

MOUNTAINHEATH ENVIRONMENTAL ANALYSTS & CONSULTANTS

379/1

DETERMINATION OF THE
ENANTIOMERIC FORMS OF
MECOPROP

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1. INTRODUCTION

Mecoprop is a systemic herbicide used to control weeds in cereal crops. It has been detected in drinking water at levels close to or exceeding the EEC limit for individual herbicides of 0.1 ug/L. Mecoprop is currently sold as a racemic mixture of enantiomers of which only one form, the D+ isomer is herbicidally active and which is contained in the racemate in equal proportion to the L- form.

Current methods in use in the Water Industry based upon gas chromatography/mass spectrometry (GC-MS) do not allow separation and quantification of the optical isomers and thus non-herbicidal mecoprop is being determined together with the enantiomer with herbicidal activity. It may well be that some contraventions of the EEC limit for this compound are not in breach of the regulations as in fact the L- form has no herbicidal activity but is being measured together with the D+ form and reported as mecoprop.

The D+ form is known as Mecoprop P and some manufacturers are attempting to enrich the proportion of this form in commercial products so that herbicidal activity is increased but the total amount of racemic mecoprop is reduced.

It is important therefore to be able to separate the enantiomers and determine them separately.

2. OBJECTIVES

The objective of this work was to develop an analytical technique to separate the enantiomers and, this achieved, to carry out work to establish the method according to the guidelines of the Drinking Water Inspectorate.

3. SOURCES OF STANDARDS AND WATER

- a. Mecoprop (racemate) was obtained from Promochem Ltd as certified material.
- b. Mecoprop P (D+ form) was obtained from Rhone-Poulenc and was certified by analysis in a laboratory following GLP standards.
- c. Distilled water - obtained from a conventional laboratory still.
- d. Tap water - Stevenage tap water, calcium content approximately 100 mg/l.

4. SELECTION OF ANALYTICAL TECHNIQUES

The technique selected was based on the draft SCA method for acidic herbicides and is conventional in most respects apart from the choice of column. C18 cartridges (1g) were treated with methanol, water and water at pH 2 before the sample (1.0L) was drawn through under vacuum. The sample was pre-treated with sodium hydroxide solution (pH 12) to convert mecoprop to the free acid form. The sample was left overnight to hydrolyse and was then acidified to pH 2.0 ± 0.1 . The free acid was then methylated with diazomethane and analysed by GC-MS. The column chosen for the GC-MS separation was a 30m α -dex chiral column (Sigma-Aldrich-Supelchem) designed to separate optical isomers. The ion fragments 142, 169 and 228 were measured. The 142 fragment was used for quantification and the 169 and 228 fragments as qualifier ions. The GC was a Hewlett-Packard Model 5890 and the MS a Hewlett-Packard mass selective detector 5972 (MSD). Automatic injection was employed and processing was by HP Vectra VL2 4/66

5. ANALYTICAL METHOD

See Appendix 1

6. DISCUSSION OF RESULTS

The separation of the D+ and L- enantiomers of mecoprop was satisfactory using the α -dex column. Other chiral columnss would, no doubt, be equivalent.

The recoveries found were rather poor overall with the distilled water samples giving better recoveries than the tap water samples. The limit of detection was calculated using a low spike injected on different occasions and gives a much higher (although perhaps more realistic) estimate of the LOD than using a set of blanks run on one occasion for the calculation. Neither the distilled nor the tap water contained any significant level of either enantiomer of mecoprop.

The individual results used to calculate the means and standard deviations are listed below:

DAY	0.02	Distilled (ug/L)		unspiked	Tap (ug/L)	
		0.10	1.0		0.1	1.0
1	0.0084	0.082	0.65	0.001	0.052	0.44
1	0.011	0.078	0.93	0.000	0.037	0.64
2	0.0065	0.045	0.61	0.000	0.024	0.37
2	0.010	0.065	0.43	0.001	0.037	0.42
3	0.0093	0.054	0.70	0.000	0.035	0.61
3	0.0090	0.052	0.66	0.001	0.039	0.48

4	0.0096	0.054	0.63	0.001	0.049	0.45
4	0.010	0.047	0.61	0.000	0.053	0.82
5	0.011	0.063	0.72	0.000	0.045	0.54
5	0.015	0.070	0.71	0.001	0.066	0.59
6	0.011	0.055	0.83		0.055	0.67
6	0.0099	0.060	0.79		0.068	0.54
7	0.010	0.066	0.57		0.026	0.48
7	0.006	0.040	0.61		0.038	0.45
8		0.052	0.54		0.028	0.38
8		0.049	0.67		0.044	0.43
9			0.73			
9			0.94			

7. CONCLUSIONS

1. The technique used is satisfactory for the determination of mecoprop.
2. The recoveries were poorer than expected but are adequate. Small variations in the cartridge extraction procedure might improve these and the use of an internal standard might improve the experimental error.
3. No correction for recovery has been made. The results could be adjusted if required.
4. For effluents and samples with high solids content, the cartridge extraction procedure is inappropriate,. The D+ enantiomer can be separated by GC-MS on a chiral column (on the column used, the D+ enantiomer emerges before the L-form)

APPENDIX A

DETERMINATION OF THE ENANTIOMERIC FORMS OF MECOPROP USING C18 CARTRIDGE EXTRACTION AND GC-MS WITH A CHIRAL COLUMN

1. Performance characteristics of the method

- | | | |
|------|----------------------------|--|
| 1.1 | Substance determined | Mecoprop D+ and L- forms. |
| 1.2 | Drinking waters | Drinking waters |
| 1.3 | Basis of the method | The sample is extracted by adsorption on a C18 cartridge and after elution and methylation, the mecoprop enantiomers are separated on a chiral column and determined by GC-MS. |
| 1.4 | Range of application | Up to 2 ug/L. The upper limit maybe extended by dilution of the sample or by taking a smaller original volume. |
| 1.5 | Calibration curve | The method is linear run over the range of application. (See Fig 1) |
| 1.6 | Standard deviation | See Table 1. |
| 1.7 | Limit of Detection | See Table 1. |
| 1.8 | Sensitivity | Depends upon the instrument in use (See fig 2) |
| 1.9 | Bias | Extraction efficiencies are less than 100%, see Appendix 1. Correction for recovery can be made by comparing samples with extracted standards or by arithmetic correction. |
| 1.10 | Interferences | Using an efficient column and suitable m/z values no interference is likely. |
| 1.11 | Time required for analysis | 10 samples may be analysed per day. |

2. Principle

The sample (1L) is made alkaline with sodium hydroxide to pH 12 and allowed to stand overnight at ambient temperature. Any mecoprop esters present are hydrolysed to the sodium salt of the free acid under these conditions. The pH of the sample is then adjusted to 2.0 ± 0.1 and the mecoprop extracted with a pre-prepared C18 cartridge. The cartridge is dried and then eluted

with ethyl acetate. The ethyl acetate is removed by evaporation under a stream of nitrogen and the residue methylated with an ethereal solution of diazomethane. The extract is evaporated to incipient dryness and made up to 1ml. with hexane before analysis by GC-MS using a chiral column and suitable selected mass fragments.

3. Interferences

Co-extracted compounds in principle may interfere. With the technique used however and the even numbered ion used for quantitation interference is effectively eliminated.

4. Hazards

Hexane, methanol, diethyl ether, ethyl acetate and acetone are flammable; n-hexane is toxic, i-hexane or pentane may be substituted. Sulphuric acid is corrosive and attacks body tissues. Sodium hydroxide and its solution is caustic. The diazomethane is toxic, carcinogenic and possibly explosive. The sulphonamide reagent used in its preparation has been shown to produce tumours in laboratory animals. Skin contact, ingestion and inhalation should be avoided. All operations involving diazomethane preparation or use should be carried out in a fume cupboard.

Diethyl ether is narcotic.

Diethyl ether may form explosive peroxides. Commercial diethyl ether is stabilised but material exceeding its shelf life should not be used.

Mecoprop and its solutions are toxic.

5. Reagents

All reagents must be of sufficient purity that they do not give rise to interfering peaks in the chromatograms ultimately obtained. A blank figure of <10% of the lowest value of interest is normally acceptable. This should be verified by running procedural blanks with each batch of samples analysed.

5.1 Sodium hydroxide solution (10%).

Dissolve 100±5g sodium hydroxide AR in approx 800ml distilled water (CARE! Heat is evolved!). Allow to cool to ambient temperature and dilute to 1.0 L with distilled water.

5.2 Sulphuric acid (50%)

Add 500±10ml concentrated sulphuric acid (AR) to about 400 ml of distilled water with swirling and cooling (CARE! Heat is evolved!). Cool to ambient temperature and dilute to 1.0L with distilled water.

5.3 Methanol - Pesticide grade

5.4 Acetone - Pesticide grade

5.5 Water - Distilled/deionised

5.6 Water, pH2. Using water (5.5) add dilute sulphuric acid until the pH is 2±0.1 using a pH meter.

- 5.7 Ethyl acetate - Pesticide grade
- 5.8 Diethyl ether - Pesticide grade
- 5.9 N-methyl-N-nitrosotoluene-4 sulphonamide AR
- 5.10 Sodium hydroxide solution (50%)
Dissolve 50±5g sodium hydroxide in 80ml of water (5.5) (CARE! Heat is evolved!). Allow to cool and make up to 100ml with water (5.5).
- 5.11 Acetic acid - glacial LR
- 5.12 Diazomethane solution
Using commercially available apparatus and reagents (5.8 - 5.11), prepare an ethereal solution of diazomethane. Use a fume cupboard. The solution should be distinctly yellow.
- 5.13 Mecoprop (racemate) stock solution - 500 mg/L
Dissolve 50±0.1mg mecoprop in 100ml acetone in a volumetric flask
- 5.14 Mecoprop (racemate) spiking solution - 10mg/L
Using a microlitre syringe, add 1 ml of the stock solution (5.13) to acetone in a 50ml volumetric flask and make up to the mark with acetone
- 5.15 Mecoprop (racemate) external standard solution (methylated - 0, 0.1, 0.2, 0.5, 1.0 mg/L
To a series of 10ml volumetric flasks, add approx. 9.0ml of hexane. Add to the flasks, aliquots of the stock solution to give a series of solutions:

0	mg/L	add	0 ul
0.05	mg/L	add	5 ul
0.10	mg/L	add	10 ul
0.20	mg/L	add	20 ul
0.50	mg/L	add	50 ul
1.0	mg/L	add	100 ul
2.0	mg/L	add	200 ul

Add approx. 2ml of an ethereal solution of diazomethane (5.12), allow to stand for 20 mins and then evaporate off the excess reagent (approx. 2ml) with a stream of purified nitrogen. No trace of yellow colour should remain. Make up to the mark with hexane. (Alternatively a commercial standard of the methyl ester of the mecoprop racemate may be used to prepare the standards. All the standards should be stored in a refridgerator and prepared freshly after 1 month.
- 5.17 Mecoprop D+ enantiomer, stock solution - 250 mg/L
Dissolve 25±0.1mg mecoprop P in 100ml acetone in a volumetric flask.
- 5.18 Mecoprop D+ enantiomer, spiking solution - 5mg/L
Using a microlitre syringe add 1ml of the stock solution (5.15) to acetone in a 50ml volumetric flask and make up to

the mark with acetone.

- 5.19 Mecoprop D+ enantiomer, external standard solutions (methylated) 0, 0.025, 0.1, 0.2, 0.5, 1.0 mg/L
To a series of 10ml volumetric flasks, add approx. 8 ml hexane. Add to the flasks aliquots of the stock solution (5.17) to give

0	mg/L	add	0 ul
0.02	mg/L	add	4 ul
0.05	mg/L	add	10 ul
0.10	mg/L	add	20 ul
0.25	mg/L	add	50 ul
0.50	mg/L	add	100 ul
1.0	mg/L	add	200 ul

Add approx. 2 ml of an ethereal solution of diazomethane (5.12), allow to stand for 20 mins and then evaporate off the excess reagent (approx 2ml) with a stream of purified nitrogen. No trace of the yellow colour should remain. Make up to the mark with hexane.

- 5.20 Nitrogen, oxygen free, filtered and dry

- 5.21 Hexane - Pesticide grade

6. Apparatus

All apparatus should be free from contamination before use. Glassware should be rinsed with acetone and allowed to dry before use.

- 6.1 Sample bottles
All glass or with PTFE lined screw caps, 1.0 litre capacity marked at 1 litre.
- 6.2 C18 sorbant (SPE) cartridges - 1g
The performance of the method can vary with different batches of cartridge material. It is necessary to test the performance of each batch before routine use. C18 Isolute cartridges (Jones Chromatography) were used in the performance testing of the method but other cartridges have also been satisfactorily used.
- 6.3 Cartridge manifold, reservoir and pump
Suitable for use with C18 cartridges and used in accordance with the manufacturers instructions. If a reservoir is used, this must be protected against implosion eg. by covering with plastic webbing.
- 6.4 Graduated test tubes, 10ml, stoppered
- 6.5 pH meter
- 6.6 Diazomethane generator - several satisfactory designs are commercially available. Use in accordance with the maker's instructions, in a fume cupboard.

- 6.7 GC-MS apparatus with a suitable data system fitted with a chiral column designed to separate enantiomers.

7. Sample storage

Samples should be extracted as soon as possible after sampling. If it is impracticable to analyse the samples at once they should be stored at about 4°C. The sample bottle should be protected from contamination by covering the cap and neck with aluminium foil. Samples should not be stored in the proximity of pure standards or their concentrated solutions.

8. Analytical Procedure

STEP	PROCEDURE	NOTES
8.1	To 1 Litre of sample in the sample bottle, add sufficient sodium hydroxide solution (5.1) to raise the pH to 12 ± 0.2 (Note a). Cap the bottle and allow to stand overnight (Note b) (Note c)	a) Typically 10-15 ml is required b) Check the pH with a pH meter c) 16hr or more is adequate for hydrolysis of mecoprop esters
8.2	To the bottle, add sufficient 50% sulphuric acid (5.2) to give a pH of 2 ± 0.1 (Note b)	
8.3	Mount a set of C18 cartridges (6.2) in a cartridge manifold. Condition the cartridges with 6 ± 0.5 ml methanol allowing the methanol to drain under gravity into the reservoir (Note d)	d) A Flow rate of 1 ml/min is satisfactory and individual adjustments are made to the manifold restrictors to achieve this flow.
8.4	When the methanol has drained, add 6 ± 0.5 ml distilled water (5.5) and allow to drain under gravity. When the water has drained, add 6 ± 0.5 ml of water, pH2 (5.6) and allow to drain under gravity.	
8.5	Connect a PTFE tube to the cartridge using an adaptor and draw the acidified sample through the cartridge at a rate of 10 ± 1 ml/min using a vacuum pump. Adjust each port individually so that the samples are drawn through the cartridges at the same rate. Allow all the 1 litre sample to pass through the cartridges (Note e)	e) Periodic adjustment of the vacuum may be necessary to maintain the flow rate, as there is a tendency for the rate to drop in the latter stages.

- 8.6 When all the sample has passed through the cartridge, disconnect the PTFE connecting tubes and draw air through the sample for 1hr±15 min (Note f)
- f) If the cartridge is not dry, water will appear in the sample. Blowing the cartridge dry with purified N₂ is more rapid although large amounts of N₂ are consumed.
- 8.7 Elute the cartridge with 3±0.1 ml of ethyl acetate (5.7) under gravity (Note g) collecting the eluate in a test tube (6.4) placed inside the manifold unit. When all the ethyl acetate has passed into the cartridge, carefully apply vacuum to allow the interstitial ethyl acetate to collect in the test tube.
- g) The flow rate is normally 0.5ml/min but should not exceed 1ml/min.
- 8.8 Release the vacuum and remove the tubes from the manifold unit. Evaporate the solvent to incipient dryness with a stream of purified nitrogen (5.2)
- 8.9 To the residue in the tube, add 1 ml ethereal diazomethane soln (5.13), swirl and allow to stand for 20±10 mins. Carefully evaporate the ether and excess reagent to incipient dryness.
- 8.10 Using a 1 microlitre syringe, add 1 ml hexane (5.21) and swirl. Transfer the extract to an auto-sampler vial ready for analysis (Note h)
- h) An internal std can be added at this stage.
- 8.11 Analysis - GC-MS determination
- 8.11.1 Set up the instrument in the EI/SIM mode in accordance with the manufacturer's instructions. Ensure by running methylated standards of the racemate that the D+ and L-forms of mecoprop can be separated. Suitable ions for this analysis are 142, 169, and 228 but other fragments may also be used for confirmation. (Note i)
- i) For the performance testing m/z=142 was used for quantification and 169 and 228 as qualifier ions.
- 8.11.2 Inject aliquots of blanks, spiked samples and sample extracts. Inject initially standards of both racemate and mecoprop P, but

mecoprop P will be used for quantification. Measure the response of the instrument corresponding to mecoprop P.

- 8.11.3 Plot a calibration line to establish the linear range of the method (Note j) j) The linearity will require rechecking at regular intervals and when any change is made to the method.
- 8.11.4 Include with batches of samples appropriate extracts for AQC purposes and such standards as are necessary to detect any instrument drift. A standard after every five samples is recommended or an internal standard used.
- 8.11.5 Check using the qualifying ions that the ratio of the fragments is correct and calculate the concentration of mecoprop P in the extracts.
- 8.12 Calculation
Concentration in extract (mg/L) =
Concentration in sample (ug/L)
(For a 1 litre sample concentrated to 1 ml)

TABLE 1**Distilled Water**

	<u>Spiking level (ug/L)</u>		
	<u>0.02</u>	<u>0.1</u>	<u>1.0</u>
Mean	0.0098	0.0583	0.685
Sw	0.0019 (7)	0.0088 (8)	0.0991 (9)
Sb	ns	ns	ns
St	0.0022 (12)	0.0119	0.131 (14)
% recovery	49	58	68
LOD	-	-	-

(Results in ug/L in the original sample. Figures in brackets are degrees of freedom)

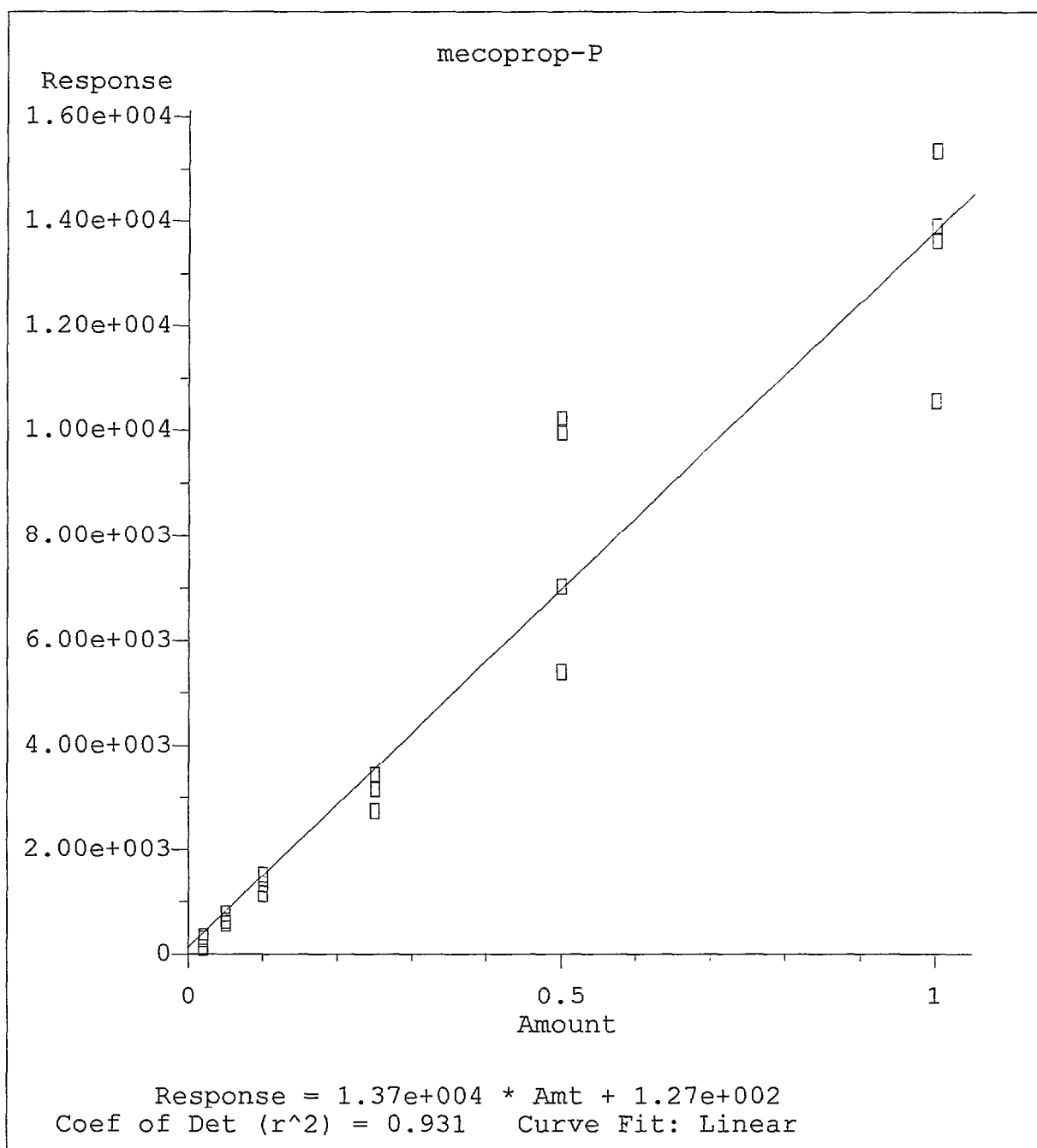
A set of 10 distilled water blanks run on a single occasion gave a mean of 0.001 ug/L with a standard deviation of 0.0005. This suggests that the minimum discernable quantity of mecoprop using the procedure is 0.002 ug/l. The limit of detection, which is a more practical measure of the capability of the method, was calculated by 4.65 Sw for 0.02ug/L spikes in distilled water. As the standard deviation of 0.02ug/L spikes is higher than that of the blank distilled water and was carried out on paired data on different days, then the figure quoted for LOD is likely to be artificially high.

TABLE 2**Tap Water**

	<u>Spiking Level (ug/L)</u>	
	<u>0.1</u>	<u>1.0</u>
Mean	0.0435	0.52
Sw	0.0095 (8)	0.117 (8)
Sb	ns	ns
St	0.0133 (11)	0.121 (15)
% Recovery	43.5	52

'10 tap water blanks gave a mean of <0.001 ug/L

Fig 1.



Method Name: C:\HPCHEM\1\METHODS\MECOPROP.M

Calibration Table Last Updated: Tue Feb 28 19:24:33 1995

FIG 2.

File : C:\HPCHEM\1\DATA\2-LD-2.D
Operator : Pam Walsh
Acquired : 26 Feb 95 1:22 am using AcqMethod MECOPROP
Instrument : Mountainh
Sample Name: DWI mecoprop data
Misc Info : method accreditation data
Vial Number: 10

