Exposure to Chloropropanols via Polyamines

PROJECT REPORT AND REPORT ON OBJECTIVE 1: Development of methods of analysis for 3 Chloropropanol Isomers

Prepared for the Drinking Water Inspectorate

DWI 70/2/148

August 2003

LGC

Setting standards in analytical science

Exposure to Chloropropanols via Polyamines

PROJECT REPORT AND REPORT ON OBJECTIVE 1: Development of methods of analysis for 3 Chloropropanol Isomers

Prepared for the Drinking Water Inspectorate

DWI 70/2/148

August 2003

Contact Point:

Steve Kippin

Tel: 020 8943 7500

Prepared by:

Richard Brown

Jo Peet

Nash Vadukul

Approved by:

Derek Craston

_____

Date:

_____

Contents

1. Brief

The Department of the Environment, Food and Rural Affairs appointed LGC to conduct a research contract into exposure to chloropropanol (CP) isomers via polyamines.

The objectives of the research are:

1. To develop methods of analysis capable of achieving a detection limit of 1ug/L for the following CP Isomers: 3-monochloropropan-1,2-diol (3-MCPD); 1,3-dichloro-2-propanol (1,3-DCP); and 2,3-dichloro-1-propanol (2,3-DCP).
2. To carry out analysis of treated water entering the supply and water from consumers premises for 3 CP isomers.
3. To adapt the method developed under (1) above, for the analysis of 3 CP isomers in samples of commercially available polyamine flocculants.
4. To carry out analysis for 3 CP Isomers in samples of commercially available polyamine flocculants.
5. To report on the potential for consumer exposure to 3 CP isomers via water supplies.

1.1 Background[1]

Chloropropanol (CP) isomers are by-products of the production of hydrolysed vegetable proteins. Some CPs are carcinogenic and the European Commission's Scientific Committee on Food has made a number of recommendations concerning the reduction of CP concentrations in foodstuffs. Polyamine flocculants contain chloropropanol isomers and it is possible that consumers are exposed to low levels of CP, when polyamines are used in drinking water treatment.

The Committee on Chemicals and Materials for use in Public Water Supply (CPP) has already recommended more stringent controls on the purity and dosing concentrations for polyamine flocculants. These controls were aimed at reducing exposure to 3-monochloropropan-1,2-diol. The CPP now wishes to obtain information about levels of exposure to three specific CP that are present in commercially available polyamine water treatment flocculants.

1.2 Work Carried out by LGC

1.2.1 Objective 1 - Development of method with a detection limit of 1ug/L for 3-MCPD;1,3-DCP; and 2,3-DCP

An account of work conducted and progress achieved for objective 1 (including full methodology) is contained in the remainder of this report (Sections 2-5).

[1] Reproduced from DEFRA invitation to tender

1.2.2 Objective 2 - The analysis of treated water entering the supply and water from consumers premises for 3 CP isomers

In August 2001 the CPP contacted UK water companies to gather information on the type and dosage of polyamine flocculants in use in water treatment. Of the respondents to this request for information, three water companies indicated that they may be able to assist with providing access to sampling points for water treated with polyamine flocculants.

LGC contacted the three water companies concerned in order to arrange the collection of water samples for analysis using the method developed in Objective 1.

The first water company contacted reported that they had now decided to discontinue the use of polyamine flocculants following the CPP request for information in August 2001.

The second water company had two sites which use polyamine flocculants however the sites were currently off-line due to capital works. When the company was approached a second time, once capital works were due to be completed, the relevant person at the water company could not be determined or contacted despite numerous attempts. After significant efforts attempts to secure samples from this source were abandoned with no success.

The third water company contacted also reported that they had now stopped using polyamine flocculants and samples of treated water would therefore not be available.

CPP then extended the scope for obtaining samples of polyamine treated water to Scotland and Northern Ireland and a potential site was identified in Northern Ireland.

Contact was also made with the Polyelectrolyte Producers Group (PPG) who agreed that some members of the group may be able to identify down-stream users of polyamines where sample collection may be possible. However, further attempts to secure such samples were fruitless. Additionally, it was unlikely that any samples available would have been from the UK and they would therefore have been of little relevance to the project.

After significant effort was made to secure samples from a variety of sources and locations it was concluded in July 2003, with the agreement of DWI, that there appeared to be little prospect of obtaining samples of treated water for analysis from the UK. (Although a potential supply was identified in Northern Ireland it was considered that a single source was insufficient to provide a robust and representative analysis of the potential for consumer exposure.) Objective 2 of the project could therefore not be completed.

1.2.3 Objectives 3, 4 and 5

Work conducted under Objective 2 lead to the conclusion that polyamines were now unlikely to be in use in water treatment in the UK, DWI requested that Objectives 3, 4 and 5 of the project now be discontinued.

Work carried out on objective 3, prior to its discontinuation, included the sourcing and acquiring of a commercially available polyamine flocculant.

2. Report on Objective 1: Method Development

2.1 Initial Work

The initial method development work for all three isomers was based on an extraction stage followed by direct GCMS analysis of the three compounds.

2.1.1 Development of Extraction Conditions

Initial trials using demineralised water spiked at 500 ppb extracted into ethyl acetate or dichloromethane (100mLs to 10mL) resulted in very poor recoveries. The 1,3-DCP and 2,3-DCP were only detected at ~10% of original concentration and the 3-MCPD was not detected at all. Acidification of the water to pH 2 prior to extraction did not improve recoveries.

The use of C18 solid phase extraction cartridges was also tried using 10mL of a 5 ppm spiked water sample. This was eluted into 2 mL of ethyl acetate and run on the GCMS method. Peaks were obtained for all 3 components, but the recoveries were poor at 30%, 20% and 4% for the 1,3-DCP, 2,3-DCP and 3-MCPD respectively.

The next stage in the development of the extraction step was to salt out the water prior to extraction with ethyl acetate. Sodium chloride was added (38g) to 100mL of spiked water, and then 3 x 10mL of ethyl acetate used to extract the components.

All three solvent phases were combined and run through saturated sodium sulphate before concentrating the extract to either 10mL or 1mL using a Turbovap 500 evaporator. The final volume was transferred to a volumetric flask prior to GCMS analysis.

2.1.2 GCMS SIM Method

Initial chromatography conditions trialed proved unsuccessful using a variety of non-polar columns, as poor resolution between the 2,3-DCP and 3-MCPD was evident. However a GCMS method for the separation of the 3 standard reference materials in ethyl acetate was developed using selective ion monitoring using a polar column with the conditions shown in Table 2.1.

Table 2.1: Initial Conditions for Gas Chromatography-Mass Spectrometry

Method name	SIM1.M
Instrument	HP6890/5972 MSD SS/ GCMS/1
Column	CP WAX 52CB 25m x 320um x 1.2um film
Column reference	WCOT/320/H/6
Carrier gas	Helium @ 5 psi constant pressure
Temp prog	100ºc ramp @ 5ºc/min to 200ºc/2min
Injection mode	2 uL Splitess @ 280ºC purge at 0.6mins 50mL/min
MS mode	SIM using 79,81 ions at 3-12mins 1,3-DCP 62,64 ions at 12-15mins 2,3-DCP 61,79,81 ions at 15-22 mins 3-MCPD
Solvent delay	3 mins
EM volts used	Autotune value +200

2.1.3 Recovery Data

Good recovery data, see Table 2.2, was obtained for both dichloropropanols however the 3-MCPD recovery was still unacceptable.

Table 2.2: Recovery data for the three CP isomers for spiked water samples extracted with ethyl acetate after salting out

Component	Spike level µg/L	Recovery µg/L	% Recovery
1,3-DCP	367	436	118
2,3-DCP	703	631	90
3-MCPD	401	27	6.7
1,3-DCP	36.7	47	128
2,3-DCP	70.3	66	94
3-MCPD	40.1	ND	-
1,3-DCP	3.67	6	166
2,3-DCP	7.03	8.2	116
3-MCPD	4.01	ND	-

The data for the lowest concentration spike was obtained from a 100mL to 1mL concentration step , and the peaks detected were sufficient in response to suppose that a 1ppb detection limit would be achievable.

The method was found to be linear in the range 50-5,000 ppb with a limit of detection in the region of 50 ppb.

2.2 Further Work

2.2.1 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol

The initial DCP method described in Section 2.1 was further developed with a larger sample size (500mL) in order to improve sensitivity and detection limits. The chromatography conditions were optimised in order to sharpen the peaks, which again improved the limits of detection, and a deuterated internal standard (d_5 -1,3-DCP) was used to improve reliability of the quantification.

The method was then validated, the results of which are presented in Section 3.

2.2.2 3-monochloropropan-1,2-diol

Due to the poor recoveries obtained from the development work described in Section 2.1, an alternative method for 3-MCPD was developed involving improved isolation, extraction and the formation of a volatile derivative suitable for GCMS analysis.

A method that had previously been developed at LGC to extract 3-MCPD from poultry was considered. The method uses a solid phase Extrelut™ packing to absorb the aqueous samples and then elute the 3-MCPD with diethyl ether. Recoveries in the region of 70-105% had been previously obtained with poultry samples.

Standard and spiked sample solutions (20mL) of 3-MCPD in water were placed in separate beakers and the contents of an Extrelut 20 refill pack added as described in LGC's original poultry method, using the slurry to pack a glass column.

After the removal of non-polar compounds with hexane:diethyl ether (90:10), the 3-MCPD was then eluted with 250mL of diethyl ether at a flow rate of approximately 8mL per minute. The final volume of ether was reduced to 5mL using a Turbovap concentrator before transfer and making up to 10mL in a volumetric flask. 1mL portions of standards and samples were reacted with Heptafluorobutylimidizole (HFBI) to form the heptafluorobutyryl derivative for GCMS analysis.

The initial work was carried out on demineralised water which gave excellent linearity. Upon repeat of this work with chlorinated water it was found that not all calibration points fitted a linear line. This was found to be due to the diethyl ether not being totally dry, therefore the diethyl ether eluting from the Extrelut column was dried by passing it through a column containing anhydrous sodium sulphate prior to Turbovap evaporation.

The method was then validated, the results of which are presented in Section 3, the full text of the method is presented in Section 5.

3. Report on Objective 1: Method Validation

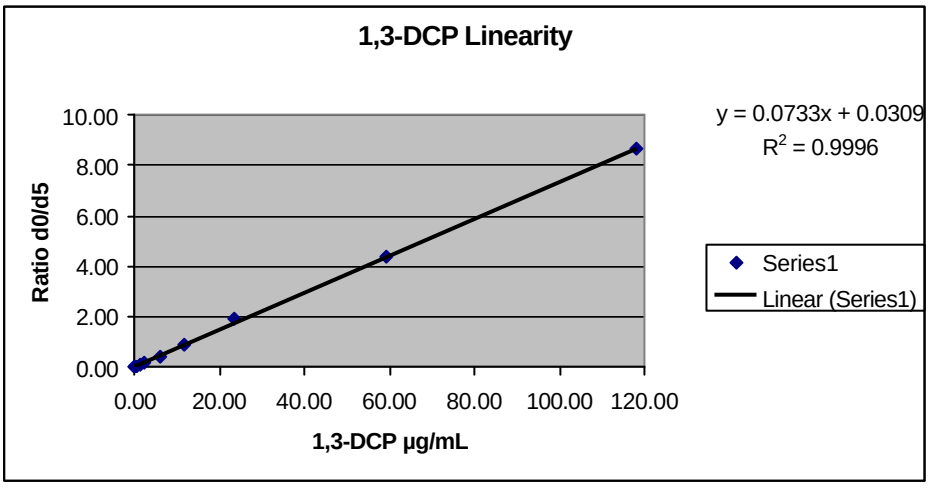
3.1 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol

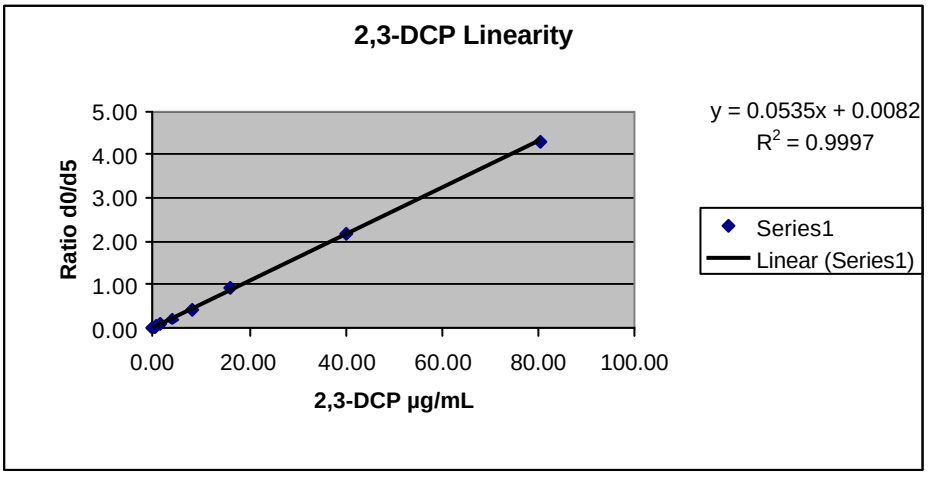
500 mL of chlorinated water was spiked in duplicate at three levels with 1,3-DCP and 2,3-DCP.

The spiked samples were neutralised with ascorbic acid and saturated with sodium chloride. The samples were then extracted three times, according to the method presented in Section 4, with ethyl acetate and concentrated to low volume (1mL) using a Turbovap evaporator. The samples were injected on the GCMS and the amounts of 1,3- and 2,3-DCP calculated.

3.1.1 Linearity

Seven point calibration lines were prepared for each analyte. The response of the method was shown to be linear in the range 0 – 118µg/mL (equivalent to 0 – 236 µg/L) with correlation coefficient (r^2) = 0.9996 for 1,3-DCP. For 2,3-DCP the response was shown to be linear in the range 0 – 80 µg/mL (equivalent to 0 – 160 µg/L) with correlation coefficient (r^2) = 0.9997.





3.1.2 Recoveries

The following recoveries were obtained:

Table 3.1: Recovery data for 1,3-DCP obtained for spiked chlorinated water samples

	Spike level µg/L	Recovery 1,3-DCP µg/L	% Recovery 1,3-DCP
Spike 1a	1.18	0.82	69
Spike 1b	1.18	0.88	75
Spike 2a	5.91	4.12	70
Spike 2b	5.91	4.54	77
Spike 3a	29.6	21.5	73
Spike 3b	29.6	21.8	74

Table 3.2: Recovery data for 2,3-DCP obtained for spiked chlorinated water samples

	Spike level µg/L	Recovery 2,3-DCP µg/L	% Recovery 2,3-DCP
Spike 1a	0.804	0.56	70
Spike 1b	0.804	0.70	87
Spike 2a	4.02	2.76	69
Spike 2b	4.02	2.70	67
Spike 3a	20.1	13.7	68
Spike 3b	20.1	13.4	67

Mean Recovery: 72.8% for 1,3-DCP and 71.2% for 2,3-DCP

3.1.3 Limit of Detection

The limit of detection was estimated from the signal/noise ratio in the lower standard solution (0.06 µg/mL). The limit of detection for 1,3-DCP and 2,3-DCP (assuming a 500mL sample) = 0.02 µg/L.

3.1.4 Limit of Quantitation

The limit of quantitation was estimated from the signal/noise ratio in the lower standard solution (0.06 µg/mL). The limit of quantitation for 1,3-DCP and 2,3-DCP (assuming a 500mL sample) = 0.2 µg/L.

The GCMS chromatograms obtained during the method validation stage are presented in Appendix 1.

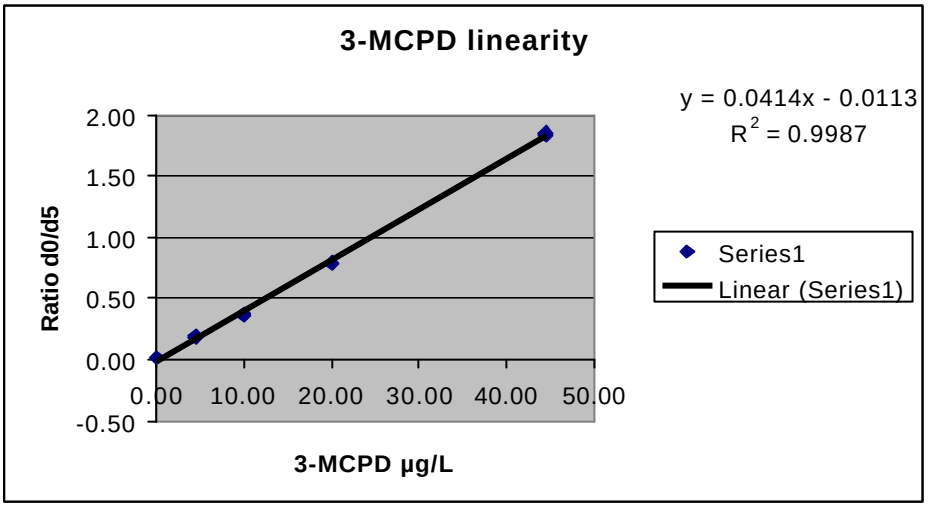
The detailed analytical method for 1,3-DCP and 2,3-DCP is presented in Section 4.

3.2 3-Monochloropropan-1,2-diol

500 mL of chlorinated water was spiked in duplicate at three levels with 3-MCPD. The 3-MCPD was extracted from 20 mL portions of these spiked samples in duplicate according to the method presented in Section 5. A volatile derivative (Heptafluorobutyryl) was prepared using HFBI for GCMS analysis and the amount of 3-MCPD calculated.

3.2.1 Linearity

A five point calibration line was prepared for 3-MCPD. The response of the method was shown to be linear in the range 0 – 60 µg/L, with a correlation coefficient (r^2) = 0.9987.



3.2.2 Comparative Recoveries

Standards were prepared in chlorinated water and 3-MCPD was extracted using the method. As such the recoveries are comparative rather than absolute. The following recoveries were obtained:

Table 3.2: Comparative recovery data for 3-MCPD obtained for spiked chlorinated water samples

	Spike level µg/L	Recovery of 3-MCPD µg/L	% Recovery 3-MCPD
Spike 1a	3.58	3.46	97
Spike 1b	3.58	3.78	106
Spike 2a	8.95	5.97	67
Spike 2b	8.95	7.22	81
Spike 3a	26.9	27.7	103
Spike 3b	26.9	26.5	99

3.2.3 Limit of Detection

The limit of detection was estimated from the signal/noise ratio in the lower standard solution (4.5 µg/L). The limit of detection for 3-MCPD (assuming a 20mL sample) = 0.43 µg/L.

3.2.4 Limit of Quantitation

The limit of quantitation (LOQ) was estimated from the signal/noise ratio in the lower standard solution (4.5 µg/L). The limit of quantitation for 3-MCPD (assuming a 20mL sample) = 1.45 µg/L.

The GCMS chromatograms obtained during the method validation stage are presented in Appendix 2.

The detailed analytical method for 3-MCPD is presented in Section 5.

4. Method for the determination of 1,3-dichloro-2-propanol (1,3-DCP) and 2,3-dichloro-1-propanol (2,3-DCP) in chlorinated drinking water using Capillary Gas Chromatography with Mass Spectrometry

4.1 Introduction

The method was optimised and validated for the determination of 1,3-dichloro-2-propanol (1,3-DCP) and 2,3-dichloro-2-propanol (2,3-DCP).

4.2 Principle

Chlorinated drinking water samples are neutralised with ascorbic acid and saturated with sodium chloride. The samples are then extracted three times with ethyl acetate and concentrated to low volume using Turbovap evaporators.

The extracts are injected on capillary gas chromatography-mass spectrometry system and the amounts of 1,3- and 2,3-dichloropropanols calculated.

4.3 Method Detection Limits

The method has been found to have a limit of detection of 0.02 µg/L for 1,3-DCP and 2,3-DCP (assuming a 500mL sample).

4.4 Reagents

Reagents of recognised analytical grade only should be used:

- Chlorinated water (ElgaStat™ purified water, treated with sodium hypochlorite)
- 1,3- and 2,3-dichloropropanols (1,3-DCP and 2,3-DCP) (Acros)
- d_5 – 1,3-dichloro-iso-propyl alcohol (internal standard) (Qm_x Laboratories Ltd)
- Sodium sulphate, anhydrous
- Ethyl acetate
- Ascorbic acid (0.4% w/v)
- Sodium chloride

4.5 Preparation of Standard Solutions

4.5.1 1,3-DCP and 2,3-DCP stock solution - A

Weigh approximately 100mg 1,3- and 2,3-DCP (record the weight to 4 figures) and make up to 100mL in a volumetric flask with ethyl acetate to give a solution of approximately 1 mg/mL.

4.5.2 Intermediate 1,3-DCP and 2,3-DCP standard solution - B

Pipette 5mL of standard solution A into a 50mL volumetric flask containing approximately 15mL of ethyl acetate. Dilute to volume with ethyl acetate and mix thoroughly to give a solution containing 100µg/mL.

4.5.3 1,3-DCP and 2,3-DCP spiking solution - C

Pipette 2.5mL of intermediate standard solution B into a 25mL volumetric flask containing approximately 5mL of acetonitrile. Dilute to volume with acetonitrile and mix thoroughly to give a solution containing 10µg/mL.

4.5.4 d_5–1,3-dichloro-iso-propyl alcohol - D

Weigh approximately 60mg d_5 -1,3-DCP (record the weight to 4 figures) and make up to 100mL in a volumetric flask with ethyl acetate to give a solution of approximately 600 µg/mL.

4.6 Preparation of Calibration Standards

Prepare a set of calibration standards from the 1,3-DCP and 2,3-DCP standard solution (4.5.2) covering a range 0, 0.5, 1, 2, 5, 10, 20 and 50 µg/mL in ethyl acetate.

4.7 Apparatus

- Gas chromatograph with mass spectrometer. (See Table 4.1 for details of instrumentation and conditions.)
- 1000 mL glass separating funnels
- Glass drying columns with a sintered glass disc.
- Pipettes
- Analytical balance (4 figures)
- Pasteur pipettes
- Volumetric glassware of varying sizes
- Turbovap evaporator with a controlled flow of dry nitrogen gas.
- 2mL chromatography vials
- 10 mL graduated test tubes

4.8 Laboratory Samples

On receipt, samples should be assigned a unique sample reference number (eg via a LIMS system). Samples are to be refrigerated until required for analysis. Samples should be allowed to reach ambient temperature prior to commencing extraction and analysis.

4.9 Procedure

4.9.1 Samples

1. Transfer 500 mL of the sample into the 1000mL separating funnel

2. Add 2 mL of ascorbic acid and mix thoroughly.

3. Add 70 mL ethyl acetate and shake vigorously for 1- 2 minutes releasing the pressure. Allow the mixture to stand so that two layers separate.

4. Fill a drying column with about 30g of anhydrous sodium sulphate.

5. Transfer the upper layer from step 3. above to the drying column and collect the eluate into a Turbovap tube.

6. Re-extract the aqueous layer with further 70 mL of ethyl acetate and repeat the process from step 3. above twice.

7. Rinse the drying column with approximately 10mls of ethyl acetate and collect the rinsing into the combined extracts.

8. Evaporate the combined extracts on the Turbovap evaporator at 30°C to about 5 mL under a stream of dry nitrogen. Do not allow the extract to evaporate to dryness.

9. Quantitatively transfer the concentrated extract to a 10 mL graduated test tube.

10. Evaporate the solution under a stream of dry nitrogen at room temperature to 1 mL .

11. Add 25 µL of d_5 –1,3 Dichloro-iso-propyl alcohol and mix the contents in the test tube.

12. Transfer the sample from step 11. above to a 2 mL vial for GCMS analysis.

4.10 Determination

Standards and samples are to be injected onto the gas chromatograph which must be operated using the conditions outlined in Table 4.1 (conditions for Gas Chromatography-Mass Spectrometry). The standards should be injected in ascending order both before and after any samples are injected. The blank sample should also be bracketed around the samples.

Table 4.1: Conditions for Gas Chromatography-Mass Spectrometry

Gas Chromatograph	Fitted with an on-column injector and autosampler.
Column	ZB -WAX; 30m x 0.25mm i.d, 0.25µm film thickness or equivalent.
Injector Temp	On-column - oven track mode
Carrier Gas	Helium, constant flow 1.0 mL/minute
Temperature Programme	60°C for 1 minute 18°C/min to 230°C (5 min hold)
Total Run Time	15.44 minutes
Injection Volume	On-column – 0.5µl
Detector	Single Quadropole MS
Transfer Line Temperature	250°C
Selected Ions	79 1,3-DCP 80 1,3-DCP 81 1,3-DCP 62 2,3 -DCP 63 2,3 -DCP 64 2,3 -DCP 82 d_5 –1,3-DCP

4.11 Calculations

4.11.1 Internal Standard Method

1. Measure the areas of the d_5–1,3-dichloro-iso-propyl alcohol (m/z 82), 1,3-DCP(m/z 79), and 2,3-DCP (m/z 62) peaks.

2. Calculate the ratio of the 1,3- and 2,3-DCP (m/z 79 and 62) to d_5 -1,3-DCP (m/z 82) peak areas.

3. Construct a calibration graph for the standards by plotting the peak area ratio against the concentration in µg/mL of 1,3-DCP and 2,3-DCP in the standard solutions

4. Calculate the slope of the calibration line.

5. Divide the peak area ratio of the unknowns by the value of the slope to give the concentration of 1,3-DCP and 2,3-DCP for the unknown samples.

4.11.2 Final Presentation of Results

Results are expressed as µg/L and are quoted to 1 decimal place.

4.12 Quality Assurance and Control

Include a reagent blank, a spike-recovery sample and a duplicate sample in each batch of samples for analysis.

4.13 Confirmation of Peak Identity

Where a positive result for a sample or blank is obtained the operator should confirm retention time and spectral quality against the calibration standards. Any positive compounds shall be within ± 0.5 minute of the respective calibration standard.

Measure the ratio of the responses at m/z 79 and 81 for 1,3-DCP and m/z 62 and 64 for 2,3-DCP in the standards. The ratios for positive samples should be within ± 10% of those obtained for the standards.

5. Method for the Determination of 3-Monochloropropan-1,2-diol (3-MCPD) in Chlorinated Drinking Water using Capillary Gas Chromatography with Mass Spectrometry

5.1 Introduction

The method was optimised and validated for the determination of 3-monochloropropan-1,2-diol (3-MCPD) in chlorinated drinking water.

5.2 Principle

The first step in the extraction of 3-MCPD from chlorinated drinking water involves the pipetting of a known volume onto the contents of an Extrelut™ refill pack. The sample extract/ Extrelut™ slurry is then transferred to a glass chromatography column and the non-polar components eluted using a mixture of hexane and diethyl ether. The 3-MCPD is subsequently eluted from the column using diethyl ether and the extract concentrated by removal of solvent. A portion of the concentrated sample extract is then taken for derivatisation and analysis by capillary gas chromatography-mass spectrometry.

5.3 Method Detection Limits

The method was found to have a limit of detection of 0.4µg/L (0.4 ppb), when a 20 mL sample volume was taken for analysis.

5.4 Reagents

Reagents of recognised analytical grade only should be used:

- Chlorine-free water (ElgaStat™ purified water).
- Chlorinated water (ElgaStat™ purified water, treated with sodium hypochlorite).
- 3-Monochloropropane-1,2-diol (3-MCPD).
- d_5 -3- Monochloropropane-1,2-diol (d_5-3-MCPD) (internal standard).
- Sodium sulphate, anhydrous.
- Heptafluorobutyrylimidazole (HFBI) - stored frozen.
- Extrelut™ 20 refill packs.
- 2,2,4-trimethylpentane (*iso*-octane).
- Diethyl ether.
- Hexane.
- Hexane + diethyl ether (90 + 10). Add 100mL of diethyl ether to 900mL hexane and mix well.

5.5 Preparation of Standard Solutions

5.5.1 3-MCPD stock solution - A

Weigh approximately 100mg 3-MCPD (record the weight to 4 figures) and make up to 10mL in a volumetric flask with ethyl acetate to give a solution of approximately 10 mg/mL.

5.5.2 Intermediate 3-MCPD standard solution - B

Pipette 100µl of standard solution A into a 25mL volumetric flask containing approximately 15mL of chlorinated water. Dilute to volume with water and mix thoroughly to give a solution containing 40µg/mL.

5.5.3 3-MCPD standard and spiking solution - C

Pipette 1mL of intermediate standard solution B into a 1000mL volumetric flask containing approximately 500mL of chlorinated water. Dilute to volume with water and mix thoroughly to give a solution containing 40µg/L.

5.5.4 d_5 -3-MCPD internal standard stock solution - D

Weigh approximately 100mg d_5 -3-MCPD (record the weight to 4 figures) and make up to 10mL in a volumetric flask with chlorine-free water to give a solution of approximately 10 mg/mL.

5.5.5 d_5 -3-MCPD working internal standard stock solution - E

Pipette 250µl of internal standard solution D into a 250mL volumetric flask containing approximately 150mL of chlorine-free water. Dilute to volume with water and mix thoroughly to give a solution containing 10µg/mL.

5.6 Preparation of Calibration Standards

Prepare a set of calibration standards from the 3-MCPD standard and spiking solution (5.5.3) so that they can be extracted in parallel with the samples. The volumes in Table 5.1 are indicative and are based upon the 3-MCPD standard and spiking solution – C, being 40µg/L. The actual concentration of each standard should be calculated based on the true concentration of this solution.

Table 5.1: Calibration Standards

Nominal 3-MCPD concentration (µg/L)	Volume of 3-MCPD Std (5.5.3) (mL)	Volume of chlorinated water (mL)	Volume of *d5*-3-MCPD Std (5.5.5) (µL)
0	0.0	20.0	100
4	2.0	18.0	100
10	5.0	15.0	100
20	10.0	10.0	100
40	20.0	0.0	100

5.7 Apparatus

- Gas chromatograph with mass spectrometer. See Table 5.2 for details of instrumentation and conditions.
- Glass chromatography columns 40cm length x 2cm i.d., with sintered glass disc and tap.
- Glass drying columns with a sintered glass disc.
- Automatic pipettes.
- Analytical balance, (4 figures).
- Pasteur pipettes
- Filter papers, Whatman No.1
- Glass funnels
- Volumetric glassware of varying sizes
- Turbovap evaporator with a controlled flow of dry nitrogen gas.
- 4mL glass vials with screw caps
- 2mL chromatography vials
- Reacti-Block for derivatisation reaction at 70°C.

5.8 Laboratory Samples

On receipt, samples should be assigned a unique sample reference number (eg via a LIMS system). Samples are to be refrigerated until required for analysis. Samples should be allowed to reach ambient temperature prior to commencing extraction and analysis.

5.9 Procedure

5.9.1 Samples

1.Pipette 20.0 mL of the aqueous sample into a 250 mL beaker. Add 100µl internal standard solution E.

2. Add the contents of an Extrelut™ refill pack to the sample and mix thoroughly with a spatula. Check the bottom of the beaker to ensure thorough mixing.

3. Add the mixture to the chromatography column, tap briefly to compact the contents, top with a 1cm layer of sodium sulphate and leave for 15-20 minutes.

4. Elute the non-polar components with 80 mL hexane + diethyl ether (90+10). Allow unrestricted flow. Close the tap when the solvent reaches the sodium sulphate layer. Discard the collected solvent.

5. Elute the 3-MCPD with 250 mL diethyl ether at a flow rate of about 8 mL per minute. This requires a steady and rapid stream of separate drops with solvent movement in the column just apparent. Collect 250 mL of eluate in a 250 mL volumetric flask.

6. Fill a drying column with about 50g of anhydrous sodium sulphate and pass the ether through it, collecting the dried ether in a 250 mL conical flask. If required at this stage, it has been found that it is safe to leave the extract in a refrigerator overnight.

7. Add approximately 15g sodium sulphate to the flask and leave for 10 to 15 minutes only.

8. Filter the eluate into a 250mL Turbovap tube through a filter paper.

9. Concentrate the extract to about 5mL under a stream of dry nitrogen at 30°C. Do not allow to evaporate to dryness.

10. Quantitatively transfer the concentrated extract to a 10mL volumetric flask with diethyl ether. Make up to volume with diethyl ether.

11. Add a small quantity of sodium sulphate (spatula tip quantity) to the flask, shake well and leave for 10 to 15 minutes, but no longer.

12. Transfer 1mL of the sample to a 4 mL vial.

13. Evaporate the solution just to dryness at room temperature under a stream of dry nitrogen.

14. Immediately add 1.0 mL 2,2,4-trimethylpentane.

15. Add 0.05mL HFBI at room temperature and immediately seal.

16. Shake for several seconds and heat at 70°C for 20 minutes.

17. Allow the mixture to cool to room temperature then add 1mL of water.

18. Shake for 30 seconds, allow the phases to separate, then repeat shaking.

19. Place a small quantity (spatula tip full) of sodium sulphate in a 2mL vial. Transfer the 2,2,4-trimethylpentane phase, from 9.18, to this vial, shake, and let stand for 2 to 5 minutes.

20. Transfer the 2,2,4-trimethylpentane, from 9.19, to a 2mL vial for GCMS analysis.

5.9.2 Blank Test

Follow the above procedure for the preparation of a test portion (steps 1–20) substituting 20mL of chlorinated water for the test sample (step 1), adding 100µl of the 10 mg/L d_5-3-MCPD internal standard solution E at the same stage.

5.9.3 Calibration Standards

Take 20 mL portions of the standard solutions prepared previously (Table 5.1) through the complete extraction procedure (steps 1 – 20).

5.10 Determination

Standards and samples are to be injected onto the gas chromatograph which must be operated using the conditions outlined in Table 5.2 (Conditions for Gas Chromatography-Mass Spectrometry). The standards should be injected in ascending order both before and after any samples are injected. The blank sample should also be bracketed around the samples.

Table 5.2: Conditions for Gas Chromatography-Mass Spectrometry

Gas Chromatograph	Fitted with either an on-column or split/splitless injector and an autosampler.
Column	DB1; 50m x 0.32mm i.d, 0.25µm film thickness or equivalent.
Injector Temp	Split/Splitless :270°C; On-column – ambient/oven track mode
Carrier Gas	Helium, constant flow 1.0mL/minute
Temperature Programme	50°C for 2 minutes 4°C/min to 130°C (zero hold) 10°C/min to 280°C 270°C for 3 minutes
Total Run Time	40 minutes
Fil/Mul Delay	780 seconds
Data Acquisition Time	20 minutes
Injection Volume	Splitless - 2.0µl; On-column – 0.5µl
Split (if used)	Splitless injection with a 40 second splitless period; 30:1 split
Detector	Single Quadropole MS
Transfer Line Temperature	250°C
Selected Ions	453 3-MCPD 291 3-MCPD 289 3-MCPD 275 3-MCPD 253 3-MCPD 257 d_5 -3-MCPD

5.11 Calculations

5.11.1 Internal Standard Method.

1. Measure the areas of the d_5-3-MCPD (m/z 257) and 3-MCPD (m/z 253) peaks.

2. Calculate the ratio of the 3-MCPD (m/z 253) to d_5-3-MCPD (m/z 257) peak areas.

3. Construct a calibration graph for the standards by plotting the peak area ratio against the concentration in µg/L of 3-MCPD in the standard solutions

4. Calculate the slope of the calibration line.

5. Divide the peak area ratio of the unknowns by the value of the slope to give the concentration of 3-MCPD for the unknown samples.

5.11.2 Final Presentation of Results

Results are expressed as µg/L and are quoted to 1 decimal place.

5.12 Quality Assurance and Control

Include a reagent blank, a spike-recovery sample and a duplicate sample in each batch of samples for analysis.

5.13 Confirmation of Peak Identity

Where a positive result for a sample or blank is obtained the operator should confirm retention time and spectral quality against the calibration standards. Any positive compounds shall be within ± 0.5 minute of the respective calibration standard.

Measure the ratio of the responses at m/z 291, 289, 275, 253 relative to the response at m/z 453 for the standards. The ratios for positive samples should be within ± 10% of those obtained for the standards.

The 3-MCPD peak will elute after approximately 14 to 16 minutes. The isomeric chloropropanol, 2-MCPD, may elute just after 3-MCPD in some samples. 2-MCPD has a spectrum almost identical to 3-MCPD but lacks the ion at m/z 453.

5.14 References

This method was originally designed for the analysis of food and has been modified for water.

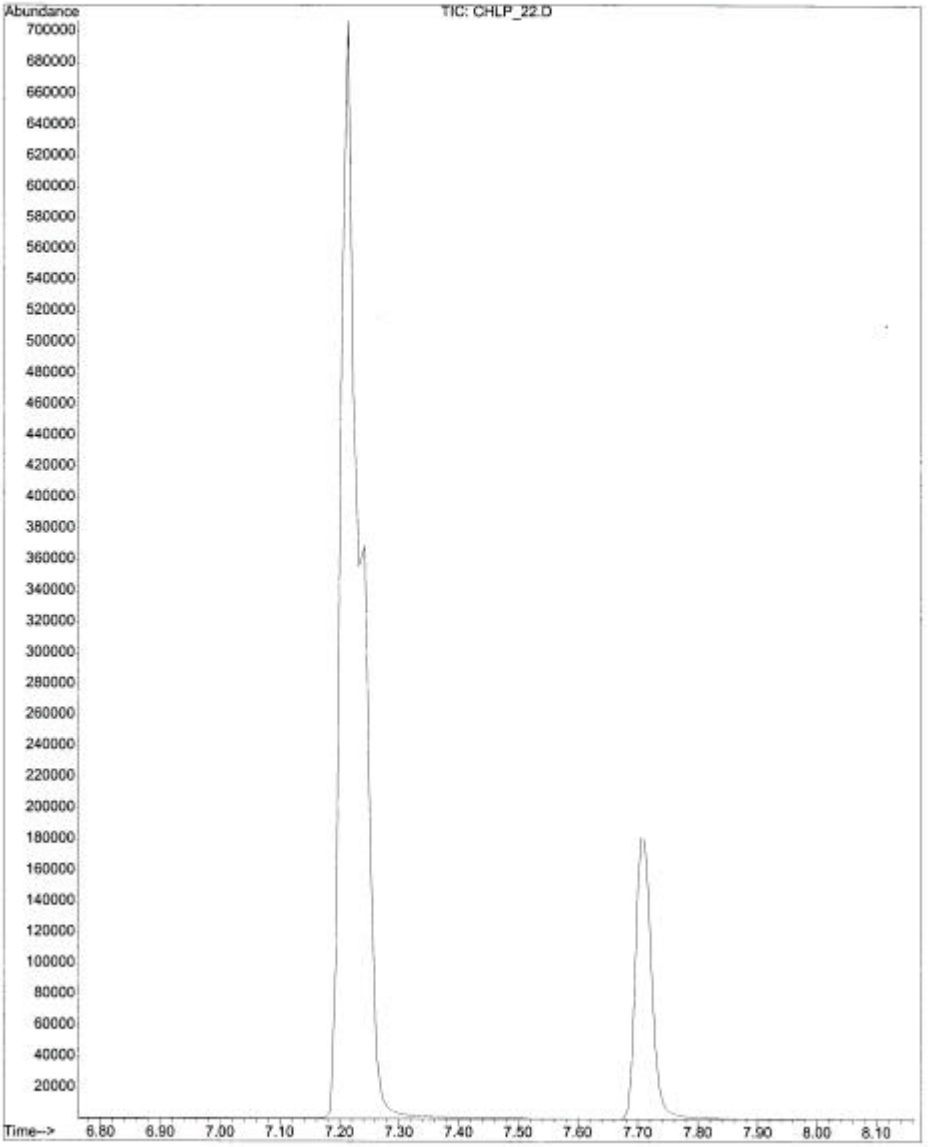
- Determination of chlorpropanols in protein hydrolysates. J. Chromatography, 1992, 589, 109-119. Van Bergen, C.A., Collier, P.D., Cromie, D.D.O., Lucas, R.A., Preston, H.D., Sissons, D.J.
- Sensitive method for the determination of 3-chloropropane-1,2-diol and 2-chloropropane-1,3-diol by capillary gas chromatography with mass spectrometric detection. J. Chromatography, 1998, 802, 325-333. Meierhans, D.C., Bruehlmann, S., Meili, J., Taeschler, C.
- MAFF collaboratively tested procedure.

APPENDIX 1

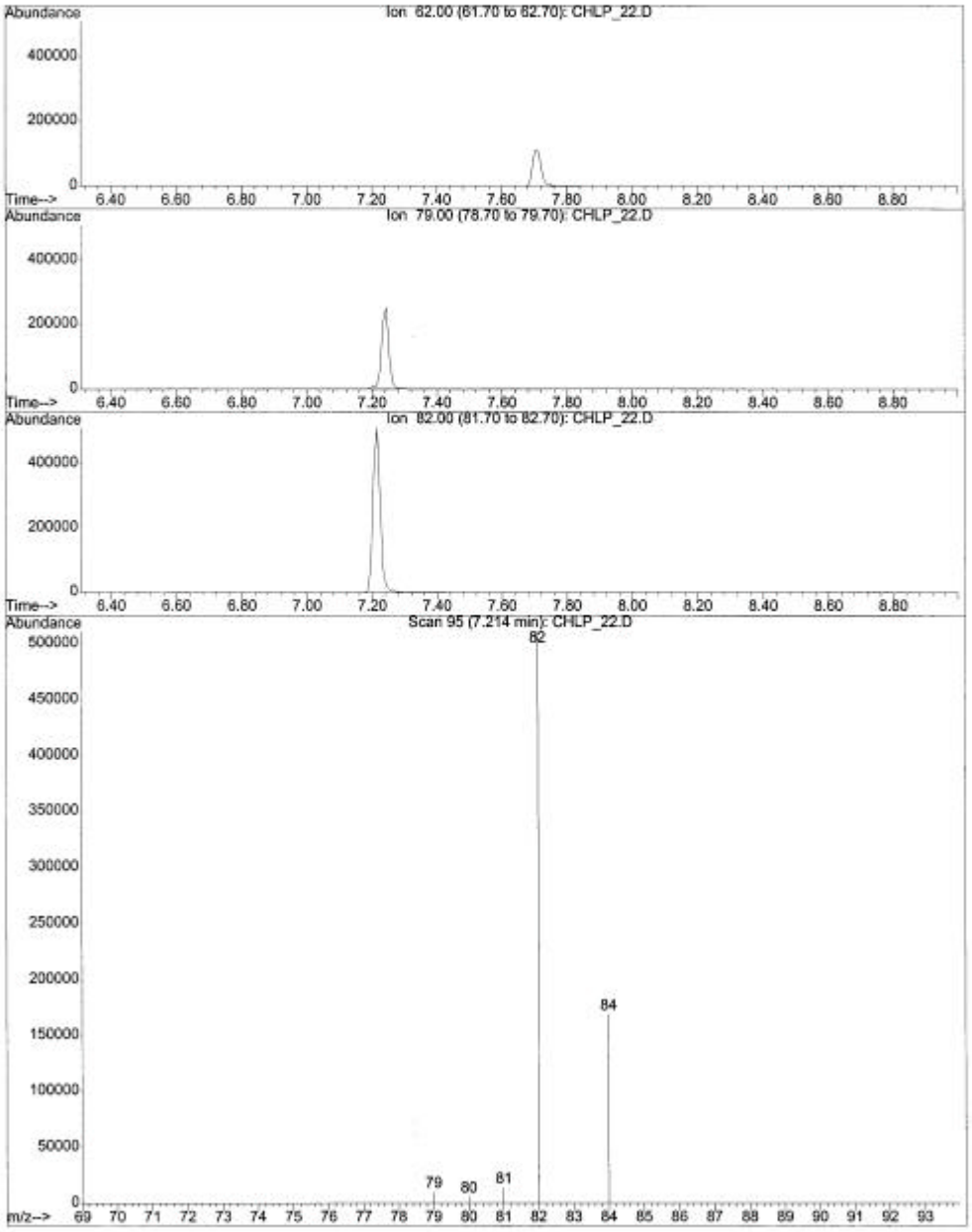
Chromatograms for 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol

1. Total Ion Chromatogram (TIC) showing 1,3-DCP-d5, 1,3-DCP-d0 and 2,3-DCP-d0
2. Extracted Ion Chromatogram (EIC) showing m/z 62, m/z 79 and m/z 82 with mass spectrum of 1,3-DCP-d5 (Internal Standard)
3. Extracted Ion Chromatogram (EIC) showing m/z 62, m/z79 and m/z 82 with mass spectrum of 1,3-DCP-d0 (Analyte)
4. Extracted Ion Chromatogram (EIC) showing m/z 62, m/z79 and m/z 82 with mass spectrum of 2,3-DCP-d0 (Analyte)

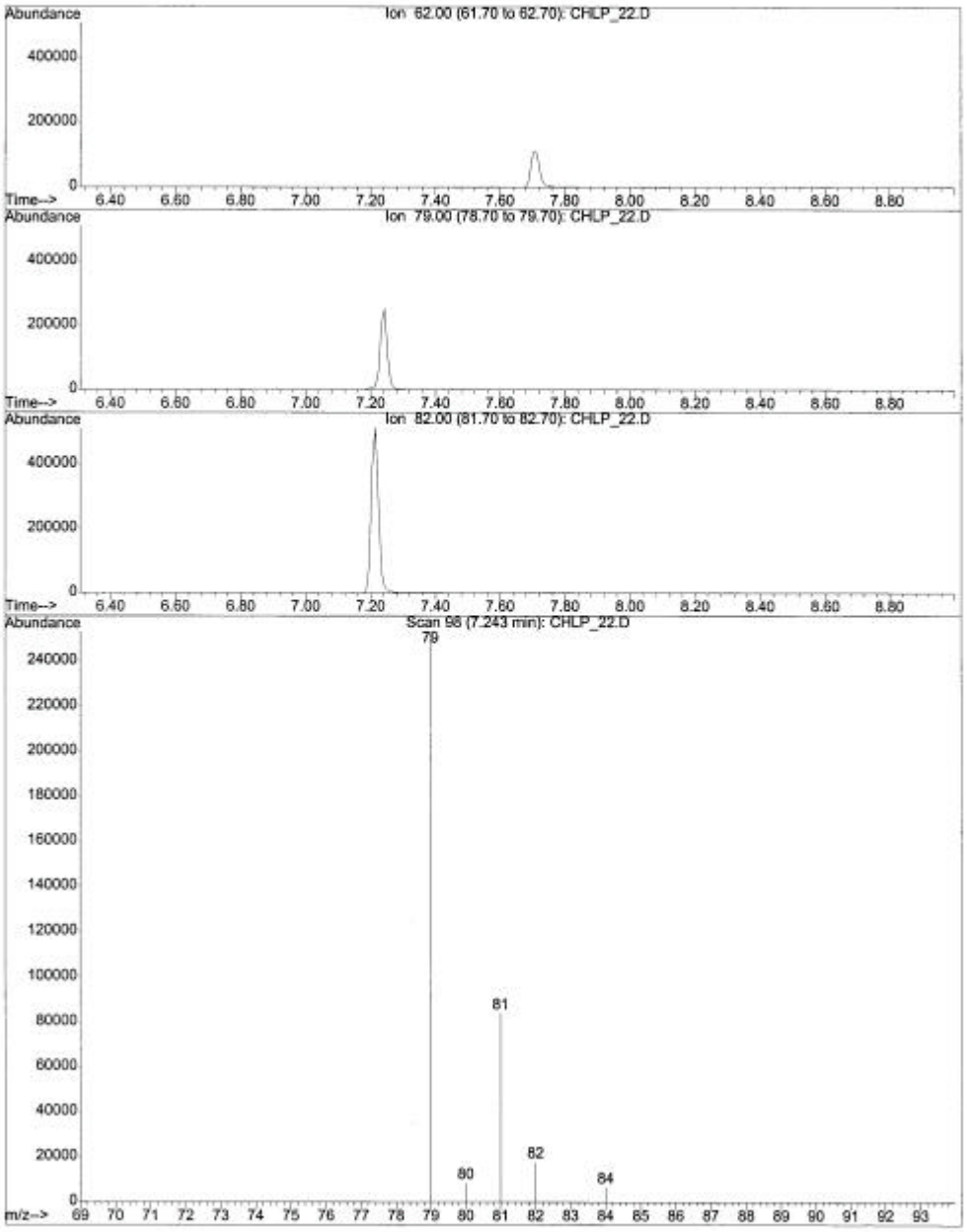
File : D:\HPCHEM\1\DATA\020724\CHLP_22.D
Operator : N.VADUKUL
Acquired : 24 Jul 2002 22:04 using AcqMethod CHLORP1T
Instrument : GC/MS Ins
Sample Name: STD 5
Misc Info :
Vial Number: 14



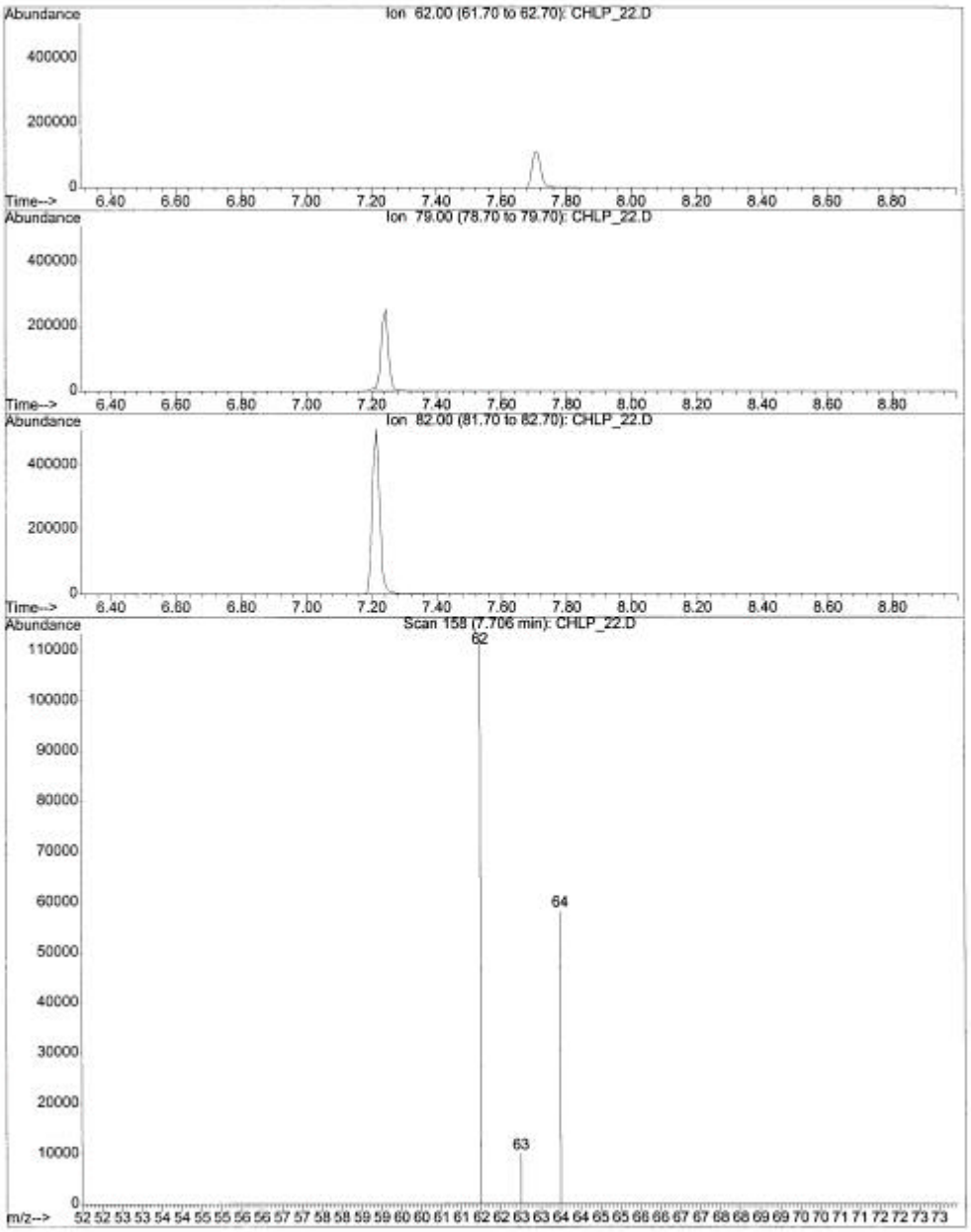
```
File       : D:\HPCHEM\1\DATA\020724\CHLP_22.D
Operator   : N.VADUKUL
Acquired   : 24 Jul 2002  22:04     using AcqMethod CHLORP1T
Instrument :    GC/MS Ins
Sample Name: STD 5
Misc Info  :
Vial Number: 14
```



```
File       : D:\HPCHEM\1\DATA\020724\CHLP_22.D
Operator   : N.VADUKUL
Acquired   : 24 Jul 2002  22:04     using AcqMethod CHLORP1T
Instrument :    GC/MS Ins
Sample Name: STD 5
Misc Info  :
Vial Number: 14
```



```
File        : D:\HPCHEM\1\DATA\020724\CHLP_22.D
Operator    : N.VADUKUL
Acquired    : 24 Jul 2002  22:04     using AcqMethod CHLORP1T
Instrument :    GC/MS Ins
Sample Name: STD 5
Misc Info   :
Vial Number: 14
```

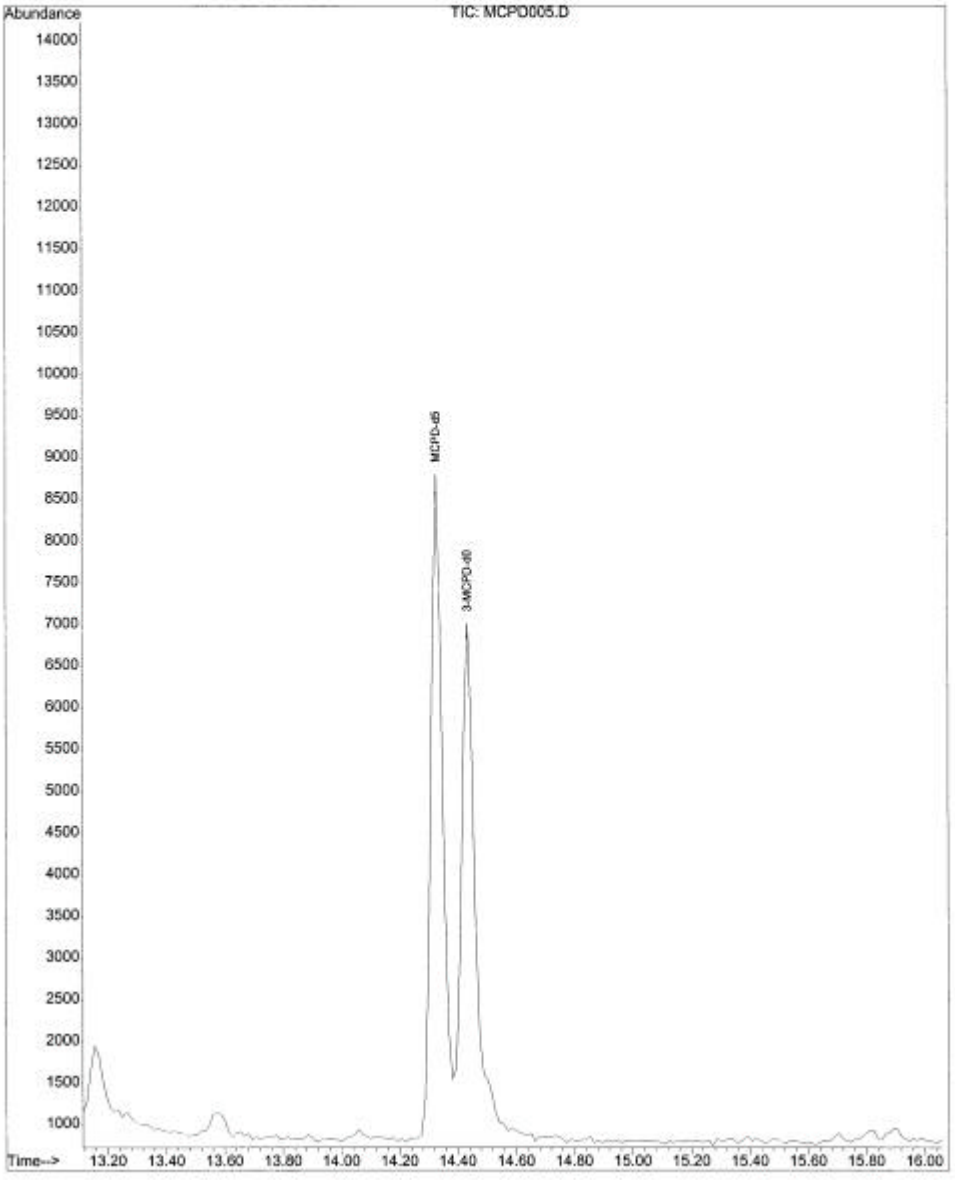


APPENDIX 2

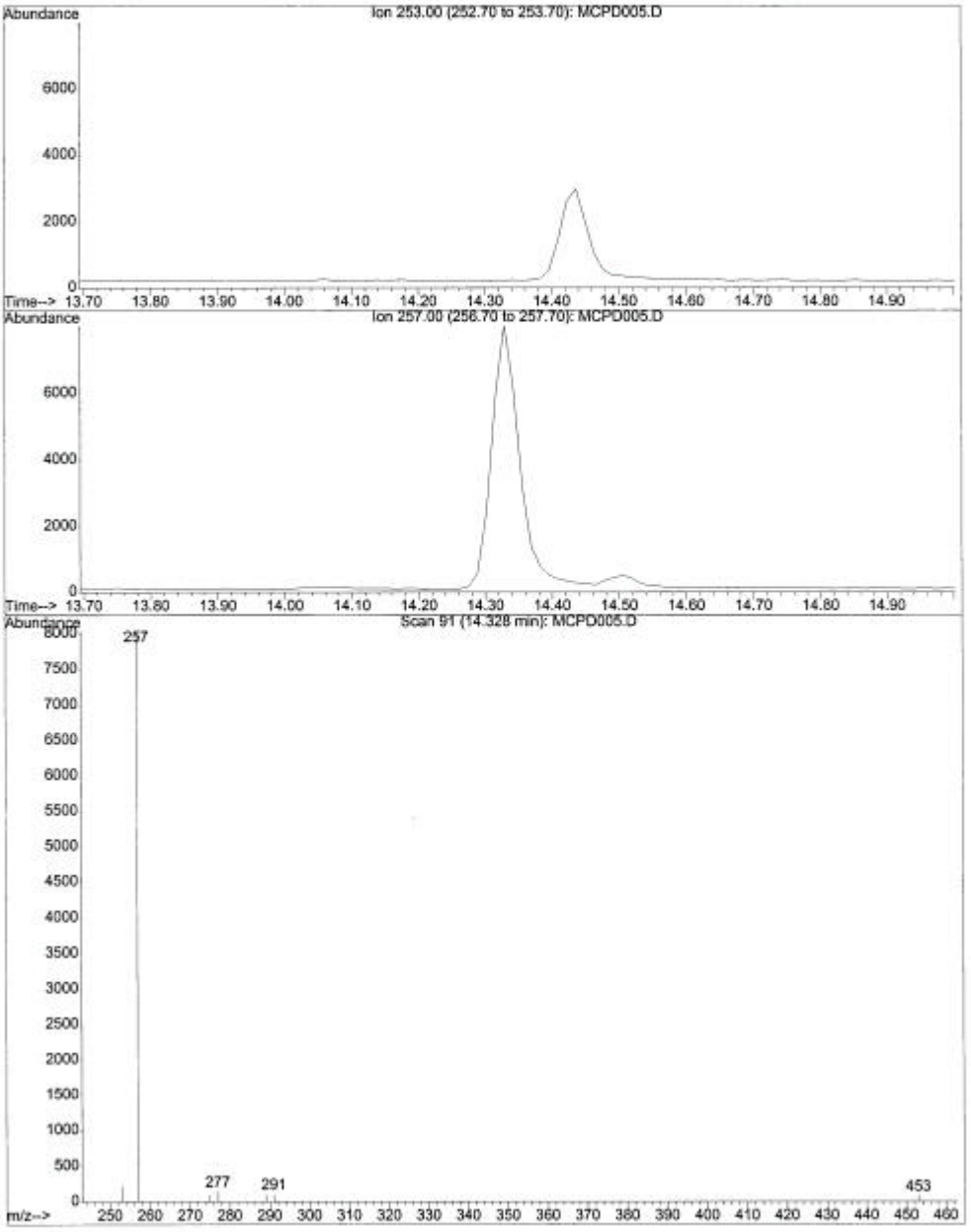
Chromatograms for 3-monochloropropan-1,2-diol

1. Total Ion Chromatogram (TIC) showing 3-MCPD-d5 and 3-MCPD-d0
2. Extracted Ion Chromatogram (EIC) showing m/z 253 and m/z 257 with mass spectrum of 3-MCPD-d5 (Internal Standard)
3. Extracted Ion Chromatogram (EIC) showing m/z 253 and m/z 257 with mass spectrum of 3-MCPD-d0 (Analyte)

```
File       : C:\HPCHEM\1\DATA\021008\MCPD005.D
Operator   : Richard Brown
Acquired   :  8 Oct 2002  18:02     using AcqMethod 3MCPD
Instrument :   GC/MS Ins
Sample Name: 10µg/L 3-MCPD + 50µg/L 3-MCPD-d5
Misc Info  :
Vial Number: 83
```



```
File       : C:\HPCHEM\1\DATA\021008\MCPD005.D
Operator   : Richard Brown
Acquired   :  8 Oct 2002  18:02     using AcqMethod 3MCPD
Instrument :    GC/MS Ins
Sample Name: 10µg/L 3-MCPD + 50µg/L 3-MCPD-d5
Misc Info  :
Vial Number: 83
```



```
File       : C:\HPCHEM\1\DATA\021008\MCPD005.D
Operator   : Richard Brown
Acquired   :  8 Oct 2002  18:02     using AcqMethod 3MCPD
Instrument :    GC/MS Ins
Sample Name: 10µg/L 3-MCPD + 50µg/L 3-MCPD-d5
Misc Info  :
Vial Number: 83
```