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Analytical Method for the Determination of Acid Herbicides in Sandstone and Chalk Aquifer Materials

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ANALYTICAL METHOD FOR THE DETERMINATION OF ACID HERBICIDES IN SANDSTONE AND CHALK AQUIFER MATERIALS

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Summary

WRc is conducting a research programme for the National Rivers Authority on the occurrence of pesticides in major aquifers. Several analytical methods for pesticides in aquifer materials have been developed as part of the contract. This report describes a multi-residue analytical method for the determination of acid herbicides in sandstone and chalk aquifer materials. The method is based on the principle of extraction into a solution of calcium hydroxide, partition into dichloromethane after acidification and determination by Gas Chromatography-Mass Spectrometry (GC-MS). as the methyl esters or ethers. The method has estimated limits of detection for each herbicide of between 0.02 and 0.09 $\mu g\ kg^{-1}$, depending on recoveries, moisture content and interferences from the aquifer material. The format of the description of the method follows that adopted by the Standing Committee of Analysts in the series 'Methods for the Examination of Water and Associated Materials'.

1 Performance characteristics of method	1.1	Substances determined	Mecoprop, MCPA, Dichlorprop, 2,4-D, 2,4-DB, Ioxynil and Bromoxynil. This method may be suitable for the determination of other acid herbicides pesticides but has only been tested with the herbicides listed above.
	1.2	Types of samples	Sandstone and chalk aquifer materials.
	1.3	Basis of method	Extraction of sample into basified water, followed by acidification and solvent extraction with dichloromethane. Determination by gas chromatography-mass spectrometry (GC-MS).
	1.4	Range of application	Tested up to 10 $\mu g\ kg^{-1}$.
	1.5	Calibration curve	Has been shown to be linear up to 10 $\mu g\ kg^{-1}$.
	1.6	Standard deviation	See Table 1.
	1.7	Limit of detection	The determinands can be detected down to the 0.02-0.09 $\mu g\ kg^{-1}$ range.
	1.8	Sensitivity	Dependent on the determinand and the instrument in use.
	1.9	Bias	Extraction efficiencies are normally less than 100%. Bias will vary with the extraction efficiency of any particular determinand. Correction for recovery should be made using results for spiked samples but this may not completely eliminate systematic errors. The results for spiked samples obtained during the performance test are given in Table 1.

1.10	Interferences	See 3 below.
1.11	Time required for analysis	About ten samples/seven days - total time

2 Principle

Aquifer material is freeze dried (if necessary), ground to a powder and extracted with an aqueous solution of calcium hydroxide. The basic aqueous extract is acidified and extracted with dichloromethane. The dichloromethane extract is concentrated by Turbo-Vap technique and methylated. Concentrations of the herbicides in the final extract are determined by gas chromatography-mass spectrometry (GC-MS).

3 Interferences

No specific interferences have been noted during the method validation. However the occurrence of compound(s) which are extracted and co-elute with the determinand and contain the quantitation ion may interfere. If interferences are suspected, additional work, not described here, may be necessary. This might include variations in the GC or the MS conditions.

4 Hazards

All reagents must be COSHH assessed before use and the recommended control procedures implemented. All the solvents are harmful and all except dichloromethane are flammable. Caution must be exercised when preparing the pesticide stock and working calibration standards solutions; skin contact, ingestion and inhalation must be avoided. Good laboratory procedure must be followed at all times.

DIAZOMETHANE REAGENT PREPARATION IS A HAZARDOUS OPERATION AND SHOULD ONLY BE CARRIED OUT IN A GLASS FRONTED FUME CUPBOARD.

5 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks in the GC-MS analysis of extracts. Purity must be checked by analysing procedural blanks with each batch of samples analysed.

5.1 Methanol, High-Performance liquid chromatography (HPLC) grade.

5.2 Dichloromethane, HPLC grade.

5.3 Acetone, glass-distilled grade.

5.4 Ethyl Acetate, HPLC grade.

5.5 Distilled or deionized water, pesticide free.

5.6 Nitrogen gas, dry oil-free passed through an activated carbon filter.

5.7 Hydrochloric acid - concentrated (d_{20}1.18), AnalaR Grade.

5.8 Dilute Hydrochloric acid (50% v/v). Prepared as follows: slowly, with constant stirring and cooling pour 400 ± 5 ml of concentrated acid (5.7 above) into about 400 ml of water. Allow to cool to room temperature and make up to 1000 ± 10 ml in a measuring cylinder. Mix and store in a glass-stoppered bottle.

Hydrochloric acid is corrosive and care is needed.

5.9 Calcium Hydroxide, technical grade.

5.10 Methylation reagents.

5.10.1 Ethanoic potassium hydroxide solution.

AnalaR potassium hydroxide (0.4g) is dissolved in 10 ml of 90% aqueous ethanol (9 ml absolute ethanol plus 1 ml deionized water).

5.10.2 Diazald solution.

Diazald (N-methyl-N-nitroso-toluenesulphonamide, Aldrich) (2.14 g) is dissolved in fresh AnalaR diethyl ether (30 ml).

5.11 Standard solutions. These must be stored in a cool dark place and prepared freshly every six months.

5.11.1 Phenoxyacid pesticide stock solution (100 ng μl^{-1})

Prepare from pure or certified material. Accurately weigh approximately 10 mg. (9-12 mg with a tolerance of ±0.1 mg) of each pesticide into a 100 ml volumetric flask, dissolve and make up to volume with acetone.

5.11.2 Internal standard (2,4-dichlorophenylacetic acid) stock solution (100 ng μl^{-1}).

Accurately weigh approximately 10 mg (9-12 mg with a tolerance of ± 0.1 mg) of the acid into a 100 ml volumetric flask, dissolve and make up to volume with acetone.

WARNING

CAUTION MUST BE EXERCISED WHEN PREPARING THE STOCK SOLUTIONS. SKIN CONTACT, INGESTION AND INHALATION MUST BE AVOIDED.

5.11.3 Working calibration standard solutions.

Prepare from the stock solution. Add the appropriate volume of stock pesticide solution (5.11.1) and 250 ± 3 µl of internal standard solutions (5.11.2) to about 5 ml dichloromethane in a 10 ml volumetric flask. Methylate as in step 8.7.1. Remove the excess diazomethane with a gentle stream of nitrogen, concentrate to near dryness and make up to volume with ethyl acetate.

Volume of stock pesticide solution added (µl)	Concentration of pesticide in standard (ng μl^{-1})	Corresponding* concentration in sample (µg kg^{-1})
5 ± 0.1	0.05	0.02
10 ± 0.2	0.1	0.04
20 ± 0.4	0.2	0.08
50 ± 0.8	0.5	0.20
100 ± 1.0	1.0	0.40

* - the corresponding concentration of the internal standard in sample is 1 µg kg^{-1}.

Note: Standards at concentrations 2.0 and 5.0 ng μl^{-1} should also be prepared and analysed when initially establishing the linear range of the GC-MS.

5.11.4 Internal standard spiking solution (10 ng μl^{-1})

Add 1.0 ± 0.01 ml of internal standard stock solution (5.11.2) to about 5 ml of methanol in a 10 ml volumetric flask and make up to volume with methanol.

6 Apparatus

Glassware should be clean (dilute acid washed, rinsed with acetone and finally rinsed with deionized or distilled pesticide-free water) and dry.

6.1 Reagent preparation - Syringes (10 µl, 100 µl, 500 µl and 1 ml). Volumetric flasks (10 ml and 100 ml). Measuring cylinder (1 l). Analytical balance (5 place).

6.2 Aquifer material preparation - Bricklayers hammer and bolster. Freeze drier (Edwards mini-fast 3400 is suitable).

Impeller-type cutting mill with 4 mm sieve (Glen Creston type SM1 mill is suitable). Plastic bags.

6.3 Moisture determination - Porcelain dishes (2). Analytical balance (5 place). Microwave oven (power rating 700 w). Desiccator.

6.4 Extraction and filtration - Quickfit widemouth stoppered conical flasks (1 l). Buchner flask (2 l). Buchner funnels (12.5 cm). Vacuum line. Whatmann glass fibre filters (GF/F and GF/D, 12.5 cm). Orbital shaker fitted with a platform suitable for 1 l conical flasks, (Gallenkamp model is suitable). Top pan balance (3 place). Quickfit widemouth stoppered 250 ml round bottomed flasks. pH meter with accuracy of ± 0.1. Freezer to accommodate 250 ml round-bottomed flasks.

6.5 Concentration and methylation - Zymark Turbo-Vap evaporator. Wheaton vials (1 ml). 250 ml Zymark Turbo-vap tubes. Diazomethane generation kit (Aldrich Diazald kit is suitable).

6.6 Gas Chromatograph - Mass Spectrometer (GC-MS) - A GC-MS system with data system operated in accordance with the manufacturer's instructions. Suitable conditions are:

GC conditions: 30 m DB-1 capillary column (J&W Scientific), 0.25 µm film thickness, 0.32 mm internal diameter. Carrier gas (helium) flow rate 1 ± 0.1 ml $minute^{-1}$. Column temperature programme: 60 °C for three minutes, then 8 °C $minute^{-1}$ temperature ramp to 300 °C.

MS conditions: ion source operated at 200 °C. The mass spectrometer is operated in Selected Ion Recording (SIR) mode and calibrated for SIR using perfluoro-tributylamine ('heptacosa').

Typical retention times and ions monitored:

Pesticide	retention time (minutes)	m/z monitored quantification	m/z monitored confirmation
Mecoprop	22.5	169	228
MCPA	22.6	214	141
Dichlorprop	23.5	162	189
2,4-D	23.7	234	199
2,4-DB	26.8	101	162
Bromoxynil	23.6	291	276
Ioxynil	27.3	385	370
Internal standard	21.7	159	183

7 Sample storage and preservation

Core samples of chalk taken using a percussion drilling rig with U100 corebarrel. Core samples of sandstone are taken using an air-flush rotary drilling rig with a triple corebarrel capable of taking cores at least 75 mm in diameter and, where possible, using a mylar corebarrel liner.

The sample, complete with corebarrel liner, is enclosed in double-lined polythene sleeving, sealed, and placed in a freezer for transit. Samples should be analysed as soon as possible upon receipt at the laboratory, if not they should be stored in a freezer.

8 Analytical procedure

Step	Procedure	Notes
8.1	Sample Pretreatment.	
8.1.1	With chalk cores, using a core pusher the core sample is removed from the corebarrel liner. With sandstone cores, the corebarrel liner is cut from the core. Between 25 and 50 mm of each end of the core sample is removed and discarded. (see note a).	a. If no further work is to be immediately carried out,the sample must be sealed in a plastic bag and returned to the freezer.
8.1.2	The core sample is broken into small lumps (see note b) with a bricklayers hammer and bolster.	b. To facilitate grinding maximum size of the lumps should be about 50 mm.
8.1.3	For chalk, the broken core sample is freeze dried over weekend (or until the moisture content is negligible,	c. The knife mill must be cleaned between batches of samples by brushing and

i.e. <0.3%) and then ground to a homogeneous powder in a knife mill so as to pass through a 4 mm sieve (see notes c, d).

For sandstone, the broken core sample is ground as above, but without the freeze drying (see notes c, d, and e).

washing (with deionized or distilled water) to remove aquifer material from inside the housing the rotary head, blades and sieve.

d. If after step 8.1.2 or 8.1.3 no further work is to be immediately carried out, the sample shall be sealed in a glass or stainless steel container and returned to the freezer.

e. If the broken sandstone core sample contains too much moisture to be ground directly, the sample will be freeze dried, but the results will then be calculated as for a chalk sample.

8.2 Extraction

8.2.1 Accurately weigh 250 ± 0.5 g of powdered sample into a wide mouthed 1 l conical flask. Record the weight (W) in kg. Add 500 ± 10 ml deionized water, stopper and mix well. In a fume cupboard slowly add 25 g calcium hydroxide, mix well and leave unstoppered for approximately 15 minutes to let any reaction subside. Stopper and place the flask on an orbital shaker and shake at about 150 revolutions per minute for 1 hour.

8.3 Filtration

8.3.1 On the bottom of a buchner filter place a GF/F glass fibre filter and a GF/D glass fibre prefilter on the top of it. Wet with deionized water and keep the apparatus under vacuum. Filter the sample under vacuum. Carefully wash all the solid sample

f. When filtering and washing do not allow the filter cake to go to dryness.

into the buchner funnel using the minimum amount of deionized water. Wash the filter cake with a further three 50 ± 5 ml portions of water (see notes f and g).

g. If aquifer particles are observed in the filtrate the filtrate must be filtered again.

8.3.2 Transfer the filtrate and washings into a clean 1-l wide mouth conical flask (see note h).

h. The filtrate should be processed further as soon as practicable; prolonged storage at high pH may lead to degradation of some of the determinands.

8.4 Acidification

Add dropwise approximately 10 to 15 ml of 50% (v/v) hydrochloric acid to the aqueous filtrate and adjust to pH 1.2 ± 0.2.

Add 25 ± 0.5 µl of the internal standard spiking solution (from step 5.11.4) to the sample (250 ng equivalent to 1 µg kg^{-1} in aquifer material).

8.5 Solvent extraction

8.5.1 Transfer the acidified aqueous filtrate to a 2-litre separating funnel pre-rinsed with dichloromethane. Add 100 ml dichloromethane to the funnel and shake for 2 minutes (see note i). Collect the organic layer into a 250 ml round bottomed flask. Add a further 100 ml dichloromethane to the aqueous filtrate and shake for a further 2 minutes. Add the organic layer to the round bottomed flask and discard the aqueous layer.

i. The separating funnel should be regularly vented to avoid solvent pressure building up.

8.5.2 Place the organic extract in a freezer overnight to freeze water content.

8.6 Concentration

8.6.1 Turbo-Vap concentration

Filter organic extract through silanised glass wool, pre-washed

with dichloromethane, into 250 ml Turbo-Vap tube. Concentrate the sample at approximately 38 °C with nitrogen at medium flow rate to a final volume of 5 ± 0.5 ml.

8.6.2 Transfer 0.5 ml portions of concentrated extract to 1 ml Wheaton vial. Wash turbo-vap tube with approximately 6 ml (4 to 8 ml) of dichloromethane and gradually transfer washings to the vial. Blow down to 0.5 ml under gentle stream of nitrogen.

8.7 Methylation

8.7.1 Diazomethane preparation.

WARNING

DUE TO THE PROPERTIES AND TOXIC NATURE OF DIAZOMETHANE IT IS STRONGLY RECOMMENDED THAT COMMERCIALLY AVAILABLE APPARATUS IS USED FOR ITS PREPARATION AND EXTREME CAUTION IS EXERCISED IN ITS USE. THE DIAZOMETHANE SHOULD BE PREPARED FRESH DAILY AND ONLY IN SUFFICIENT QUANTITY FOR IMMEDIATE USE.

The diazomethane apparatus is assembled, ensuring it is clean and dry. To the reaction vessel is added ethanoic potassium hydroxide solution (10 ml) and to the dropping funnel a solution of diazald in diethyl ether (30 ml).

8.7.2 The water bath is heated to 65-70 °C and the reaction carefully initiated by addition of a small amount (few ml) of the diazald solution. A steady rate of distillation is maintained by dropwise addition of the remainder of the diazald solution. The diazomethane-diethyl ether solution is collected in a suitable flask.

8.7.3 Transfer about 0.5 ml of the diazomethane solution to the Wheaton vial from step j. If after about 10 minutes the yellow colour

8.6.2. Cap the vial and allow to stand for 1 hour (see note j).

of the diazomethane is discharged, add a further volume of diazomethane solution.

8.7.4 Remove the excess diazomethane with a gentle stream of nitrogen (until yellow colour disappears).

8.7.5 Add 100 µl of ethyl acetate to the extract and concentrate the extract to 100 µl using a gentle stream of nitrogen.

8.7.6 If the extracts are not to be examined immediately by GC-MS the vials must be capped and stored in a freezer.

8.8 GC-MS

8.8.1 In conjunction with step 6.5 the GC-MS conditions should be optimised to give the best signal-to-noise ratio.

8.8.2 Run the first GC-MS calibration standard until the ratios of the internal standard peak area to those of the determinands are no more than 20% different from the previous run. Complete the calibration of the GC-MS by running the calibration standards (0.05, 0.1, 0.2, 0.5 and 1.0 ng μl^{-1}). This will give a calibration corresponding to phenoxyacid concentrations in the sample from 0.02 to 0.4 µg kg^{-1}. For concentrations outside this range run the appropriate standards. (See note in 5.11.3)

8.8.3 Check that there is no sample carry over by injecting a solvent blank after the highest concentration standard.

8.8.4 Inject the sample extracts.

8.8.5 Check there has been no significant drift in the calibration by repeating a calibration standard at the end of the run or after each six samples (for longer runs). If the differences between the

peak area ratios for the calibration standard and the repeat standard are greater than 20%, then repeat the GC-MS analysis of samples which are affected.

Check that the peak area of internal standard in the extract is at least 50% of the average area in the standard solutions. If not, the whole analysis should be repeated with a fresh sample.

8.9 Moisture determination (sandstone samples).

Accurately (± 0.01 g) weigh lumps of sample from step 8.1.2 (about 50 g) into a tared shallow porcelain dish, in duplicate. Place the sample and dish in a microwave oven and heat on maximum for 30 minutes.

Cool the sample and dish in a desiccator and reweigh. Return the sample and dish to the microwave oven and heat on maximum for a further 10 minutes. Cool the sample and dish in a desiccator and reweigh. If the difference in weight between the first and second weighing after drying is greater than 0.01 g then return the sample to the oven for a further 10 minutes, and repeat until the difference is less than 0.01 g.

Calculate the % moisture:

Moisture (M%) = 100 x (1-[dry weight/wet weight])

8.10 Calculation of concentrations

8.10.1 Calibration

For each calibration standard, control sample and sample, divide the pesticide peak area by the internal standard peak area to obtain the peak area ratio. (Peak areas of the quantification ions.)

Determine the linear regression of the calibration data from the peak area

ratio and concentration for each pesticide (ng μl^{-1}). Obtain the gradient and intercept. Prepare a graphical representation of the calibration data. Visually assess that the calibration is linear.

8.10.2 Concentration in the extract.

The concentration of pesticide, C_E, in the extract can be calculated using the following equation:

$$C_E = \frac{(A_R - I)}{G} \text{ ng } \mu l^{-1}$$

where A_R = area ratio
I = intercept
G = gradient

8.10.3 Recovery

The recovery is determined from spiking experiments. Typical recoveries are quoted in Table 1 (see note k).

k. The results indicate that recoveries obtained for spiked samples analysed in the same batch as the samples will provide more accurate estimates of concentrations than mean recoveries.

8.10.4 Concentrations in sandstone

Concentration of each pesticide, C (corrected for recoveries), in sandstone samples, which are not dried before sampling is calculated using the following equation:

$$C = \frac{C_E \times 10}{W \times R} \text{ µg kg}^{-1}$$

where W = weight of sample (kg)
R = recovery as %

8.10.5 Concentrations in chalk

For chalk samples (and samples of sandstone which have to be dried

before sampling) the calculation is as in 8.10.4, but the concentration is related to the dry aquifer material, C_{DW}:

$$C_{DW} = \frac{C_E \times 10}{W \times R} \quad \mu g\ kg^{-1}$$

To obtain concentration C in the sample C_{DW} needs to be corrected for the moisture content.

$$C = \frac{C_{DW} \times (100 - M\%)}{100} \quad \mu g\ kg^{-1}$$

where M% = % moisture in sample

8.11 Control samples

To check for interferences, recoveries and accuracy, at least one blank, one spiked control sample and one duplicate sample should be analysed by the entire procedure with each batch of analyses.

Table 1 Estimates of recoveries and standard deviations for spiked samples of chalk and sandstone

	Concentration of spiked sample, µg kg^{-1}		MCPP	MCPA	DCPP	2,4-D	2,4-DB	Bromox.	Iox.
Mean concentration found, µg kg^{-1}	0.2		0.196	0.100	0.187	0.091	0.144	0.137	0.120
	1.0		0.735	0.458	0.826	0.395	0.598	0.574	0.487
	1.0 QC		**1.086**	**0.619**	**0.765**	**0.561**	**0.834**	**0.460**	**0.230**
	10.0		9.00	5.74	10.85	5.28	7.51	7.71	7.91
Recoveries, %	0.2		98.0	50.0	93.5	45.5	72.0	68.5	60.0
	1.0		73.5	45.8	82.6	39.5	59.8	57.4	48.7
	1.0 QC		**108.6**	**61.9**	**76.5**	**56.1**	**83.4**	**46.0**	**23.0**
	10.0		90.0	57.4	108.5	52.8	75.1	77.0	79.1
Standard deviations, µg kg^{-1}	0.2	SW	0.036	0.011	0.013	0.017	0.011	0.033	0.022
		SB	0.011	0.005	0.057	0.009	0.065	0.023	0.034
		ST	0.037	0.012	0.058	0.019	0.066	0.040	0.040
		DegF	5	4	2	4	2	4	3
	1.0	SW	0.074	0.061	0.124	0.048	0.063	0.039	0.051
		SB	0.066	0.098	0.275	0.098	0.094	0.105	0.114
		ST	0.100	0.115	0.302	0.109	0.113	0.112	0.125
		DegF	5	4	4	4	4	3	4
	1.0 QC	SW	0.064	0.066	0.204	0.109	0.287	0.062	0.038
		SB	0.063	0.053	0.0	0.116	0.0	0.024	0.068
		ST	0.090	0.084	0.204	0.159	0.287	0.066	0.077
		DegF	5	5	7	5	7	6	4
	10.0	SW	0.90	1.17	3.06	1.23	0.61	1.18	2.01
		SB	0.75	0.41	3.54	0.0	0.12	3.70	4.40
		ST	1.17	1.24	4.67	1.23	0.63	3.88	4.83
		DegF	4	5	3	5	5	2	2

MCPP - Mecoprop, DCPP - Dichlorprop, Bromox. - Bromoxynil, Iox. - Ioxynil.

SW, SB, ST - within-batch, between-batch and total standard deviations, respectively.

DegF - Degrees of freedom calculated by analysis of variance.

QC - Quality control samples analysed with batches of chalk and sandstone samples for profiling.

Note: The initial validation experiment consisted of three batches of chalk samples and three batches of sandstone samples, each batch included duplicate samples spiked at 0.1, 1.0 and 10.0 µg kg^{-1} and duplicate unspiked samples. Results from three of the batches had to be rejected because the recovery of the internal standard was poor (<50%) and the recoveries of the determinands varied widely. The pattern of results suggested that the variation may have been related to the control of pH, which was kept between 0.5 and 2.0. Consequently, the method was modified slightly to include more precise pH control (pH 1.2 ± 0.2; see step 8.4 in the method). The QC samples (four batches of duplicate samples spiked at 1.0 µg kg^{-1} and unspiked chalk or sandstone) and the samples of chalk and sandstone analysed for profiling were processed with the modified acidification procedure, step 8.4, and the recovery of the internal standard was consistently above 50%.

Comparisons of the statistical evaluation of the results for samples spiked with 1.0 µg kg^{-1} show that the standard deviations, particularly the between-batch variations, were generally better for the QC samples then for the earlier samples. The acidification at pH 1.2 ± 0.2 resulted in better mean recoveries for four compounds (results for MCPP and 2,4-DB were statistically significant) but the recoveries of bromoxynil and ioxynil were significantly lower. It is possible that a precisely controlled pH value other than 1.2 would give an optimum overall recovery for the range of determinands investigated.