berlin,* 1980.

[25] D. B. Harper, D. O'Hagen, *Nat. Prod. Rep*. 1994, 11, 123.

[26] T. Tamura, M. Wada, N. Esaki, K. Soda, *J. Bacteriol.* 1995, 177, 2265.

[27] L. E. Twigg, D. R. King, *Natural Toxins,* 1996, 4, 122 - 127

[28] L. E. Twigg, D. R. King, *Native Australian Fauna,* 1991, 61, 412 - 430.

[29] B. A. Kimball, E. A. Mishalanie, *J. Chromatogr.* 1993, 634, 289-296.

[30] M. Molina, F. S. Rowland, *Nature,* 1974, 249, 810 - 812.

[31] J. D. Farman, B. G. Gardiner, J. D. Shanklin, *Nature,* 1985, 315, 207 - 210.

[32] M. J. Prather, R. T. Watson, *Nature,* 1990, 344, 729 - 734.

[33] T. J. Wallington, M. D. Hurley, J. C. Ball, E. W. Kaiser, *Environ. Sci.Technol.*1992, 26, 1318.

[34] J. Franklin, *Chemosphere*, 1993, 27, 1565.

[35] A. McCulloch, *Env. Int.* 1995, 21, 353 - 362.

[36] D. J. Bowden, S. L. Clegg, P. Brimblecombe, *Chemosphere,* 1996, 32, 405 - 420.

[37] S. Schwarzbach, *Nature,* 1995, 376, 297 - 298.

[38] H. Franklin, A. Klein, D. Renschen, *Nature,* 1996, 382, 34.

[39] M. L Hewitt, K. R. Munkittrick, I. M. Scott, J. H. Carey, K. R. Solomon, R. S. Mark, Environ. *Tox. Chem.* 1996, 15, 894.

[40] P. W. Gabbadon, F. Chapman, *Progressive Fish-Culturist*, 1996, 58, 23.

[41] A. E. David, A. M. Scott, *Environ. Sci. Technol*. 2000, 34, 632 - 637

[42] D. A. Blake, R. S. Rozman, H. F. Cascorbi, J. C. Krantz, *Biochem. Pharmacol*. 1967, 16, 123.

[43] R. A. V. Dyke, *Environ. Health Perspect*. 1977, 21, 121.

[44] T. S. Sutton, D. D. Kolbin and L. D. Gruenke, *Anesth. Analg*. 1991, 73, 180.

[45] P. L. Visscher, C. W. Culbertson, R. S. Oreland, *Nature,* 1994, 369, 729.

[46] A. Jordan, H. Frank, *Atmos. Environ*. 1999, 33, 4525 - 4526.

[47] J. Mowrer, J. Nordin, *Chemosphere* 1987, 16, 1181 - 1192

[48] D. T. Williams, G. L LeBel, F. M. Benoit, *Chelsea, MI*, 1990, pp 3 -19.

[49] A. Klein, Ph.D. Dissertation, University of Bayreuth, 1997.

[50] T. H. Eichhold, E. B. Hookfin, Y. O. Taiwo, *Pharm. Biomed. Anal.* 1997, 16, 459 - 467.

[51] H. M. Stevens, A. C. Moffat, V. D. Janet, *Forensic Sci*. 1976, 8, 131 - 137.

[52] H. M. Stahr, W. B. Buck, P. F. Ross, *J. Assoc. Off. Anal. Chem.* 1974, 57, 405 - 07.

[53] Y. M. Ho, G. W. Miller, Environ. *Sci. Technol*. 1970, 4, 492 - 495.

[54] W. J. Allender, *J. Anal. Toxicol.* 1990, 14, 45 - 49.

[55] D. G. Burke, D. K. T. Lew, X. Cominos, *J. Assoc. Off. Anal. Chem*. 1989, 72, 503 - 507.

[56] E. J. Hugghins, H. H. Casper, C. D. Ward, *J. Assoc.Off. Anal. Chem*. 1988, 71, 579 - 581

[57] I. Okuno, G. E. Connolly, P. J. Savarie, C. P. Breidenstein, D. Wildl, *J. Assoc. Off. Anal. Chem*. 1984, 67, 549 - 553.

[58] H. H. Casper, T. L. McMahon, G. D. Paulson, *J. Assoc. Off. Anal. Chem.* 1985, 68, 722 - 725.

[59] I. Okuno, D. L Meeker and R. R. U. S. Felton, *J. Assoc. Off. Anal. Chem*. 1982, 65, 1102 - 1105.

[60] H. M. Stahr, *J. Assoc. Off. Anal. Chem*. 1977, 60, 1434 - 1435.

[61] J. E. Peterson, *Bull. Environ. Contam. Toxicol*. 1975, 13, 751 - 757.

[62] L. E. Twigg, D. R. King, L. H. Bowen, G. R. Wright and C. T. Eason, *Nat. Toxins,* 1996, 4, 122 - 127.

[63] T. Vartiainen, P. Kauranen, *Anal. Chim. Acta* 1984, 157 (1), 91- 97.

[64] J. Tannock, J. Rhod. *Agric. Res.* 1975, 13 (1), 55 - 62.

[65] M. Mori, H. Nakajima and Y. Seto, *J. Chromatogr.* A 1996, 736, 229 - 234.

[66] H. Ozawa, T. Tsukioka, *Anal. Chem*. 1987, 59, 2914 - 2917.

[67] B. A. Kimball, E. A. Mishalanie, *J. Chromatogr*. 1993, 634, 289 - 296.

[68] B. A. Tomkins, *Anal. Lett*. 1994, 27, 2703 - 2718.

[69] H. Ozawa, T. Tsukioka, *J. Chromatogr*. 1989, 473, 251 - 259.

[70] D. Zehavi, J. N. Seiber, *Anal. Chem*. 1996, 68, 3450 - 3459.

[71] F. Hartmut, D. Renschen, A. Klein, H. Scholl. *J. High Resol. Chromatogr.* 1995, 18, 83 - 88.

[72] J. Armin, F. Hartmut, *Environ. Sci. Technol,* 1999, 33, 522 - 27.

[73] E. W. Chad, M. C. Thomas, N. S. James, *Environ. Sci. Technol*. 1999, 33, 1747 - 1751.

[74] L. D. Gruenke, L. A. Waskell, Biomed, *Environ. Mass Spectrom*. 1988, 17, 471 - 475.

[75] L. Witte, H. Nau, J. H. Fuhrhop, *J. Chromatogr*. 1977, 143, 329 - 334.

[76] D. Karashima, S. Takahashi, A. Shigematsu,. T. Furukawa, T. Kuhara, I. Matsumoto, *Biomed. Mass Spectrom*. 1976, 3, 41 - 44.

[77] D. A. Both, M. Jemal, *J. Chromatogr*. 1992, 596, 85 - 90.

[78] A. Preiss, J. Kruppa, J. Buschmann, C. Mugge, *J. Pharm. Biomed. Anal*. 1998, 16, 1381 - 1385.

[79] D. D. Breimer, H. C. J. Ketlaars, J. M. V. Rossun, *J. Chromatogr*. 1974. 88, 55 - 63.

[80] F. Hartmut, R. Dirk, K.Armin, S. Harad, *J. High Resol .Chromatogr.* 1995,18, 83 -88.

[81] B. F. Scott, M. Alaee, *Water Qual.Res. J. Canada*, 1998, 33, 279 - 293.

[82] M. N. Sarrion, F.J. Santos, M.T.Galceran, *J. Chromatogr. A* 1999, 859, 159 - 171.

[83] J. C. Ireland, L. A. Moore, H. pourmoghaddas, A. A. Stevens, *Biomed. Environ. mass spectrum.* 1988, 17, 483 - 486

[84] R. A. Landowne, S. R Lipsky, *Anal. Chem.* 1963, 35, 532 - 535.

[85] US Environmental Protection Agency, EPA Method 552.2 National Exposure Research Laboratory, Cincinnati, OH, 1995.

[86] L. O. O. Korhonen, *J. Chromatogr*. 1984, 287, 399 - 406.

[87] Y. Zimeng, G. N. Henderson, M. O. James, P.W. Stacpoole, *J. Chromatogr. B* 1997, 703, 75 - 84.

[88] L. M. Vonsydow, A. T. Nielsen, A. B. Gromvall, *Environ. Sci. Technol*. 2000,34, 239 - 245.

[89] Z. Yan, G. N. Henderson, M. O. James, P.W. Stacpoole, *J. Pham. Biomed. Anal.* 1999, 19, 309 - 318.

[90] H, Michitsuji, A. Ohara, S. Yamada, *Shojinkai igakushi*, 1992, 31, 167 - 75.

[91] Z. Yan, G. N. Henderson, *J. Pham. Biomed. Anal.* 1999, 19, 309 - 318.

[92] H. P. Faculty, *J. Chromatogr.* 1985, 337, 126 - 130.

[93] R. V. D. Hoeven, R. H. Drost, R. A. A. Maes,. *J. Chromatogr.* 1979, 164, 106 - 108.

[94] V. D. Chmil, N.I.Glembitskii, Khim, *Selsk. Khoz*, 1977, 15, 42 - 44.

[95] L. M. Kedik, I. S. Novikova, *Gigienai Sanitariya*, 1988, 52 - 53. M. Ogata, T. Saeli, *Int. Arch. Arbeitsmed.* 1974, 33, 49

[96] R. V. D. Hoeven, R. H. Drost, R. A. A. Maes, *J. Chromatogr*. 1979, 164, 106 - 108.

[97] A. C. Ray, L. O. Post, J. C. Reagor, *J. Assoc. Off. Anal. Chem*. 1981, 64 (1), 19 - 24.

[98] D. M. Collins, J. P. Fawcett, C. G. Rammell, *Bull. Environ. Contam. Toxicol*. 1981, 26, 5, 669 - 673.

[99] D. Collins, *New Zealand J. Sci.* 1983, 26, 283 - 286.

[100] H. L. Kramer, *J. Assn. Off. Anal. Chem.* 1984, 7 (6), 1058 - 1061.

[101] M. L Baron, C. M. Bothroyd, G. I. Rogers, A. Staffa, I. D. Rae, *Phytochemistry*, 1987, 26 (8), 2293 - 2295.

[102] B. V. ickery, M. L. Vickery, J. T. Ashu, *Phytochemistry,* 1973, 12, 145 - 147.

[103] I. Okuno, D. L Meeker, *J. Assn. Off. Anal. Chem*. 1980, 63, 49.

[104] J. A. Peterson. *Bull. Environ. Contam. Toxicol*. 1975, 13, 751.

[105] J. J. M. Meyer, N. Grobbelaar,. *J. Plant Physiol*. 1990, 135 (5), 546 - 549.

[106] P. P. Minnaar, G. E. Swan, R. I. McCrindle, W. H. J. D. Beer, T. W. Naude *J. Chromatogr. Sci.* 2000, 38 (1), 16 - 20.

[107] M. Imbenotte, A. Brice, F. Erb, J. M. Haguenoer, *Talanta.* 1984, 31(2), 147 - 149.

[108] M. Ogata, Y. Yamazaki, *Acta. Med, Okayama*, 1979, 33, 479 – 481

[109] S. Husain, R. Narshima, S. N. Alvi, R. N. Rao, *J. Chromatogr*. 1992, 600, 316.

[110] S. Hashimoto, A. Otsuki, *J. High Resolq. Chromatogr*. 1998, 21 (1), 55 - 58.

[111] A. F. Kingery, H. E. Allen*, J. Chromatogr. A* 671 (1994) 231- 237.

[112] M. N. Lakshmy, etc. *J. Chromatogr. A* 1994, 671, 309 - 313.

[113] N. Simonzadeh, *J. Chromatogr.* 1993, 634, 125 - 128

[114] Dionex. Detrmination of Trifluoroacetic acid (TFA) in peptides. Application Note 115, 1-6.

[115] H. Nakazawa, M. Nagase, T. Onuma, *Bunseki-Kagaku*, 1987, 36 (6), 396 - 398.

[116] L. A. Kaine, J. B. Crowe, K. A. Wolnik, *J. Chromatogr.* 1992, 602, 141 - 147.

[117] T. L. Bott, L. J. Standley, *Water-Res. Apr* 1999, 33 (6), 1538 - 1544.

[118] R. Kawaguchi, K. Fujii, M. Morio, O. Yuge, M. D. H. Delawar, *J. Med. Sci*. 1989, 38 (1), 27 - 34.

[119] D. C., Hankins, E. D., Kharasch, *J. Chromatogr. B* 1997, 692, 413 - 418.

[120] S. Sakado, *Analyst,* 1989, 114, 1637 - 1640

[121] Y. W. Ko, T. J. Gtrmm, F. Changhua, *J. Anal. Chem.* 2000, 366, 244 – 248.

[122] C. Y. Kuo, *J. Chromatogr. A* 1998, 804, 265 - 272

[123] C. Sarzanini, M. C. Bruzzoniti, E. Mentasti, *J. Chromatogr. A* 1999, 850, 197 - 211.

[124] R. Roehl, R. Slingsby, N. Avdalovic, P. E Jackson, *J. Chromatogr. A* 2002, 245 - 254.

[125] Y. Liu, C. Charan, *Journal of AOAC, International*, 1999, 82, 689 - 704.

[126] L. Narayanan, A. P. Moghaddam, A. G. Taylor, G. L. Sudberry, J. W. Fisher, *J. Chromatogr. B* 1999, 729, 271 - 277.

[127] L. M. Nair, S. Nordhaus, J. M. Anderson, *J. Chromatogr. A* 1994, 671, 309 - 313.

[128] K. Ya-Wen, Y. J. Gremm, G. Abbt-Braun, J. Fresennius, *Anal. Chem.* 2000, 366, 244 - 248.

[129] P. Jandik, G. Bonn, *Capillary Electrophoresis of Small Molecules and Ions*, VCH,

New York, 1993.

[130] S. M. Cousin, P. R. Haddad, W. Buchberger, *J. Chromatogr*. A 1994, 671, 397.

[131] D. Martnez, F. Berrull, M. Calull, *J. Chromatogr*. A 1998, 827, 105 - 112.

[132] Standard Methods for the examination of water and waste water 6251APHA, AWWA and EPCF, Washington, DC,19th ed, 1995.

[133] R. C. Barth, P. S. Fair, *J. AWWA* 1992, 84, 94.

[134] H. Arora, M. W. LeChevallier, Dixon, K.L. *J. AWWA* 1997, 89, 60.

[135] L. A. Berrueta, B. Gallo, F. Vicente, *Chromatogaphia* 1995, 40, 474.

[136] I. Liska, D. Barcelo, M. Grasserbauer, *Trends Anal. Chem*. 1996, 15, 326.

[137] M. W. F. Nielen, Ph.D. Thesis, Free University, *Amsterdam*.1987.

[138] M. W. F. Nielen, J. deLong, R. W. Frei, U. A. T. Brinkman, *Int. J. Environ. Anal. Chem.* 1986, 25, 37.

[139] F. Guan, H. F. Wu, Y. Luo, *J. Chromatogr. A* 1996, 719, 421 - 426

[140] F. Guan, Z. C. Miao, Y. Liu, G. Li, Z. Du, *Junshi Yixue Kexueyuan Yuankan* 1997, 21 (2), 116 - 119.

[141] R. L De-Moraes-Moreau, M. Haraguchi, H. Morita, *J. Med. Bio. Res*.1995, 28 (6), 685 - 692.

[142] M. Mazurek, J. Czuprynski, Z. Witkiecicz, *Akad. Tech*. 1997, 46 (10), 33 - 41.

[143] B. Vickery, M. L Vickery, J. T. Ashu, *Phytochemistry* 1973, 12 (1), 145 - 147.

Chapter 2

General Experimental

The following instrumentation, reagents and procedures were used throughout the project, except where indicated otherwise. The term Milli-Q water in this work describes water purified using a Milli-Q (Millipore, Bedford, MA, USA) water purification system. General information on the chemicals used is listed in Table 2.1. The preservation and extraction procedures followed were adapted from literature procedures [1][2].

2.1 Instrumentation

2.1.1 Chromatograph

The block diagram of the instrumentation used in this project is provided in Figures 2.1 and 2.2. The ion chromatograph employed in this work was a Dionex (Sunnyvale, CA, USA) Model DX-600 instrument equipped with a GP50 gradient pump, a CD25 conductivity detector with ASRS suppression, an EG40 eluent generator, an AS50 auto sampler, an AD25 absorbance UV detector Data were acquired by using Dionex Peaknet 6.3 software installed on a Dell P-III 550 MHz computer.

2.1.2 Columns

Dionex IonPac AG17 (4×50 mm) guard column and an IonPac AS17 (4×250 mm) analytical column were employed for all separations. Dionex On-Guard II sample pre-treatment cartridges (2.5mL) in the Ag form were used for sample clean up.

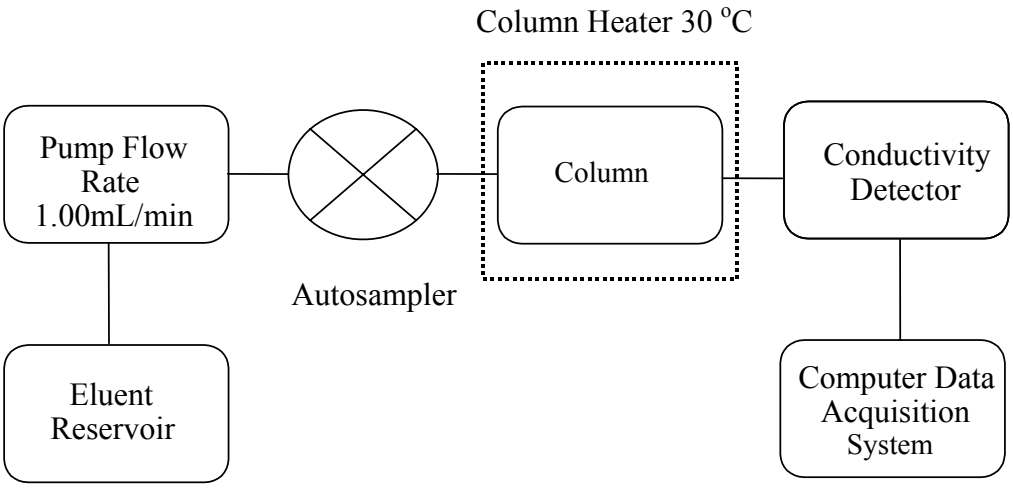


Figure 2.1. Block diagram of instrument- configuration 1

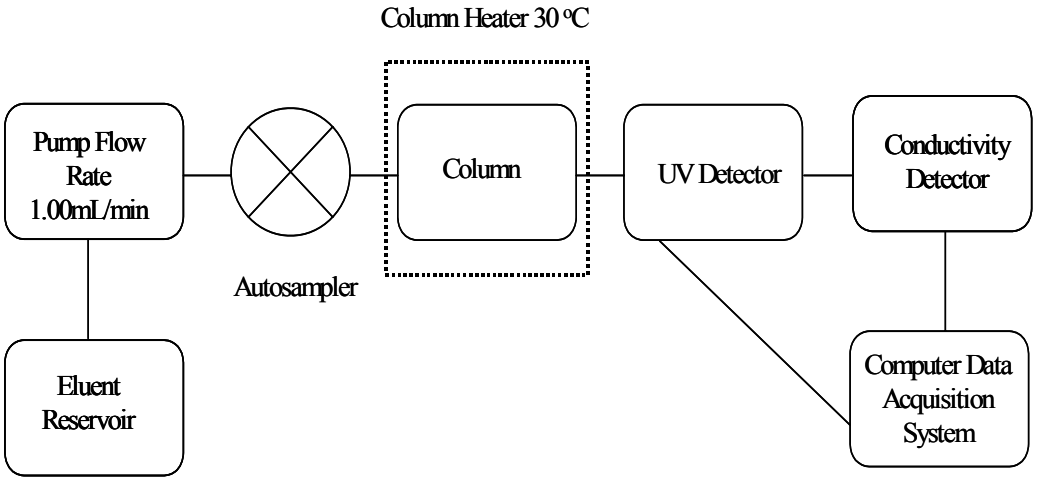


Figure 2.2. Block diagram of instrument-configuration 2

2.2 Reagents

Table 2.1. General chemicals

Compound	Formula	Grade	Supplier
Monofluoroacetic acid	CH_2FCOOH	99%	BDH
Difluoroacetic acid	CHF_2COOH	98%	BDH
Trifluoroacetic acid	CF_3COOH	98%	BDH
Monochloroacetic acid	$CH_2ClCOOH$	97%	Fluka
Dichloroacetic acid	$CHCl_2COOH$	98%	Aldrich
Trichloroacetic acid	CCl_3COOH	99%	BDH
Monobromoaceic acid	$CH_2BrCOOH$	99%	Fluka
Dibromoacetic acid	CBr_2COOH	98%	Fluka
Tribromoacetic acid	CBr_3COOH	98%	Fluka
Potassium fluoride	NaF	97%	BDH
Sodium perchlorate	$NaClO_4 \cdot H_2O$	LR	Ajax
Sodium chloride	NaCl	95%	BDH

Table 2.1. (cont.) General chemicals

Compound	Formula	Grade	Supplier
Potassium nitrite	KNO_3	AR	Ajax
Sodium sulphate	Na_2SO_4	98%	BDH
Sodium formate	HCOONa	AR	Ajax
Silver nitrate	AgNO3	AR	Ajax
Potassium hydroxide	KOH	97%	Sigma
Phophase	PO_4^-	95%	Fluka
Acetic acid	HC_3OOH	AR	Ajax
Sodium hydroxide	NaOH	AR	Sigma
Phthalate acid	$C_6H_6N_2O_2$	90%	BDH
Sodium thiocyanate	NaSCN	98%	Aldrich
Hydrobromic acid	HBr	AR	Aldrich
Methanol	CH_3OH	AR	BDC
Sodium sulfate	Na_2SO_4	AR	Prolabo
Sodium thiosulfate	Na_2S_2O3	AR	BDH

2.3 Experimental

2.3.1 Preparation of standard solutions

Individual stock solutions containing 1000 mg/L of MFA, DFA, TFA, MCA, DCA TCA, MBA, DBA and TBA, were prepared from AR grade or reagent grade chemicals, in the form of their acid or sodium salt forms, as obtained from Aldrich (Milwaukee, WI, USA) Fluka (USA) and BDH (Australia). These stock solutions were provided in sealed amber glass ampoules and were used to prepare the composite solutions used in the method development. Milli-Q water (Millipore, Milford, MA, U.S.A.) was used to prepare all solutions, including eluents, stock solutions, and standard solutions. The stock solutions were transferred into plastic bottles, and kept refrigerated until required.

Working standards were prepared by diluting the necessary amount of the stock standard to achieve the different concentrations required.

2.3.2 Instrumental conditions

In all experiments, the eluent was pumped through the system at a flow rate of 1.00 μL/min, while the injection volume used was 50 μL, unless otherwise stated. Injections were performed in triplicate to ensure reproducibility of the results. The column was kept at a constant temperature of 30 ^{0}C. Gradient elution was achieved using varying concentrations of KOH produced with the Dionex EG40 eluent

generator. The gradient profile was optimised according to the different separation required.

2.4 Sample resource and preparation

2.4.1 Water Samples

Water samples were supplied by Analytical Services Tasmania, a division of the Department of Primary Industries, Water and Environment, and filtered through a nylon acrodisc syringe filter prior to analysis. The syringe filter was manufactured by Gelman Sciences of Ann Arbor, USA. The specific filter used was VacuCap 60 with a pore size of 0.45 μm. The filtrate was then spiked with different concentrations of each fluoroacetate of the standard MDTFA's mixture, containing 0.1, 0.2 and 0.4 ppm of each fluoroacetate prior to analysis.

2.4.2 Carrot samples

Carrot baits were prepared according to a published method [3] in which the sample was homogenised in a blender and soaked in a standard aqueous solution of 1.5 % MFA for 20 min. The supernatant liquid was removed and the sample partially dried for 2 h under nitrogen. For analysis, a 1.00 g portion was added to 100 mL of water, the mixture shaken for 1 h on a reciprocating shaker, followed by ultrasonic digestion for 3 h at 20º C. Finally, the supernatant liquid was filtered through a 0.45 μm nylon syringe filter, prior to MFA analysis.

An actual field bait carrot sample, which had dried for three months after exposure to 1080 (MFA), was also analysed. An accurately weighed portion comprising about 1 g of the sample was divided into small pieces and added to 100 mL of deionised water. The remaining steps for the extraction were as described above.

2.4.3 Plant Samples

Ten grams of fresh, chopped leaves were added to 100 mL of hot deionized water (75 ^{o}C) and left to stand for 15 minutes, followed by cooling to room temperature [4]. Deionised water was added to make up the initial 100 mL. After being subjected to ultrasonic vibration for 3 hours, the supernatant liquid was drawn off and filtered through a 0.45 μm nylon syringe filter. The filtrate was divided into two portions, with the first portion being analysed directly and the second portion being spiked with 0.8 ppm of each fluoroacetate standard before analysis.

2.4.4 Soil Samples

Soil samples GSS- 5 and GSS-2, (mountainside soil containing decayed vegetable matter from the base of trees of Mount Wellington, Tasmania, Australia) supplied by Geology Department of the University of Tasmania and 27648 (2g) (alluvial soil from the Savage River, Tasmania, Australia) supplied by Analytical Services Tasmania, a division of the Department of Primary Industries, Water and Environment. Soil samples were extracted with 20 mL of Milli-Q water in 50 mL glass centrifuge tubes by shaking for 1 hr on a reciprocating shaker (100 rpm),

followed by ultrasonic digestion for 3 hours. After centrifuging for 10 min, the supernatant liquid was filtered through a 0.45 μm syringe filter, and analysed [5].

2.4.5 Urine Samples

The urine sample was supplied by Analytical Services Tasmania, a division of the Department of Primary Industries, Water and Environment and kept refrigerated at 2 ^{o}C for three days after being collected from the carcass of a possum prior to analysis. The possum was known to have consumed a carrot bait soaked in MFA, DFA and TFA solution. Samples were diluted 10 times with Milli-Q water, followed by filtration through 0.45 μm nylon syringe filters.

References

[1]. K. Cerschueren, *Handbook of Environmental Data on Organic Chemicals*. Second Edition 1983.

[2]. A. D. Eaton, L. S. Clesceri and A. E. Greenberg, *Standard Methods for the Examination of Water and Wastewater.* 1995.

[3]. J. D. Coleman, T.L. Montague, C.T. Eason and H.L. Statham. *The management of problem browsing and grazing mammals in Tasmania*, Second edition, Commissioned by the Browsing Animal Research Council.

[4]. L. E. Twigg, D. R. King, *Natural Toxins*. 1996, 4, 122 - 127.

[5]. G. E. Raymen, F. R. Higginson, Australian Laboratory Handbook of soil and water Chemical Methods. 1992.

Chapter 3

Simultaneous determination of monofluoroacetate, difluoroacetate and trifluoroacetate in environmental samples by ion chromatography

3.1 Introduction

The polyfluoroacetates comprise mono-(MFA), di-(DFA), and tri-fluoroacetate (TFA) and these species may be present in many environmental samples due to their use in various industrial, commercial and medical applications. Trace amounts are found in drinking water, chemical waste, animal products and plants. MFA is used as the active chemical in some rodenticides and was first reported for this application shortly after the end of the Second World War [1]. This compound is still commonly referred to under the laboratory serial number, "1080" (sodium fluoroacetate), assigned by the Economic Investigations Laboratory, US Fish and Wildlife Service, at Patuxet, MD, USA. LD_{50} values for MFA are extremely low, ranging from 66 μg/kg (dog, oral) to 714 μg/kg (human male, oral) [2], and it has been used as a vertebrate pesticide for more than 50 years, particularly throughout North America, Australia and New Zealand with peak usage occurring in the 1960's [3]. Every year, about 80 tonnes of carrot, laced with MFA is laid as baits in Australia.

DFA is reported as being acutely toxic [4], but no value for its LD_{50} could be found in a current literature review. The LD_{50} for TFA is reported to be 200 mg/kg. Few reports on the biodegradation of DFA and TFA are available, although Visscher *et al*. [5] reported instances of

reductive defluorination of TFA under methanogenic and sulfate-reducing conditions. It was found that TFA was sequentially defluorinated to DFA, MFA, and acetic acid, with the latter component ultimately yielding methane. TFA is also produced as a by-product of the metabolism of the anaesthetic, halothane [5]. As all fluoroacetates are toxic, the availability of a reliable method for their determination is considered important.

The standard method for determining fluoroacetates is liquid-liquid extraction followed by gas chromatography (GC) using electron-capture detection. This method is described in US-EPA method 552 [6] and is applicable to the determination of six halogenated acetic acids in drinking, ground and raw water. Even though the detection limits for the acids are in the low µg/L range, this method is complicated and time-consuming, with two significant problems being evident First, the high polarity of fluoroacetates makes these substances unsuitable for direct GC analysis and derivatisation to their alkyl esters is necessary prior to analysis. Second, the fluoroacetates have high water solubility, which makes their separation from water difficult and is often the cause of low recoveries [7, 8]. HPLC has also been used for the determination of MFA and TFA in baits [9, 10, 11], but due to their low UV absorptivities appropriate derivatisation is necessary to enhance their sensitivity to UV or fluorescence detection. These derivatisation reactions are often difficult to drive to completion, so that reaction times of up to 10 hours are normally needed [12]. Although the derivatisation time was reduced to less than 30 min by Collins *et al* [9, 10], extraction and drying procedures prior to derivatisation were still needed since the derivatisation reaction occurred only in non-aqueous solution, making this method unsuitable for the analysis of large numbers of samples. Determination of

fluoroacetates can be achieved using ion-interaction or anion-exchange chromatography with detection by UV absorbance at 210 nm or by a method utilising the refractive index [13]. Currently these methods cannot be used for trace analysis due to sensitivity problems.

Ion chromatography (IC) with suppressed conductivity detection is particularly well-suited to the separation of small ions such as fluoroacetates. Several IC methods using both ion-exchange and ion-exclusion as the separation principle (the latter approach being possible due to the differing pKa values of the analytes, see Table 3.3) have been described in the literature [14, 15, 16, 17, 18]. However, none of these methods are capable of simultaneous determination of all three fluoroacetates at the levels found commonly in environmental samples. We have therefore investigated the use of anion-exchange IC utilising gradient elution with hydroxide eluents, coupled with suppressed conductivity detection. Using this approach, simplified sample preparation procedures can be used and appropriate detection limits can be realised. Samples analysed include river water and carrot baits.

3.2 Experimental

3.2.1 Reagents

Stock solutions containing MFA, DFA and TFA were prepared from AR grade reagents obtained from Aldrich (Milwaukee, WI, USA). Purified water was produced with a Milli-Q system (Millipore, Milford, MA, U.S.A.) and used to prepare all solutions, including eluents, stock solutions, and standard solutions. The details of chemicals and solutions used are provided in Chapter 2.

3.2.2 Apparatus and operating conditions

The details on the ion chromatograph used for this work are given in Chapter 2. The optimised gradient profile is shown in Table 3.1.

3.2.3 Preparation of samples

The details on the preparation of water, carrot samples are described in Chapter 2.

3.3 Results and discussion

3.3.1 Optimization of gradient elution for MDTFA's separation

Potassium hydroxide gradients were produced using a Dionex EG40 eluent generator. The gradient profile was optimised empirically using isocratic eluents to establish elution behaviour of the analytes, and common inorganic and organic ions likely to be present in environmental samples [19]. This approach was taken due to the complex and unknown nature of sample matrices, with further optimisation being undertaken if peak overlap occurred. In general, MFA and DFA were retained weakly and were separated poorly from other singly charged anions such as formate, fluoride, acetate and chloride, whilst TFA was retained strongly. For these reasons, it was necessary to use a very weakly eluting eluent composition at the start of the separation and to rapidly increase the eluent strength so that TFA could be eluted in a reasonable time (< 35 min), enabling common inorganic and organic ions to be separated from the target ions. The optimised gradient conditions are provided in Table 3.1, while Table 3.2 lists retention times of the fluoroacetates and potential interferents [20] under the optimal gradient conditions. Using the optimised gradient profile, the three fluoroacetates were resolved from each other and also from other common inorganic and organic anions. A

representative chromatogram obtained for a standard mixture of fluoroacetates using this gradient profile is illustrated in Figure 3.1.

Table 3.1. Gradient profile for separation of MFA, DFA and TFA by anion-exchange IC

Time (min)	Flow (ml/min)	KOH (mmol/L)	Comment
Initial	1.0	0.5	
0	1.0	0.5	Sample injection
10	1.0	2.5	
30	1.0	20	
30	1.0	0.5	End of step gradient
35	1.0	0.5	

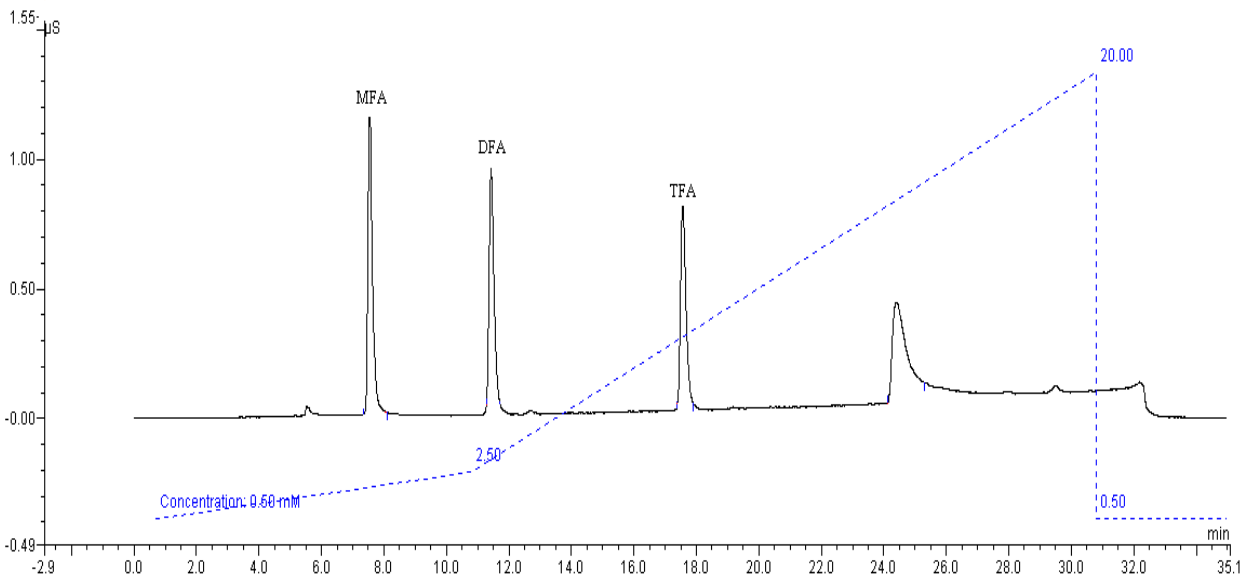


Figure 3.1. Ion chromatogram for MFA, BFA and TFA standard solution, 0.5 mg/L of each acid. Conditions: Column, Dionex Ion Pac AS17 (4×250 mm); Eluent, see Table 3.1; Injection Volume, 50 µL; Detection, suppressed conductivity ASRS®-ULTRA.

3.3.2 Interference study

The common anionic species expected to be present in real samples include chloride, fluoride, sulfate, nitrate, nitrite, perchlorate, and other organic anionic species. Using the separation conditions outlined in Table 3.1, MFA, DFA and TFA can be adequately separated from other common inorganic compounds, with minimal interference. Table 3.2 details the retention times for different inorganic and organic anions (0.5 ppm of each ion) under these separation conditions. The resolutions between Bromate - DFA and Bromide -TFA are 1.63 and 1.52 respectively.

Table 3.2. Retention time of interference species and target compounds

Inorganic anions	Retention time (min)	Organic anions	Retention time (min)
F^-	6.08	Acetate	6.36
BrO_3^-	12.69	Format	7.64
Cl^-	13.20	MFA	8.01
NO_2^-	14.70	DFA	12.06
Br^-	18.67	TFA	18.15
NO_3^-	19.47	Phthalate	13.24
SO_4^-	28.53	Succinate	25.62
ClO_4^-	29.22	Tartrate	26.97
PO_4^-	24.95	Oxalate	29.94

3.3.3 Analytical performance characteristics

The use of an eluent generator coupled with suppressed conductivity detection allowed very low detection limits to be achieved and the results displayed in Table 3.3. Linear calibration

plots ($R^2 > 0.9996$) were obtained for each of the fluoroacetates over the range 2.5-6400 μg/L. Typical calibration graphs for MFA, DFA and TFA as shown in Figures 3.2a, 3.2b and 3.2c. Using a signal to noise ratio of 3, the detection limits for MFA, DFA, and TFA were 21 μg/L, 38 μg/L, and 36 μg/L, respectively. Replicate injections (MFA, BFA and TFA concentration: each 0.05 ppm) over a period of 30 days gave percentage relative standard deviation (% RSD) values for the peak area of 0.83%, 0.56%, and 0.48% for MFA, DFA and TFA, respectively. Both UV absorbance and conductivity detection was employed simultaneously. Comparison of these detection techniques are illustrated in Figures 3.3a and 3.3b, and indicate that the sensitivity of UV detection was much lower than that for suppressed conductivity detection. However, the UV detector was more selective and therefore was of some value in the analysis of real samples. The physical properties of MDTFA including ε values at a wavelength of 190 nm, are shown in Table 3.4.

Table 3.3. Linearity and detection limits for haloacetates under optimum conditions

Analyte	Linearity	R^2	Detection limit (μg/L)
MFA	0.5369x-0.0041	0.9995	21
DFA	0.5236x- 0.0090	0.9998	38
TFA	0.4423x+0.0036	0.9989	36

where x is the conductivity (μS) for different concentrations of haloacetic acid

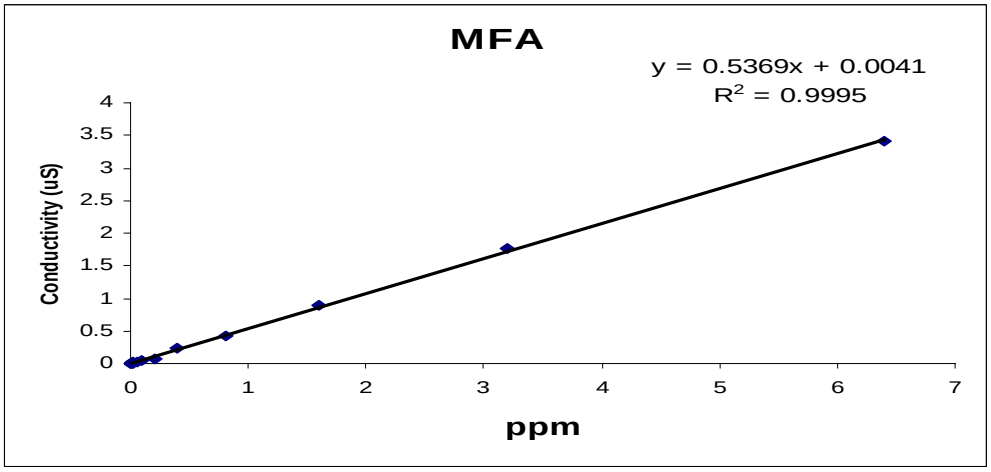


Figure 3.2a Typical calibration graph for MFA

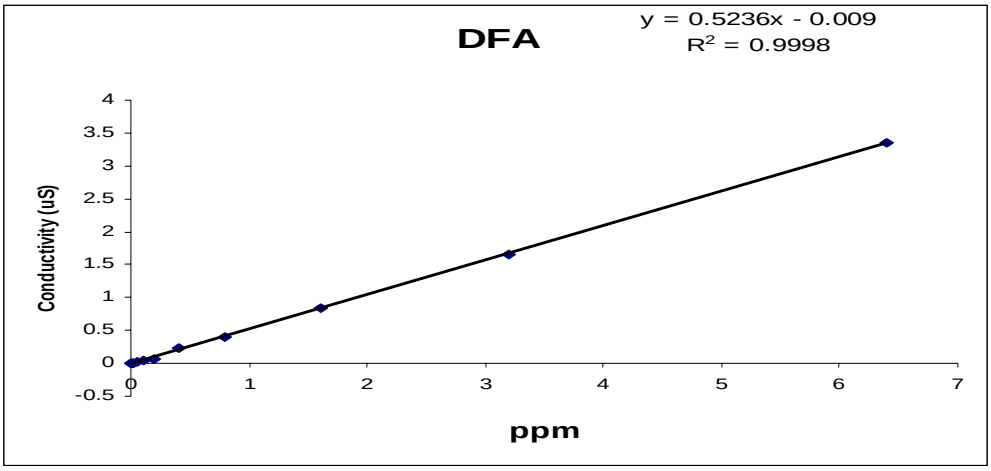


Figure 3.2b Typical calibration graph for DFA

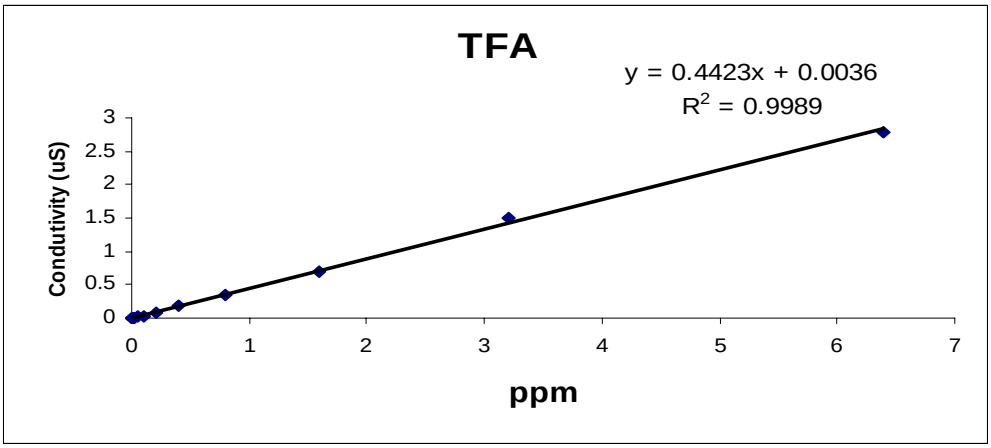


Figure 3.2c Typical calibration graph for TFA

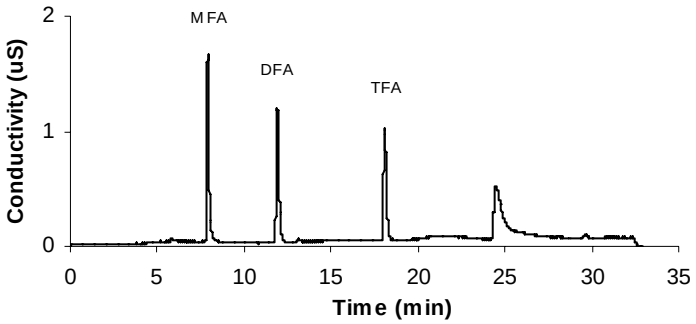


Figure 3.3 (a) MDTFA standard solution containing 0.8ppm each of MFA, DFA and TFA, Conductivity detection. Conditions: see Figure. 3.1

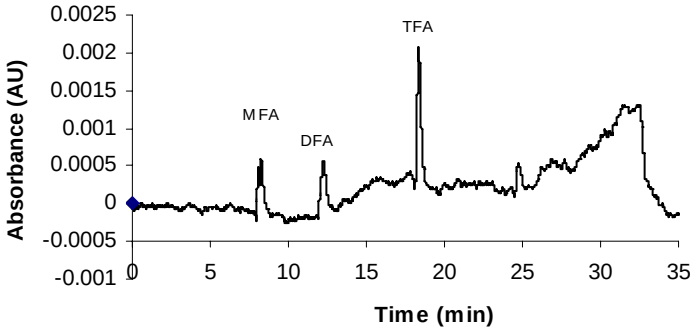


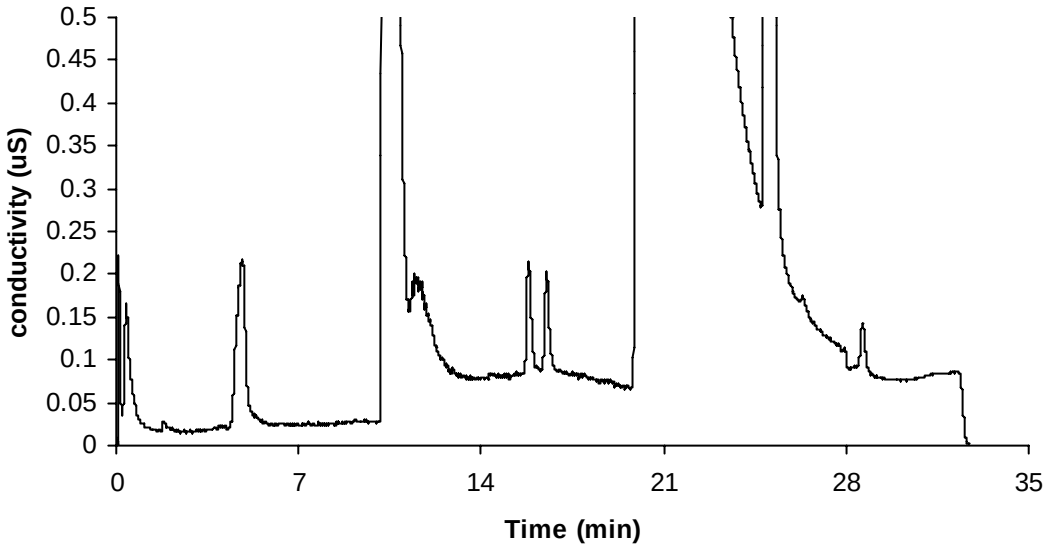
Figure 3.3(b) MDTFA Standard solution containing 0.8ppm each of MFA, DFA and TFA, UV detection. Conditions: see Figure. 3.1

Table 3.4. Physical properties of target analytes

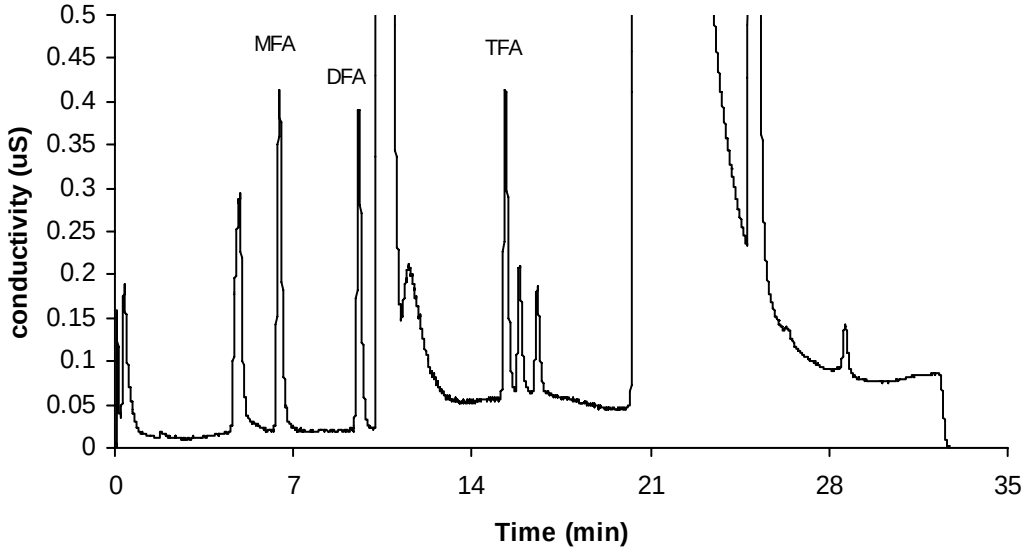
Name	Abbreviation	Formula	pK_a	ε ($L.mol^{-1}.cm^{-1}$)
Monofluoroacetate	MFA	CH_2FCO_2H	2.59	1.74×10^3 (187nm)
Difluoroacetate	DFA	CHF_2CO_2H	1.33	1.57×10^3 (187nm)
Trifluoroacetate	TFA	CF_3CO_2H	0.50	4.47×10^3 (190nm)

3.3.4 Applications

Environmental concerns are high in areas where MFA baits have been laid, particularly with regard to MFA being leached from baits and entering watercourses and soil. After the carrot bait has been ingested by an animal, the MFA will enter the body tissues, and finally be excreted in the urine etc. Three water samples supplied by Analytical Services Tasmania was analysed, however no MFA could be detected in these samples. In order to establish that the proposed method could be used for such samples, the water samples were spiked with the fluoroacetates. A representative chromatogram of before-spiked and after-spiked river water samples are shown in Figure 3.4 (a) and (b). Table 3.5 lists the recoveries for each of the fluoroacetates. Figure 3.4 shows well-defined peaks for MFA, DFA and TFA with good separation from chloride, which was the most probable interferent present in these samples. Two smaller peaks were eluted after TFA, but no attempt was made to identify these analytes.



(a)



(b)

Figure 3.4 Ion chromatogram of before-spiked and after-spiked with MFA, DFA and TFA in river water samples. Condition: see Fig. 3.1.Table 3.4 shows that the recovery values for the spiked MFA, DFA and TFA were in the range 96-102%.

Table 3.5. Water Samples Recovery of MDTFA (Spiked 0.2 ppm)

Sample No.	MFA (%)	DFA (%)	TFA (%)
30811	104.7	98.6	98.5
30840	96.4	98.6	97.1
28800	98.8	97.3	98.5

Analysis of carrot baits is also of interest in order to determine the rate of loss of MFA from the baits and hence their practical lifetime. Two kinds of carrot baits were analysed, the first being a bait prepared freshly from raw carrots according to an established method, and the second being an aged sample taken from the field after exposure for three months. The chromatogram for the freshly prepared sample before soaked in 1.5% MFA standard is shown in Figure 3.5 (a), after soaked in 1.5%, MFA standard is shown in Figure 3.5 (b). and produced an MFA level of 37 ppm ± 0.5 ppm, determined from triplicate determinations, (RSD 3.5%). The chromatogram for the aged bait sample is illustrated in Figure 3.6 and depicts a more complex chromatogram than was obtained for the freshly prepared sample, presumably due to the presence of contaminants and degradation products arising from field exposure. A further complication arises from the use the dye, Monsperse Blue, which is an aqueous dispersion of a copper phthalocyanine blue pigment in a surfactant based system containing propylene glycol added as a marker to identify toxic baits. The level of MFA in the aged bait was 88 ± 0.5ppm (RSD 3.8%), which while appearing to be an increase over the fresh bait was in fact due to the aged bait having a very much lower water content than the fresh bait. However, this indicates that MFA in carrot baits is very persistent, and not prone to degradation.

A urine sample was collected from a carcass of a possum after it had consumed a carrot bait soaked in MFA, DFA and TFA solution. The chromatogram for the urine sample is detailed in Figure 3.7, and produced an MFA level of 2.1 ppm ± 0.07 ppm, a DFA level of 1.8 ppm ± 0.09 ppm, and a TFA level of 5.1 ppm ± 0.3 ppm, determined from triplicate determination, (RSD for MFA, DFA and TFA were 2.7%, 3.0 % and 2.9%, respectively). The chromatogram shows MFA, DFA and TFA completely separated from interference peaks, although the MFA peak appears above another large interferent, however the start and end positions are well defined. The results indicate that MFA, DFA and TFA enter the animal's kidney after the carrot bait was consumed.

Figure 3.8 (a) details the results of the soil sample that was supplied by the Analytical Services Tasmania Laboratory, a section of the Department of Primary Industry, Water and Environment, Tasmania, Australia. Figure 3.8 (b) details the results of the soil sample were spiked with a standard mixture containing 4.0 ppb of each of the three fluoroacetates. The recoveries for MFA, DFA and TFA were 95%, 96% and 92% respectively. Although the MFA peak was close to formate, the resolution between MFA and formate was calculated to be 0.71. The remaining two fluoroacetates are well separated from possible interferences and are easily quantified.

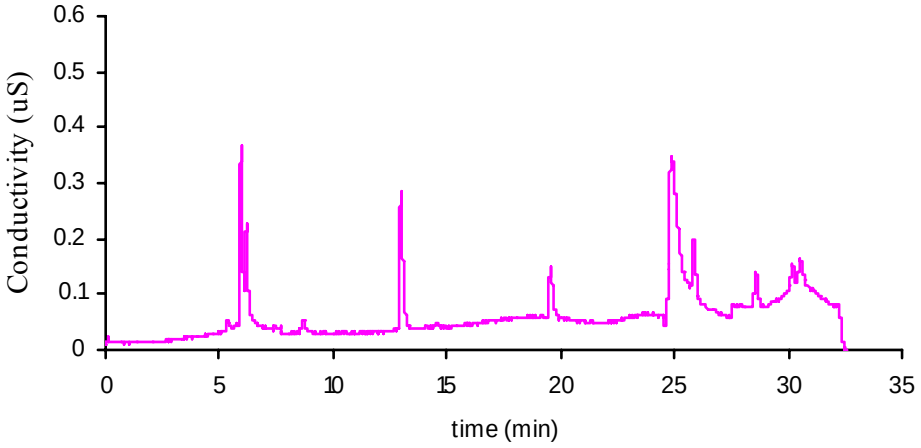


Figure. 3.5 (a) Ion chromatogram of carrots before soaked in 1.5 % MFA standard solution. Conditions: see Figure. 3.1

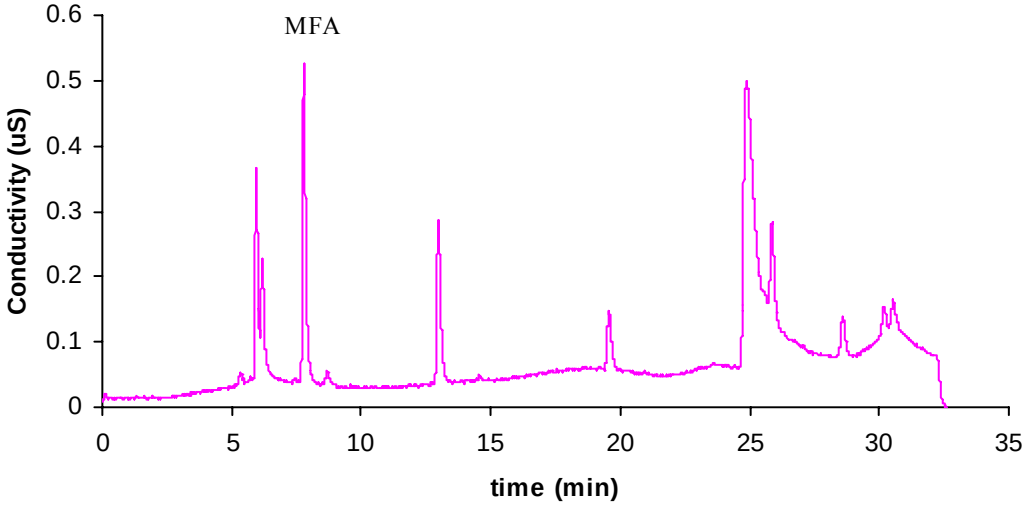


Figure. 3.5 (b) Ion chromatogram of carrots soaked in 1.5 % MFA standard solution. Conditions: see Figure. 3.1

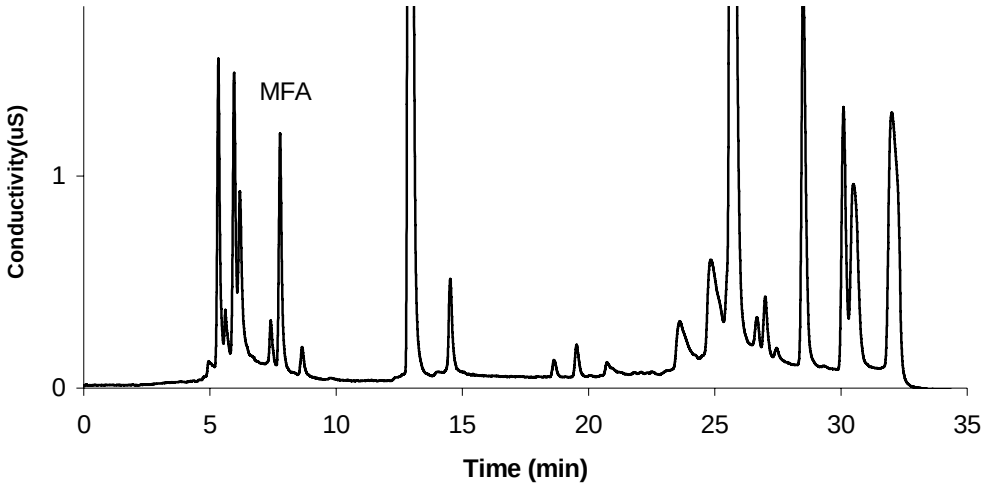


Figure 3.6 Ion chromatogram of field carrot baits. Conditions: see Figure. 3.1.

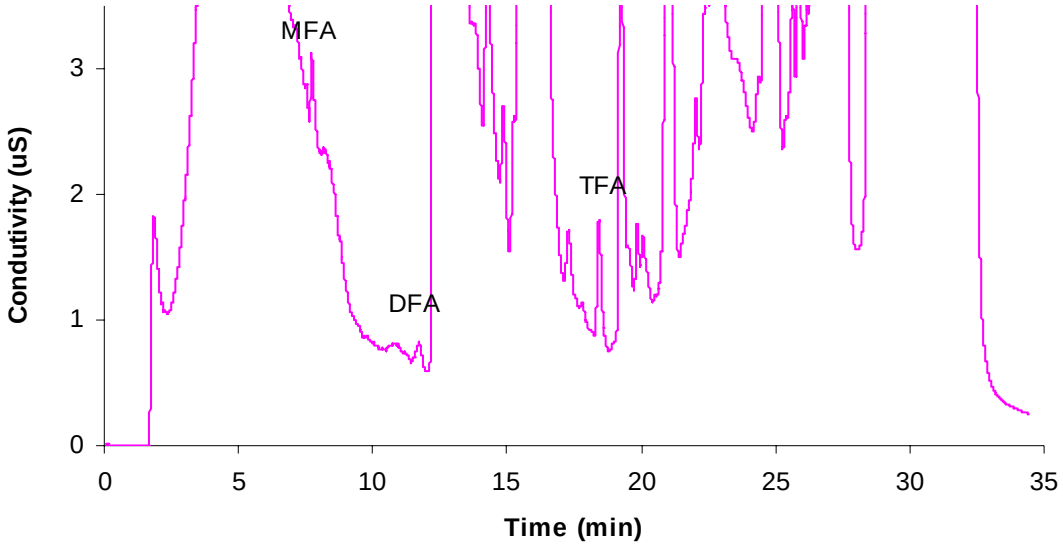


Figure 3.7 Ion chromatogram of urine sample. Conditions: see Figure. 3.1.

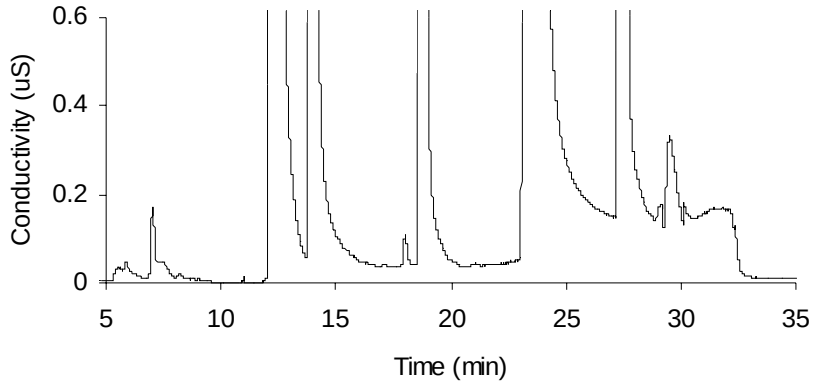


Figure 3.8 (a) Ion chromatogram of soil sample before-spiked with MFA, DFA and TFA . Conditions: see Figure. 3.1.

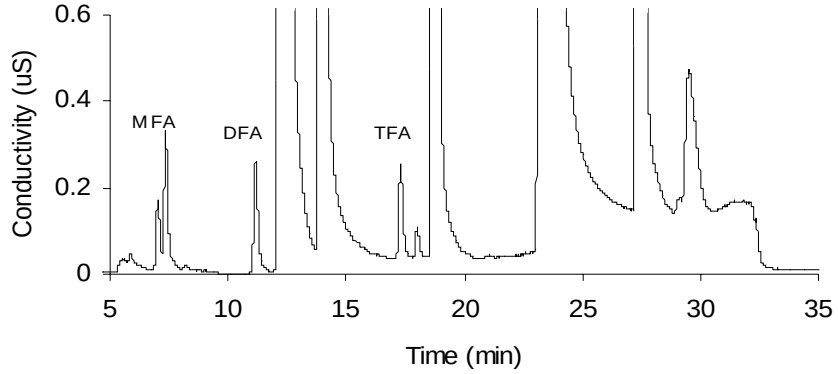


Figure 3.8 (b) Ion chromatogram of soil sample after-spiked with MFA, DFA and TFA. Conditions: see Figure. 3.1.

3.4 Conclusions

Simultaneous determination of fluoroacetates was achieved using anion-exchange ion chromatography with suppressed conductivity detection. Under optimised conditions, MFA, DFA and TFA were rapidly and completely separated with high detection sensitivity. The method suffers from no significant interferences from concurrent inorganic and organic ions, and was successfully used to analyse MFA in carrot and water samples. Compared to existing alternative methods for fluoroacetates, IC offers a straightforward and convenient approach to the routine analyses of fluoroacetates.

3.5 Reference

1. E. R. Kalmbach, *Science*, 1945**,** 102, 232 - 233.
2. N. I. Sax, R. J. Lewis, Dangerous Properties of Industrial Material, Van Nostrand Reinhold, New York, 7th Ed., 1989, 3, 1747 - 3066.
3. B. A. Kimball, E. A. Mishalanie, *J. Chromatogr*. 1993, 634, 289 - 296.
4. Chemwatch Newsletter Quarterly CD Release 2003/2
5. P. T. Visscher, C.W. Culbertson, R. S. Oreml, *Nature*, 1994, 369, 729.
6. Method 552, The Determination of haloacetic acids in Drinking Water by Liquid/Liquid Extraction and Gas Chromatography with Electron Capture Detection, US Environmental Protection Agency, Cincinnati, OH, 1989**.**
7. H. Ozawa, T. Tsukioka, *Anal. Chem.* 1987, 59, 2914 - 17.
8. H. Ozawa, T. Tsukioka, *J. Chromatogr*. 1989, 473, 251-259.
9. D. M. Collins, J.P. Fawcett, C.G. Rammell, *Bull. Environ. Contam. Toxicol*. 1981, 26, 669-73.
10. D. Collins, *New Zealand Journal of Science*, 1983, 26, 283 - 286.
11. H. L. Kramer, *J-Assoc-Off-Anal-Chem.* 1984, 67, 1058 - 1061.
12. A. C. Ray, L. O. Post, J. C. Reagor, *J. Assoc. Off. Anal. Chem.* 1981, 64, 19 - 24.
13. M. Imbenotte, A. Brice, F. Erb, J.M. Haguenoer, *Talanta*, 1984**,** 31, 147 - 149.
14. L. M. Nair, R. Saari-Nordhaous, J.M. Anderson, *J. Chromatogr*. A, 1994, 671, 309 - 313.
15. N. Simonzadeh, *J. Chromatogr.* A, 1993, 634, 125 - 128.
16. A. F. Kingery, H. E. Allen, *J. Chromatogr.* A, 1994, 671, 231-237.
17. L. A. Kaine, J. B. Crowe and K. A. Wolnik, *J. Chromatogr. A,* 1992, 602, 141-147.
18. P. R. Haddad, P. E. Jackson, Ion Chromatography: Principles and Applications, Elsevier Amsterdam, (1990), 489 - 713.
19. S. F. Mou, C. N. Liu, Ion Chromatography: Methods and Applications, (2000), 229-265.

Chapter 4

Simultaneous determination of monofluoroactetes difluoroactetes and trifluoroactetes in plant samples

4.1 Introduction

The toxic substance in *chapetalum cymosum*, a plant known as "gifblaar" or poison leaf, has been identified as monofluoroacetic acid (MFA) [1] *D.cymosum* is one of the most poisonous plants in southern Africa, causing the sudden death of ruminants in Gauteng, Mpmalanga, North West Province, and Northern Province, as well as in Zimbabwe, Botswana, and Namibia [2]. *D.cymosum* is reported as the fourth most economically important plant poisoning syndrome of livestock in South Africa [2]. The mortality of cattle following the ingestion of *D.cymisum* leaves, especially during the months of March and August to November, has been reported by various authors [3, 4].

MFA is produced by three genera of plants in Australia as a chemically mediated defense strategy against herbivors [5, 6, 7]. Although most of these plants are restricted to the south-west corner of Western Australia, three species are found in northern and central Australia; namely *Gastrolobium brevipes*, *G. grandiflorum* and *Acacia georginae*. In the Northern Territory, *G. grandiflorum* occurs mainly in the Top End but it is also patchily distributed down to the southern edge of the Tanami Desert bioregion in central Australia [8]. The distribution of *G. brevipes* is also patchy, and is restricted to range habitat south of the Tanami Desert between 23^{o} and 26^{o} S. Although the toxic component was not identified at the time, *G. grandiflorum* from central Australia was known to be toxic as early as the 1870´s when it was responsible for stock losses [9]. Air-dried leaves of *G. grandiflorum* are known

to contain up to 185 µg/g of MFA [10]. Leaves of *A.georginae* are less toxic, containing about 25 µg/g [11]. Interestingly, these two species are considerably less toxic than the species from southern Western Australia where many species contain 400 - 2600 MFA µg/g in plant tissues [5, 7, 9, 13,]. Plants of the genus *Gastrolobium* (Legumnosac) also produce considerable quantities of MFA as a chemically mediated defense medium [5, 12, 13]. As a consequence of this, native animals have developed varying degrees of tolerance to this potent poison [5, 13]. The distribution of these plants is described by Twigg and King [5].

Estimates of the concentration of fluoroacetate in most of the 40 species of *Gastrolobium* were undertaken in the early 1970's using colorimetric techniques [14], However, while it was the only technique available at the time, this procedure lacks sensitivity and precision. In addition, the concentration of plant secondary compounds like fluoroacetate generally varies between the different parts of the plants [15], but this has never been examined in any detail for the toxic *Gastrolobium*. The persistence of fluoroacetate in the environment where these *Gastrolobium* occur is also unknown. MFA is used extensively as a predacide and rodenticide. Carrot baits containing 1080 are used for animal control. The poison remains in the carcase of the dead animal and enters the environment as the carcase decays. Thus, it is important to understand the fate of fluoroacetates in the environment. As yet, MFA has not been detected in plant material in Tasmania, although MFA has been detected in plant material in the mainland of Australia. This is sufficient to kill half a million animals. Its effects are prolonged and distressing. Animals stagger around, frightened, disoriented and convulsing, sometimes for days, until they succumb to central nervous system collapse, coronary failure, or are attacked by predators that they cannot fend off. The poison can be extensively transmitted from animal to animal, and then into plants and the environment [16].

Colorimetric procedures have been used for the analysis of MFA in *D.cymosum* [17] and other *Dichapetalum* species [18]. These procedures rely on the formation of coloured complexes with the fluoride or MFA ions, which are then determined spectrophotometrically. The conversion of organic fluorine to inorganic fluoride by ashing followed by colorimetric estimation can possibly lead to erroneous results, particularly in cases where the organic fluorine is present in amounts comparable to, or smaller than, the inorganic fluoride in the plant. The presence of iron or of plant pigments in extracts is known to interfere with the colour produced as a result of MFA. Although these interferences can be removed by adsorption on charcoal, by chromatography on solocic acid [19], or by distillation [20]), the introduction of these clean-up procedures was considered unnecessarily complex and a possible source of error.

Thin-layer chromatographic (T.L.C) methods are, at best, only semiquantitative, as they usually rely on visual comparisons of intensities of spots on thin-layer plates. A T.L.C. method has been used for the estimation of sodium monofluoroacetate in animal tissue [21], and while the method could probably be modified to allow estimation of MFA in plant samples. Such a method was regarded as unsatisfactory for the reason stated above, and also because the small concentrations found in plant materials may not be detectable unless very large samples are extracted.

Gas-liquid chromatography (G.L.C.) has been increasingly applied as a quantitative analytical tool and has been extensively used for the estimation of carboxylic acids, either as the free acids, or a more volatile derivative for the reason stated above, and also because

of the small concentrations found in plant materials not be detectable unless very large samples are extracted.

The determination of MFA in *Acacia georginae* [22], and in *Gastrolobium grandiflorum* [23] was carried out by G.L.C. of extracted MFA. Ethanol was used as the extraction solvent, and an elaborate column chromatographic clean-up was required to remove interfering substances. More recently, the estimation of MFA in animal tissues and stomach contents has been made by converting it to a methyl ester for G.L.C. determination has been reported [24]. Although a G.L.C. method was considered suitable for the purposes, the lack of information on sample preparation, and on extraction efficiencies and recoveries, as well as the need to reduce the number of operations required prior to chromatography, meant that a thorough investigation of these aspects of the analysis was required before the G.L.C. method could be considered suitable.

Gas chromatography confirmed the relatively high concentrations of fluoroacetate found in *Gastrolobiums*, (0.1-3875 µg/g (ppm) dry weight), with young leaves and flowers containing the highest concentrations [25]. However, there was considerable variation between individual plants of at least two coefficients of variation ranging from 94% to 129%. Sample preparation required extraction with deionised water or an alcohol/water mixture (1:1), and removal of interfering compounds on a strongly basic ion-exchange column. MFA was extracted from tea leaves using one of two methods: (1). 10 g samples were left to stand in hot water for 15 min (simulates domestic use of tea), and (2). 1 g of tea leaves were exposed to a multiple alkali infusion for 30 min to enhance the release of MFA. The guar gum samples were extracted for 30 min with hot alkaline water followed by the addition of isopropyl alcohol to coagulate the gel formed in the water. MFA in the aqueous

extract was acidified with hydrochloric acid and converted to the dichloroanilide derivative by using N,N'-dicyclohexylcarbimide (DCC) and 2,4-dichloroaniline (DCA). The derivative was extracted with ethyl acetate, purified on a silica cartridge, eluted with toluene and quantified by gas chromatography with electron-capture detection [26]. The limits of detection were reported as 0.0015 μg/g (ppm) in plant material.

High performance liquid chromatography (HPLC) has also been used confirm the presence of MFA in plant samples. AC-610 organic acid analysis column at ambient temperature with 0.02 M H_3PO_4 as an eluent and ultraviolet detection at 210 nm, was utilised to quantitate MFA. Retention time of MFA is 21.38 min. with the complete run time being 40 minutes.

A recent literature review did not reveal any current work on the determination of DFA and TFA in plant material, nor any published paper employing ion chromatography to determine MFA in plant samples. DFA is reported as being acutely toxic [27]. The LD_{50} for TFA is reported as 200 mg/kg. Few reports on the biodegradation of DFA and TFA are available. Visscher *et al* [28] reported instances of reductive defluorination of TFA under methanogenic and sulfate-reducing conditions. TFA was sequently defluorinated to DFA, MFA, and acetic acid, which was cleaved to yield methane. Under aerobic conditions, the authors also report the production of fluoroform. DFA and TFA could be important for the next research step for plant material in toxicity studies.

This research investigated the use of anion-exchange IC utilising gradient elution with hydroxide eluents, coupled with suppressed conductivity detection. Using this approach, simple sample preparation procedures can be used to produce fast, sensitive, accurate, and economical results. Simultaneous determination of MFA, DFA and TFA has been achieved,

using this method, which suits different kinds of plant material include *Bilobum, Calvcinum* and *Spinosum.*

4.2 Experimental

4.2.1 Instrumentation

Figure 2.2 Block diagram of instrumentation are given in Chapter 2, Section 2.1.

4.2.2 Reagents

The details of chemicals and solutions used is given in Chapter 2, Section 2.2.

4.2.3 Apparatus and operating conditions

The details of ion chromatograph used for this work are given in Chapter 2, Section 2.3.

The optimised gradient profile and ion chromatogram of standard mixture MFA, DFA and TFA is shown in Table 3.1 in Chapter 3.

4.2.4 Preparation of samples

The details of preparation of plant samples are given in Chapter 2, Section 2.3.

4.3 Results and discussion

Optimization of gradient elution for MDTFA separation, interference study, MDTFA standard linearity, detection limits, and reproducibility; detail description is given in Chapter 3, Section 3.3.

4.4 Applications

All plant samples were collected from the Mundaring Weir area ($31^{o}50'$S:$115^{o}55'$E), 35 km from the Perth CBD, which is a restricted access water catchment area. The Vertebrate Pest Research Section, Agriculture Protection Board, Forrest Field, Western Australia, provided

young spring growth plant samples. Simultaneous determination of MFA, DFA and TFA in plant material was performed using the procedure designed in this research. The analysis of plant-A is given in Figure 4.1, and shows MFA, DFA and TFA concentration of 11, 3.5 and 0.22 ppm, with recoveries of 110%, 99% and 91% respectively from spiked samples. As can be observed, all the target peaks are completely separated from adjacent interference. Table 4.1 identifies the plant types and their corresponding coded reference, A to G inclusive, and lists the recovery percentage and concentration (μg/g) for each.

After preparing the samples (see Chapter 2), all were divided into two portions, one portion spiked with a 0.8 ppm standard mixture of each fluoroacetate (MFA, DFA and TFA). Each portion was analysed separately using ion chromatography using the conditions optimised in Section 3.3 of Chapter 3. MFA was found to be present in all of the plant samples, with the concentrations ranging from 10 - 788 ppm for undried plant material, with recoveries in the range 85% - 115%. Figure 4.2 illustrates the chromatogram for plant–C, which was found to contain 145 ppm MFA, with a recovery of 110%. DFA and TFA were not identified in this sample. DFA was present in most of the analysed undried plant samples with a concentration range of 3.5 - 17 ppm, with a recovery range of 85% - 110%, as shown by Figures 4.3 and 4.4. Note that DFA was well separated from the following large peak, which is chloride, an ion frequently in environmental samples. The results in Table 4.1 indicate that TFA was present in some plant samples, in a concentration range from 0.22 - 1.2 ppm. The recovery of the compound was 88%-102%. The foregoing determinations used samples on an “as received”, undried basis.

Plant extracts contain formic and acetic acids, and other volatile fatty acids. The peak indicating the retention time for formic acid (7.64 min) is located just ahead of the MFA

(8.01 min) peak. Formic acid in high concentrations will interfere with the separation of MFA, and especially if the MFA concentration is less than 10 μg/mL. In such a case dilution steps will be required to enable reliable quantitation. To optimise the dilution ratio for this purpose, dilutions of 10:1, 5:1, and 2:1 were analysed separately. A dilution of 2:1 was found to provide an acceptably reduced height of the interference peak. The target peak was also reduced, but sensitivity was adequate. No dilution was required for the analysis of DFA or TFA in this low concentration range. In the situation just cited, two determination runs will be required.

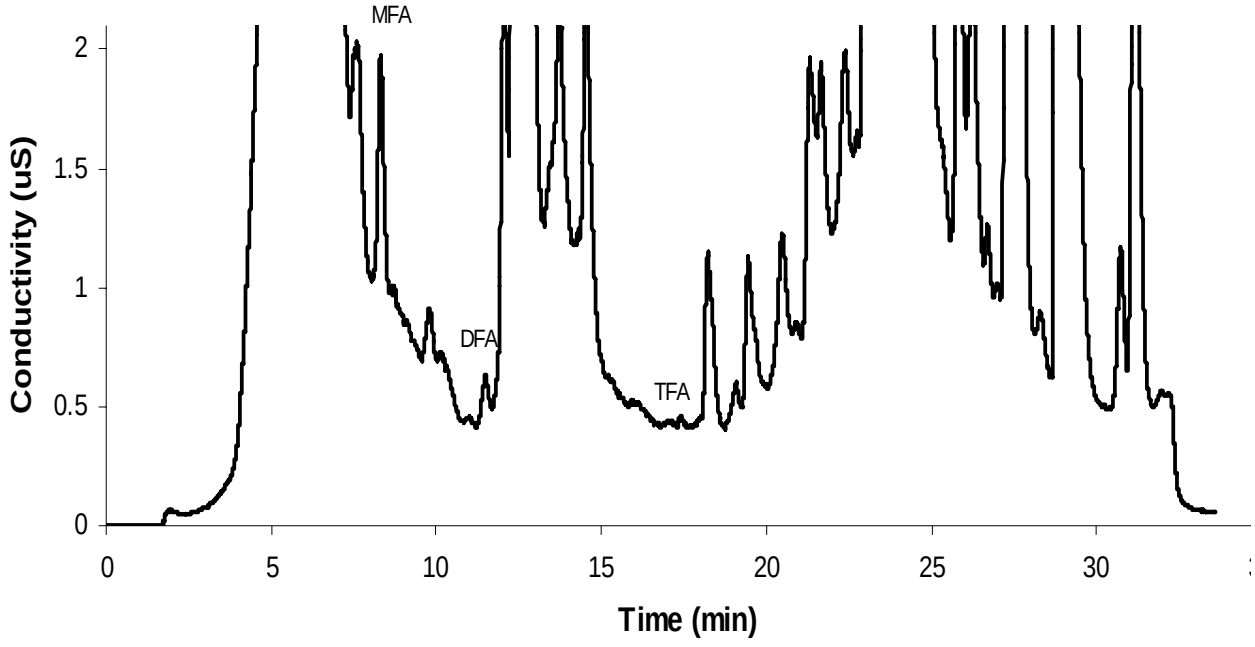


Figure 4.1. Ion chromatogram of Plant-A. MFA, DFA and TFA in *Bilobum* material.

Conditions: see Figure.3.1.

Table 4.1. Contents (μg/g) and recovery (%) of MDTFA in plants

Sample Name*	MFA				DFA				TFA			
	Retention time(min)	Area	Result (μg/g)	Recovery %	Retention time (min)	Area	Result (μg/g)	Recovery (%)	Retention time(min)	Area	Result (μg/g)	Recovery %
Plant-A	7.59	0.145	11.0	110	11.5	0.0940	3.50	99	17.4	0.0190	0.220	91
Plant-B	7.63	8.90	788	89	ND**	ND	ND	89	ND	ND	ND	88
Plant-C	7.41	1.66	145	110	ND	ND	ND	85	ND	ND	ND	89
Plant-D	7.39	0.135	10.0	85	11.1	0.190	7.80	99	17.0	0.0280	1.20	86
Plant-E	7.29	0.121	8.60	94	11.0	0.218	8.90	93	ND	ND	ND	98
Plant-F	7.24	0.186	14.0	115	11.2	0.391	17.0	87	ND	ND	ND	102
Plant-G	7.20	0.168	13.0	95	11.0	0.342	15.0	110	ND	ND	ND	95

*Plant types: A, B and C are *Bilobum*, D and E are *Calvcinum*, F and G are *Spinosum*.

** ND: Not Detected.

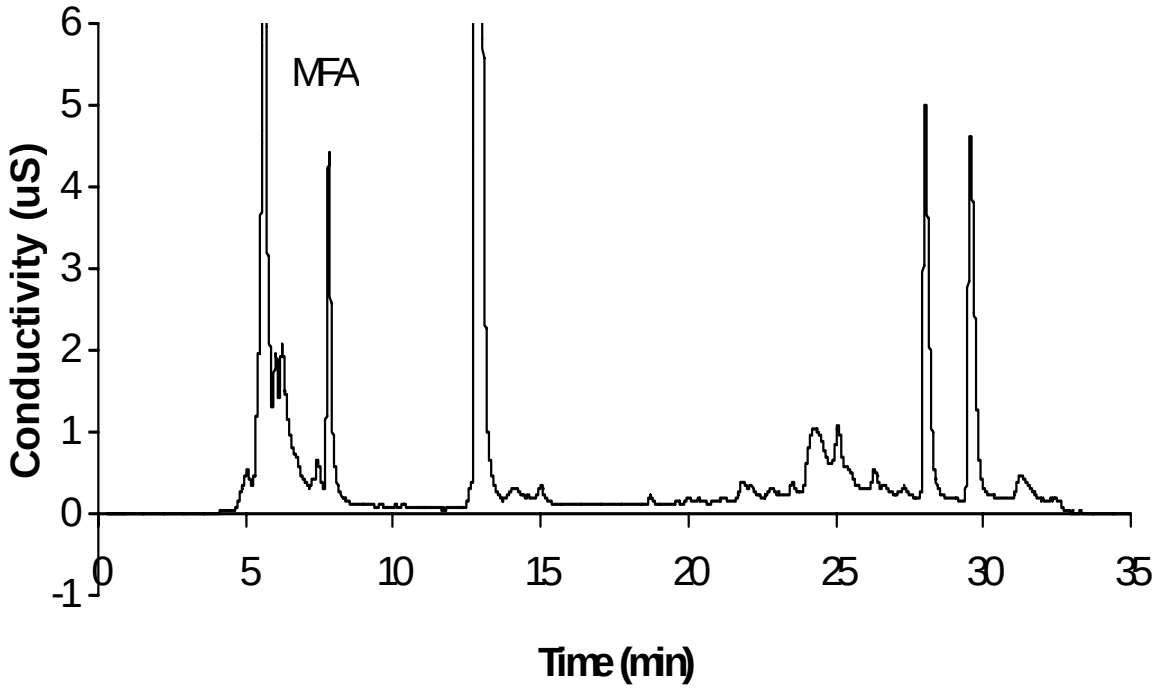


Figure 4.2. Ion chromatogram of Plant-C. MFA in *Bilobum* material. Conditions: see Figure.3.1.

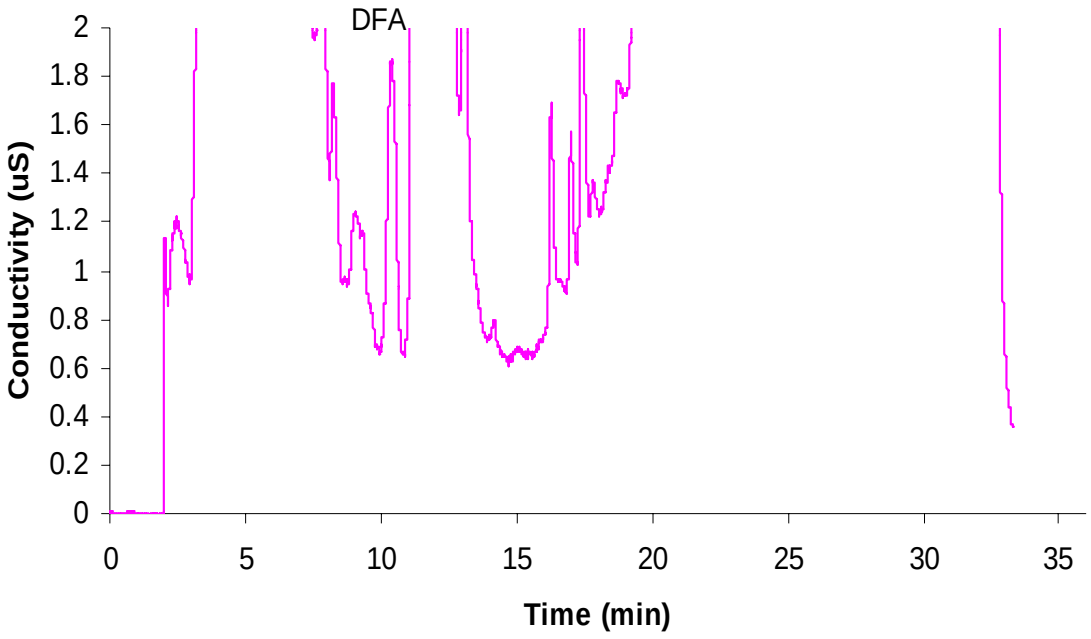


Figure 4.3. Ion chromatogram of Plant-G. DFA in *Bilobum* material. Conditions: see Figure.3.1.

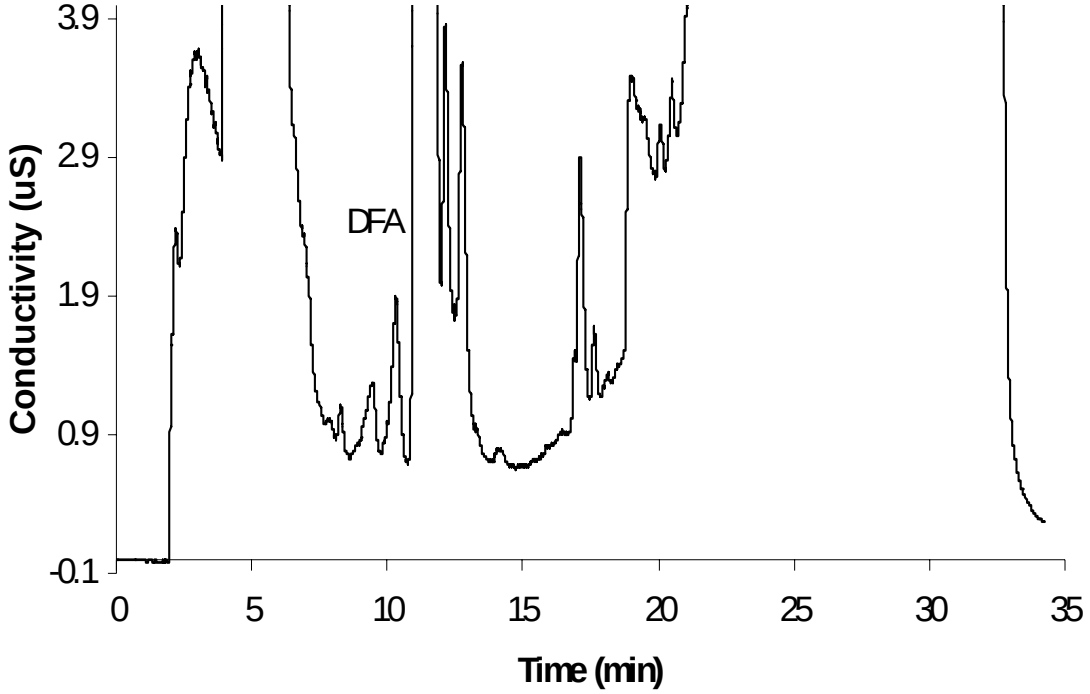


Figure. 4.4. Ion chromatogram of Plant-F. DFA in *Bilobum* material. Conditions: see Figure.3.1.

4.5 Conclusions

The method described combines simplicity and minimum sample preparation with adequate speed and precision for the simultaneous determination of MFA, DFA and TFA in the presence of other organic and inorganic anions. Using a reasonable dilution method it is possible to eliminate formic acid and other volatile fatty acids from interference with the target ions. This IC procedure was well suited to the analysis of organic acids in different plant material samples. Under the conditions described in this method, large numbers of samples can be analysed in a relatively short period of time (35 min per sample). This makes the technique useful for isolation and identification of the toxic components of plant material. Seven real plant samples from three sources were successfully analysed by the developed method.

4.6 References

1. J. S. C. Marais, *Monofluoroacetic acid, the toxic principle of Cifb*; *aar*, Dichapetalum cymosum. Onderstepoort *J. Vet.Sci. Anim.ind*. 1944, 20 (1), 67-73
2. T. S. Kellerman, T.W.Naude, The distribution, diagnoses and estimated economic impact of plant poisonings and mycotoxicoses in South Africa. Onderstepoort *J. Vet. Res*.1996, 63, 65-90.
3. D. G. Steyn, Vergiftiging van Mens en Dier. Van Schaiks, Pretoria, South Africa, 1949,pp 144 - 145 .
4. T. S. Kellerman, J. A. W. Coetzer, T, W. Naude,. *Plant Poisonings and Mycotoxicoses of livestock in Southern Africa*. Oxford University Press, Cape Town, South Africa, 1988, 108 - 110.
5. L. E. Twigg, D. R. King, The impact of fluoroacetate-bearing vegetation on native Australian fauna: A review. Oikos 1991, 61, 412 - 430.
6. M. D. Crisp, P. H. Weston, Cladistics and legume systematics, with an analysis of the Bossiaeae, Brongniartieae and Mirbelieae. In 'Advances in Legume Systematics, Part 7, *Phylogeny*'1994, pp, 65-130 (Royal Botanic Gardens, Richmond Press: Surrey,UK.) .
7. L. E. Twigg, D. R. King, L. H. Bowen, G. E. Wright, C. T. Eason. The fluoroacetate content of some species of the toxic Australian plant genus, *Gastrolobium*, and its environmental persistence. *Natural Toxins* 1996a, 4, 122 - 127.
8. D. E. Albrecht, A.W. Duguid, P. K. Latz, H. Coulson, M. J. Barritt, Vascular plant checklist for the southern bioregions of the Northern Territory; nomenclature, distribution and conservation status. Parks and Wildlife Commission of the Northern Territory, Alice Springs.
9. Poison plants of Western Australia: the toxic species of Gastrolobium and Oxylobium. *Western Australian Department of Agriculture bulletin* 1971, 3772, 1 - 66 .

10. Isolation and identification of the toxic principle of Gasteolobium grandiflorum. Queensland, *J. Agricul. Sci*. 1964, 21, 1 - 14.

11. P. B. Oelrichs, T. McEwan, Isolation of the toxic principle of Acacia georginae. *Nature* 1961, 190, 808 - 809.

12. L. E. Twigg, D.R King, *Westen Australian Department of Agriculture Bulletin* 1971, 3772, 1 - 66 .

13. L. E. Twigg, The Royal Society of New Zealand, *Miscellaneous Series* 1994, 28, 97 - 115.

14. Poison plants of Western Australia: The toxic species of Castrolobium and Oxylobium. *Western Australian Department of Agriculture Bulletin* 3772:1- 66.

15. R. G. Cates, D. E. Rhodes, *Biochem Syst Ecol* 1977, 5, 185 - 193.

16. The 1080 Story; http://www.trump.net.au/~glazfolk/valley/1080.htm (2002)

17. N. Louw, floormetabolisme in Dichapetalum cymosum. M.Sc. thesis, University of Pretoria. 1968.

18. B. Vickery, M. L. Vickery, Fluoride metabolism *in Dichapetalum cymosum. Phytochem.* 1972, 11, 1905.

19. A.O.A.C. 1970. Methods of Analysis of the Association of Official Analytical Chemists, 11th ed., Washington, D.C., P. 504.

20. B. Viclery, M. L. Viclery, J. T. Ashu, *Analysis of plants for fluoroacetic acids. Phytochem.* 1973, 12, 145.

21. V. A. Perry, Thin layer chromatographic determination of sodium monofluoroacetate. J. *Ass. off. anal. chem*. 1970, 53, 737.

22. P. B. Oelrochs, T. McEwan, The toxic principle of Acacia georginae. *QdJ.agric. Sci*. 1962, 19, 1.

23. T. McEwan, Isolation and identification of the toxic principle of Gastrolobium grandiflorum. *QdJ. Agric. Sci*. 1964, 21, 1

24. M. H. Stahr, W. B. Buck, P. F. Ross, Gas-liquid chromatographic determination of sodium monofluoroacetate. *J. Ass. off. Anal. Chem*. 1974, 57, 405.

25. L. E. Twigg, D. R. King, *Natural Toxins*. 1996, 4, 122 - 127.

26. H. Ozawa, T. Tsulioka, Gas chromatographic determination of sodium monofluoroacetate in water by derivatisation with dicycohexylcarbodiimide. *Anal Chem* 1987, 59, 2914 - 2917.

27. Chemwatch Newsletter Quarterly CD Release 2003/2

Chapter 5

Simultaneous determination of fluoroacetates, chloroacetates and bromoacetates in soil samples by ion chromatography

5.1 Introduction

Haloacetates generally refer to MDTFA, MDTCA and MDTBA, and exist widely in the biosphere. These arise from photochemical degradation of halogenated hydrocarbons from direct anthropogenic emissions. Furthermore, there is also evidence of natural sources, although these are quantitatively uncertain. Other possible sources of haloacetates are waste incineration [1], and the break down of fluorinated pesticides [2,3].

MDTFA typically includes mono-(MFA), di-(DFA) and tri-fluoroacetate(TFA). MFA is produced naturally at toxic levels in southern hemisphere plants [4]. TFA is believed to be predominantly an atmospheric oxidation product of chlorofluorocarbon (CFC) substitutes, such as 1,1-dichloro-2,2,2-trifluoroethane (HCFC-123), 1-chloro-1,2,2-tetrafluoroethane (HCFC-124), and 1,1,1,2-tetrafluoroethane (HFC-134a) [5,6,7]. Few reports on the biodegradation of TFA are available [8].

MDTCA, which cover mono-(MCA), di-(DCA), tri-chloroacetates (TCA), can be formed via atmospheric breakdown of the airborne C2-chlorocarbons, trichloroethene, tetrachloroethene, and 1,1,1-trichloroethane, which are all used as solvents for degreasing and dry-cleaning [9]. However, the industrial production of 1,1,1-trichoroethene has progressively decreased over the past decade.

MDTBA, including mono-(MBA), di-(DBA) tri-bromoacetate (TBA), are suggested to be atmospheric degradation products of brominated hydrocarbons, released into the atmosphere by marine organisms[10].

Haloacetates are highly soluble in water; consequently hydrometeors introduce these toxins to the soil. In 1998, rain and fog water samples taken in Northeast Bavaria, Germany, were subjected to gas chromatographic analysis and concentrations of up to 11 μ/L, for MFA, DFA, TFA, MCA, DCA, TCA, MBA and DBA [11] were obtained. Also, trace amounts of these acids are found in drinking water as chlorination by-products [12]. The occurrence of haloacetates at such high levels in the environment indicates their presence in the soil is to be expected.

Some haloacetates are to present as natural plant products [13,14]. Over the past 15 years, the number of haloacetates-containing agricultural chemicals increased in number faster than non-haloacetate agrochemicals. These compounds are primarily used as herbicides (48%), insecticides (23%), and fungicides (18%). Dichloro-, trichloro-, bromochloro-, dibromo-, tribromoacetic acids were found to be carcinogenic, even at low concentrations [15]. Professional reports indicate that organic fluorine has been detected in the blood of individuals from the general public as well as industrial workers [16,17].

Due to their toxicity, and the wide existence of MDTFA, MDTCA and MDTBA in the environment, federal regulation for their monitoring is being considered, and active quantification methods are required to be developed. The standard method for their determination [18] requires a lengthy procedure, based on a liquid-liquid extraction with methyl *tert*-butyl ether, followed by esterification with diazomethane and gas chromatographic analysis. The low detection limits (0.5 μg/L) achievable compensate for this time consuming procedure. Pre-concentration, followed by ion chromatographic

separation has been used for the determination of MBA, DBA, MCA, DCA and TCA in drinking water samples [12]. Anion-exchange chromatography has been used for the determination of MCA, DCA, TCA, TFA, MBA and DBA in peptide and herbicide samples [19]. Recently, a liquid chromatographic method, coupled with negative ion electrospray mass spectrometry, has been proposed by Hashimoto and Otsuki [20]. The method, when applied to the samples of haloacetic acids containing DCA, MBA, TBA, MCA and MBA, requires extraction of the analytes with methyl *tert* -butyl ether coupled with a controlled stream of dry nitrogen to increase concentration. Although the limits of detection achieved were good (0.003-0.070 μg/L), few details about the chromatographic separation of the mixture of analytes are provided. Rochl and Slingsby reported the application of IC-ESI-MS for the determination of MCA, DCA, TCA, MBA, DBA and TBA in water [21], however co-elution problems among MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA and TBA were not resolved.

The purpose of this section was to develop an ion chromatographic method for the separation and analysis of MFA, DFA, TFA, MCA, DCA, TCA, MBA and DBA in soil. The optimisation of the chromatographic method and the gradient elution conditions have been applied to these nine compounds, resulting in complete separation which is free from interference. The conflict from chloride, which commonly occurs in soil, and bromide that is much less common, were studied and overcome.

5.2 Experimental

5.2.1 Reagents

Stock solutions containing mono-fluoroacetate, di-fluoroacetate, tri-fluoroacetate, mono-chloroacetate, di-chloroacetate, tri-chloroacetate, mono-bromoactate, di-bromoacetate, and tri-bromoacetate were prepared from ACS grade, or analytical reagent grade chemicals, obtained from Aldrich (Milwaukee, WI, USA). Milli-Q water (Millipore, Milford, MA, U.S.A.) and used to prepare all solutions, including eluents, stock and standard solutions. All other chemicals employed were AR grade unless otherwise specified. The details of chemicals and solutions used are provided in Section 2.2 of Chapter 2.

5.2.2 Apparatus and operating conditions

The details of ion chromatograph used for this work are given in Section 2.1 and 2.2 of Chapter 2.

5.2.3 Preparation of samples

The details of preparation of the soil samples are detail in Section 2.4 of Chapter 2.

5.3 Results and discussion

5.3.1 Optimisation of gradient elution for the separation of fluoroacetates, chloroacetates and bromoacetates

The chromatographic behaviour of some of the haloacetates is very similar, while others are quite different. In order to separate the nine haloacetate compounds in a single run, with a suitable run time, the gradient conditions used required further optimisation.

A potassium hydroxide eluent, produced with the EG40 eluent generator, was chosen as a means of preparing the gradient eluent, since it can be easily suppressed, allowing for conductivity detection. To obtain the optimum separation condition for the fluoroacetates, chloroacetates and bromoacetates, fourteen gradient profiles were designed and tested. Of these, some lacked satisfactory separation, while others had unacceptably long retention times. Four were chosen (A, B, C, and D) for a more detailed study. The variations in potassium hydroxide concentration for each of the profiles are shown in Figure 5.1, and detailed in Table 5.1.

The optimisation of the gradient profiles, with the aim of obtaining the best separation with the shortest retention times, was performed empirically through a detailed study of the retention factors (k′). The relationship between the retention factor (k′), and retention time is expressed as: $k' = (t_{Rn} - t_0) / t_0$; $t_{Rn} = t_{R1}, t_{R2}$, where t_0 is the injection time of the solvent and t_{Rn} is the retention times of the ions being analysed. The relationship between them for the various haloacetic acids is illustrated in Figure 5.2. The experimental k′ values for gradient profiles A, B, C and D are summarised in Table 5.2, and detailed in Figure 5.3.

From Figure 5.3, it can be seen that for Profiles A and C, not only is the separation efficiency lower, but, the separation time is longer than for Profiles B and D. Further more, a comparison of Profiles B and D, indicates that D has an uncommonly short analysis time and the separation efficiency also is better than B. As a result, Profile D was selected for the subsequent study, as it gave the best separation conditions with the shortest analysis time. Ion chromatograms for Profile D are shown in Figure 5.4. The peaks appearing between analytes labelled 7 and 8 are gradient system peaks of unknown origin.

Table 5.1. Optimised gradient profile for separation of nine haloacetatic acid by anion-exchange IC

Time (min)	Flow (mL/min)	KOH (mmol/L)	Comment
Initial	1.0	0.5	
0	1.0	0.5	Sample injection
12	1.0	1.8	
15	1.0	9.0	
25	1.0	9.0	
26	1.0	60	
40	1.0	80	
40	1.0	0.5	
50	1.0	0.5	End of step gradient

Table 5.2. Retention factor (k′) of nine haloacetates under four gradient profiles

Profile	MFA	MCA	DFA	MBA	TFA	DCA	DBA	TCA	TBA
A	0.62	1.36	1.57	1.78	3.46	3.62	4.26	5.61	7.69
B	0.62	1.38	1.60	1.77	3.57	3.75	4.42	5.44	7.08
C	0.53	1.21	1.41	1.60	4.30	4.39	4.82	5.81	7.85
D	0.61	1.38	1.60	1.81	2.56	2.84	3.61	5.06	6.65

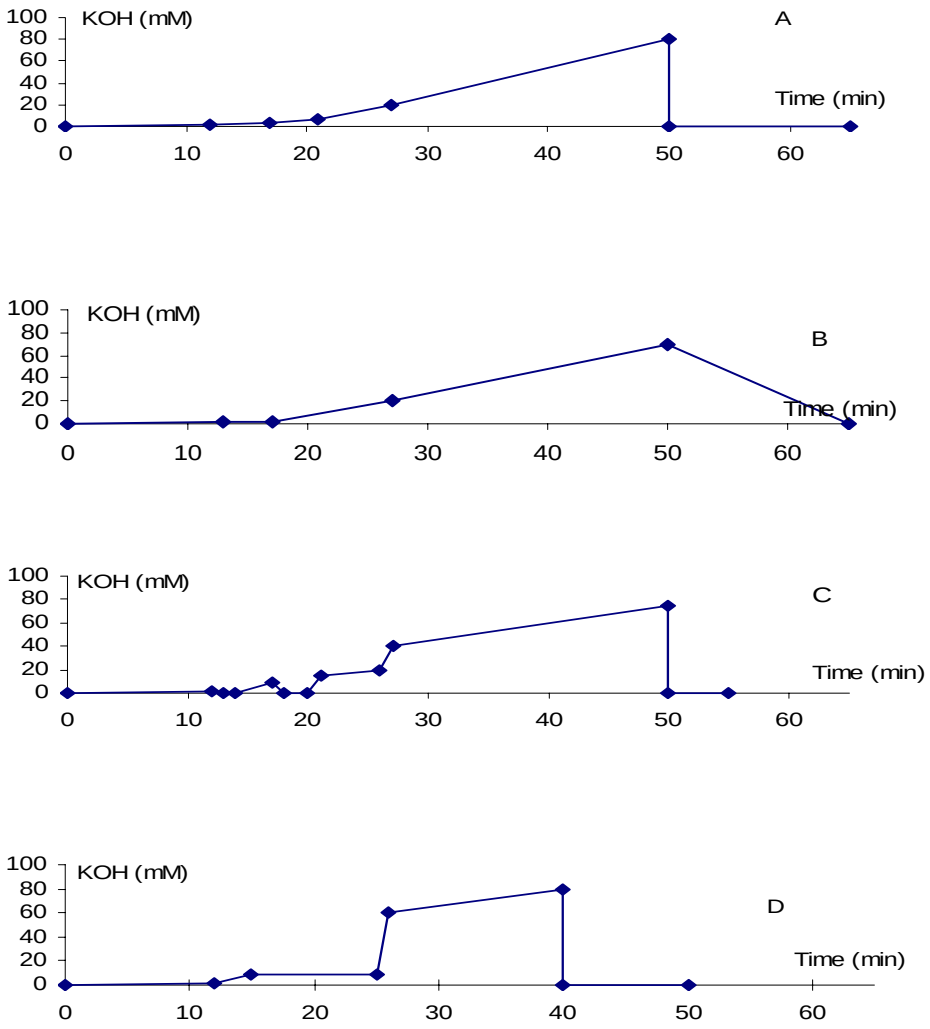


Figure 5.1. Gradient profiles A, B, C, and D

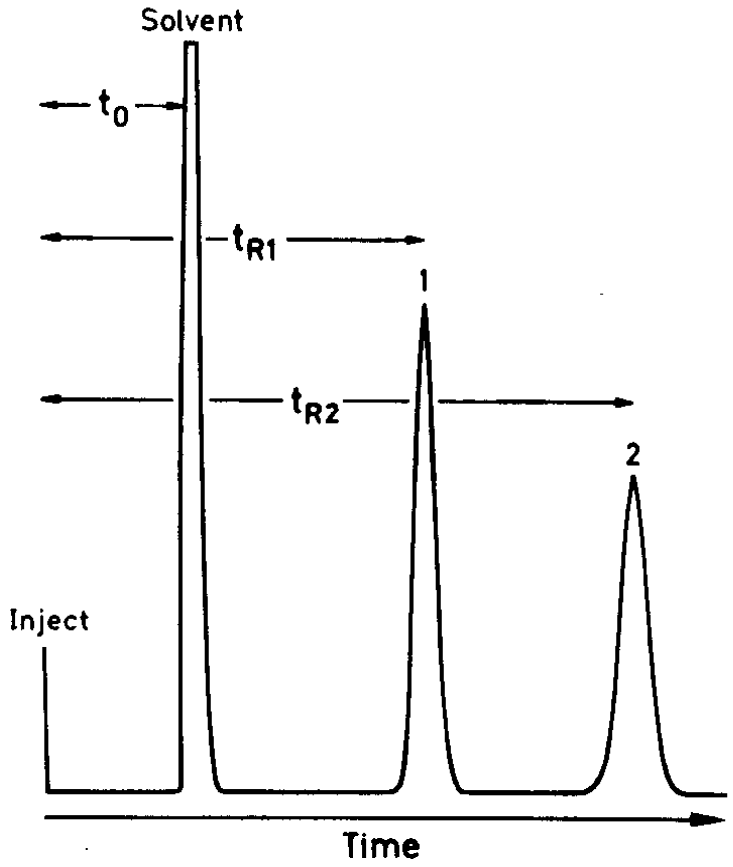


Figure 5.2. The relationship between the retention factors (k′), and retention times of t_0, t_{Rn}.

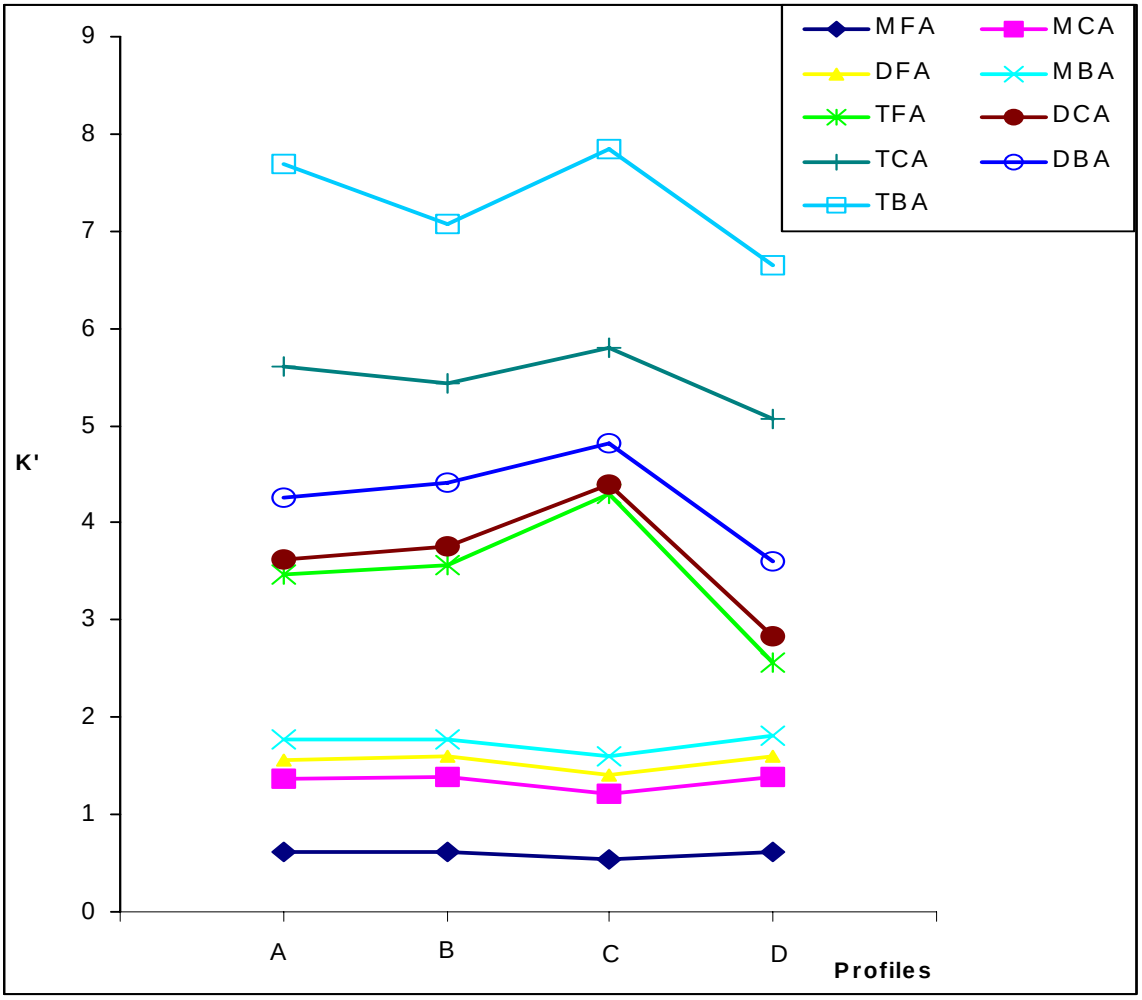


Figure 5.3. Retention factor (k′) of nine haloacetates under four gradient profiles.

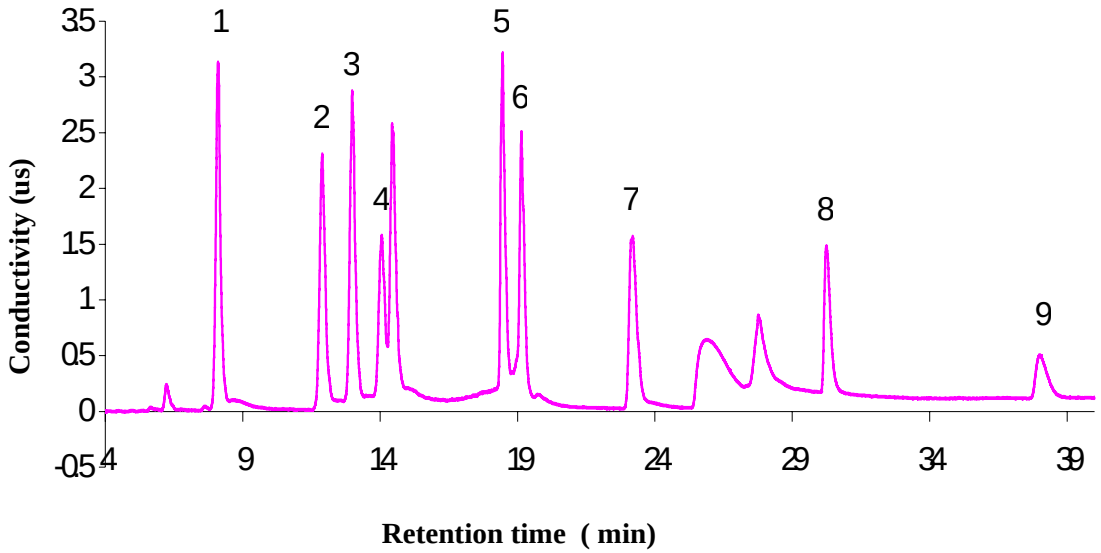


Figure 5.4. Gradient elution separation of haloacetates. gradient profiles D. column: Dionex Ion Pac AS17 (4×250 mm) plus AG17 Guard column; Injection volume, 50 µL; Detection, suppressed conductivity ASRS®- ULTRA. Suppressor current of 50 mA-100 mA; column temperature of 30°C; pump flow rate of 1.00 mL/min. Dionex Peaknet 6.3 software. Analyte concentrations: 1.0 ppm. Peaks: 1.Monofluoroacetate 2. Monochloroacetate 3. Difluoroacetate 4. Monobromoacetate 5. Trifluoroacetate 6. Dichloroacetate 7. Dibromoacetate 8. Trchloroacetate 9. Tribromoacetate

5.3.2 Retention order

Under the above experimental conditions, the nine haloacetate compounds were eluted in the following order:

MFA<MCA<DFA<MBA<TFA<DCA<DBA<TCA<TBA

Their retention times and other relative physiochemical properties are listed in Table 5.3 From their pK_a values, which decrease in the order MFA>DFA>TFA and in the order F> Cl> Br, it is clear that the haloacetic acids are completely ionised under the conditions for gradient Profile D. The relationship between the pK_a and retention time for fluoroacetates, chloroacetates and bromoacetates is an inverse relationship such that a low pK_a corresponds to a long retention time. Within the group of nine haloacetates there are three subgroups namely, MFA, MCA, MBA; DFA, DCA, DBA, and TFA, TCA, TBA. The general retention time order for the three subgroups is nFA<nCA<nBA, where n, may be mono-, di- or tri-. This general trend can be attributed to the physical size of the F^-, Cl^- and Br^- ions.

However, when the analytical system contains all nine ions: namely MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA and TBA, the pKa value alone does not correlate with or predict with the retention order. Generally speaking, in ion-chromatography the elution order is mainly affected by ionic charge, size and solubility, providing the elution conditions remain the same. For haloacetates, all carry a unit negative charge, which means that the hydrated size polarisability and phase solubility of the ions are the main factors that affect the retention order, see Table 5.3.

Table 5.3. Retention order of haloacetates and some relative physical chemistry properties.

Analyte in retention order	Formula	Retention time (min)	Relative ion size	pk_a value [1]	Limiting equivalent ionic[1] conductance
MFA	CH_2FOOH	8.00	201	2.59	44.4
MCA	$CH_2ClCOOH$	11.83	236	2.85	39.7
DFA	CHF_2COOH	12.94	235	1.33	
MBA	$CH_2BrCOOH$	14.00	251	2.90	39.2
TFA	CF_3COOH	18.48	269	0.50	
DCA	$CHCl_2COOH$	19.40	305	1.26	38.3
DBA	$CHBr_2COOH$	23.25	335	1.39	
TCA	CCl_3COOH	30.25	374	0.52	36.6
TBA	CBr_3COOH	38.29	419	-0.15	

5.3.3 Interference study

The common anionic species expected to be present in field samples include chloride, fluoride, sulfate, nitrate, nitrite, phosphate and formate. Their retention times, as well as those of the target ions, fluoroacetates, chloroacetates and bromoacetates, were determined under the operating condition described in Section 5.3.1, and the obtained results are detailed in Table 5.4.

Table 5.4. Retention times for interference species and target compounds

Interference Ions	Retention time (min)	Target Ions	Retention time (min)
F^-	5.70	MFA	8.00
CH^3COO^-	6.15	MCA	11.83
$HCOO^-$	7.59	DFA	12.94
Cl^-	14.50	MBA	14.00
NO_2^-	16.25	TFA	18.48
Br^-	18.97	DCA	19.40
NO_3^-	19.80	DBA	23.25
SO_4^-	27.73	TCA	30.25
PO_4^-	27.99	TBA	38.29

Many field samples contain a high concentration of chloride ion, which has a retention time of 14.50 min, and is close to that of MBA (14.00). Thus, the presence of chloride is a possible source of interference in this analysis. As a first step, different chromatographic conditions were investigated to remove this problem, however this resulted in reduced detection limits, and the appearance of other interferences. Likewise, the retention times for TFA, Br^- and DCA are 18.48, 18.97 and 19.40 min, respectively. Consequently, the retention time for bromide ion lies between those of TFA and DFA. At concentrations less than 5 ppm, the separation of bromide from TFA and DFA is possible, however there exists a potential interference in samples containing bromide in excess of 5 ppm. This means that practical methods need to be developed to eliminate any interference caused by the presence of chloride and bromide ions in samples. Three possible solutions to this problem were examined.

5.3.3.1 Silver chloride precipition

The solubility product constants (K_{sp} values) for the slightly soluble silver salts possible from the ions present in these solutions are:

$$K_{AgCH_3COO} = 1.94\times 10^{-3},\quad K_{AgCl} = 1.77\times 10^{-10},\quad K_{AgBr} = 5.35\times 10^{-13}$$

So, if $[Ag^+] = 1.0$ mM, then

$$[Cl^-] = K_{AgCl}/[Ag^+] = 1.77\times 10^{-7}\text{M}$$

$$[Br^-] = K_{AgBr}/[Ag^+] = 5.35\times 10^{-10}\text{ M}$$

$$[CH_3COO^-] = K_{AgCH_3COO}/[Ag^+] = 1.94\text{ M}$$

This means that if a 1 mM equilibrium concentration of $AgNO_3$ is maintained in to a sample solution containing chloride, bromide, and acetate ions, the Cl^- and Br^- ions will be precipitated much earlier than CH_3COO^-. The resulting free ion concentrations of Cl^- and Br^- will be so low that they will not interfere with the determination of the target analytes. One of the limitations of the method is the introduction of NO_3^- as the Cl^- and Br^- ions are removed. From Table 5.4, the retention time of NO_3^- is close that of to DCA. If the concentration of NO_3^- is higher than 0.05mM, it will become another source of interference. It was found that the method could tolerate a maximum NO_3^- concentration of 15 ppm introduced by the removal of Cl^- and Br^- interference by $AgNO_3$. Fortunately, in most environmental samples, the concentration of Br^- is typically very low and only ppb levels of $AgNO_3$ need to be added.

5.3.3.2 Solid-phase extraction with a silver cartridge

The sample pretreatment using Dionex OnGuard II Silver Cartridges was also performed as a means of sample clean up prior to analysis. These cartridges were chosen since they are suitable for matrix elimination and some specific solid phase extraction methods. Matrix elimination is a method of sample preparation that removes interfering species from the sample matrix by specifically binding them to a solid phase, leaving the analyte for subsequent determination. The cartridge contains a high capacity, strong acid cation-exchange resin in the silver form. The OnGuard II silver cartridge is capable of removing Cl^-, Br^-, I^-, AsO_4^{3-}, CrO_4^{2-}, CN^-, MoO_4^{2-}, SO_3^-, S^{2-}, PO_4^{3-}, SeO_3^{2-}, $SeCN^-$ and WO_4^{2-} by precipitation. This sample clean up method has been successfully employed for reducing interferences as a result of the presence of chloride and bromide matrix ions. The 2.5 mL cartridge has a capacity of 5.0-5.5 meq, on a water-swollen basis, which means that approximately 23 mL of a 1% by weight NaCl solution may be treated with each cartridge [22].

In order to assess the effects of this silver cartridge on target analytes, recoveries for the ions MFA, DFA, TFA, MCA, DCA, TCA, MBA, DCA and TCA were determined. A standard mixture solution containing 0.4 ppm of each of the fluoroacetates, 0.4 ppm of each of the chloroacetates and 1.2 ppm of each of the bromoacetates, was prepared.

Prior to use, the silver cartridge was flushed with 15 mL of Milli-Q water and 15 mL of methanol to remove trace ionic and organic contaminants from the cartridge and to condition the packing. Using a 5 mL syringe, the sample was passed through the silver cartridge at a flow rate of 2 mL/min, with the first 6 mL of effluent being discarded, while the next 4 mL of sample was collected for analysis. If the volume of the sample was less

than 10 mL, then sufficient Milli-Q water was used to flush the sample through the cartridge, thus making the volume up to 10 mL

Each standard solution was analysed using this pretreatment method in triplicate and the recovery of each haloacetate calculated, with the results being shown in Table 5.5. The average recovery for the target ions was in the range of 95.2% to 101.6%, with %RSD ranging from 0.75% to 4.50%. From these results it can be concluded that the use of a silver cartridge to remove Cl^- and Br^-does not result in the loss of target ions.

Table 5.5. Recovery of analytes after treating by a silver cartridge, calculated using three replicate determinations

Analyte	Average Recovery (%)	Relative standard deviation (RSD) (%)
MFA	98.5	0.75
DFA	101.6	1.48
TFA	101.2	3.36
MCA	97.7	2.80
DCA	98.0	13.46
TCA	101	1.50
MBA	100.8	4.21
DBA	95.6	1.15
TBA	95.2	5.97

5.3.3.3 *Various detectors*

Figure 5.5 illustrates the UV spectra of MBA and potassium chloride, over the wavelength range of 200-400 nm. It can be observed from the spectra that chloride has a weak UV absorbance, while MBA has a relatively strong absorbance. Use of a UV detector has the potential to negate any interference caused by Cl^-, when MBA is being quantified.

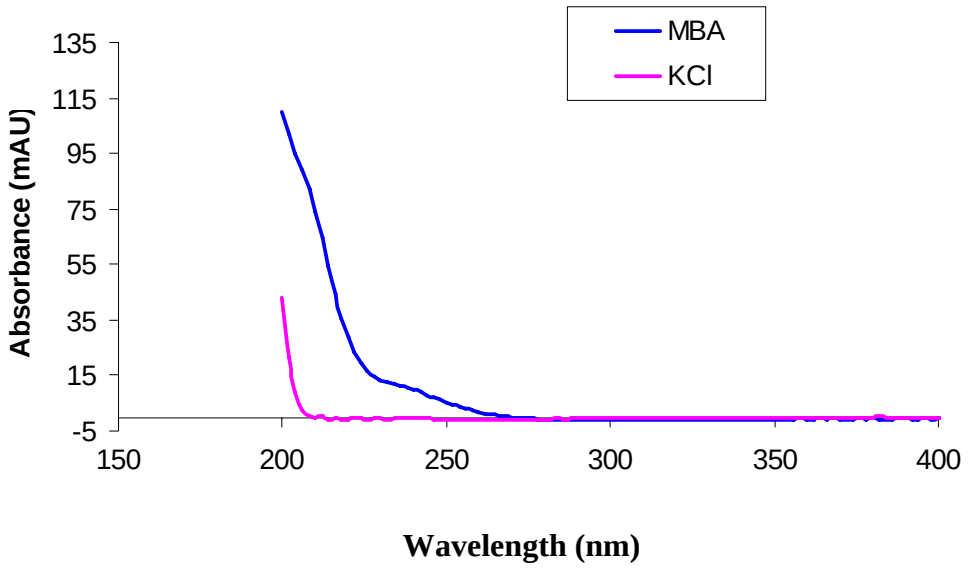


Figure 5.5. UV spectra of MBA and KCl. Concentration: 1mM; L =1 cm.

5.3.4 *Linearity and detection limits*

Under the aforementioned optimum conditions, the linearity ranges and detection limits of the method for nine haloacetates were determined, and the results displayed in Table 5.6. Linear calibration plots ($R^2 > 0.9993$) were obtained for each of the target ions over the range 2.5-6400 µg/L. Using a signal to noise ratio of 3, the detection limits of the various haloacetates were in the range 21-160 µg/L

Table 5.6. Linearity and detection limits for haloacetates under optimum conditions.

Analyte	Linearity	R^2	Detection limit (μg/L)
MFA	0.9938x-0.0075	1.0000	21
DFA	0.8756x-0.0252	0.9995	40
TFA	0.9261x-0.0138	0.9999	40
MCA	0.7466x-0.0132	1.0000	28
DCA	0.6810x-0.0065	0.9993	48
TCA	0.4220x-0.0026	0.9994	86
MBA	0.4557x-0.0103	0.9996	67
DBA	0.5303x-0.0100	0.9990	55
TBA	0.2120x-0.0062	0.9999	160

where x is the conductivity (μS) for different concentrations of haloacetic acid

5.4 Analyses of soil samples

Using the developed method, Figure 5.6 depicts the results of the soil sample, GSS-2, which was mountainside soil containing decayed vegetable mater from the base of trees of Mountain Wellington of Tasmania, Australia, spiked with a standard mixture containing 0.8 ppm of each haloacetate. The GSS-2 soil sample was found to contain only a small amount of chloride, so was not filtered though the On-Guard pre-column. Although the MBA peak (peak 4) was close to that of the chloride, there was adequate resolution, with the valve between the two calculated to be 1.12. The remaining eight haloacetates are well separated from possible interferences and are easily quantified.

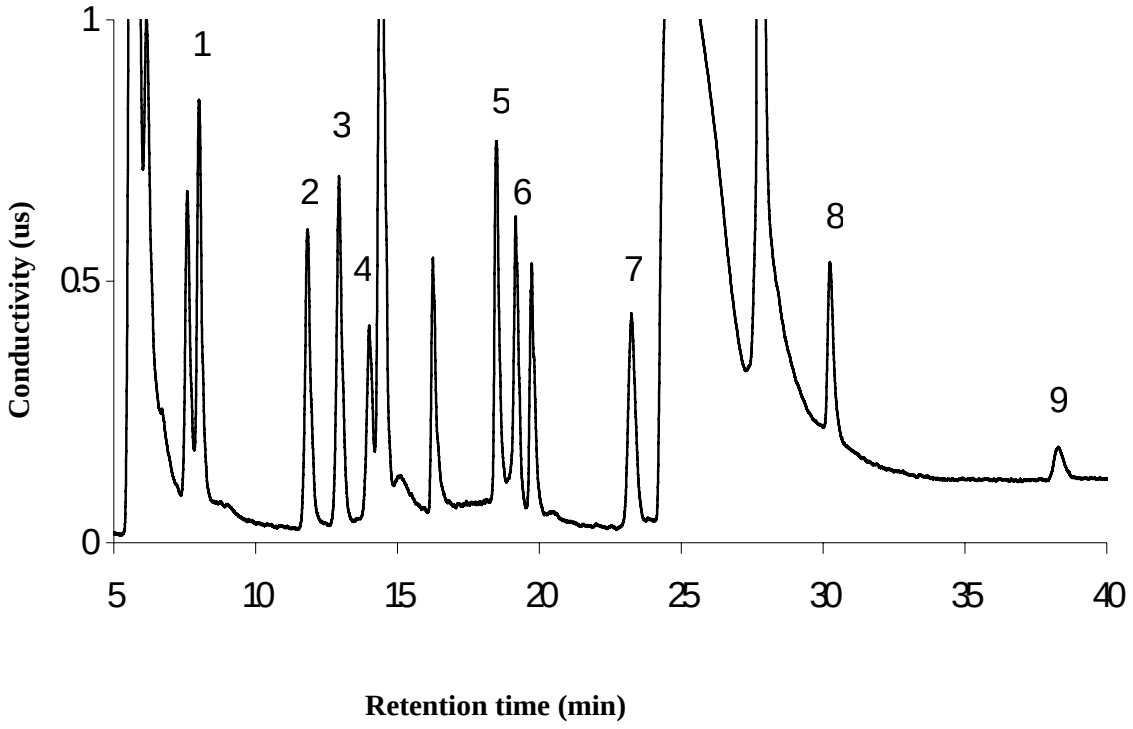


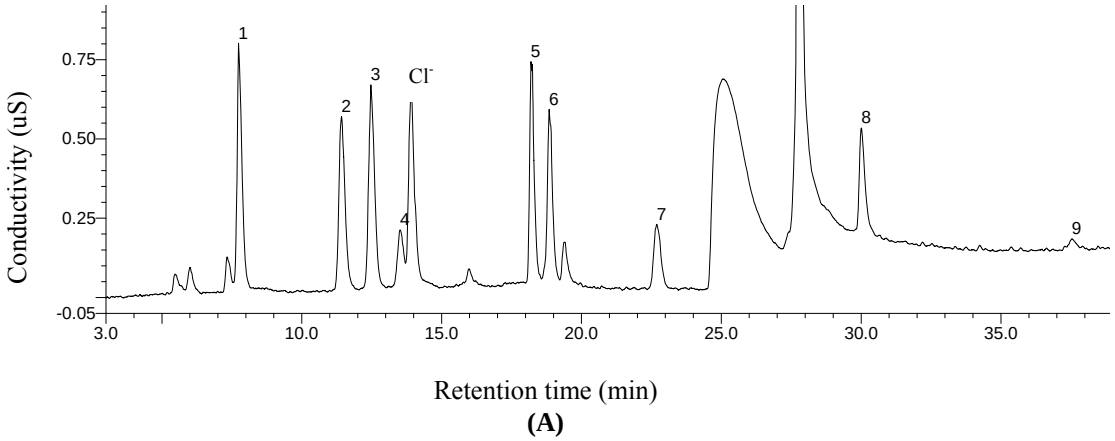
Figure 5.6. Ion chromatogram of soil sample GSS-2 before and after spiked with 0.4 ppm chloroacetates, 0.4 ppm fluoroacetates and 0.8 ppm bromoacetates, using conductivity detection 1.Monofluoroacetate, 2.Monochloroacetate, 3.Difluoroacetate, 4.Monobromoacetate, 5.Trifluoroacetate, 6.Dichloroacetate, 7.Dibromoacetate, 8.Trichloroacetate 9.Tribromoacetate. Chromatographic conditions as for Fig. 5.4.

A standard mixture of 0.4 ppm of each of the fluoroacetates, chloroacetates and bromoacetates was used to spike the soil sample, GSS-5, which was subsequently analysed without pre-treatment with the OnGuard II silver cartridge. The resulting chromatogram is displayed in Figure 5.7A, in which it can be observed that the peak for MBA is almost fully concealed by the chloride ion. The remaining eight haloacetates are completely separated from any interference. Consequently, the chloride ion must be eliminated to enable quantification of the MBA. This elimination was accomplished by pre-treating the spiked GSS-5 soil sample, with the OnGuard II silver cartridge. The resulting chromatogram is shown in Figure 5.7B, which illustrates the removal of the chloride peak, leaving the MBA peak clearly visible and free from interference. It is also noted that two unknown peaks at retention times of 6.0 and 7.5 min, increased after pretreatment with the silver cartridge, however the cause was not investigated. There was a small peak at the chloride retention time, after treatment, which could be residual chloride, but this also was not investigated further.

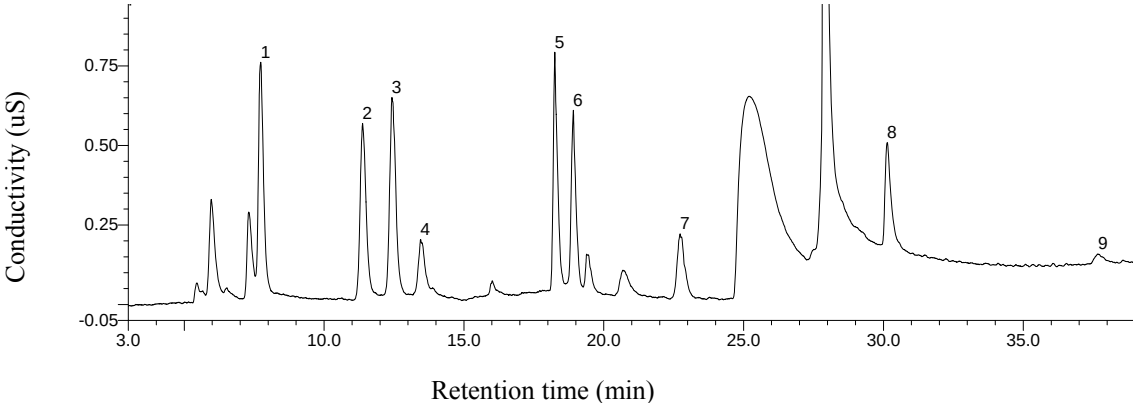
The same process was used for the analysis of soil sample number 27648 (Alluvial soil from the Savage River, Tasmania, Australia). This sample was spiked with 0.8 ppm bromide, and 0.4 ppm of both TFA and DCA. Figures 5.8A and 5.8B illustrate the results before and after pre-treatment using the Dionex OnGuard II silver cartridge. The recovery for TFA was calculated to be 100.2%, while that for DCA was 98.8%. This demonstrates that the OnGuard II silver cartridge is very effective at reducing interference brought about by the presence of halide ions.

Finally, soil sample GSS-3 was spiked with a standard containing 0.4 ppm of MBA. This sample was analysed using the optimised method employing both conductivity and UV detection. The resulting ion chromatogram from the conductivity detector is displayed in Figure 5.9. Since, chloride and MBA cannot be separated, the MBA is obscured by the

chloride peak. However, the use of the UV detector allows quantification of the MBA at 210 nm due to the extremely low molar absorptivity of Cl^- at this wavelength. The resulting ion chromatogram using UV detection is shown before and after spiking with MBA in Figures 5.10 (a) and 5.10 (b), and Figure. 5.10 (b) and clearly indicates that the Cl^- peak is almost completely separated from peak 4 (MBA). The MBA recovery was found to be 91%. The molar absorptivity of Br^- is higher than Cl^- and the conditions described would apply to Br^-, only for soil samples having a lower concentration of Br^- than Cl^-.

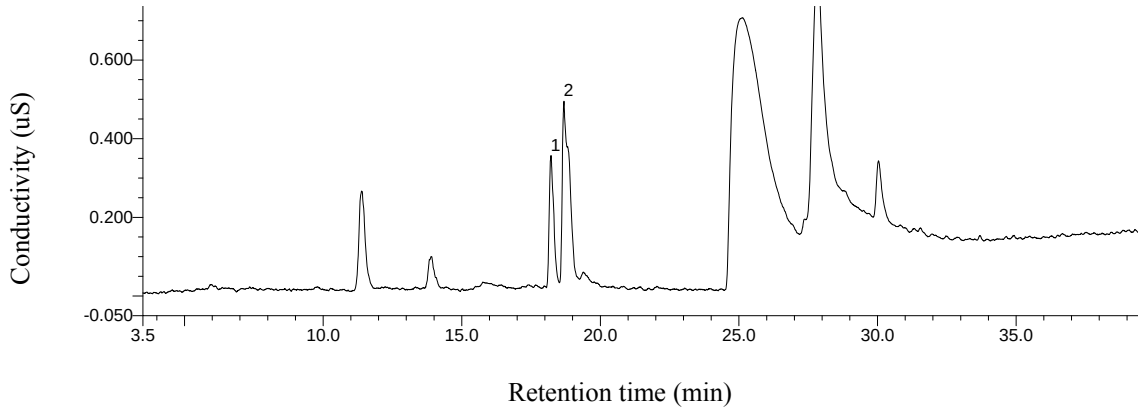


(A)

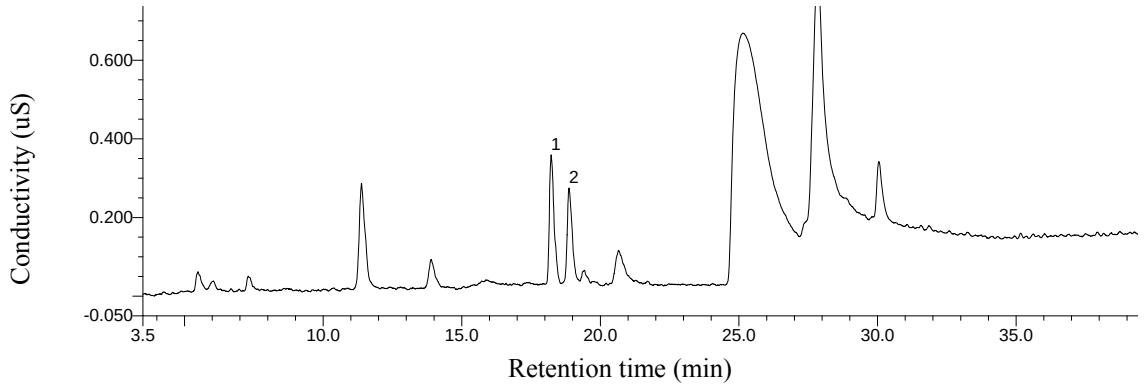


(B)

<u>Figure 5.7A and 5.7B</u>. A and B Ion chromatogram of soil GSS-5, 7A before pretreatment and 7B after pretreatment with silver cartridge. Peak: 1. Monofluoroacetate 2. Monochloroacetate, 3. Difluoroacetate 4. Monobromoacetate 5.Trifluoroacetate 6.Dichloroacetate 7. Dibromoacetate 8.Trichloroacetate 9.Tribromoacetate. Experimental conditions as for Figure 5.4.



(a)



(b)

Figure 5.8a and 5.8.b. Ion chromatograms of soil samples number 27648, (a) before and (b) after filtering with silver cartridge. Experimental conditions as for Fig. 5.4.

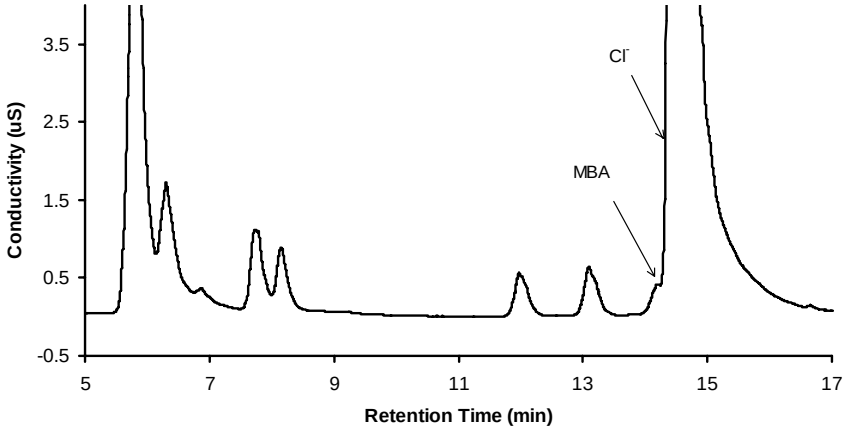


Figure 5.9. Ion chromatograms of soil sample GSS-3, using conductivity detection. Conditions as for Figure 5.4.

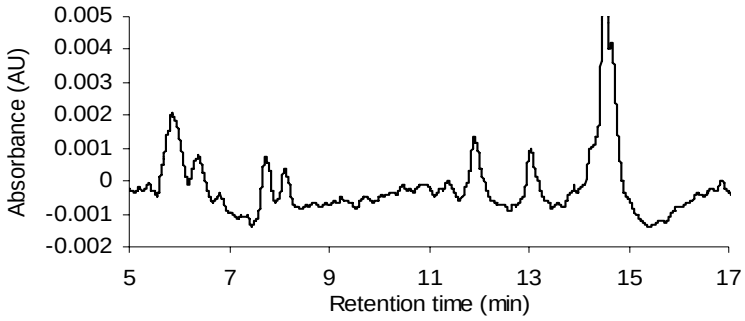


Figure 5.10 (a) Ion chromatograms of soil sample GSS-3, using UV detection. Before spiking with Monobromoacetate. Wavelength, 210 nm. Experimental conditions as in the text for Figure 5.4

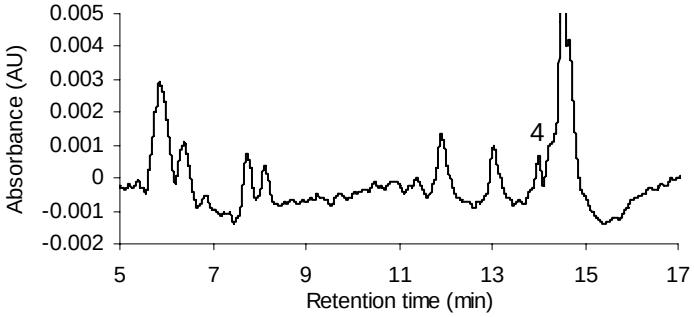


Figure 5.10 (b) Ion chromatograms of soil sample GSS-3, using UV detection. Peak: 4. Monobromoacetate. Wavelength, 210 nm. Experimental conditions as in the text for Figure 5.4.

5.5 Conclusions

By optimising the gradient profile, the nine haloacetate compounds can be completely separated by ion chromatography with a suitable run time and high resolution. The retention mechanism of the haloacetates was discussed, and it was found that elution order increased with solvated ion size as expected. Use of the OnGuard II silver cartridge or UV detection method enabled the easy elimination of interferences caused by Cl^- and Br^- in soil samples. Three soil samples from various sources were analysed by the developed method for the nine haloacetates, with complete recovery of spikes being possible.

5.6 References

[1] J. Mowrer, J. Nordin, *Chemosphere* 1987, 16, 1181 - 1192.

[2] D. A. Eillis, S. A. Marbury, *Environ. Sci. Technol*. 2000, 34, 632 - 637.

[3] B. D. Key, R. D. Howell, C. S. Criddle *Environ. Sci. Technol*. 1997, 31, 2445 - 2454.

[4] L. E. Twigg, D. R. King, *Natural Toxins*. 1996, 4, 122 - 127.

[5] J. Franklin *Chemosphere* 1993, 27, 1565 - 1601.

[6] E.O. Edney, B. W. Gay, D. J. Driscoll, *J. Atmos. Chem***.** 1991, 12, 105 - 120.

[7] E. O. Edney, D. J. Driscoll. *Int.j.Chem. Kinet***.** 1992, 24, 1067 - 1081.

[8] M. Imbenotte, A. Brice, F. Erb, J. M. Haguenoer, *Talanta*, 1984, 31, 147 - 149.

[9] A. Jordan, H. Frank, *Atmos. Environ***.** 1999, 33, 4525 - 4526.

[10] A. Klein, Ph. D. Dissertation, *University of Bayreuth*, 1997.

[11] A. Rompp, O. Kkemm, *Environ. Sci.Technol*. 2001, 35, 1294 - 1298.

[12] C. Sarzanini, M. C. Bruzzoniti, *J. Chromatog. A*. 1999, 850, 197 - 211.

[13] G. Bringmann, R. Kuehn. *Water Res*. 1980, 14, 231 - 241.

[14] A. G. Berenda, J. C. Boutonnet, C. G. de Rooij. *Environ. Toxicol. Chem*. 1999, 18, 1053 - 1059.

[15] J. J. Rook, *Water Treatment Exam*. 1974, 23, 234.

[16] Belisle, *J. Science* 1981, 212, 1509.

[17] F. D. Gilliland, J. S. Mandel, *Am. J. Ind. Med.* 1996, 29,560.

[18] A. E. Greenberg, L. S. Clesceri, A. D. Eaton (Eds.), Standard Methods For the Examination Of Water and Wastewater, 6233, Disinfection by-products: haloacetic acids and trichlorophenol, 18th ed, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, 1992, pp. 6-66.

[19] L. M. Nair, R. Saari-Nordhaus. *J. Chromatogr. A*. 1994, 671, 309 - 313.

[20] S. Hashimoto, A. Otsuki, *J High Resolut. Chromatogr*. 1998, 21, 55.

[21] R. Rochl, R. Slingsby, N. Avdalovic, P. E. Jackson. *J. Chromatogr.* A. 2002, 956, 245 - 254.

[22] DIONEX Corporation, 2000, 2001 Document No 031688, Revision 03, 23 August 2001.

Chapter 6

General Conclusions

6.1 Separation and determination of MFA, DFA and TFA

Simultaneous determination of fluoroacetates was achieved using anion-exchange ion chromatography with suppressed conductivity detection. Under optimised conditions, MFA, DFA and TFA were rapidly and completely separated with high detection sensitivity. The method suffers from no significant interferences from concurrent inorganic and organic ions, and was successfully used to analyse MFA, DFA and TFA in carrot baits, water, urine, soil and plant samples. The method described combines simplicity and minimal sample preparation with adequate speed and precision for the simultaneous analysis of MFA, DFA and TFA in the presence of other organic and inorganic anions. Using the dilution method, elimination of formic acid and other volatile fatty acids interference was possible allowing quantitation of the target ions

6.2 Separation and determination of MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA and TBA

Also optimising the gradient profile, the nine haloacetates including MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA and TBA can be completely separated by ion chromatography with a suitable run time and with high resolution. The retention mechanism of the haloacetates has been discussed, and it was found that elution order increased with ion size. Using the OnGuard II silver cartridge, or this silver nitrate

precipitation method, or by using on alternative detection method, enabled the easy elimination of interferences caused by Cl^- and Br^- in soil samples. Three soil samples from various sources were analysed by the developed method for the nine haloacetates, while complete recovery of spikes was possible. Compared to existing alternative methods for fluoroacetates, chloroacetates and bromoacetates, ion chromatography offers a straightforward and convenient approach to routine analyses. The procedure was well suited to analyse organic acids in different environmental material samples. Under the conditions described in the methods, large numbers of samples can be analysed in a relatively short period of time.

6.3 Further research

Further work is needed to refine the method. In particular improvements to the procedure for preparation of plant and urine samples with a view to eliminating interference material which is exchangeable and absorbable with ion chromatographic column. Preliminary experiments show that Sep-Pak Cartridge C_{18} can be use as a “chemical filter” to retain the interfering materials while the analyte passes through unretained. In this project, water Sep-Pak Cartridges were used, but because of limited study time, further experiments are need. After refining the above procedure, the ion chromatographic column life will be extended.

Additional work directed to refining the gradient elution conditions for MFA, DFA, TFA, MCA, DCA, TCA, MBA, DBA and TBA separation, would lead to improved baseline

stability, reduced retention time, and allow the analysis of more complex environmental samples.