Environment Agency

The Determination of Phenoxy Acid Herbicides and Related Pesticides in Raw and Potable Waters by Liquid Chromatography

Methods for the Examination of Waters and Associated Materials

The Determination of Phenoxy Acid Herbicides and Related Pesticides in Raw and Potable Waters by Liquid Chromatography.

Methods for the Examination of Waters and Associated Materials

This booklet contains six methods for the determination of Phenoxy acid herbicides in waters.

2 using SPE sample preparation LCMS
2 using on line enrichment SPE LCMS
2 using Direct Injection LCMSMS

Each method has been validated in only one laboratory and consequently details are included for information purposes only as an example of the type of procedures that are available to analysts. Information on routine multi-laboratory use of these methods would be welcomed to assess their full capabilities.

Whilst this booklet may report details of the materials actually used, this does not constitute an endorsement of these products but serves only as an illustrative example. Equivalent products are available and it should be understood that the performance characteristics of the method might differ when other materials are used. It is left to users to evaluate methods in their own laboratories.

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soils (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series “Methods for the Examination of Waters and Associated Materials” and their continuing
revision is the responsibility of the Standing Committee of Analysts (established 1972 by the Department of the Environment). At present, there are eight working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical, Inorganic and physical methods
4 Metals and metalloids
5 Solid substances
6 Organic impurities
7 Biological, biodegradability and inhibition methods
8 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. If users wish to receive copies or advanced notice of forthcoming publications or obtain details of the index of methods then contact the Secretary on the SCA’s web-page:- http://www.standingcommitteeofanalysts.co.uk/Contact.html

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary. Users should ensure they are aware of the most recent version they seek.

Rob Carter
Secretary
April 2017

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; “Safe Practices in Chemical Laboratories” and “Hazards in the Chemical Laboratory”, 1992, produced by the Royal Society of Chemistry; “Guidelines for Microbiological Safety”, 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and “Safety Precautions, Notes for Guidance” produced by the Public Health Laboratory Service. Another useful publication is “Good Laboratory Practice” produced by the Department of Health**.**

The Determination of Phenoxy Acid Herbicides and Related Pesticides in Raw and Potable Waters by Liquid Chromatography

1 Introduction

Phenoxy Herbicides are a family of pesticides related to the growth hormone indoleacetic acid. They are selective to broad leafed plants meaning that when used they induce rapid, uncontrolled growth, eventually killing them. When sprayed on crops such as wheat or corn, they selectively kill just the broad-leaf plants in a field i.e the weeds, leaving the crops relatively unaffected.

The monitoring requirements for all pesticides in drinking water are derived from the Water Safety Plans (WSP) of each individual water company. The WSP's are based on extensive risk assessments and risk management plans in a water supply chain from catchment to consumer.

They include control measures, monitoring and incident and emergency plans. As each water catchment area is unique, the monitoring requirements of each area and hence water company will also vary.

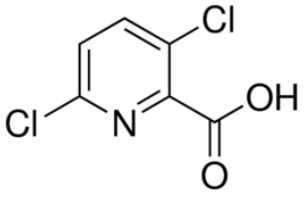
Analysis of herbicides from the phenoxy acid group are identified from the risk assesments and as such will vary by catchment and water supplier.

Methods are based on un-spiked and spiked samples and have variations in sample preparation, concentration or enrichment prior to being analysed by liquid chromatography mass spectrometry or tandem mass spectrometry. The methods described in this booklet are very similar but subtle differences are reported in the procedures.

Structures and description

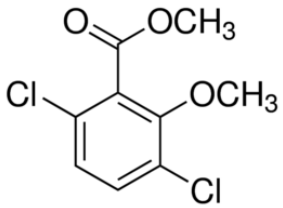
The following is a list of determinands contained in this document, their structures, CAS numbers and other relevant information sources to aid in the use of this document

Clopyralid



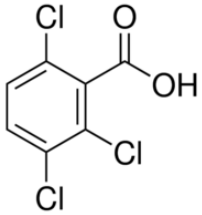
IUPAC name: 3,6-Dichloro-2-pyridinecarboxylic acid
Formula: $C_6H_3Cl_2NO_2$
CAS No.: 1702-17-6
pKa: 2.01
Molecular weight: 192.00

Dicamba



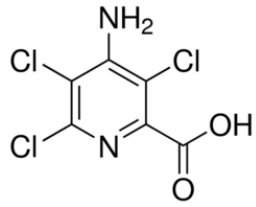
IUPAC name: 3,6-Dichloro-2-methoxybenzoic acid
Formula: $C_8H_6Cl_2O_3$
CAS No.: 1918-00-9
pKa: 1.87
Molecular weight: 221.04

2,3,6 – Trichlorobenzoic acid



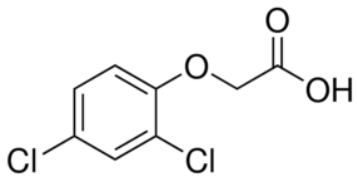
IUPAC name: 2,3,6-Trichlorobenzoic acid
Formula: $C_7H_3Cl_3O_2$
CAS No.: 50-31-7
pKa: 1.80
Molecular weight: 225.46

Picloram



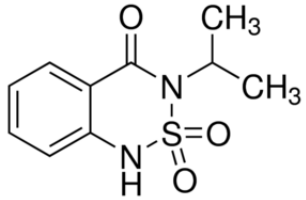
IUPAC name: 4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid
Formula: $C_6H_3Cl_3N_2O_2$
CAS No.: 1918-02-1
pKa: 2.30
Molecular weight: 241.46

2,4-D



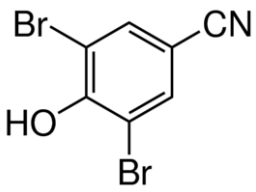
IUPAC name: 2,4-Dichlorophenoxyacetic acid
Formula: $C_8H_6Cl_2O_3$
CAS No.: 94-75-7
pKa: 2.73
Molecular weight: 221.04

Bentazone



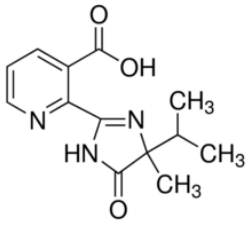
IUPAC name: 3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Formula: $C_{10}H_{12}N_2O_3S$
CAS No.: 25057-89-0
pKa: 3.30
Molecular weight: 240.28

Bromoxynil



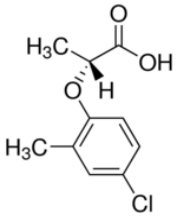
IUPAC name: 3,5-dibromo-4-hydroxybenzonitrile
Formula: C_7H_3BrNO
CAS No.: 1689-84-5
pKa: 3.86
Molecular weight: 276.91

Imazapyr



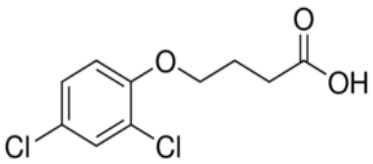
IUPAC name: (RS)-2-(4-Methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl) pyridine-3-carboxylic acid
Formula: $C_{13}H_{15}N_3O_3$
CAS No.: 81334-34-1
pKa: 3.80
Molecular weight: 261.28

MCPP (Mecoprop)



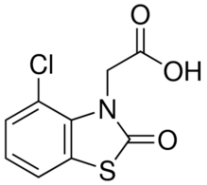
IUPAC name: (R)-2-(4-chloro-o-tolyloxy) propionic acid
Formula: $C_{10}H_{11}ClO_3$
CAS No.: 16484-77-8
pKa: 3.10
Molecular weight: 214.65

2,4-DB



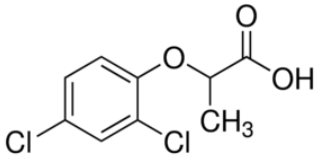
IUPAC name: 4-(2,4-dichlorophenoxy) butanoic acid
Formula: $C_{10}H_{10}Cl_2O_3$
CAS No.: 92-84-6
pKa: 2.73
Molecular weight: 249.09

Benazolin



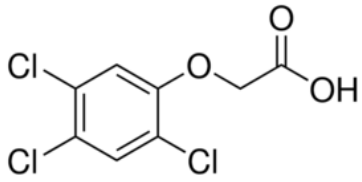
IUPAC name: 4-chloro-2,3-dihydro-2-oxo-1,3-benzothiazol-3-ylacetic acid
Formula: $C_9H_6ClNO_3S$
CAS No.: 813-05-6
pKa: 3.04
Molecular weight: 243.67

Dichlorprop



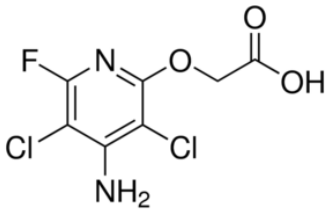
IUPAC name: (R)-2-(2,4-dichlorophenoxy) propanoic acid
Formula: $C_9H_8Cl_2O_3$
CAS No.: 120-36-5
pKa: 3.10
Molecular weight: 235.06

2,4,5-T



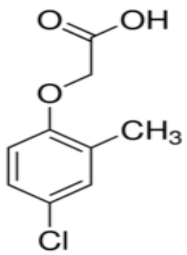
IUPAC name: 2,4,5-Trichlorophenoxyacetic acid
Formula: $C_8H_5Cl_3O_2$
CAS No.: 93-76-5
pKa: 2.88
Molecular weight: 255.48

Fluroxypyr



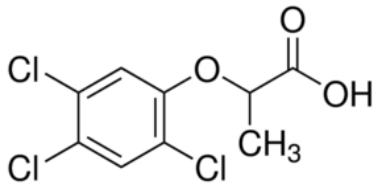
IUPAC name: [(4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetic acid
Formula: $C_7H_5Cl_2FN_2O_3$
CAS No.: 69377-81-7
pKa: 2.94
Molecular weight: 255.03

MCPA



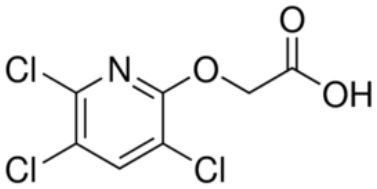
IUPAC name: (4-Chloro-2-methylphenoxy)acetic acid
Formula: $C_9H_9ClO_3$
CAS No.: 94-74-6
pKa: 3.07
Molecular weight: 200.62

Fenoprop



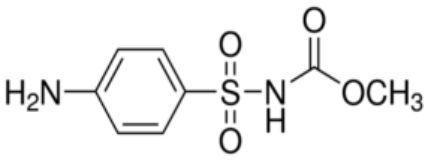
IUPAC name: 2-(2,4,5-Trichlorophenoxy)propionic acid
Formula: $C_9H_7Cl_3O_3$
CAS No.: 93-72-1
pKa: 2.84
Molecular weight: 269.51

Triclopyr



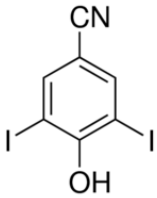
IUPAC name: [(3,5,6-Trichloro-2-pyridinyl)oxy]acetic acid
Formula: $C_7H_4Cl_3NO_3$
CAS No.: 55335-06-3
pKa: 2.68
Molecular weight: 256.47

Asulam



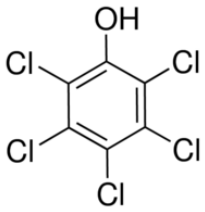
IUPAC name: N-(4-Aminophenyl)sulfonylcarbamic acid methyl ester
Formula: $C_8H_{10}N_2O_4S$
CAS No.: 3337-71-1
pKa: 4.82
Molecular weight: 230.24

Ioxynil



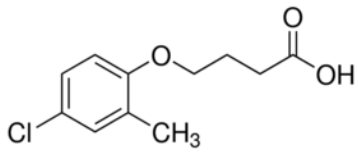
IUPAC name: 4-hydroxy-3,5-diiodobenzonitrile
Formula: $C_7H_3I_2NO$
CAS No.: 1689-83-4
pKa: 3.90
Molecular weight: 370.91

PCP



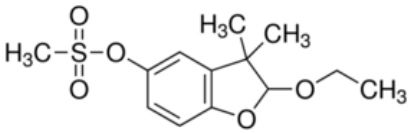
IUPAC name: 2,3,4,5,6-Pentachlorophenol
Formula: C_6HCl_5O
CAS No.: 87-86-5
pKa: 4.74
Molecular weight: 266.34

MCPB



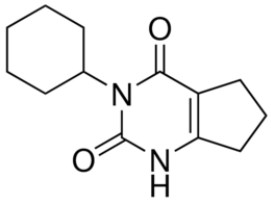
IUPAC name: 4-(4-Chloro-2-methylphenoxy) butyric acid
Formula: $C_{11}H_{13}ClO_3$
CAS No.: 94-81-5
pKa: 4.50
Molecular weight: 228.67

Ethofumesate



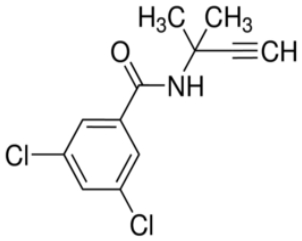
IUPAC name: 2-Ethoxy-3,3-dimethyl-2,3-dihydro-1-benzofuran-5-yl methanesulfonate
Formula: $C_{13}H_{18}O_5S$
CAS No.: 26225-79-6
pKa: Unknown
Molecular weight: 286.34

Lenacil



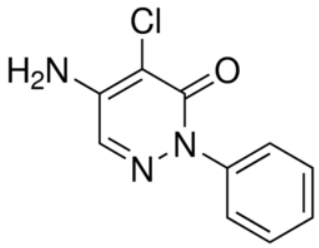
IUPAC name:3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(3H)-dione
Formula: $C_{13}H_{18}N_2O_2$
CAS No.: 2164-08-1
pKa: 10.3
Molecular weight: 234.29

Propyzamide



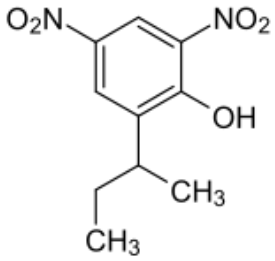
IUPAC name: 3,5-Dichloro-N-(1,1-dimethylpropynyl)benzamide
Formula: $C_{12}H_{11}Cl_2NO$
CAS No.: 23950-58-5
pKa: 12.3
Molecular weight: 256.13

Chloridazon



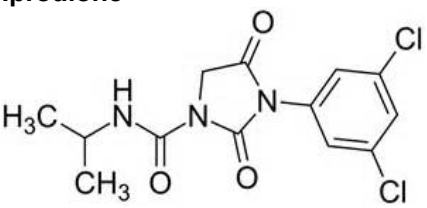
IUPAC name: 5-Amino-4-chloro-2-phenyl-3(2H)-pyridazinone
Formula: $C_{10}H_8ClN_3O$
CAS No.: 1698-60-8
pKa: Unknown
Molecular weight: 221.64

Dinoseb



IUPAC name: (RS)-2,4-Dinitro-6-sec-butylphenol
Formula: $C_{10}H_{12}N_2O_5$
CAS No.: 88-85-7
pKa: 4.62
Molecular weight: 240.21

Iprodione



IUPAC name: 3-(3,5-Dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
Formula: $C_{13}H_{13}Cl_2N_3O_3$
CAS No.: 36734-19-7
pKa: Unknown
Molecular weight: 330.17

Determinands covered by the methods in this book

Method identifier	A	B	C	D	E	F
Preparation technique	Online enrichment	SPE	Online enrichment	Direct Injection	SPE	Direct Injection
Instrumental technique	LCMSMS	LCMS	LCMSMS	LCMSMS	LCMSMS	LCMSMS
Instrument supplier*	Agilent (6410)	Agilent (1956)	Agilent	Agilent	Agilent	Sciex/Dionex
2,3,6-Trichlorobenzoic acid				X		
2,4,5-T	X		X	X		
2,4-D	X	X	X	X	X	X
2,4-DB	X	X		X	X	X
Asulam				X		
Benazolin	X		X	X		X
Bentazone	X	X	X	X	X	X
Bromoxynil	X		X	X	X	X
Chloridazon			X			
Clopyralid	X	X		X		Not included due to performance
Dicamba	X	X	X	X	X	X
Dichlorprop	X		X	X	X	X
Dinoseb				X		
Ethofumesate			X			
Fenoprop (2,4,5-TP)			X	X		
Fluroxypyr	X	X	X	X		X
Imazapyr				X		
Ioxynil	X		X	X	X	X
Iprodione				X		
Lenacil			X			
MCPA	X	X	X	X	X	X
MCPB	X	X	X	X	X	X
MCPP (Mecoprop)	X	X	X	X	X	X
PCP	X		X	X		
Picloram	X	X	X			
Propyzamide			X			
Triclopyr	X	X	X	X	X	X
2,4-DPA (S)						Listed in method but not performance data
4-Chlorophenyl acetic acid						
2-Bromo-4-Cyano phenol						

* Note: this book in no way endorces a particular instrument manufacturer or supplier, this is listed as a guide only to the configuration set up in the specific analytical sections to enhance understanding.

Sample Stability

Laboratories have provided details of the stability of samples and extracts were appropriate as a guide to holding time. Where no maximum time period is provided, this stage does not form part of the incorporated method.

Method identifier	A	B	C	D	E	F
From sampling to preparation	n/a	28 days	n/a	n/a	14 days	n/a
Extract stability	n/a	7 days	n/a	n/a	28 days	n/a
Sampling to analysis	21 days	35 days	21 days	21 days (exception 7 days for iprodione and 14 days for dinoseb)	42 days	21 days

Note: On embarking on the use of these methods, a laboratory should ensure the correct pH can be achieved for their water types following these instructions and amend accordingly for use within their own system. Dissolved organic carbon concentrations and hardness levels of waters may affect the performance of the method.

2 References

Methods for the Examination of Waters and Associated Materials: The determination of Acidic Herbicides, Bromoxynil, Ioxynil and Pentachlorophenol in waters 1997. (No. 186). Aldrich Technical Information Bulletin Number AL-180.

A The determination of phenoxy acid herbicides and associated compounds in raw and potable waters using liquid chromatography with mass spectrometric detection.

A1 Performance characteristics of the method

A1.1	**Substances determined**	Bentazone, Benazolin, Bromoxynil, Clopyralid, Dicamba, Fluroxypyr, Dichlorprop, 2,4-DB 2,4-D, Imazapyr, Ioxynil, MCPA, MCPB, Mecoprop, Picloram, Pentachlorophenol (PCP), Triclopyr, 2,4,5-T.
A1.2	**Type of sample**	Raw waters, drinking waters and process waters.
A1.3	**Basis of method**	The Determinands are pre-concentrated on-line by the use of a re-useable solid phase cartridge prior to analysis on a liquid chromatography with mass spectrometric detection.
A1.4	**Range of application**	Typically, up to 0.200 µg L^{-1}
A1.5	**Standard deviation**	See Table A1**.**
A1.6	**Limit of detection**	Typically, 0.005 µg L^{-1}, based on low level (0.02 µg L^{-1}) standard solutions.
A1.7	**Bias**	See Table A1.

A2 Principle

The sample, after dilution if required, and addition of internal standard is acidified and filtered through 0.2 µm syringe filter prior to loading on a pre-conditioned polymeric solid phase extraction column where the determinands are retained. The cartridge is then switched in line with the analytical column on LC system and the determinands are eluted on to the analytical column using a gradient LC method and detected by LC mass spectrometric detection.

A3 Interferences

Any co-extracted material which has a LC retention time similar to any of the above determinands and which gives a detector response at the monitored masses will interfere. However, none are known at the m/z values selected.

A4 Hazards

Skin contact or inhalation of all reagents and their solutions specified in this method should be avoided. Methanol and Acetone are highly flammable; these solvents should be handled

away from sources of ignition. Acid herbicides are toxic and suspected carcinogens. Formic acid and Acetic acid are corrosive.

A5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during the analysis and distilled, deionised or similar grade water should be used throughout. A procedural blank should be run with each batch of samples to check for interferences. All solutions should be mixed well prior to use.

A5.1 - **Water** LCMS Grade, or Deionised

A5.2 - **Acetone**

A5.3 - **Methanol**

A5.4 - **Acetonitrile**

A5.5 - **Formic Acid** LCMS Grade

A5.6 - **Acetic Acid** LCMS Grade

A5.7 - **Sodium Thiosulphate pentahydrate**

A5.8 - **Individual Acid Herbicide stock calibration solutions (1000 mg L^{-1}).** Weigh 50.0 mg of acid herbicide (after purity correction) into 50 mL volumetric flask dissolve and make up to volume with Acetone.
Individual Acid Herbicide stock Aqc solutions can be prepared in a similar way. The Solutions should be stored in a freezer and have an expiry date of 2 years.

A5.9 - **Mixed Intermediate calibration solution (100 µg L-1).** Add 10 µL of stock calibration standard solutions to 100.0 mL volumetric flask and make to volume with methanol.The solution may be stored between 1 - 10 °C and have an expiry date of 6 months.

A5.10 - **Mixed Intermediate Aqc solution (200 µg L^{-1}).** Add 20 µL of stock Aqc standard solutions to 100.0 mL volumetric flask and make to volume with methanol.The solution may be stored between 1 - 10 °C and have an expiry date of 6 months.

A5.11 - **Stock internal standard solution (100 mg L^{-1}).** Weigh 10mg of pure 2,4-Dichlorophenyl Acetic Acid (DCPAA) into a 100 mL volumetric flask dissolve and make to mark with acetone. The solution should be stored in a freezer and have an expiry date of 2 years.

A5.12 - **Working internal standard solution (500 µg L^{-1}).** Add 250µL of stock DCPAA (A5.11) into 50 mL volumetric flask and make to volume with methanol. The solution may be stored between 1 - 10 °C and have an expiry date of 6 months

A5.13 - 0.1% Acetic Acid. Pour LCMS water into 500 mL volumetric flask until 2cm below line add 500 µL Acetic Acid. Mix and make to volume with LCMS Water.

A5.14 - **1% Formic Acid**. Pour 500 mL of LCMS water into 1000 mL volumetric flask add 10 mL Formic acid. Mix and make to volume with LCMS water.

A5.15 - **Calibration standards** were prepared by first acidifying one litre of ultrapure water by adding 5 mL of formic acid. 50 mL of acidified water was then added to each of five 60 mL amber bottles, and 50 µL of internal standard were added to each bottle. The calibration standards were then prepared in the five 60 mL bottles per this matrix:

Bottle Number	Volume of Mixed Intermediate Standard added (µL)	Final Concentration of Standard (µg L^{-1})
1	100	0.20
2	50	0.10
3	20	0.04
4	10	0.02
5	0	0.00

A5.16 AQC solution was prepared by measuring 50 mL of acidified water to 60 mL amber bottle; add 50 µL of internal standard and 25 µL AQC intermediate solution.

A6 Apparatus

In addition to normal laboratory glassware the following may be required.

A6.1 - **Amber glass sample bottles**. Nominal 1 litre capacity with PTFE –lined screw plastic cap.

A6.2 - **Regenerated cellulose filter** 0.2 µm 15 mm.

A6.3 - **2.5mL Luer slip plastic syringes**.

A6.4 - **Solid phase extraction cartridge** PLRP-S (15-25 µm) Cartridges 10*2 mm

A6.5 - **On line enrichment set up**, Quaternary LC pump (Agilent 1200 series), autosampler capable of injecting 1.5 mL (or 2*750 µL), 6 port/2 position valve, 6 position selection valve.

A6.6 – **Liquid chromatograph** (Agilent 1200 series) fitted with a Binary pump, column oven connected to a triple quad mass spectrometer (Agilent 6410) capable of at least unit resolution and operating in MRM mode, with a data station. The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

A6.6.1 On line extraction conditions:

Mobile Phase	A: 1% Formic acid B: Acetonitrile
SPE cartridge	PRLP-s 10x2 mm, 15-25 µm
Temperature	Ambient
Flow (load)	1 mL min^{-1}

Gradient Program

Time	Gradient (%B)	Flow Rate (mL min^{-1})
0.0	0	1.0
3.0	0	1.0
3.5	0	0.5
4.5	100	0.5
18.0	100	0.5
18.2	100	1.0
25.0	100	1.0
25.5	0	1.0

Injector Program

Command:
VALVE mainpass
EJECT def. amount into seat, max. speed
DRAW def. amount from sample 750 µL speed 800
VALVE mainpass
WAIT 1.5 min

A6.6.2 LC Conditions

Analytical column	Zorbax C-18 Eclipse Plus, 2.1 mm x 150 mm x 3.5 µm (p/n 959763-902)
Column temperature	60°C
Injection volume	Injection program, 2 X 750 µL, for a total of 1.5 mL
Mobile phase	A = 0.1% Acetic acid B = Acetonitrile
Run time	29.0 min
Flow rate	0.25 mL min^{-1}

.

Gradient Program

Time (min)	Gradient (%B)
0	15
1.00	15
1.01	25
2.00	25
17.00	70
18.00	100
20.00	100
21.00	15

A6.6.3 MS Conditions

Acquisition parameters	ESI mode, pos/neg ionization; MRM (7 time segments)
Gas temperature	250 ^{o}C
Drying gas	8 L min^{-1} Nitrogen
Nebulizer pressure	40 psig
Vcap voltage	3000 V

LC-MS ions, Electrospray ionization.

Compound/ Determinand	Expected retention times (min)	Polarity Mode	Qualifier Transition	Quantitation Transition
DCPAA Internal std.	12.65	Negative ion	205.0>161.0	202.9>159.0
Clopyralid	5.3	Positive ion	194.0>148.0	192.0>146.0
Picloram	6.05	Positive ion	243.0>197.0	241.0>195.0
Imazapyr	6.55	Positive ion	262.2>234.3	262.2>217.2
Dicamba	9.03	Negative ion	221.0>177.0	219.0>175.0
Benazolin	9.5	Negative ion	242.0>198.0	242.0>170.0
Fluroxypyr	9.76	Negative ion	255.0>197.0	253.0>195.0
Bentazone	11.5	Negative ion	239.0>197.0	239.0>132.0
2,4-D	12.45	Negative ion	221.0>163.0	219.0>161.0
Bromoxynil	12.45	Negative ion	276.0>81.0	274.0>79.0
MCPA	12.65	Negative ion	201.0>143.0	199.0>141.0
Triclopyr	13.5	Negative ion	256.0>198.0	254.0>196.0
Ioxynil	14.1	Negative ion	369.8>214.9	369.8>126.9
Dichlorprop	14.3	Negative ion	235.0>163.0	233.0>161.0
245-T	14.5	Negative ion	254.9>196.9	252.9>194.9
MCPP	14.5	Negative ion	215.0>143.0	213.0>141.0
24-DB	15.3	Negative ion	249.0>163.0	247.0>161.0
MCPB	15.45	Negative ion	229.0>143.0	227.0>141.0
PCP	19.7	Negative ion	264.9>264.9 266.9>266.9	262.9>262.9

A7 Sample collection and preparation

Samples may be taken in amber glass with caps (lined with an appropriate inert material) containing sodium thiosulphate (3% m/v, giving a final concentration of 30 mg/l). Stability data indicate that samples may be stored for up to 21 days in glass bottles prior to analysis. Samples should be stored at 3°C ± 2°C.

A8 Analytical procedure

A8.1 Prepare standards and AQC as listed A5.15 and A5.16. Shake well, then filter 2 mL using regenerated cellulose filter into a labelled 2 mL amber vial. Note that 2 mL amber vials need to be filled to allow 1.5 mL to be removed.

A8.2 Shake sample bottles and measure 50 mL sample into labelled 60 mL amber bottle. Add 50 µL of internal standard. Shake well and filter 2 mL using regenerated cellulose filter into labelled 2 mL amber vial. Raw water samples may need a dilution to overcome matrix suppression effects of early eluting compounds.

A8.3 Set up LC and MS as above. Run at least two 0.2 µg L^{-1} standards to update MRM time segments.

A8.4 Run calibration standards and construct a calibration graph of response versus amount for each determinand, monitoring ions given in section A6.6.

A8.5 Analyse sample extracts and from calibration graph calculate amount of determinand in sample.

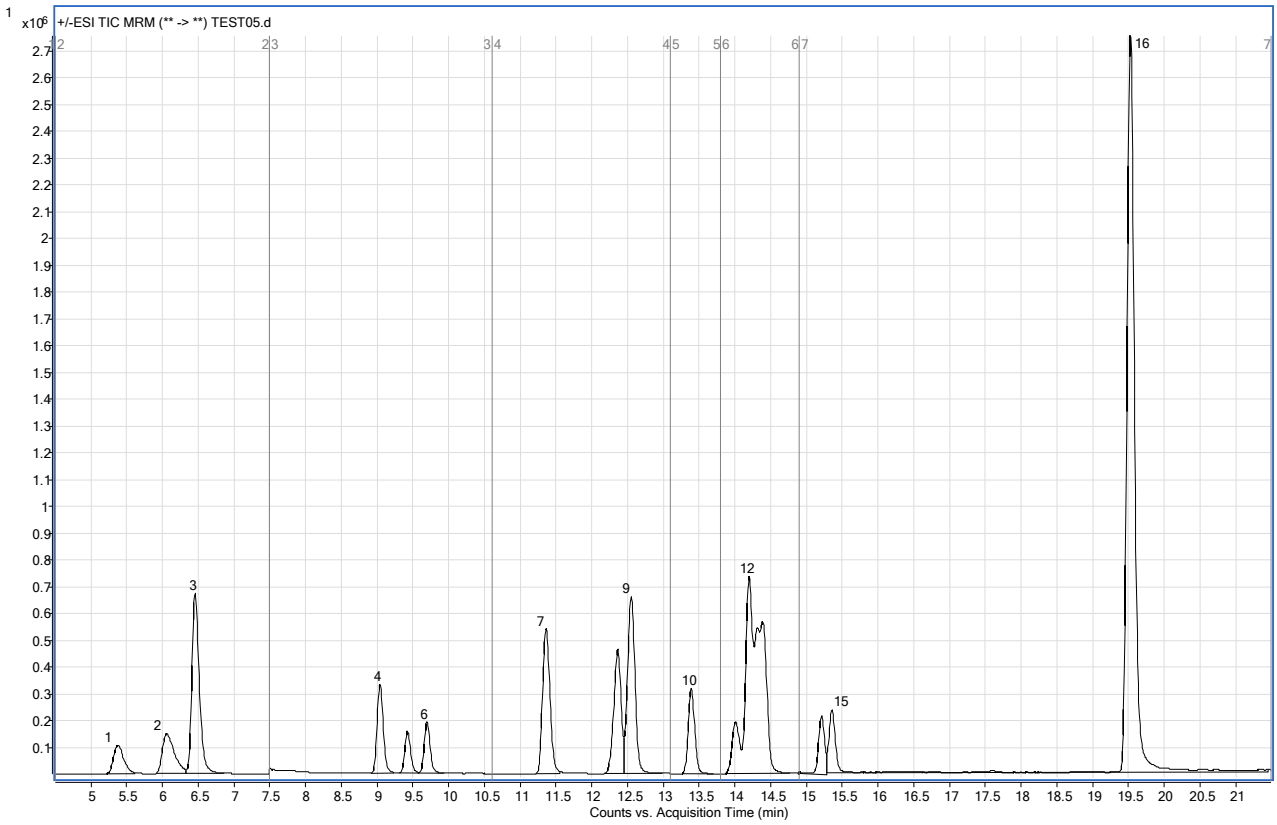
A9 Calculation

The concentration of each acid herbicide is given by an internal standard procedure:-

$$\mathbf{C} = \frac{\mathbf{A.\,ISstd.\,Cstd}}{\mathbf{Astd.\,IS}}\ \mu g\ L^{-1}$$

A = peak area in sample
Astd= peak area in standard
IS = peak area of internal standard in sample
ISstd = peak area of IS in standard
Cstd = concentration of analyte in standard, (µg L^{-1})

Figure A1 Typical Chromatogram



Key	Compound
1	Clopyralid
2	Picloram
3	Imazapyr
4	Dicamba
5	Benazolin
6	Fluroxypyr
7	Bentazone
8	2,4-D/Bromoxynil
9	MCPA/DCPAA
10	Triclopyr
11	Ioxynil
12	Dichlorprop
13	2,4,5-T/MCPP
14	2,4-DB
15	MCPB
16	PCP

Table A1 Performance Data

Compound/ Determinand	LOD	Sample*		Standards			
				0.02 µg L^{-1}		0.1 µg L^{-1}	
	µg L^{-1}	SD	Bias %	SD	Bias %	SD	Bias %
Imazapyr	0.005	0.005	-3.1	0.002	1.5	0.005	-3.6
Dicamba	0.005	0.005	-5.6	0.001	3	0.005	-2.4
MCPP	0.005	0.002	-1.4	0.001	2	0.004	0
MCPA	0.005	0.005	-3.4	0.001	2	0.006	-2.4
Dichlorprop	0.005	0.002	-2.0	0.001	1	0.004	-1.8
2-4 D	0.005	0.004	-1.5	0.001	3.5	0.005	-1.9
Bromoxynil	0.005	0.004	-3.0	0.001	5	0.005	-2.8
Triclopyr	0.005	0.003	-3.6	0.001	3.5	0.006	-3.2
MCPB	0.005	0.004	-1.0	0.001	4.5	0.004	-0.4
2,4,5-T	0.005	0.003	-0.5	0.001	4.5	0.004	-0.5
Fluroxypyr	0.005	0.004	-2.8	0.001	5.5	0.005	0.3
2,4-DB	0.005	0.002	-2.5	0.001	4	0.005	-2.3
Bentazone	0.005	0.005	-3.5	0.001	-2.5	0.005	-0.7
Ioxynil	0.006	0.005	0.0	0.001	0.5	0.004	-1.4
Clopyralid	0.020	0.015	-12.8	0.001	13	0.012	9
Picloram	0.015	0.007	-6.1	0.003	4.5	0.011	-0.1
PCP	0.006	0.004	-1.5	0.001	-6	0.003	-5.2
Benazolin	0.005	0.004	-3.8	0.001	6.5	0.006	-0.7

*Final treated water from ground water, typical hardness 195 mg L-1 CaCO3
LOD Sw of Low Standard*5
(where Sw is within batch standard deviation)

Data provided by South East Water

B The Determination of Phenoxy Acid Herbicides in Waters by Solid Phase Extraction and Liquid Chromatography Mass Spectrometry

B1 Performance Characteristics of the Method

B1.1	**Substances determined**	Clopyralid, Picloram, Dicamba, Fluroxypyr, Bentazone, 2,4-D, MCPA, Mecoprop, Triclopyr, 2,4-DB and MCPB.
B1.2	**Types of Sample**	River, reservoir, ground and drinking waters.
B1.3	**Basis of method**	Samples are acidified, then prepared using Solid Phase Extraction (SPE) and examination by liquid chromatography mass spectrometry (LC-MS)
B1.4	**Range of application**	Up to 0.2 µg L^{-1}. The upper limit may be extended by dilution of sample extract or by taking a smaller sample volume.
B1.5	**Calibration curve**	This method is linear over the range of application
B1.6	**Internal standards used**	Dicamba-d3, Bentazone-d6, 2,4-D-d3, Mecoprop-d3 and MCPB-d6 See Table B1
B1.7	**Standard deviation and Bias**	See Table B2
B1.8	**Limit of detection**	See Table B2

B2 Principle

50 mL of sample is spiked with deuterated internal standards; then pH is adjusted to 2.0 by the addition of hydrochloric acid. The sample is then extracted using a styrene divinyl benzene solid phase extraction (SPE) cartridge. The cartridge is dried, and then eluted with solvent. The extract is taken to dryness and re-constituted using mobile phase. The herbicides are determined by LC-MS operating in negative mode with selected ion monitoring (SIM). Calibration standards and procedural blank are extracted and analysed alongside test samples. Quantification is performed using extracted standard with internal standard as part of the instrument software system.

B3 Interferences

Any co-extracted material which is not removed by the SPE procedure, which has a similar retention time to the analyte and gives a response for that particular ion under the particular fragment or conditions, will interfere.

B4 Hazards

Acid herbicides are toxic.
Hydrochloric acid is an irritant and corrosive
Formic acid is harmful, corrosive and flammable.
Methanol is toxic and flammable
Acetone and ethyl acetate are irritants and flammable.
Acetonitrile is harmful and flammable
Skin contact or inhalation of all reagents and their solutions specified in this method should be avoided.

B5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks or raised background levels during the LC-MS analysis. Procedural blanks should be run with each batch of samples analysed.

B5.1 **Ultra-Pure Water** (UPW)

B5.2 **Water**

B5.3 **Sodium Sulphite**

B5.3.1 **Sodium sulphite solution**
Add 3.5 g sodium sulphite to 1 Litre UPW. Prepare weekly.

B5.4 **Methanol** LC-MS grade

B5.5 Ethyl Acetate

B5.6 Acetone HPLC grade

B5.7 Formic Acid LC-MS grade

B5.7.1 2.5mM formic acid as LC solvent
Add 95 µL formic acid to 1 Litre UPW and use as aqueous mobile phase

B5.7.2 Make-up Solvent
Weigh 475 g 2.5 mM formic acid solvent into clean bottle. Weigh 19.6 g Acetonitrile and add to the formic acid (5% acetonitrile in 2.5 mM formic acid solvent)

B5.8 5 M Hydrochloric acid (AR grade)

B5.8.1 1.25 M HCl
Prepare by slowly adding 250 mL of 5 M HCl to approximately 750 mL UPW, after allowing to cool make up to 1 L with UPW

B5.8.2 pH 2 Water
Add 17.5 mL of 1.25 M HCl to 982.5 mL UPW

B5.9 Acetonitrile (LCMS grade)

B5.10 Standard Solutions

B5.10.1 Stock Standard solution of Acid Herbicides
Prepare a mixed stock solution of Acid Herbicides in methanol at a concentration of 1.0 mg L^{-1}. They can be purchased as certified reference materials, or as individual solutions.

B5.10.2 Stock AQC solution of Acid Herbicides
Prepare a mixed stock solution of Acid Herbicides in methanol at a concentration of 0.5 mg L^{-1}. They can be purchased as certified reference materials, or as individual solutions.

B5.10.3 Stock solution of Internal Standard
Prepare a mixed stock solution of Deuterated herbicides in methanol at a concentration of 1.0 mg L^{-1}. They are purchased as individual solutions.

B5.10.4 Spiking Standard solution of Acid Herbicides

Prepare by diluting Standard stock solution in methanol to a concentration of 0.05 mg L^{-1}.

B5.10.5 Spiking AQC solution of Acid Herbicides

Prepare by diluting AQC stock solution in methanol to a concentration of 0.025 mg L^{-1}.

B5.10.6 Spiking solution of Internal Standard

Prepare by diluting Internal standard stock solution in methanol to a concentration of 0.05 mg L^{-1}.

B6 Apparatus

Apparatus should be free from contamination before use. Glassware should be washed, rinsed with dilute acid followed by deionised water and allowed to dry.

B6.1 **Sample Bottles**, food grade PET, 500 or 1000 mL.

B6.2 **Volumetric flasks**, measuring cylinders, pipettes, culture tubes, vials. Various sizes.

B6.3 **Glass autosampler vials**. 0.3 mL for the LC-MS.

B6.4 **Styrene divinylbenzene (SPE) cartridges** (25 mg) sorbent mass. The performance of the method may vary with different batches of cartridge material or supplier. It is important that different batches are performance tested before routine use. The performance testing of the method was undertaken using ENV+ cartridges.

B6.5 **Cartridge manifold** and pump or automated sample extraction device. Suitable for use with ENV+ cartridges

B6.6 **Plastic SPE reservoirs**, cartridge adapters and taps. Suitable for use with ENV+ cartridges.

B6.7 **Turbo-Vap**, solvent evaporator

B6.8 **LC-MS equipment**. LC-MS capable of operating with electrospray and negative ion collection, with selected ion monitoring (SIM).

B6.8.1 LC Conditions

Column	Ultra-Aqueous C18, 10 cm x 2.1 mm ID, 3 µm material size. With matched guard, 1 cm.

	LC Binary pump	0.3 mL min^{-1} run time 35.5 min.
	Mobile Phase	A = 2.5mM Formic Acid B = Acetonitrile
	Gradient Programme	See Table B2
	Injection volume	40 µL
	Column Oven	35°C

B6.8.2 MS Conditions

Drying gas flow	Nitrogen, 13.0 L min^{-1}.
Drying gas temperature	350°C
Nebulizer pressure	60 psig
VCap (negative)	2500 V
Acquisition parameters	Diagnostic fragment ions are given in table B3

B7 Sample Collection and Preservation

Samples should be taken in food grade PET bottles. They should be dechlorinated as soon as possible using the sodium sulphite solution. They should be extracted as soon as possible after sampling, if this is not possible they should be stored in a dark cold store at 3°C ± 2°C.

B8 Analytical Procedure

B8.1 SPE cartridge preparation, use manifold

B8.1.1 Condition an ENV+ cartridge with 600 µL acetone followed by 600 µL ethyl acetate then 600 µL methanol. The solvents should be allowed to flow through the cartridge under gravity.

B8.1.2 Add 600 µL of pH2 water, followed by a second aliquot of 600 µL. Immediately add an adapter, a closed tap and then attach a reservoir. The cartridge should not be allowed to dry out

B8.2 Sample Treatment

B8.2.1 Pour 50 mL of sample into the reservoir. Add 200 µL of internal standard spiking solution, label cartridge with unique sample number.

B8.2.2 Add 1750 µL of 1.25 M HCl solution.

B8.3 Standard Treatment

B8.3.1 Pour 50 mL of natural water into each reservoir (4 required). Add 200 µL of internal standard spiking solution, label one cartridge as BLK

B8.3.2 To one reservoir add 200 µL spiking standard solution, label cartridge as STD

B8.3.3 To one reservoir add 20 µL spiking standard solution, label cartridge as LOW

B8.3.4 To one reservoir add 20 µL spiking standard solution, label cartridge as AQC

B8.3.5 Add 1750 µL of 1.25 M HCl solution.

B8.4 Extraction

B8.4.1 Open the taps on the reservoirs, a vacuum of up to 5 inches Hg may be required, and draw the samples through the cartridges to waste.

B8.4.2 Remove each cartridge as soon as the sample has been extracted The cartridges should not be allowed to dry at this stage. For samples which contain a significant amount of particulate the vacuum may require to be increased to aid extraction.

B8.4.3 Replace cartridges onto the manifold and wash with 600 µL pH 2.0 water. Then dry cartridges with at least 15 inches Hg (or equivalent) until visibly dry (about 8 minutes).

B8.4.4 Put labelled culture tubes into rack inside manifold, being careful to position correctly labelled tube below its labelled cartridge.

B8.4.5 Elute by adding 600 µL methanol, allowing the solvent to flow through. Elution through PTFE needles minimizes metal ion content in the extracts. Then add 600 µL ethyl acetate and allow to flow through. Finally add 60 µL acetone. Apply a vacuum to draw off last drops of solvent and to dry the cartridges.

B8.5 Evaporation

B8.5.1 Place culture tubes into Turbo-Vap and evaporate samples just to dryness

B8.5.2 Dissolve the residue to 200 µL with make-up solvent

B8.5.3 Transfer extract to 0.3 mL vial and cap. If vialled extracts cannot be analysed immediately, they must be stored under refrigeration, they must be analysed within 7 days.

B8.6 Set up LC and MS as specified in B6.8

B8.7 Run calibration standards followed by blanks, samples and AQC.

B8.8 The calibration standards are used to set up the calibration curve, using response versus amount.

B8.9 All compounds are quantified using the main ion for each analyte, and calculated using the calibration graph. B9 shows the calculation. Table B4 shows LC-MS ions

B8.10 Confirmation of positive results is by examination of the qualifier ion for each analyte. See Table B4

B8.11 If a significant interference occurs for a particular analyte, the qualifier is considered (with caution) if it meets the performance criteria

---

B9 Calculation

The concentration of each acid herbicide is given by an internal standard procedure:-

$$\mathbf{C} = \frac{\mathbf{A.\,ISstd.\,Cstd}}{\mathbf{Astd.\,IS}}\ \mu g\ L^{-1}$$

A = peak area in sample
Astd= peak area in standard
IS = peak area of internal standard in sample
ISstd = peak area of IS in standard
Cstd = concentration of analyte in standard, (µg L^{-1})
C = calculated concentration of analyte in sample (µg L^{-1})

Figure B1 Typical chromatogram

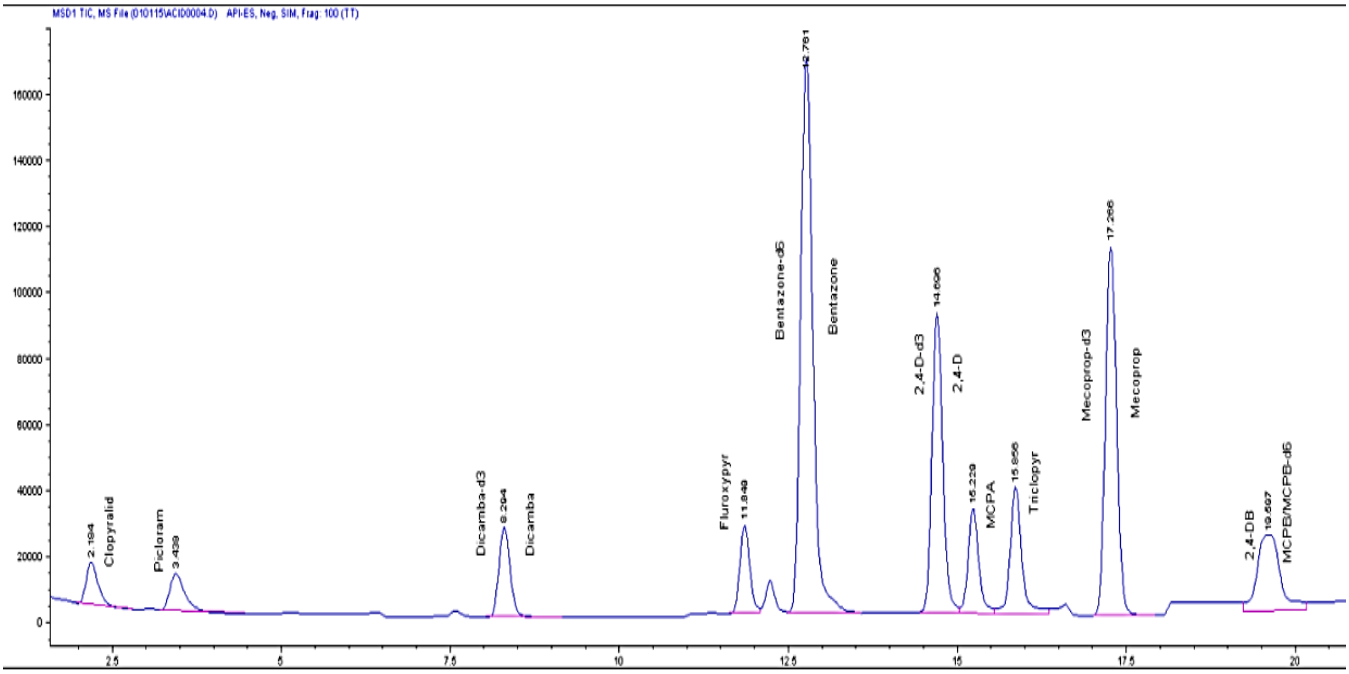


Table B1 Internal Standard and Associated Phenoxy Acid Herbicides

Internal Standard			
Dicamba-d3	Clopyralid	Picloram	Dicamba
Bentazone-d6	Fluroxypyr	Bentazone	
2,4-D-d3	2,4-D	MCPA	
Mecoprop-d3	Triclopyr	Mecoprop	
MCPB-d6	2,4-DB	MCPB	

Table B2 Binary Pump Timetable

Time	Solvent B %	Flow mL min^{-1}
0.00	5.0	0.300
11.00	35.0	0.300
22.00	50.0	0.300
24.00	50.0	0.300
25.00	95.0	0.300
27.00	95.0	0.500
28.00	5.0	0.500
34.00	5.0	0.300

Table B3 LC-MS ions, negative ion Electrospray ionization.

Compound	Quantifier Ion	Fragmentor Voltage	Qualifier ions	Fragmentor Voltage	Relative Abundance %	Retention time	Polarity Mode
Clopyralid	190	100	192	100	63.6	2.18	Negative
Picloram	195	140	139	80	166.6	3.44	Negative
Dicamba-d3	180	100	178	100	156.3	8.28	Negative
Dicamba	175	100	177	100	66.6	8.30	Negative
Fluroxypyr	195	160	253	80	102.0	11.84	Negative
Bentazone-d6	245	160	246	160	12.4	12.725	Negative
Bentazone	239	160	140	160	12.7	12.791	Negative
2,4-D-d3	164	160	222	100	101.8	14.67	Negative
2,4-D	161	160	219	100	109.0	14.71	Negative
MCPA	141	180	143	180	31.6	15.23	Negative
Triclopyr	196	150	198	150	89.3	15.85	Negative
Mecoprop-d3	144	180	216	100	135.8	17.23	Negative
Mecoprop	141	180	213	100	161.5	17.28	Negative
2,4-DB	161	140	163	140	70.8	19.49	Negative
MCPB-d6	147	135	-	-	-	19.56	Negative
MCPB	141	160	227	100	139.8	19.68	Negative

Table B4 Standard deviation and Recoveries

Standard deviations on three water types spiked at 0.1 μgL^{-1} level, with > 10 degrees of freedom

Determinand	LOD	Sample*		Standards			
		0.1 µg L^{-1}		0.02 µg L^{-1}		0.1 µg L^{-1}	
	µg L^{-1}	SD	Bias %	SD	Bias %	SD	Bias %
Clopyralid	0.005	0.0046	0.2	0.0020	-6.2	0.0052	1.8
Picloram	0.005	0.0070	-7.0	0.0011	-9.9	0.0036	-1.2
Dicamba	0.006	0.0041	-0.9	0.0017	-20.5	0.0034	-2.0
Fluroxypyr	0.005	0.0035	-6.9	0.0012	-15.9	0.0038	0.6
Bentazone	0.003	0.0029	4.3	0.0008	-3.8	0.0021	1.7
2,4-D	0.003	0.0019	3.0	0.0008	-1.4	0.0015	2.2
MCPA	0.002	0.0027	2.8	0.0011	-3.2	0.0020	1.6
Triclopyr	0.005	0.0025	-9.8	0.0018	-8.9	0.0033	2.3
Mecoprop	0.002	0.0025	1.8	0.0008	-6.3	0.0017	0.7
2,4-DB	0.008	0.0057	-1.3	0.0019	-11.4	0.0045	1.4
MCPB	0.003	0.0031	2.7	0.0009	-5.3	0.0034	1.7

Limits of detection (LOD) based on 3 x Within Batch Standard deviation (Sw) of a 0.04 µg L^{-} sample. *Sample is surface water, standards are ground water. SD is total standard deviation

Data provided by Wessex Water

C The Determination of Phenoxy Alkanoic Acid Herbicides and Other Compounds in Raw and Potable Waters by Online Enrichment Liquid Chromatography Mass Spectrometry (LCMSMS)

C1 Performance Characteristics of the Method

C1.1	**Substances determined**	Dicamba, MCPP, MCPA, Dichlorprop, Bromoxynil, Triclopyr, Pentachlorophenol (PCP), Fenoprop, MCPB, Fluroxypyr, Bentazone, Benazolin, 2,4-D, 2,4,5 T, 2,4 DB, Propyzamide, Ioxynil, Picloram, Chloridazon, Ethofumesate, Lenacil.
C1.2	**Types of Sample**	Raw and Potable Waters
C1.3	**Basis of method**	Online enrichment followed with analysis by Liquid Chromatography Mass Spectrometry (LCMSMS).
C1.4	**Range of application**	Up to 0.5 µg L^{-1} (Greater with dilution)
C1.5	**Calibration curve**	This method is linear over the range of application
C1.6	**Standard deviation**	See Table C1
C1.7	**Limit of detection**	See Table C1

C2 Principle

Phenoxy alkanoic acid herbicides (PAA) and additional herbicides are enriched using an on-line system then analysed using LCMS-MS. The compounds are identified by their specific mass transitions and retention times in comparison to known standards. They are quantified by comparison with a 7 point calibration curve, using internal standards, (MCPA-D_6 , 2,4-$D^{13}C_6$, Bentazone D_6, Dicamba$^{13}C_6$, Dichlorprop D_6, MCPP D_6, PCP $^{13}C_6$, ,Chloridazon-D_5 and Propyzamide D_3)

C3 Interferences

Any compounds present after enrichment which have similar properties and give rise to the same ions may interfere.

C4 Hazards

Methanol and Acetone are toxic by inhalation and ingestion and are flammable. All the phenoxyalkanoic herbicides are toxic.

Formic acid is corrosive. All the above reagents should be handled only in a fume cupboard, wearing Nitrile gloves and safety spectacles.

C5 Reagents

All reagents are HPLC-MS grade except where stated. All deionised water is prepared using a Milli- Q™ System. All reagents should be mixed thoroughly where required.

C5.1 Methanol

C5.2 Acetone

C5.3 0.1% Formic Acid – Using an appropriate autopipette add 2 mL of Formic acid to 2000 mL of deionised water. Mix thoroughly.

C5.4 Acetonitrile

C5.5 Standard Solutions

C5.5.1 Stock Mixed Calibration Solution 100 ng μL^{-1}

Expiry date as stated by the manufacturer

This is stored under refrigeration at 3°C ± 2°C once opened

C5.5.2 Calibration Spiking Solution 50 µg L^{-1}

Into a 50 mL volumetric flask containing approximately 30 mL of acetone add 25µl of 100 ng/µL of Stock Solution using a 100µl microsyringe. Make up to volume with acetone.

C5.5.3 Working Calibration Standards

0 ng L^{-1}, 30 ng L^{-1}, 50 ng L^{-1}, 100 ng L^{-1}, 200 ng L^{-1}, 300 ng L^{-1} and 500 ng L^{-1}.

To separate 50mL volumetric flasks and using an appropriate autopipette add 0 µL, 30 µL, 50 µL, 100 µL, 200 µL, 300 µL and 500 µL of 50 µg L^{-1} Calibration spiking solution to approximately 25 mL of DI water and make up to the mark. Mix thoroughly and transfer to suitably labelled, separate 44 mL sample vials. Standards are then treated as samples according to the analytical procedure.

These seven standards are used to produce a multiple point calibration curve and may be stored at 3°C ± 2°C for up to 21 days.

C5.6 Internal Standard Preparation

C5.6.1 Stock Solutions

Expiry date as stated by the manufacturer
100 mg L^{-1} MCPA-D6
100 mg L^{-1} 2,4-D-13C6
100 mg L^{-1} Bentazone D6
100 mg L^{-1} Dicamba 13C6
100 mg L^{-1} Dichlorprop D6
100 mg L^{-1} MCPP D6
100 mg L^{-1} PCP 13C6
100 mg L^{-1} Chloridazon D5
100 mg L^{-1} Propyzamide D3

C5.6.2 50 µg L^{-1} Internal Standard Spiking Solution

Add 50 µL of each Stock Solution to approximately 50 mL of acetone in a 100 mL volumetric flask and make up to volume. The solution is stable for 6 months if refrigerated at 3°C ± 2°C and kept in the dark.

C5.7 AQC Stock Mixed AQC Solution 100 ng μL^{-1}

Expiry date as stated by the manufacturer
This is stored under refrigeration at 3°C ± 2°C once opened

C5.7.1 AQC Spiking Solution 50 µg L^{-1}

Into a 50 mL volumetric flask containing approximately 30 mL of acetone add 25µl of 100 ng μL^{-1} of Stock Solution using a 100µl microsyringe. Make up to volume with acetone.

C5.7.2 Working AQC Standards 100 ng L^{-1}.

Into a 50mL volumetric flasks and using an appropriate autopipette add 100µl, of 50 µg L^{-1} AQC spiking solution to approximately 25 mL of DI water and make up to the mark. Mix thoroughly and transfer to a suitably labelled 44 mL sample vial. AQCs are then treated as samples according to the analytical procedure.

C6 Apparatus

In addition to normal laboratory glassware and equipment the following may also be required
Apparatus should be free from contamination before use. Glassware should be washed, rinsed with dilute acid followed by deionised water and allowed to dry.

C6.1 Solid phase cartridges, PLRPs or equivalent

C6.2 LCMS system

C6.3 Column- Eclipse Plus C18 column, 2.1 x 150 mm 3.5 um 600 bar (Agilent)

C6.4 Chromatography conditions.

LC Binary pump
Mobile Phase A 0.1% Formic Acid in Water
Mobile Phase B Acetonitrile
Injection volume: 1800 µL.
Flow: 0.3 mL min^{-1}.

Time (minutes)	Mobile phase A (%)	Mobile phase B (%)
0.00	95	5
4.00	95	5
4.1	95	5
10.00	68	32
22.00	35	65
24.00	35	65
24.10	95	5
26.00	95	5

LC Quat Pump- Online SPE.
Injection volume: 1800 µL.

Time (minutes)	Flow Rate mL min^{-1}	Mobile phase A (%)
0.00	0.5	100
5.00	0.5	100
16.9	0.5	100
19.00	2.0	0
20.9	2.0	0
21.0	2.0	100
23.0	2.0	100
23.1	1	100

C6.5 Source Parameters

	Value(+)	Value(-)
Gas Temp	275	275
Gas Flow(L min^{-1})	8	8

Nebulizer(psi)	45	45
SheathGasHeater	300	300
Capillary(V)	3500	2000
Vcharging	500	750

C 6.6 LC-MSMS ions, positive/negative ion Electrospray ionization.

Compound	Precursor ion	Product Ion	Fragmentor Voltage	CE	Approx Retention time	Polarity Mode
2,4,5-T	255	197	75	7	17.4	Negative
2,4-D	219.1	161	80	7	15.8	Negative
2,4-D 13C6	225.1	167	80	7	15.8	Negative
2,4-DB	247.1	161	65	7	18.18	Negative
Benazolin	244	170	87	21	13.6	Positive
Bentazone	239	132	113	25	15.1	Negative
Bentazone-d6	245.1	132	136	25	15	Negative
Bromoxynil	275.9	79	130	40	15.4	Negative
Chloridazon	222	104	120	15	11.7	Positive
Chloridazon-d5	227	109	120	15	11.7	Positive
Dicamba	219	175	60	0	13.8	Negative
Dicamba 13C6	225	181	60	0	13.8	Negative
Dichlorprop	233.1	161	75	5	17.3	Negative
Dichlorprop d6	239.1	164	75	5	17.3	Negative
Ethofumesate	304.1	121.1	90	16	19.8	Positive
Fenoprop	266.85	195	143	17	18.9	Negative
Fluroxypyr	253	195	90	9	13.7	Negative
Ioxynil	369.9	127	135	50	16.9	Negative
Lenacil	235.1	153.1	80	10	14.7	Positive
MCPA	199.2	141.1	90	11	15.8	Negative
MCPA-d6	205.1	147	83	9	15.8	Negative
MCPB	227.2	141.1	65	5	18.3	Negative
MCPP	213	140.9	78	9	17.4	Negative
MCPP d6	219	146.9	78	9	17.4	Negative
PCP	262.9	262.9	60	1	22	Negative
PCP 13C6	268.9	268.9	60	1	22	Negative
Picloram	241	195	75	18	10.9	Positive
Propyzamide	254.1	228.2	125	9	19.5	Negative
Propyzamide d3	257.1	231.2	125	9	19.5	Negative
Triclopyr	254.1	196.1	65	6	16.6	Negative

C7 Sample Collection and Preservation

Samples should be collected in 44 mL amber sample vials. Sample vials should not be rinsed out prior to sampling and vials should be filled to the top. Upon receipt samples are acidified with 50 µL of Formic acid and clearly marked H+ to indicate acidification. Samples should be refrigerated at 3ºC ± 2ºC on receipt and are stable for 21 days after sampling date.

C8 Analytical Procedure

C8.1 Add 90 µL of ISTD Solution to a 2 mL glass autosampler vial.

C8.2 Transfer 1800 µL of sample to the same glass vial and cap. Samples may need to be filtered and/or diluted if high in particulate matter.

C8.3 The LC-MS is set up according to the manufacturers instructions. Samples and standards are run using method conditions described. Retention times may require adjusting periodically due to column usage, Dynamic windows may need altering accordingly

C8.4 Analyse blank and AQC solutions using the entire procedure as described, replacing the sample as appropriate.

C8.5 The system is calibrated using the calibration standards and blank. Calibration is linear across this range. Calibration is by peak area determinations. The calibration format is linear with a minimum of 3 points plus the blank. Analyse the sample and from the calibration graph, determine the amount of Acid Herbicides in the sample. If the response exceeds the calibration range, the analysis may be repeated using a smaller quantity of sample diluted with deionised water

---

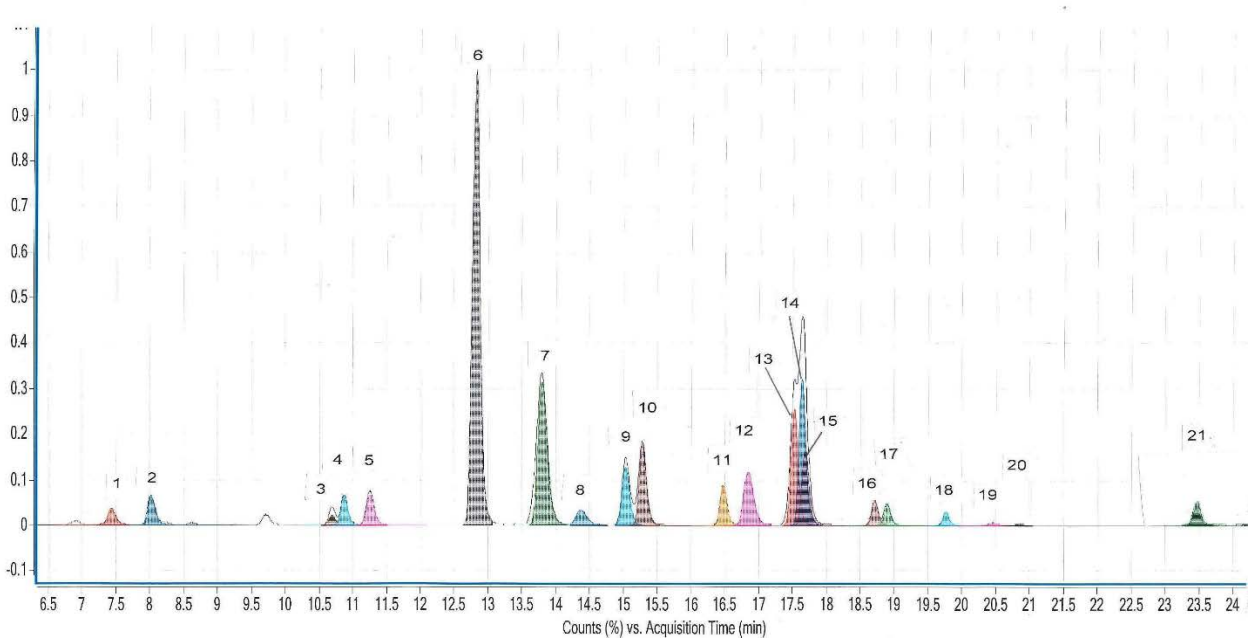
C9 Calculation

The concentration of each acid herbicide is given by an internal standard procedure:-

$$\mathbf{C} = \frac{\mathbf{A}.\mathbf{ISstd}.\mathbf{Cstd}}{\mathbf{Astd}.\mathbf{IS}} \ \mu g\ L^{-1}$$

A = peak area in sample
Astd= peak area in standard
IS = peak area of internal standard in sample
ISstd = peak area of IS in standard
Cstd = concentration of analyte in standard, (µg L^{-1})

Figure C1 Typical chromatogram



Key	Compound
1	picloram
2	chloridazon
3	benazolin
4	fluroxypyr
5	dicamba
6	lenacil
7	bentazone
8	bromoxynil
9	2,4-D
10	MCPA
11	triclopyr
12	ioxynil
13	dichlorprop
14	MCPP
15	2,4,5-T
16	2,4-DB
17	MCPB
18	fenoprop
19	propyzamide
20	ethofumesate
21	PCP

Table C1 Performance data

LOD: 3 x Std deviation (within batch) of 0.020 µg L^{-1} spiked sample

Determinand	LOD	LOD Sample *	Standards			
		0.02 µg L^{-1}	0.035 µg L^{-1}		0.35 µg L^{-1}	
	µg L^{-1}	SD	SD	Bias %	SD	Bias %
Clopyralid	0.0057	0.00190	0.00224	-1.05	0.02132	4.62
Dicamba	0.012	0.00373	0.00281	-8.53	0.00939	-1.01
MCPP	0.0032	0.00107	0.00102	-6.55	0.00462	-7.70
MCPA	0.0042	0.00663	0.00131	-8.22	0.00706	-11.69
Dichlorprop	0.0030	0.00099	0.00048	-0.81	0.00512	-9.28
Bromoxynil	0.0068	0.00224	0.00213	-11.22	0.01367	-0.64
Triclopyr	0.0038	0.00125	0.00092	-5.75	0.00683	-10.05
PCP	0.018	0.00591	0.00265	-11.49	0.00877	-5.62
Fenoprop	0.0069	0.00228	0.00315	6.43	0.0256	-8.01
Lenacil	0.0035	0.00117	0.00108	-7.23	0.00728	-4.74
Chloridazon	0.0057	0.00184	0.00160	-2.10	0.00678	-1.80
MCPB	0.0057	0.00139	0.00121	-5.70	0.00772	-9.85
Fluroxypyr	0.012	0.00172	0.00136	-1.31	0.01592	-6.15
Bentazone	0.0032	0.00076	0.00080	-8.31	0.00604	-5.02
Benazolin	0.0042	0.00237	0.00231	-1.62	0.01643	-5.01
2,4-D	0.0030	0.00125	0.00051	-0.62	0.00323	-10.64
2,4,5-T	0.0068	0.00117	0.00115	-1.78	0.00673	-9.82
2,4 DB	0.003	0.00113	0.00163	-1.10	0.00828	-6.83
Propyzamide	0.018	0.00323	0.00419	-16.55	0.01525	-2.96
Ioxynil	0.0069	0.00151	0.00096	-19.79	0.00725	-1.28
Picloram	0.0035	0.00337	0.00223	-5.70	0.01291	-1.86
Ethofumesate	0.0057	0.00623	0.00386	-5.96	0.01123	-0.79

*Soft Final water
Method Provided by Northumbrian Water

D - The Determination of Benzonitrile, Phenoxy Alkanoic Acid Herbicides and other Compounds in Treated and Raw Water using Direct Aqueous Injection and High Performance Liquid Chromatography with Multiple Reaction Monitoring Mass Spectrometry Detection.

D1 Performance characteristics of the method

D1.1	**Substances determined**	2,3,6-Trichlorobenzoic acid (2,3,6-TBA), 2,4-D, 2,4-DB, 2,4-DP (Dichlorprop), 2,4,5-T, 2,4,5-TP (Fenoprop)' Asulam, Benazolin, Bentazone, Bromoxynil, Clopyralid, Dicamba, Dinoseb, Fluroxypyr, Imazapyr, Ioxynil, Iprodione, MCPA, MCPB, MCPP (Mecoprop), Pentachlorophenol (PCP), Picloram, Triclopyr
D1.2	**Type of sample**	Raw and treated waters.
D1.3	**Basis of method**	Acid herbicides are analysed by direct aqueous injection using liquid chromatography (LC) with mass spectrometric (MS) detection. A triple quadruple mass spectrometer is used employing positive and negative ion electrospray with multiple reaction monitoring (MRM).
D1.4	**Range of application**	Typically, up to 0.25 µg L^{-1}. The range may be extended (see section D8.4).
D1.5	**Standard deviation**	See Table D1.
D1.6	**Limit of detection**	Typically, 0.005 µg L^{-1}, based on a low level (0.01 µg L^{-1}) standard solution.
D1.7	**Bias**	See Table D1.

D2 Principle

Phenoxy alkanoic acid herbicides (PAA) and additional herbicides are analysed by direct aqueous injection using LCMS-MS. The compounds are identified by their specific mass transitions and retention times in comparison to known standards. They are quantified by comparison with a 7 point calibration curve, using internal standards, (Bentazone-D_6, Carbendazim-D_4, Dicamba$^{13}C_6$, 2,4-D$^{13}C_6$, Dichlorprop-D_6, MCPA-D_6). To the sample, after dilution if required, is added a known amount of sodium thiosulphate, formic acid and internal standard. Acid herbicides are then determined by LC MS using positive and negative ion electrospray and multiple reaction monitoring detection.

D3 Interferences

Any compound that elutes under the conditions used, and has similar chromatographic and mass spectrometric properties to the determinands will interfere.

The MS-MS technique of using a specific precursor to product ion reduces the risk of positive bias.

D4 Hazards

Acid herbicides are considered harmful as a solid material; although in most laboratories obtain as dilute solutions in a common solvent.

Methanol is toxic and flammable; acetonitrile is irritant and flammable. Solutions of the internal standard, e.g. 2,4-D $^{13}C_6$, are considered to be irritant and dangerous to the environment. Formic acid is corrosive and causes burns, contact with eyes and skin should be prevented.

Waste solvents should be discarded according to documented procedures.

Appropriate safety procedures should be followed at all times.

D5 Reagents

All reagents should be of sufficient purity so that they do not give rise to significant interfering peaks in the chromatographic analysis. This should be checked for each batch of chemicals and reagents and verified by running procedural blanks with each batch of samples analysed. Analytical grade solvents for high performance liquid chromatography or pesticide use are normally suitable unless otherwise stated. Water should be distilled, deionised or of similar grade quality. Reagents should be stored appropriately in accordance with supplier's instructions and in suitable containers. Laboratory prepared reagents are mixed thoroughly to ensure homogeneity and are stored at 5 °C ± 3 °C.

D5.1 Acetonitrile.

D5.2 Methanol.

D5.3 Formic acid.

D5.4 Ascorbic acid (30 g L^{-1}). Dissolve 30 g of ascorbic acid in approximately 900 mL of ultra-pure water. Mix well. Make to 1000 mL with ultra-pure water. Mix well. This solution is used to de-chlorinate the sample and may be stored for up to one month.

D5.5 Sodium thiosulphate solution (18 g L-1). Dissolve 9 g of sodium thiosulphate pentahydrate in approximately 450 mL of ultra-pure water. Mix well. Make to 500 mL

with ultra-pure water. Mix well. This solution is used to matrix modify the sample and may be stored for up to one month.

D5.6 Working solutions of tap water. To 7 separate 40 mL vials (each containing 25 mL of tap water) add 25 µL of ascorbic acid (30 g L^{-1}) and sodium thiosulphate solution (18 g L^{-1}). These solutions should be prepared on the day of use.

D5.7 Stock calibration individual acid herbicide standard solution (1000 mg L^{-1}). Dissolve 25 mg of the individual acid herbicides in 25.0 mL of acetonitrile. Cap and mix well. This solution may be stored for up to 12 months.

D5.8 Intermediate calibration acid herbicide standard solutions (5 mg L^{-1}). Add 100 µL of the stock calibration acid herbicide standard solution to approximately 15 mL of methanol. Mix well. Make to 20 mL with methanol. Cap and mix well. This solution may be stored for up to 12 months.

D5.9 Spiking calibration acid herbicide standard solution (0.025 mg L^{-1}). Add 500 µL of intermediate calibration acid herbicide standard solution to approximately 90 mL of methanol. Mix well. Make to 100.0 mL with methanol. Cap and mix well. This solution may be stored for up to six months.

D5.10 Working calibration acid herbicide standard solutions. For example in the concentration range of 0.02 - 0.25 µg L^{-1}. To each of the seven working solutions of tap water add 0, 20, 50, 100, 150, 200 and 250 µL of spiking calibration acid herbicide standard solution. Mix well. Each vial containing 25 mL of tap water contains 0, 0.50, 1.25, 2.50, 3.75, 5.00 and 6.25 ng of the acid herbicide. To each vial, add 50 ± 1 µL of internal standard spiking solution. Mix well. These solutions contain 2.50 ng of the individual internal standards. These solutions should be prepared on the day of use.

D5.11 Stock internal standard solution (100 mg L^{-1}). For example, add 10.0 $\pm$ 0.1 mg of 2,4-D $^{13}C_6$ to approximately 90 mL of acetonitrile and mix well. Make to 100.0 mL with acetonitrile. Mix well. This solution may be stored for up to 12 months.

D5.12 Spiking internal standard solution (0.05 mg L^{-1}). Add 50 µL of stock internal standard solutions to approximately 90 mL of methanol and mix well. Make to 100.0 mL with methanol. Mix well. This solution may be stored for up to 12 months.

D6 Apparatus

In addition to normal laboratory glassware the following may be required.

D6.1 Liquid chromatograph. Fitted with a quadrupole mass spectrometer capable of operating in MRM mode, with a data station. The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

Liquid Chromatograph

Analytical Column: Agilent Zorbax Eclipse Plus C18, 150 mm long x 2.1 mm internal diameter, 3.5 µm particle size, part number 959763-902.
Oven Temperature: 40 °C
Injection volume: 100 µL
Total Run Time: 24.75 minutes
Mobile phase A: Water containing 0.01 % formic acid.
Mobile phase B: Acetonitrile containing 0.01 % formic acid.

Gradient Programme

Time (minutes)	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate (mL min^{-1})
0.00	95	5	0.30
0.50	95	5	0.30
11.00	60	40	0.30
16.00	40	60	0.30
17.00	35	65	0.30
20.00	5	95	0.30
21.00	5	95	0.30
21.10	95	5	0.30
21.25	95	5	0.45
24.25	95	5	0.45
24.50	95	5	0.30
24.75	95	5	0.30

Mass Spectrometer

Ionisation Mode : Positive and Negative Ion Electrospray
Drying Gas Temperature : 200 °C
Drying Gas Flow : Nitrogen at 8 L min^{-1}
Nebuliser Pressure : 40 psi
Shealth Gas Temperature : 300 °C
Shealth Gas Flow : Nitrogen at 11 L min^{-1}

Capillary Voltage : Variable
Charging (Nozzle) Voltage : Variable
Detection Mode : Multiple Reaction Monitoring

Delta EMV : 400 V
Quadrupole Temperatures : 100 °C (MS1 & MS2)

Compound	Precursor ion	Product Ion	Fragmentor Voltage	CE	Approx Retention time	Polarity Mode
2,3,6-TBA	223.0	179.0	55	0	8.7	Negative
2,4,5-T	253.0	195.0	85	10	16.0	Negative
2,4-D	219.0	161.0	95	8	14.3	Negative
2,4-D 13C6	225.0	167.0	95	8	14.3	Negative
2,4-DB	247.0	161.0	95	4	16.8	Negative
Asulam	231.0	156.0	100	5	5.9	Positive
Benazolin	242.0	170.0	85	6	11.3	Negative
Bentazone	239.1	132.1	135	24	13.4	Negative
Bentazone-d6	245.1	132.1	135	24	13.4	Negative
Bromoxynil	275.9	79.0	130	38	14.4	Negative
Carbendazim-d4	196.1	164.0	100	15	5.7	Positive
Clopyralid	192.1	146.0	70	19	4.4	Positive
Dicamba	219.0	175.0	55	0	10.3	Negative
Dicamba 13C6	225.0	181.0	55	0	10.3	Negative
Dichlorprop	233.0	161.0	95	8	15.9	Negative
Dichlorprop-d6	239.0	164.0	95	8	15.9	Negative
Dinoseb	239.1	194.0	145	20	20.0	Negative
Fenoprop	267.0	195.0	85	8	17.4	Negative
Fluroxypyr	253.0	195.0	90	4	11.9	Negative
Imazapyr	262.1	220.0	95	15	7.5	Positive
Ioxynil	369.9	126.9	140	40	15.8	Negative
Iprodione	330.0	245.0	110	10	18.7	Positive
MCPA	199.0	141.0	105	12	14.5	Negative
MCPA-d6	205.0	147.0	105	12	14.5	Negative
MCPB	227.1	141.0	100	2	16.9	Negative
MCPB-d6	233.1	147.0	100	2	16.9	Negative
MCPP	213.0	141.0	105	8	16.0	Negative
PCP	264.9	35.0	130	30	20.1	Negative
PCP 13C6	272.9	35.0	130	30	20.1	Negative
Picloram	241.0	195.0	95	21	6.1	Positive
Triclopyr	254.0	196.0	90	5	15.2	Negative

D6.2 Internal Standard Alignment

Analyte	Associated Internal Standard and Surrogates For Data Reprocessing
2,3,6-Trichlorobenzoic Acid	2,4-D 13C6
2,4,5-T	Dichlorprop-d6
2,4-D	2,4-D 13C6
2,4-DB	Dichlorprop-d6
Asulam	Carbendazim-d4
Benazolin	2,4-D 13C6
Bentazone	Bentazone-d6
Bromoxynil	2,4-D 13C6
Clopyralid	Dichlorprop-d6
Dicamba	Dicamba 13C6
Dinoseb	MCPA-d6
Dichlorprop	Dichlorprop-d6
Fenoprop	Dichlorprop-d6
Fluroxypyr	2,4-D 13C6
Imazapyr	Dichlorprop-d6
Ioxynil	Dichlorprop-d6
Iprodione	Dichlorprop-d6
MCPA	MCPA-d6
MCPB	MCPA-d6
MCPP (Mecoprop)	MCPA-d6
Pentachlorophenol	MCPA-d6
Picloram	Carbendazim-d4
Triclopyr	Dichlorprop-d6

D7 Sample collection and preservation

Samples may be collected in 500 mL amber glass bottles with a screw cap lid containing a polytetrafluoroethylene liner. Samples should be collected in bottles containing 0.5 mL of ascorbic acid solution. Samples are stable for 21 days (exception 7 days for iprodione and 14 days for dinoseb).

D8 Analytical procedure

Step	Procedure
D8.1	Add Vs mL (typically, 25 ± 1 mL) of sample to a 40 mL vial that contains 25 ± 1 µL ascorbic acid solution. If the sample contains particulate material, it may need to be filtered To this bottle add 50 µL spiking internal standard solution. This equates to 2.5 ng of internal standard in 25 mL of sample. Add 25 ± 1 µL sodium thiosulphate solution. Add 25 µL formic acid.
D8.2	The solution is now ready for LC-MS determination.
D8.3	Analyse blank and AQC solutions using the entire procedure described in sections D8.1 - D8.2 replacing the sample with water.
D8.4	Analyse the sample extract and from the calibration graph, obtain the amount, Av, of acid herbicide in the bottle and then calculate the concentration, Cs, of acid herbicide in the sample. If the response exceeds the calibration range, the analysis may be repeated using a smaller volume of sample (D8.1) and making the volume to 25 mL with water.

D9 Calculation

From the calibration graph determine the amount, Av, of acid herbicide in the vial and determine the concentration, Cs, in the sample using the equation:

$$Cs = (Av \times DF \times 1000) / Vs \quad \mu g\ L^{-1}$$

Where

Cs is the concentration (µg L^{-1}) of acid herbicide in the sample;
Av is the amount (µg) of acid herbicide obtained from the graph;
Vs is the volume (mL) of sample analysed (D8.1);
DF is the dilution factor if appropriate.

Figure D1 Typical Chromatogram

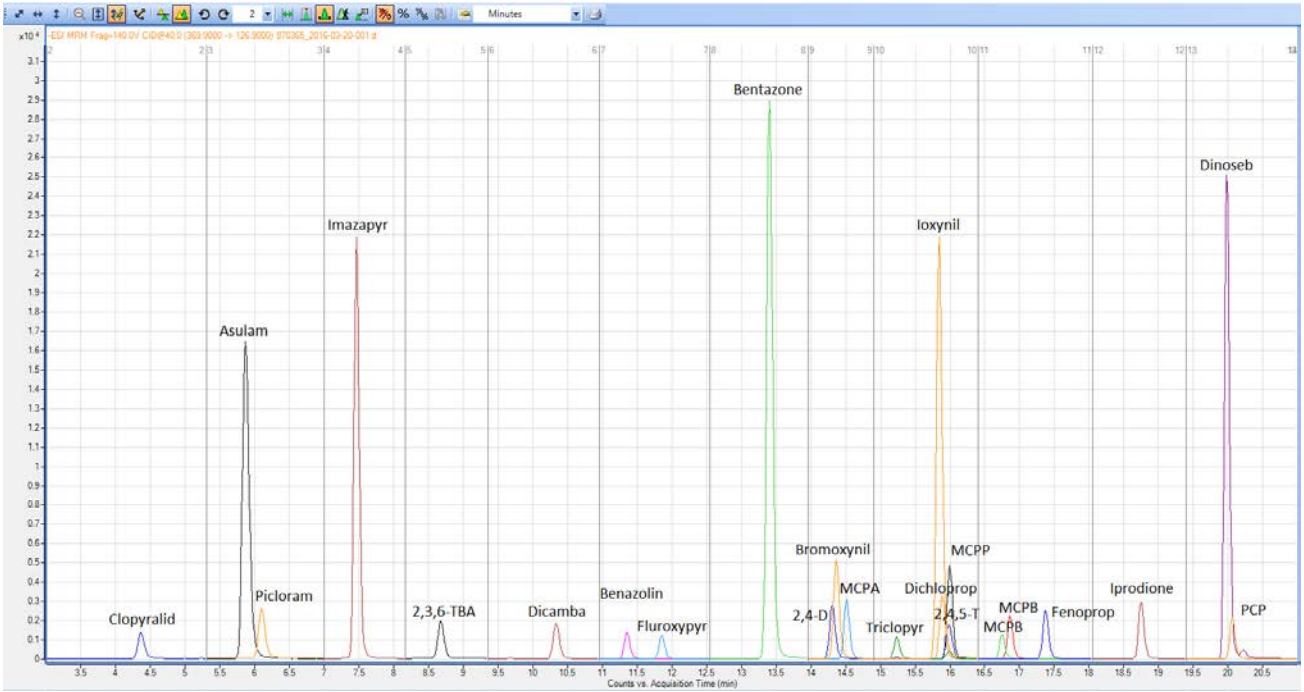


Table D1 Performance Data

Analyte	LOD	AQC Sample		Standards			
	0.01 µg L^{-1}	0.10 µg L^{-1}		0.05 µg L^{-1}		0.20 µg L^{-1}	
	µg L^{-1}	SD	Bias %	SD	Bias %	SD	Bias %
2,3,6-TBA	0.0048	0.0038	0.38%	0.0023	-0.53%	0.0071	-0.02%
2,4,5-T	0.0029	0.0056	-4.01%	0.0046	-2.62%	0.0079	1.69%
2,4,5-TP	0.0027	0.0064	4.18%	0.0025	1.26%	0.0051	0.68%
2,4-D	0.0035	0.0042	-5.92%	0.0029	-2.51%	0.0067	0.34%
2,4-DB	0.0047	0.0081	1.03%	0.0032	-1.33%	0.0066	1.10%
2,4-DP	0.0022	0.0038	-0.77%	0.0026	-0.36%	0.0061	0.05%
Asulam	0.0028	0.0038	0.25%	0.0015	-0.95%	0.0057	0.85%
Benazolin	0.0024	0.0043	0.30%	0.0026	0.58%	0.0073	0.60%
Bentazone	0.0014	0.0014	-1.10%	0.0011	-0.56%	0.0039	0.70%
Bromoxynil	0.0033	0.0056	1.94%	0.0032	0.91%	0.0108	3.58%
Clopyralid	0.0058	0.0061	6.91%	0.0042	-2.70%	0.0072	-1.08%
Dicamba	0.0048	0.0037	-0.69%	0.0023	0.02%	0.0079	0.71%
Dinoseb	0.0029	0.0086	-0.68%	0.0043	0.95%	0.0117	1.91%
Fluroxypyr	0.0028	0.0040	-1.40%	0.0029	0.27%	0.0072	0.24%
Imazapyr	0.0014	0.0064	-2.67%	0.0033	-1.32%	0.0076	1.37%
Ioxynil	0.0027	0.0055	0.18%	0.0033	1.01%	0.0070	2.05%
Iprodione	0.0016	0.0044	-2.10%	0.0024	-1.98%	0.0043	-0.68%
MCPA	0.0026	0.0039	-3.50%	0.0025	0.63%	0.0061	0.10%
MCPB	0.0030	0.0063	1.35%	0.0031	-0.86%	0.0076	-0.43%
MCPP	0.0019	0.0034	-3.88%	0.0020	-2.07%	0.0058	0.02%
PCP	0.0065	0.0070	3.93%	0.0043	-1.36%	0.0105	1.05%
Picloram	0.0057	0.0049	-5.65%	0.0032	0.55%	0.0087	2.26%
Triclopyr	0.0052	0.0055	0.65%	0.0036	1.45%	0.0100	0.94%

SD: S_t Total Standard Deviation

Sample Type Information

The method has been validated with "soft" upland reservoir (Langsett, YW), "medium" combined surface and ground (Banwell, Bristol) and "hard" surface (Elvington, YW) derived treated waters. The method has also been validated with a "hard" ground (Cowick borehole, YW) derived raw water and a "hard" surface (River Derwent at Elvington) derived raw water.

Data provided by ALS Environmental

E The Determination of Phenoxy Acid Herbicides in Raw and Potable Waters using Solid Phase Extraction followed by Liquid Chromatography with Mass Spectrometric Detection.

E1 Performance characteristics of the method

E1.1	**Substances determined**	Dicamba, Bentazone, Bromoxynil, 2,4-D, MCPA, Ioxynil, Dichlorprop, Mecoprop, Triclopyr, 2,4-DB, MCPB. This method has been performance tested for the phenoxy acid herbicides listed above but may be suitable for other additional acidic herbicides.
E1.2	**Type of sample**	Raw waters, drinking waters and process waters.
E1.3	**Basis of method**	Phenoxy acids is extracted from samples using solid phase extraction cartridges. Following elution using methyl-tert-butyl ether (MTBE)/Methanol (0.01% Formic acid), the eluate is analysed using liquid chromatography with mass spectrometric detection in MRM ion monitoring mode.
E1.4	**Range of application**	Typically, up to 0.200 µg L^{-1}. The range may be extended (see section E8.13).
E1.5	**Standard deviation**	See Table E1.
E1.6	**Limit of detection**	See Table E1.
E1.7	**Bias**	See Table E1.

E2 Principle

The sample, after dilution if required, is passed through a pre-conditioned styrene divinyl benzene polymer solid phase extraction column where phenoxy acid herbicides are retained. The cartridge is then dried and eluted with methyl-tert-butyl ether (MTBE) / Methanol (0.01% Formic acid). The extract is evaporated to dryness and is reconstituted to 1 mL in 20% methanol Phenoxy acid herbicides are determined by LC with MS detection using negative ion electrospray and a triple quadruple mass spectrometer with MRM.

E3 Interferences

Any compound that elutes under the conditions used, and has similar liquid chromatographic and mass spectrometric properties to phenoxy acid of interest will interfere.

The selection of several mass ion fragments (MRM's) reduces the risk of positive bias

---

E4 Hazards

Phenoxy herbicides are considered harmful as a solid material, although in most laboratories it is obtained as a dilute solution in a common solvent.

Methanol is toxic and flammable; acetone and with methyl-tert-butyl ether (MTBE) are irritant and flammable. Solutions of the deuterated surrogate standards, i.e. dicamba-d_3 and MCPA-d_3, are considered to be irritant and dangerous to the environment. Formic acid is corrosive and causes burns, contact with eyes and skin should be prevented.

Waste solvents should be discarded according to documented procedures.
Methanol, acetone and methyl-tert-butyl ether (MTBE) are highly flammable. These compounds should be handled away from sources of ignition. Phenoxy acid herbicides, methanol, methyl-tert-butyl ether (MTBE) and deuterated surrogate standards, i.e. dicamba-d_3 and MCPA-d_3, should be considered toxic and handled with adequate ventilation and appropriate personal protective equipment.

E5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during the analysis and distilled, deionised or similar grade water should be used throughout. A procedural blank should be run with each batch of samples. All standards and reagents solutions must be mixed thoroughly. Reagents are stored at ambient temperature.

E5.1 Methanol.

E5.2 Methyl-tert-butyl-ether (MTBE)

E5.3 Formic Acid

E5.4 Hydrochloric Acid, concentrated (d20 1.16).

E5.5 Water. Deionised, distilled water or HPLC grade.

E5.6 Methanol (0.01% Formic Acid). Add 50 µL of formic acid to 500 mL of methanol and mix well. This solution is stable for 1 week.

E5.7 Methyl-tert-butyl ether (MTBE) / Methanol (0.01% Formic acid). Add 50 mL of methanol to 450 mL of Methyl tert-butyl ether (MTBE). Add 50 µL of formic acid. This solution is stable for 1 week.

E5.8 0.25% Hydrochloric acid solution. Carefully add 2.5 mL of hydrochloric acid to 1000 mL of water. This solution is stable for 6 months.

E5.9 **0.02% Formic Acid.** Add 200 µL of formic acid to 1000 mL of water. This solution is stable for 1 week.

E5.10 **20% Methanol solution.** Add 200 mL of methanol to 800 mL of water. This solution is stable for 6 months.

E5.11 **Sodium Thiosulphate (anhydrous)**

E5.12 **10% Sodium Thiosulphate.** Dissolve 25 g of sodium thiosulphate (anhydrous) in approximately 150 mL of water, mix well to dissolve and make up to 250 mL with water. This solution should be stored in an amber glass bottle for up to 12 months.

E5.13 **Stock calibration phenoxy acid herbicides standard solution (1000 mg L^{-1})**. Commercially certified standard containing the appropriate phenoxy acid herbicides

E5.14 **Intermediate Calibration Spiking Standard (0.2 mg L^{-1}).** Add 20 µL of stock calibration phenoxy acid (1000 mg L^{-1}) and 0.5mL of 10% sodium thiosulphate into approximately 90 mL of water. Make up to the mark with water. This solution may be stored at 5 ± 3ºC for up to 6 months.

E5.15 **Dicamba-d3 (100 mg L^{-1}) & MCPA-d3 (100 mg L^{-1}) surrogates.** Commercially certified standards containing the deuterated dicamba-d3 and MCPA-d3.

E5.16 **Working Deuterated Internal Standard (100 µg L^{-1}).** Add 100µL of Dicamba-d3 Internal Standard (100 mg L^{-1}) and 100µL MCPA d3 Internal Standard (100 mg L^{-1}) into approximately 90mL of water and mix well. Make up to the mark with water. This solution may be stored at 5 ± 3ºC for up to 6 months.

E6 Apparatus

In addition to normal laboratory glassware the following may be required.

E6.1 **Solid phase cartridges**. Strata X 60 mg (3 mL) cartridges were used to generate the performance data. Other similar cartridges may be used, but their performance would need to be verified.

E6.2 **Manual Phase solid phase Extraction (SPE) tank** and appropriate Buchner Flask. Ideally the Buchner Flask should be located in a fume cupboard or have a Perspex shield round the flask when the vacuum pump is on.

E6.3 **Blow-down apparatus** (capable of being set at 35 ± 3 °C) that can direct a gentle stream of air into a vial.

E6.4 **LCMS system**. Fitted with quadrupole mass spectrometer capable of operating in MRM mode, with a data station. The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

Column: Zorbax Eclipse XBD-C18 2.1 x 150 mm, 3.5 um
Mobile phase A: 0.02% formic acid in Water
Mobile phase B: Methanol

Chromatography conditions.

Column Temperature: 30 ^{o}C
LC Flow Rate: 0.2 mL
Injection volume: 30 µL

Time (minutes)	Mobile Phase A (%)	Mobile phase B (%)
0	80	20
2	80	20
4	40	60
12	10	90
18.0	10	90
18.5	80	20

MS conditions

Parameter	Value
Ionisation mode	ESI negative
Delta EMV	500
Scan Type	MRM
Gas Temperature	250^{o}C
Gas Flow	10 L min^{-1}
Nebuliser	40psi
Capillary	4000V
MS1 Resolution	Widest in all cases
MS2 Resolution	Unit in all cases

Using these conditions, the following apply

Compound	Approximate retention time (mins)	Ions monitored		Polarity Mode
		Precursor	Product	
Dicamba	12.0	219.0	175.1	-ve
Dicamba-d_3	12.0	220.0	178.1	-ve
Bentazone	12.6	239.1	132.1	-ve
Bromoxynil	13.6	273.9	79.0	-ve
2,4-D	14.1	219.0	161.0	-ve

MCPA	14.4	198.9	141.1	-ve
MCPA-d_3	14.3	202.1	144.1	-ve
Ioxynil	14.5	369.9	126.8	-ve
Dichlorprop	15.3	233.0	161.1	-ve
Mecoprop	15.4	213.1	114.1	-ve
Triclopyr	14.9	254.9	197.1	-ve
2,4-DB	15.9	246.9	161.1	-ve
MCPB	16.3	227.2	141.1	-ve

E7 Sample collection and preservation

Samples may be taken in clear glass bottles with caps lined with a PTFE inert material. The volume taken should allow for at least one repeat analysis, i.e. a minimum of 200 mL of sample should be collected. Sample stability data indicate that samples may be stored for up to 14 days in glass bottles stored at 5 ^{o}C ± 3 ^{o}C and the sample extracts are stable for 28 days stored at 5 ^{o}C ± 3 ^{o}C.

E8 Analytical procedure

E8.1 Condition a solid phase cartridge using 5 mL of MTBE / Methanol (0.01% Formic acid). Elute the cartridge and discard the eluate. Do not allow the cartridge to dry out at this stage, i.e. do not allow the meniscus of the solvent to fall below the level of the cartridge packing material.

E8.2 Add 2 mL of methanol (0.01% Formic Acid) and elute the cartridge. Discard the eluate. Do not allow the cartridge to dry out at this stage.

E8.3 Add 2 mL of water and elute the cartridge. Discard the eluate. Do not allow the cartridge to dry out at this stage.

E8.4 Add 2 mL of 0.25% hydrochloric acid and elute the cartridge. Discard the eluate. Do not allow the cartridge to dry out at this stage.

E8.5 Add Vs mL (typically 100 mL of potable or untreated water) of sample (If the sample contains particulate matter, it may need to be filtered) Using a vacuum pull 100 mL of sample through the cartridge at a slow to medium flow rate. Samples should take a minimum of 5 minutes to be fully pulled through the cartridge Discard the eluate.

E8.6 Allow the column to run dry for 20 minutes. Place cartridges in centrifuge at a speed of 3000 rpm for a minimum of 2 minutes. Add 2 mL of water and elute the cartridge

E8.7 Add 2.5mL of MTBE / Methanol (0.01% Formic acid) and elute the cartridge collecting the eluate in a suitable test tube.
Remove and dispose of the cartridge

E8.8 Place the test tube in a turbo-vap at 35 °C. Evaporate the solvent in the test tube to dryness. Takes approximately 14 minutes using air or nitrogen gas

E8.9 Add 1.00 mL of 20% methanol solution to each test tube and agitate using a vortex mixer to reconstitute the components.

E8.10 Transfer the contents to a labelled 2 mL glass vial and cap. At this stage the solution may be stored in a refrigerator for up to one month before the LC-MS determination begins.

E8.11 Set up the LCMS system according to manufacturer's instructions. 100 mL of standards are extracted through the SPE extraction (E8.1 to E8.10). Using the five calibration solutions i.e. 0.02, 0.05, 0.10, 0.15 and 0.2 µg L^{-1} standards of the working calibration standard solutions construct a calibration graph of response versus amount of component, monitoring the ions referred to in E6.4.

E8.12 Analyse blanks, working calibration standards and AQC solutions using the entire procedure described in sections E8.1 – E8.11 replacing the sample suitable bottle water or tap water.

E8.13 Analyse the sample extract and from the calibration graph, obtain the amount, Av, of phenoxy herbicide acid in the vial and then calculate the concentration, Cs, of phenoxy herbicide in the sample. If the response exceeds the calibration range, the analysis may be repeated using a smaller amount of sample (E8.5) and making the volume to 100 mL with water.

E9 Calculation

From the calibration graph determine the amount, Av, of component in the vial and determine the concentration, Cs, in the sample using the equation:

$$Cs = (Av \times 100/Vs) \quad \mu g\ L^{-1}$$

Where

Cs is the concentration (µg L^{-1}) of in the sample;
Av is the amount (µg L^{-1}) of obtained from the graph; and
Vs is the sample volume taken (E8.5)

Blank correction is not normally necessary but should be considered if the blank control sample is above the limit of detection.

Figure E1 Typical chromatogram
(concentration of 0.200 µg L^{-1} extracted standard – 40 µg L^{-1} in extract)

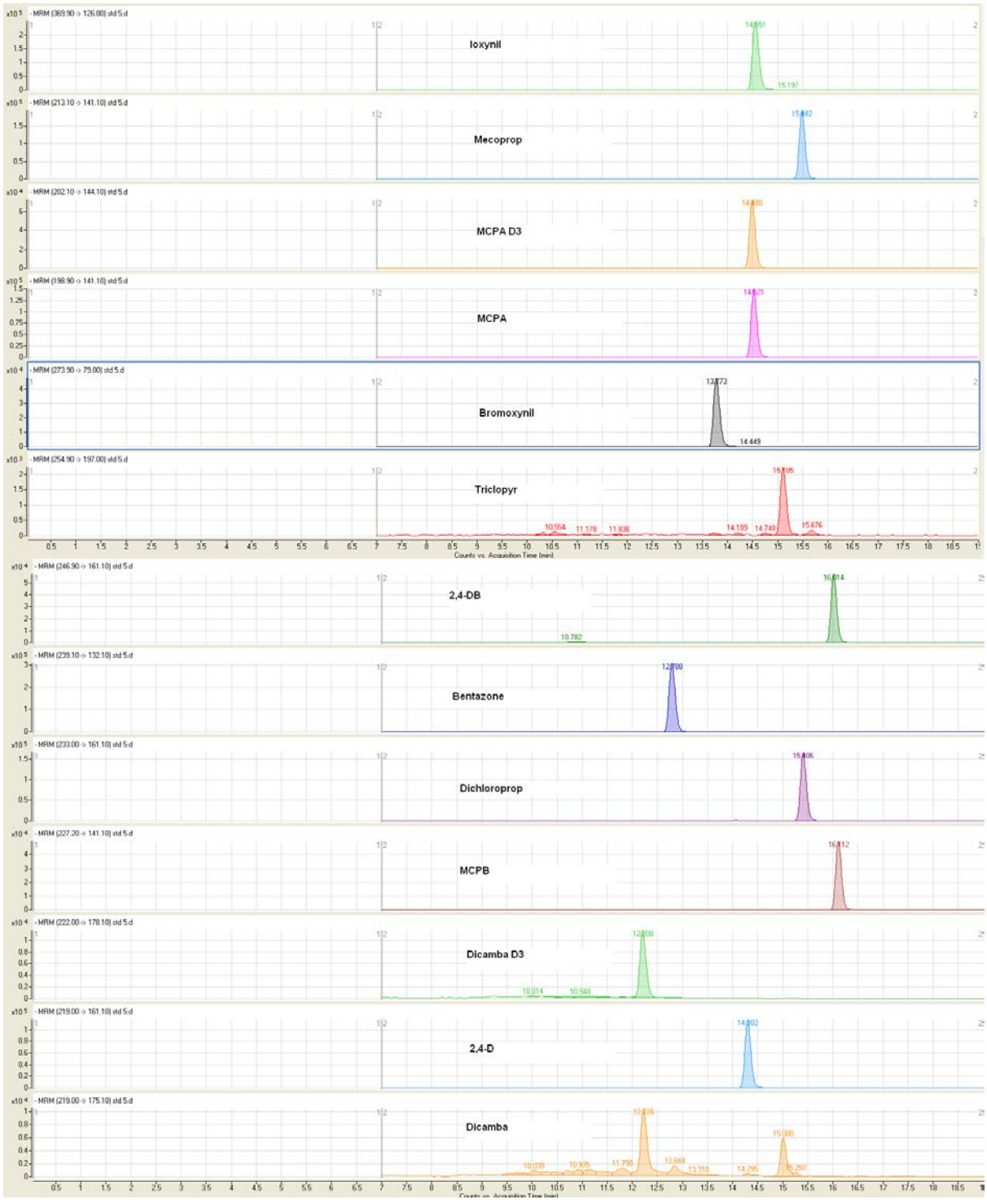


Table E1 Performance data

Determinand	LOD	Soft potable water Sample		Standards			
		0.100 µg L^{-1}		0.04 µg L^{-1}		0.18 µg L^{-1}	
	µg L^{-1}	SD	Bias %	SD	Bias %	SD	Bias %
Dicamba	0.008	0.00313	0.0	0.00135	-0.5	0.00304	1.8
Bentazone	0.004	0.00374	3.6	0.00193	5.3	0.00666	-1.7
Bromoxynil	0.005	0.00536	1.6	0.00198	1.5	0.00765	-0.9
2,4-D	0.003	0.00339	-1.8	0.00142	-3.3	0.00552	-1.9
MCPA	0.004	0.00368	-1.5	0.00169	-3.0	0.00644	-3.9
Ioxynil	0.004	0.00501	2.3	0.00202	1.5	0.00812	-0.2
Dichlorprop	0.004	0.00324	-1.8	0.00155	-2.8	0.00525	-1.6
Mecoprop	0.003	0.00169	-2.0	0.00136	-3.3	0.00462	-2.4
Triclopyr	0.015	0.00742	2.4	0.00461	4.8	0.00969	1.5
2,4-DB	0.004	0.00324	0.4	0.00158	-2.0	0.00536	-1.9
MCPB	0.005	0.00403	0.6	0.00217	0.5	0.00441	-1.4

All values expressed as µg L^{-1} unless otherwise stated.
LOD - Limit of detection (approximates to 4.65 x Sw is within batch standard deviation).
Limits of detection were determined using soft tap water.

Data provided by Scottish Water Scientific Services, Edinburgh Laboratory.

F The Determination of Phenoxy Acid Herbicides in Surface and Ground Waters using Direct Aqueous Injection Liquid Chromatography with Mass Spectrometric Detection using Multiple Reaction Monitoring

F1 Performance characteristics of the method

F1.1	**Substances determined**	2,4-D, 2,4-DB, Dichlorprop, MCPA, MCPB, Mecoprop, Dicamba, Clopyralid, Fluroxypyr, Triclopyr, Benazolin, Bentazone, Bromoxynil, Ioxynil.
F1.2	**Type of sample**	River and Groundwater.
F1.3	**Basis of method**	Pre-filtered acid herbicide samples are analysed by direct aqueous injection using liquid chromatography with mass spectrometric detection in MRM ion monitoring mode.
F1.4	**Range of application**	Typically 6-600 ng L^{-1} for all compounds except Clopyralid where the range is 25-1200 ng L^{-1}.
F1.5	**Standard deviation**	See appendix F2.
F1.6	**Limit of detection**	See appendix F2.
F1.7	**Bias**	See appendix F2.

F2 Principle

The sample is spiked with surrogate and internal standard. 10 mL of each sample is filtered and collected, 0.85 mL of Acetonitrile is then passed through the filter. The subsequent sample is then mixed and an aliquot transferred to a 4 mL vial. The sample is then analysed using LC with MS detection using negative ion electrospray and a triple quad mass spectrometer in MRM mode.

F3 Interferences

Interference can occur by sample matrix suppression of ionisation.

Any compound that elutes under the conditions used, and has similar chromatographic and mass spectrometric properties to the determinands will interfere.

The MS-MS technique of using a specific precursor to product ion reduces the risk of positive bias.

F4 Hazards

Acid herbicides standards used in this method are toxic and most are irritants to some extent. Ingestion, inhalation and skin contact should be avoided. Gloves shall be worn when weighing out solid certified standards.
Acetonitrile is an irritant and flammable. Acetone is an irritant and flammable. Acetic acid is flammable, irritant and may cause severe burns contact with the eyes and skin should be avoided.

Solutions of the internal standards, e.g. 4-Chlorophenyl acetic acid and 2-Bromo-4-cyanophenol are considered irritant and dangerous to the environment.

Waste solvents should be discarded according to appropriate waste management procedures.

Appropriate safety procedures should be followed at all times.

F5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during the analysis. A procedural blank must be run with every batch of samples.
Analytical grade solvents for high performance liquid chromatography or pesticide use are normally suitable unless otherwise stated. Water should be distilled, deionised or of similar grade quality. Reagents must be stored appropriately in accordance with supplier's instructions and in suitable containers.
Where reagents are prepared by dissolution of solid material or by dilution of stock solutions it is essential that they are thoroughly mixed before use or storage.

F5.1 Acetic Acid

F5.2 Water. Deionised, distilled water or HPLC grade.

F5.3 Acetonitrile

F5.4 Acetonitrile Mobile Phase Measure 500 mL of acetonitrile into a measuring cylinder**.** Using a 1000 µL syringe or a 0 -1 mL pipette add 500 µL (0.5 mL) of Acetic acid, transfer to the appropriate HPLC reservoir. This solution is stable for 2 months at room temperature.

F5.5 Mobile Phase Measure 500mL mL of water, free of target compounds into a 500 mL measuring cylinder. Using a 1000 µL syringe or a 0-1 mL pipettor add 500 µL (0.5 mL) of acetic acid, transfer to the appropriate HPLC reservoir. This solution is stable for 2 weeks at room temperature.

F5.6 Acetone, Glass distilled

F5.7 Stock Surrogate Standard (2,4-DPA, solid material), Commercially certified standard.

F5.8 Primary Stock Surrogate standard.

Weigh 0.02 g to an accuracy of ± 0.0004 g the certified solid material so when dissolved in 10 mL of acetonitrile it gives an approximate concentration of 2000 mg L^{-1}.

F5.9 Secondary Stock Surrogate standard.

Dispense the required volume of primary surrogate standard to prepare a standard of concentration 50 mg L^{-1} in 50 mL of acetonitrile. The exact concentration of the primary surrogate standard is required to calculate the volume dispensed.

F5.10 Stock Acid herbicide standard solution (500 mg L^{-1}).

Commercially certified standard containing the appropriate acid herbicides.

F5.11 Secondary Stock Intermediate Calibration Standard (10 mg L^{-1}, 20 mg L^{-1} Clopyralid)

Dispense 200 µL of the Stock Acid herbicide standard solution into a 10 mL volumetric flask. Make up to the mark with acetonitrile and mix thoroughly.

F5.12 Tertiary Acid Herbicides stock calibration composite standard.

Dispense 250 µL of the Secondary stock intermediate calibration standard into a 50 mL volumetric flask. Dispense 1.00 mL of the Secondary Stock Surrogate Standard into the same 50 mL volumetric flask. Make up to the mark with acetonitrile and mix thoroughly.

F5.13 Tertiary Stock Surrogate Spiking Solution (1.0 mg L^{-1})

Dispense 1.00 mL of the Secondary Stock Surrogate standard into a 50 mL volumetric flask. Make up to the mark with acetonitrile and mix thoroughly.

F5.14 Primary stock Internal Standards

Prepare individual stock primary and secondary solutions of 2-Bromo-4-Cyanophenol and 4-Chlorophenyl acetic acid as described in F5.8 above.

F5.15 Secondary internal standard

Dispense the required volumes of secondary standard to give a working standard of the following concentration in a 25 mL volumetric flask: 2-Bromo-4-Cyanophenol (25 mg L^{-1}) and 4-Chlorophenyl acetic acid (100 mg L^{-1}). The dispensed volumes will be dependent on the exact concentrations. Make up to the mark with acetonitrile and mix thoroughly.

F5.16 Tertiary internal standard

Dispense 100 µL of secondary standard to give a working standard of the following concentration in a 25 mL volumetric flask: 2-Bromo-4-Cyanophenol (0.025 mg L^{-1}) and 4-Chlorophenyl acetic acid (0.10 mg L^{-1}).

F6 Apparatus

In addition to normal laboratory glassware the following may be required.

F6.1 A 20 position vacuum manifold fitted with tube rack, individual on/off PTFE stopcocks and vacuum control valves - or equivalent, Edwards 2-stage Rotary Vacuum Pump, or equivalent, manifold tube rack.

F6.2 Disc filters. 25 mm 0.2 µm pore.

F6.3 12 mL sample reservoirs Plastic with syringe tips compatible with PTFE stopcocks from 5.1 above.

F6.4 LCMS system. Fitted with a tandem quadruple mass spectrometer capable of operating in MRM mode, with a data station. The suitability of the equipment will need to be evaluated.

F6.4.1 Instrument conditions for analysis

The following conditions have been used in generating performance data.

Column: Phenomenex Luna 150 mm x 3 mm, 3 µm C18(2)
Oven Temperature: 40 °C
Mobile phase A: Water containing 0.1% Acetic Acid
Mobile phase B: Acetonitrile containing 0.1% Acetic Acid

Chromatography conditions.
Injection volume: 200 µL.
Flow: initial 0.4 mL min^{-1}, Equilibrate for 5 minutes.

Time (minutes)	Mobile phase A (%)	Mobile phase B (%)	Flowrate (mL min^{-1})
0	90	10	0.4
1.5	90	10	0.4
10	0	100	0.7
18	0	100	0.7
18.5	90	10	0.4
18.6	End	End	End

LC-MS ions, negative ion Electrospray ionization.

Using these conditions, the following apply

Component	Approximate retention time (minutes)	Precursor ion	Product ion	Polarity Mode
2,4-D	8.68	218.9	161.1	negative
Dicamba	8.87	218.9	175.1	negative
2,4-DB	8.48	246.90	161.0	negative
Dichlorprop	8.85	232.9	161.1	negative
Bromoxynil	8.27	275.77	81.0	negative
Ioxynil	8.89	369.7	127.0	negative
Bentazone	10.31	239.0	132.0	negative
MCPA	8.39	199.0	141.1	negative
MCPB	8.51	227.1	141.1	negative
MCPP	8.61	213.0	141.1	negative
Triclopyr	8.7	253.9	196.0	negative
Clopyralid	6.36	189.9	146.0	negative
Fluroxypyr	7.41	253.0	195.0	negative
Benazolin	7.66	242.0	170.1	negative
2,4-DPA (Surrogate)	7.87	203.10	159.1	negative
4-Chlorophenyl acetic acid (I.S)	7.36	169.0	125.0	negative
2-Bromo-4-Cyano phenol (I.S)	7.24	195.9	78.9	negative

F7 Sample collection and preservation

Samples are collected in 1000 mL clear Pyrex glass bottles fitted with a PTFE lined screw cap. Samples are returned to the laboratory as soon as possible. Once received by the laboratory the samples are stored at 5°C ± 3°C prior to extraction.

Samples are stable for 21 days after collection. No significant alterations are made during sample preparation so there is no adjustment to post sampling stability.

F8 Analytical Procedure

F8.1 Shake the sample to ensure any particulate material present is thoroughly distributed within the liquid. Pour 25 mL of sample into a 25 mL graduated tube. Ensure that there is no settlement of particulates

F8.2 Dispense 50 µL of Tertiary Surrogate Spiking standard with 100 µL of acetonitrile into the 25 mL of sample. Stopper and shake to mix thoroughly

F8.3 Dispense 100 µL of Tertiary Internal Standard with 50 µL of acetonitrile into the sample. Stopper and shake to mix thoroughly. Place the collection tube in the corresponding position in the manifold rack to that of the assigned disc filter for each sample.

F8.4 Place the rack in the vacuum tank and place the manifold lid on top. Ensure all stopcocks are in the 'closed' position. Turn on the vacuum pump.

F8.5 Place a 25 mm filter disc on the vacuum manifold. Place a 12 mL reservoir in the top of the filter disc.

F8.6 Shake the sample tube to ensure even distribution of particulates, then using a 5mL pipettor transfer 2 x 5 mL $\pm$ 0.1 mL aliquots of sample to the 12 mL reservoir. Standards should be transferred into a 25 mL measuring cylinder to make the sample transfer to the 12 mL reservoirs easier and the standards should be extracted from low concentration to high to minimise contamination.

F8.7 Open the stopcock gently until the liquid starts to flow. Ensure the flow is gentle enough to avoid splashing within the collection tube.

F8.8 Stop the flow just as the last of the sample exits the main body of the reservoir (i.e. do not allow the filter to develop an air gap).

F8.9 Using the 1mL pipettor transfer 0.85 mL $\pm$ 0.05 mL of acetonitrile to the reservoir. Open the stopcock and allow the acetonitrile to completely drain.

F8.10 Turn off the vacuum and allow the pressure to equilibrate gently. Remove the tube rack from the tank.

F8.11 Use a Pasteur pipette to mix the acetonitrile thoroughly into the filtered sample/ standard and then transfer an aliquot to an autosampler vial. Label the vial appropriately.

F8.12 Analyse the prepared sample/ standard as soon as possible. Extracted samples/standards are stable for up to 3 weeks.

F8.13 If sample dilution is required, dispense the required volume of shaken sample into a 25 mL test tube using the appropriate pipettor. Make up to the 25 mL mark with water and proceed from (F8.3). Ensure the dilution factor is recorded on all appropriate paperwork and vials. If dilutions greater than x 25 are to be made then use larger volume glassware (100 mL and 200 mL volumetric flasks and follow the procedure from (F8.2).

F9 Calculation

Provided the appropriate quality checks are acceptable, sample results are calculated using the equation below:

$$X = \frac{[\,(Y * D\,) - B\,]}{R} \qquad ng.L^{-1}$$

where: X = Final result (ng L^{-1})
Y = Raw result from quantitation (ng L^{-1})
D = Dilution factor (if applicable) = ($V_{final\ extract}$ [µL]) / $V_{original\ extract}$ [µL])
B = Process blank value (ng/mL)
R = Mean % Recovery / 100

Note: The % Recovery will always be 100 so R= 1. The standards go through the same procedure as the samples.

Figure F1 Typical chromatogram

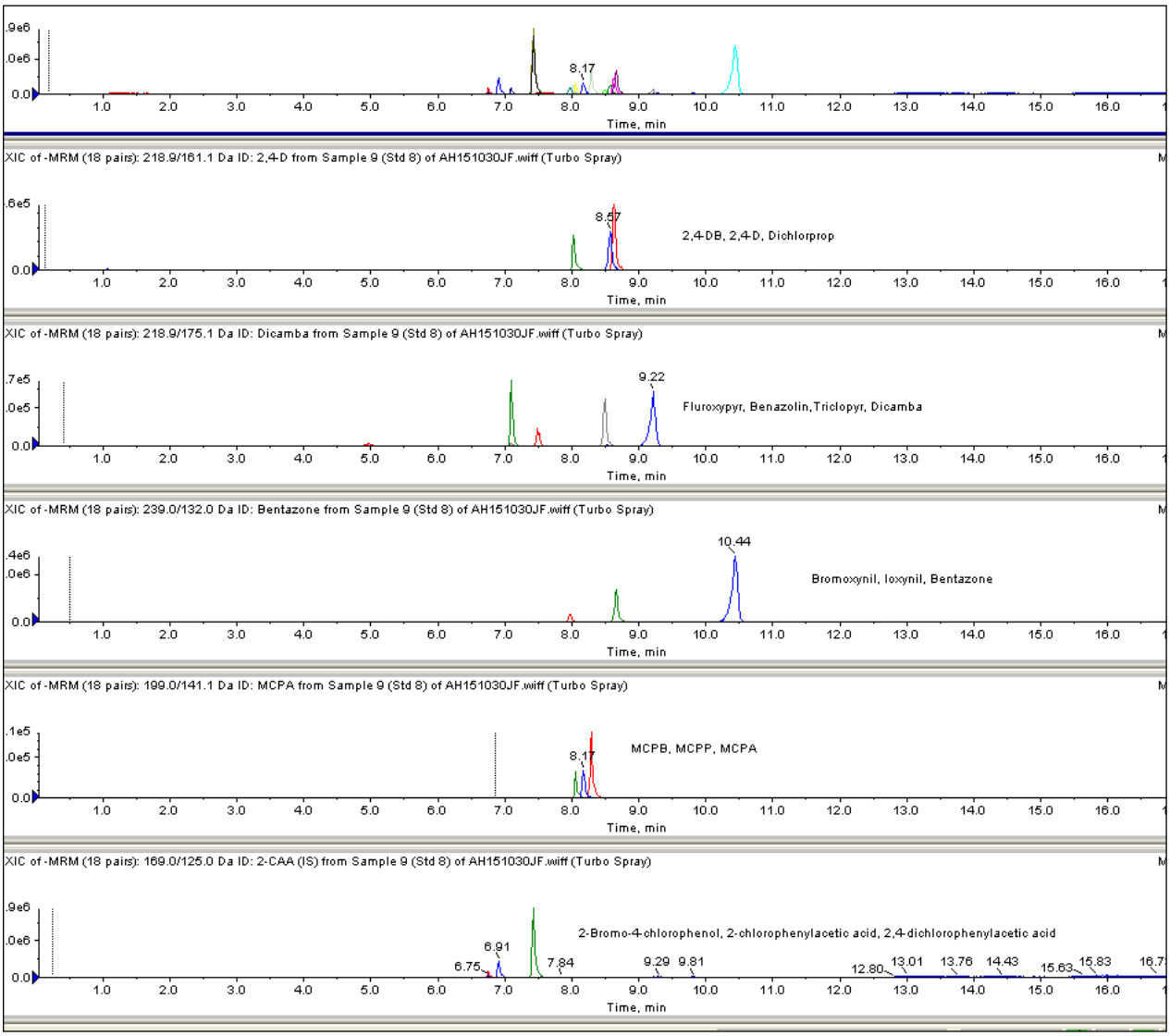


Table F1 Performance data

Determinand	LOD	Sample		Sample		Standards			
		River water		Ground water		0.06 µg L^{-1}		0.54 µg L^{-1}	
	µg L^{-1}	%RSD	*Bias %*	%RSD	*Bias %*	SD	Bias %	SD	Bias %
MCPA	0.0045	6.5	2.3	6.9	-0.6	7.0	4.08	2.6	2.47
Clopyralid	0.009	5.4	-2.7	6.6	-12.1	11.6	-0.07	4.0	-2.25
2,4 D	0.0027	5.7	10.6	6.9	5.8	7.4	5.79	2.8	1.77
Dicamba	0.0017	5.4	3.1	6.3	-2.0	9.3	3.99	3.5	1.85
2,4 DB	0.0015	6.1	-10.5	7.3	-10.9	7.5	1.71	4.7	0.90
Dichlorprop (2,4-DP)	0.0035	5.7	-1.0	5.4	-5.2	6.5	2.88	3.3	1.74
Bromoxynil	0.0021	9.5	-6.6	6.5	-5.3	4.3	4.60	7.7	-1.23
Ioxynil	0.0036	8.4	-8.1	8.7	2.4	4.9	5.32	5.8	-2.05
Bentazone	0.0013	11.7	-0.9	10.9	-6.2	6.3	1.53	4.5	-1.39
MCPB	0.0053	5.2	7.9	6.6	-3.1	4.5	3.06	3.2	0.39
Mecoprop (MCPP)	0.0014	5.1	-2.7	4.1	2.5	6.4	3.06	2.6	0.29
Triclopyr	0.0025	5.4	-8.4	6.2	-4.3	7.2	3.33	2.2	1.06
Fluroxypyr	0.0036	6.7	-5.9	6.7	-11.4	8.0	3.49	2.3	1.99
Benazolin	0.0040	6.0	-8.1	3.8	-7.6	6.7	4.38	4.3	-0.05

Validation Data	MDL	10% Cal std		90% Cal std		AQC	River spiked sample		Groundwater spiked sample	
Analyte	ng L^{-1}	%RSD	% Bias	%RSD	% Bias	% Rec	%RSD	% Exp Rec	%RSD	% Exp Rec
MCPA	4.5	7.0	4.08	2.6	2.47	99.22	6.5	102.3	6.9	99.4
Clopyralid	9.0	11.6	-0.07	4.0	-2.25	99.25	5.4	97.3	6.6	87.9
2,4 D	2.7	7.4	5.79	2.8	1.77	99.61	5.7	110.6	6.9	105.8
Dicamba	1.7	9.3	3.99	3.5	1.85	98.50	5.4	103.1	6.3	98.0
2,4 DB	1.5	7.5	1.71	4.7	0.90	97.72	6.1	89.5	7.3	89.1
Dichlorprop (2,4-DP)	3.5	6.5	2.88	3.3	1.74	100.52	5.7	99.0	5.4	94.8
Bromoxynil	2.1	4.3	4.60	7.7	-1.23	98.56	9.5	93.4	6.5	94.7
Ioxynil	3.6	4.9	5.32	5.8	-2.05	99.11	8.4	101.1	8.7	102.4
Bentazone	1.3	6.3	1.53	4.5	-1.39	98.72	11.7	91.9	10.9	93.8
MCPB	5.3	4.5	3.06	3.2	0.39	100.06	5.2	99.1	6.6	96.9
Mecoprop (MCPP)	1.4	6.4	3.06	2.6	0.29	100.72	5.1	107.9	4.1	102.5
Triclopyr	2.5	7.2	3.33	2.2	1.06	99.83	5.4	97.3	6.2	95.7
Fluroxypyr	3.6	8.0	3.49	2.3	1.99	99.78	6.7	91.6	6.7	88.6
Benazolin	4.0	6.7	4.38	4.3	-0.05	99.00	6.0	94.1	3.8	92.4

Data provided by SEPA

Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below. In addition, if users wish to receive advanced notice of forthcoming publications, please contact the Secretary.

Secretary
Standing Committee of Analysts
Environment Agency (National Laboratory Service)
Staplake Mount Starcross Exeter
EX6 8FD

http://www.standingcommitteeofanalysts.co.uk/Contact.html

Environment Agency
Standing Committee of Analysts

Members assisting with these methods

Without the good will and support given by these individuals and their respective organisations SCA would not be able to continue and produce the highly valued and respected blue book methods.

Member		Organisation
Edel	Abbott	Thames Water
Ian	Anderson	Northumbrian Water
Ian	Barnabas	Northumbrian Water
Richard	Brown	LGC
Katie	Buck	ALS Environmental
Wayne	Civil	EA
S	Connor	SEPA
Neil	Cullum	Anglian Water
Neil	Dutton	United Utilities
Dave	Evans	ALS Environmental
Daniel	Evans	Thames Water
Russell	Gibbs	Dwr Cymru Welsh Water
Sarah	Gledhill	South East Water
Anthony	Gravell	Natural Resources Wales
Toni	Hall	Wessex Water
Sam	James	Affinity Water
James	Kee	Thames Water
Kasia	Lis	South West Water
Kim	Lowe	Thames Water
Gavin	Mills	Severn Trent Water
Katie	Pardoe	Dwr Cymru Welsh Water
Laura	Pinkney	United Utilities
David	Powell	United Utilities

Chris	Pratt	Northumbrian Water
John	Quick	ALS Environmental
Matthew	Rawlinson	Affinity Water
Sarah	Roberts	UKAS
Kevin	Snaddon	Scottish Water
Adrian	Thomas	Severn Trent Water
Jim	Thomas	SEPA
Lee	Thomas	Severn Trent Water
David	Thompson	Thames Water
Sam	Towers	ALS Environmental
Ian	Townsend	South West Water
David	Turnbull	SES
Jeanette	Williams	United Utilities
Josie	Willot	South East Water
Steve	Wilson	Northumbrian Water