Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name:	Tralkoxydim
MRID # :	453020-02
Matrix:	Water
Analysis:	LC/MS

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STANDARD OPERATING PROCEDURE

RAM 334/01

RESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF TRALKOXYDIM IN ENVIRONMENTAL WATER SAMPLES.

Effective Date	:	**Review Date**	: **Annually**
Author	: **S L Hargreaves**	**Issuing Section**	: **Environmental Fate**
Reviewer	: **n/a**		
Authorised by	: _____	**R S I Joseph**	_____
		Environmental Fate	**Date**

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Summary of revisions to previous version

Version	Summary of Revisions
01	New SOP RAM - Not Applicable

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Appendices

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1. Introduction and Summary

1.1 Scope

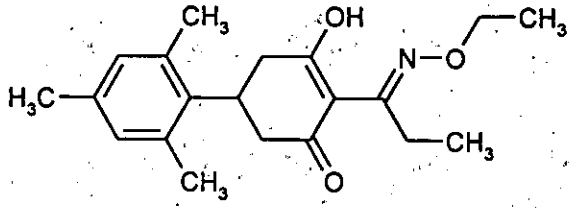
The analytical procedure described is suitable for the determination of residues of tralkoxydim (Figure 1) in environmental water samples using an external standardisation procedure. The limit of quantification has been set at 0.05 µg l^{-1}.

Figure 1 : : Tralkoxydim

IUPAC Name : 2-[1-(ethoxyimino)propyl]-3-hydroxy-5-mesitylcyclohex-2-enone

CAS Number : 87820-88-0

R Number : R162604



1.2 Method Summary

An analytical method has been developed and validated for the determination of tralkoxydim in environmental water samples. Water is passed through an Oasis® HLB solid phase extraction cartridge (SPE) on which residues of tralkoxydim are retained. The analyte is eluted using ethyl acetate. Final determination is by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (HPLC-MS) operating in single ion monitoring mode (SIM). The limit of quantification (LOQ) is 0.05 µg l^{-1}.

2. Materials

The recommended equipment and reagents are described in Appendices 1 and 2. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted provided that they can be shown to be suitable.

2.1 Apparatus

See Appendix 1 for a list of apparatus used during this method.

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2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method.

2.3 Preparation of Analytical Standards

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Weigh out accurately, using a five figure balance, sufficient tralkoxydim analytical standard to allow dilution in acetone to give a 1000 $\mu g\ ml^{-1}$ stock solution in a volumetric flask. This standard should then be diluted by serial dilution to 0.1 $\mu g\ ml^{-1}$ in acetone. These standards should be used for the fortification of the samples prior to extraction and HPLC-MS analysis.

When not in use, always store the standard solutions in a refrigerator at ≤7°C to prevent decomposition and/or concentration of the standard. Tralkoxydim analytical standards have been proven to be stable in acetone for four months when stored at ≤7°C. Analytical standards should be replaced with freshly prepared standards after this time.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate safety manual (e.g. ZENECA Laboratory Safety Manual) which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by S G Luxon, The Chemical Society, London (Ref. 1).

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2.5 Solvent Hazards

	Acetone	Ethyl acetate	Acetonitrile
Harmful Vapour	✓	✓	✓
Highly Flammable	✓	✓	✓
Harmful by Skin Absorption	×	×	✓
ZENECA Divisional Toxicity Class	4	4	3
OES Short Term (mg m^{-3})	3560	n/a	105
OES Long Term (mg m^{-3})	1780	1400	70

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

Tralkoxydim has been assigned to ZENECA Divisional Toxicity Classification 3. The toxicity classification scale rates highly toxic chemicals as class 1 and non toxic chemicals as class 5.

2.6 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples over the course of 1 day.

2.7 Work Stoppages

It is recommended that analysis is completed within a single day.

3. Analytical Procedure

3.1 Sample Collection

Water samples should be collected from a source using sampling techniques consistent with those employed for low level residue analysis. Ideally, samples should be collected directly into high density polyethylene bottles (500 ml volume or greater).

3.2 Sample Preparation Prior to Solid Phase Extraction Clean Up.

a) Accurately measure out 500 ml of each water sample to be analysed, using a measuring cylinder, and transfer to a suitable container (e.g. 500 ml round bottom flask).

Note: Should 500 ml water not be available, the stated LOQ of 0.05 µg l^{-1} may not be achievable .

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b) At least one untreated control and two control samples fortified with known amounts of tralkoxydim in acetone (not more than 0.5 ml) should be analysed with each batch of samples using the same procedure to enable verification of the method and recovery corrections to be made.

c) Filter samples through two glass fibre filters into a suitable container (e.g. 500 ml round bottom flask) to remove particulate matter and prevent blockage of SPE columns.

d) Immediately prior to loading samples onto the SPE columns, add glacial acetic acid (2.5 ml) to each sample and mix thoroughly. If less than 500 ml sample volume has been taken, adjust volume of acid accordingly e.g. add 0.5 ml of acetic acid to 100 ml water.

3.3 Sample Clean-up

a) Take one Oasis® HLB (200 mg, 6 ml) solid phase extraction cartridge for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add methanol (2 ml) and draw through under vacuum to the level of the top frit at a rate of approximately 2 ml min^{-1}, discarding the column eluate. Do not allow the cartridges to become dry. Add water (2 ml) to the top of each cartridge and draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluates. Do not allow the cartridges to become dry.

b) Attach a column reservoir to the top of each column using a suitable column connector.

c) Load samples from section 3.2 (d) onto the columns and draw through under low vacuum (approx. 200 mbar) at a rate of ~5 ml min^{-1}. Discard the column eluates. Tralkoxydim is retained on the column.

d) Remove column reservoirs and adaptors. Add ultra pure water (2 x 2 ml) to the top of each cartridge and draw through under vacuum to the level of the top frit at a rate of approximately 2 ml min^{-1}. Discard the wash. Remove any remaining droplets of water adhering to the inside of the cartridge with absorbent tissue. And dry the cartridges under high vacuum (≤500 mbar) for approximately 30 minutes.

Note : Where achievable vacuums are less than specified or apparatus does not allow sufficient air flow through the cartridges, longer drying times may be required.

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e) Add hexane to the top of each cartridge and draw through under vacuum to the level of the top frit at a rate of approximately 2 ml min^{-1}. Remove excess hexane under vacuum (≤500 mbar), discarding the wash.

f) Place collection tubes (10 ml) under each port, as required, in the manifold rack. Add ethyl acetate (2 ml) to the top of each cartridge and draw through under vacuum to the level of the top frit at a rate of approximately 2 ml min^{-1} to elute tralkoxydim.

g) Evaporate the samples to dryness in a heating block at 40°C under a stream of dry air and dissolve the residual material in acetonitrile (200 µl) with ultrasonication. Add ultra pure water (800 µl) and mix with ultrasonication. Transfer the samples to appropriate autosampler vials for analysis by HPLC-MS. The sample concentration factor is 500.

Note: The above SPE procedure has been developed using columns from the stated manufacturer, however, it is possible to carry out the procedure using similar columns from other manufacturers. In all cases it is strongly recommended that the elution profile is checked prior to commencing analysis. This will rule out any variation between manufacturers' products and between batches.

3.4 Preparation of Calibration Standards in the Presence of the Matrix

For HPLC-MS final determination it has been found that there are matrix enhancement and suppression effects. These were found to be approximately 30% enhancement for drinking water and sea water. River and ground water produced a suppression of approximately 75% and 40% respectively. To compensate for these effects a calibration standard in the presence of matrix can be prepared if deemed necessary. The decision to use a matrix standard is left to the discretion of the study director.

To prepare a matrix standard, take a second untreated control sample through the entire procedure as described in sections 3.2 and 3.3, up to point 3.3 (f). To prepare, for example, a 0.025 µg ml^{-1} matrix matched standard, add 25 µl of a 1 µg ml^{-1} tralkoxydim in acetone to the sample at point 3.3 (f). Evaporate to dryness under a stream of clean, dry air in a heating block at 40°C. Dissolve the residual material in 200 µl acetonitrile and ultrasonicate the solution thoroughly. Add ultra pure water (800 µl) and mix with ultrasonication. Transfer the samples to appropriate autosampler vials for analysis by HPLC-MS.

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4. Final Determination by HPLC-MS (SIM)

4.1 Equipment

The following conditions have been found to be suitable for analysis of tralkoxydim in water in this laboratory. Other instruments can be equally used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

Mass spectrometer	:	Hewlett Packard 1050 quaternary pump;
LC pump	:	Hewlett Packard 1050 automatic sampler;
Autosampler	:	Hewlett Packard 1050 automatic sampler;
Degasser	:	Hewlett Packard 1050 degasser;
Column oven	:	Hewlett Packard 1050 column compartment

Chromatography Conditions

Column	:	Spherisorb S3ODS1 50 x 3.2 mm
Column temperature	:	40°C
Mobile phase	:	Solvent A = Ultra pure water + 0.2% glacial acetic acid.
		Solvent B = Acetonitrile

Gradient conditions :

Time (min)	% A	% B
0.00	85	15
0.5	85	15
6.0	35	65
6.1	5	95
8.5	5	95
8.6	85	15
10.0	85	15

Stop Time : 10 minutes

Flow rate : 1.5 ml min^{-1}

Injection volume : 100 µl

Injection protocol : Analyse calibration standard after 3 to 4 sample injections.

Tralkoxydim retention time : 6.28 minutes

Mass Spectrometer Operating Conditions

Instrument : PE Sciex API III, triple quadrupole MS-MS

Ionisation mode : Ionspray (ESI) positive ion mode

Acquisition type : Single Ion Monitoring (SIM) : m/z 330 protonated molecular ion.

Protonated molecular ions m/z = 330 are generated in the ion source then monitored and used for quantitative analysis.

Typical chromatograms are shown in Appendix 4, Figures 2-17

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Should confirmation of residues be required by an alternative technique, samples may also be analysed by HPLC-MS-MS using the same instrument and chromatography conditions, but operating in Single Reaction Monitoring (SRM) mode.

Protonated molecular ions m/z 330 are generated in the ion source are selected and subjected to further fragmentation by collisional activation. The most abundant ion, m/z 138 in the resulting daughter spectrum is then monitored and used for quantitative analysis.

Typical chromatograms for HPLC-MS-MS (SRM) are shown in Appendix 4, Figures 18-21.

5. Calculation of Results

Tralkoxydim residues may be calculated in $\mu g\ l^{-1}$ for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing tralkoxydim at an appropriate concentration into the HPLC-MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak area obtained for tralkoxydim.

b) Make an injection of each sample solution and measure the peak heights or areas of the peaks corresponding to tralkoxydim.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the tralkoxydim residue in the sample, expressed as $\mu g\ l^{-1}$, using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}} \times 1000$$

PK area (SA) = Peak response for sample

PK area (STD) = Average peak response for bracketing standards

Standard Conc. = Concentration of tralkoxydim standard ($\mu g\ ml^{-1}$)

Sample Conc. = Sample concentration factor

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Example calculation of tralkoxydim recovery from drinking water:

PK area (STD) = (2645391 + 2824314) ÷ 2

= 2734852

PK area (SA) = Peak response for sample

= 1195863

Standard Conc. = 0.05 ($\mu g\ ml^{-1}$)

Sample Conc. = 500

$$\text{Residue} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}} \times 1000$$

$$\text{Residue} = \frac{1195863}{2734852} \times \frac{0.05}{500} \times 1000$$

Residue = 0.044 $\mu g\ l^{-1}$

To calculate the percentage recovery:

$$\%\ \text{Recovery} = \frac{\text{Residue } (\mu g\ l^{-1})}{\text{Fortification Level } (\mu g\ l^{-1})} \times 100$$

$$\%\ \text{Recovery} = \frac{0.044}{0.050} \times 100$$

% Recovery = 88%

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \quad (\mu g\ l^{-1})$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

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6. Control and Recovery Experiments

Control and external recovery experiments should be completed as section 3 for each set of samples analysed. Provided the recovery values are acceptable they may be used to correct any tralkoxydim residues found.

The levels of external recoveries should be decided by the residue levels expected. A minimum of one control and two external recovery experiments should be run alongside each set of samples analysed (that is untreated samples accurately fortified with a known amount of tralkoxydim prior to extraction) in the range of 0.05 - 0.5 $\mu g\ l^{-1}$.

Recovery data are generally considered acceptable when the mean values are between 70% and 110% and with a coefficient of variation of $\leq$20%.

7. Interference

7.1 Matrix

For HPLC-MS final determination it has been found that there are matrix enhancement and suppression effects. These were found to be approximately 30% enhancement for drinking water and sea water. River and ground water produced a suppression of approximately 75% and 40% respectively. To compensate for these effects a calibration standard in the presence of matrix can be prepared if deemed necessary. The decision to use a matrix standard is left to the discretion of the study director.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no reagent interference has been found.

7.3 Labware Interference

No labware interference has been observed. To eliminate the chance of any spot contamination it is recommended to thoroughly clean the solid phase extraction manifold and the sample concentration manifold before each use.

8. Method Validation

Method validation has been carried out on the procedures described in Section 3. The method validation data is shown in Appendix 3. Recovery of tralkoxydim through the analytical procedure was assessed by fortifying the water samples with tralkoxydim prior to filtering and loading samples onto Oasis® HLB SPE columns. Five recoveries were carried out at 0.05 μg l^{-1} and five recoveries at 0.5 μg l^{-1}. In addition, two controls were analysed for each water type.

8.1 Limit of Quantification and Limit of Detection

8.1.1 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70 - 110% with a c.v. of ≤ 20% has been obtained.

Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The LOQ has been set at 0.05 μg l^{-1} for HPLC-MS (SIM) determination.

8.1.2 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as four times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The LOD of this method of tralkoxydim in each water type tested is as follows:

0.002 μg l^{-1} for tralkoxydim in ground water
0.005 μg l^{-1} for tralkoxydim in drinking water
0.01 μg l^{-1} for tralkoxydim in sea water
0.02 μg l^{-1} for tralkoxydim in river water

8.2 Detector Linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of detector responses. Detector linearity graphs are given in Appendix 5.

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The linearity of the HPLC-MS detector response for tralkoxydim standard in the presence of matrix was tested in the range from 0-1.0 μg ml^{-1} concentration, equivalent to 0- 100 ng injected on column, and was found to be linear.

Standards were injected in triplicate and the mean response plotted against amount injected, using Excel Chartwizard version 7.0. The intercept was set to zero and a linear trendline fit applied (Ref.1). The data was also plotted without forcing the line through zero. The two plots were compared by application of the F-test using Microsoft Excel version 7.0. This gives a P-value of 0.73. A P-value of >0.05 is considered to show that the two response curves are statistically similar. It is therefore considered acceptable to use single point calibrations for residue calculations.

If residues beyond the tested concentration range are expected, dilute the extract appropriately to bring it within the tested linear range prior to quantification.

In these laboratories the linearity of HPLC-MS detector response for tralkoxydim standard in matrix was tested in the range from 0-1.0 μg ml^{-1} concentration and was found to be linear. If residues beyond the tested concentration range are expected, dilute the extract appropriately to bring it within the tested linear range prior to quantification.

8.3 Limitations

The method has been tested on representative water types. It can be reasonably assumed that the method can be applied for other water types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method for those water types.

9. Conclusions

The method described is suitable for the analysis of tralkoxydim residues in the ground water, drinking water, river water and sea water. Only commercially available laboratory equipment and reagents are required. The analysis of a batch of twenty samples can be completed by one person in 1 day (8 working hour periods). Untreated and fortified samples should be extracted and analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification has been set at 0.05 μg l^{-1} with final analysis by HPLC-MS (SIM).

REFERENCE COPY

10. References

1. Cardone M J, Palermo P J and Sybrandt L B : Potential error in single point ratio calculations based on linear calibration curves with a significant intercept. Anal Chem., 52 pp 1187-1191, 1980.

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Appendices

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Appendix 1 : Apparatus

UK Suppliers

Measuring cylinders, 500 ml size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Whatman Glass microfibre filters GF/C size 90 mm, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Buchner porcelain funnel, size 96 mm diameter, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Filtercones for Buchner funnel, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Quickfit adaptor with 'T'-connection, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Round Bottomed flasks, 500 ml size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Isolute Vacmaster-20™ sample processing station, available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK.

Oasis® HLB 6 ml 200 mg size solid phase extraction columns available from Waters Ltd., The Boulevard, Blackmoor Lane, Croxley Green, Watford, Herts, WD8 8YW

70 ml column reservoirs part numbers 120-1009-F available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK.

Column connection adapters, available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK.

Disposable borosilicate glass test tubes 10 ml size, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Techne Dri-block 3D heating block, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. Part number BLD-750-010Y.

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

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Crimp cap autosampler vials and caps available from Hewlett Packard Ltd., Cain Road, Bracknell, Berkshire RG12 1HN, UK.

PE Sciex AP III triple quadrupole mass spectrometer equipped with interchangeable ionspray ISP and atmospheric pressure chemical ionisation (ACPI, heated nebuliser) sources, available from Perkin Elmer Ltd, Beaconsfield Buckinghamshire, UK

Reversed phase HPLC column Spherisorb S3ODS available from Jones Chromatography Ltd., Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan CF8 8AU, UK.

US Suppliers

Measuring cylinders, 500 ml size, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Whatman Glass microfibre filters GF/C size 90 mm, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Buchner porcelain funnel, size 96 mm diameter, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Filtercones for Buchner funnel, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Quickfit adaptor with 'T'-connection, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Round Bottomed flasks, 500 ml size, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Isolute Vacmaster-20™ sample processing station, available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329.

Oasis® HLB 6 ml 200 mg size solid phase extraction columns available from Waters Corporation, 34 Maple Street, Milford, Massachusetts 01757.

70 ml column reservoirs part numbers 120-1009-F available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329.

Column connection adapters, available from Jones Chromatography USA Ltd., PO Box 280 329, Lakewood, Colorado, 8022-0329.

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Disposable borosilicate glass test tubes 10 ml size, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Techne Dri-block 3D heating block, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Crimp cap autosampler vials and caps available from Hewlett Packard Co., PO Box 1000, Avondale, PA 19311-1000.

PE Sciex AP III triple quadrupole mass spectrometer equipped with interchangeable ionspray ISP and atmospheric pressure chemical ionisation (ACPI, heated nebuliser) sources, available from Perkin Elmer Ltd., 761 Main Avenue, Norwalk, CT 06859, USA.

Reversed phase HPLC column Spherisorb S3ODS1 available from Jones Chromatography USA Ltd, PO Box 280 329, Lakewood, Colorado, 8022-0329.

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Appendix 2 : Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used.

UK Suppliers

Glacial acetic acid, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Hexane, ethyl acetate and acetonitrile, available from Romil Ltd., The Source, Convent Drive, Waterbeach, Cambridge CB5 9QT, UK.

Ultra pure water from a laboratory water purification system e.g. Elga Maxima available from Elga Ltd., High Street, Lane End, High Wycombe, Bucks HP14 3JH, UK.

US Suppliers

Glacial acetic acid, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.
Hexane, ethyl acetate and acetonitrile, available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA (Tel: 312-689-8410).

Ultra-pure water from a laboratory water purification system available from e.g.
Waters Corporation, Milford, MA, USA.

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Appendix 3 : Method Validation Data

Table 1. : Recovery Data Obtained During Method Validation

Matrix	Fortification Level ($\mu g\ l^{-1}$)	Recovery (%)	Mean (%)	RSD (%)	Range
River Water	control	ND**			
	0.05*	106, 100, 91, 102, 94	99	6.1	91-106
	0.5	100, 112, 93, 99, 104	102	7.0	93-112
Sea Water	control	ND**			
	0.05*	105, 96, 82, 94, 93	94	8.2	82-105
	0.5	91, 94, 88, 77, 78	86	7.7	77-94
Groundwater	control	ND**			
	0.05*	101, 104, 96, 101, 97	100	3.3	96-104
	0.5	90, 90, 91, 91, 113	95	10.0	90-113
Drinking Water	control	ND**			
	0.05*	87, 91, 92, 95, 95	92	3.3	87-95
	0.5	78, 77, 75, 73, 66	74	4.8	66-78

*Limit of quantification, defined by the lowest validated fortification level
ND** No residues detected in the control samples above 30% LOQ

Table 2 : Determination of HPLC-MS Matrix Effects

Matrix Type	Matrix Enhancement
Drinking water	30%
Sea Water	30%

Matrix Type	Matrix Suppression
Ground water	40%
River Water	75%

To compensate for these effects a calibration standard in the presence of matrix can be prepared if deemed necessary. The decision to use a matrix matched standard is left to the discretion of the study director.

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Appendix 4 : Representative Chromatograms

Figure 2 : Tralkoxydim Ground Water Matrix Standard, 0.05 μg ml^{-1}. Sample Concentration Factor 500.(SIM)

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10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 3437799
Height 1069980
Start Time 6.15
End Time 6.39
Integration Width 0.25
Retention Time 6.28
Integration Type A - VB

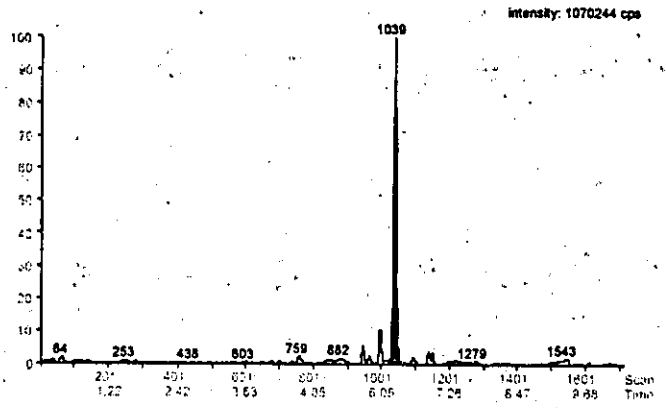

Figure 3 : Untreated Ground Water Sample, Sample Concentration Factor 500. (SIM)

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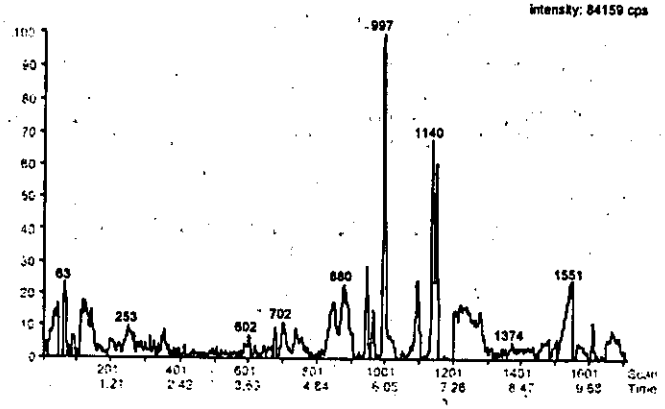
10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.38 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 0
Height 0
Start Time 0.00
End Time 0.00
Integration Width 0.00
Retention Time 0.00
Integration Type M



REFERENCE COPY

Figure 4 : Untreated Ground Water Sample Fortified at 0.05 µg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 90%. (SIM)

MacQuan, version 1.8
Printed: Thu, Mar 16, 2000 12:14
Calibration File: CalibFile Path: Sciex Data 2000:00:201-250:00222 PP604 SH 25/2/00:
Comments No comments

Page 1 of 1

00/222 - 003 J5765/32C Mon, Feb 28, 2000 19:11
J5765/32

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.38 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.30
Area 763445
Height 168578
Start Time 6.14
End Time 6.39
Integration Width 0.25
Retention Time 6.29
Integration Type A - VB

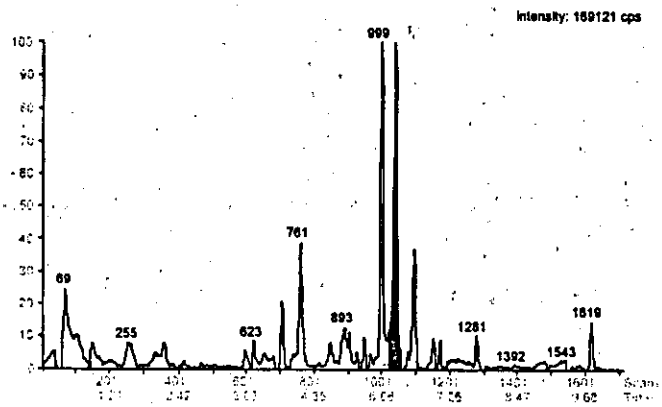


Figure 5 : Untreated Ground Water Sample Fortified at 0.5 µg l^{-1} with Tralkoxydim. Sample Concentration Factor 100. Recovery = 90%. (SIM)

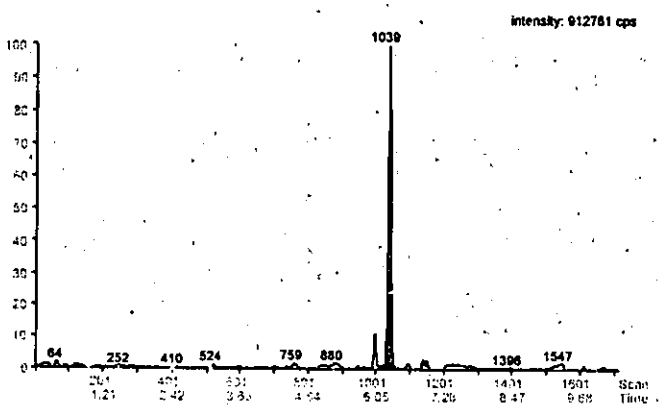
MacQuan, version 1.8
Printed: Thu, Mar 16, 2000 12:26
Calibration File: CalibFile Path: Sciex Data 2000:00:267 804 sh 9/3/00:
Comments No comments

Page 1 of 1

00/267-003 J5765/44C Thu, Mar 9, 2000 18:17
J5765/44

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.38 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 2908740
Height 912489
Start Time 6.17
End Time 6.39
Integration Width 0.21
Retention Time 6.28
Integration Type A - VB



REFERENCE COPY

Figure 6 : Tralkoxydim Drinking Water Matrix Standard, 0.05 µg ml^{-1}. Sample Concentration Factor 500. (SIM)

MacQuan, version 1.6
Printed: Thu, Mar 16, 2000 12:18
Calibration File: CalibFile Path: Solex Data 2000.00/201-250.00/227 PP804 SH 28/2/00:
Comments: No comments

Page 1 of 1

00/227 - 001 J5765/34A Mon, Feb 28, 2000 16:54
J5765/34

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.30
Area 2645391
Height 806067
Start Time 6.17
End Time 6.40
Integration Width 0.23
Retention Time 6.30
Integration Type A - VB

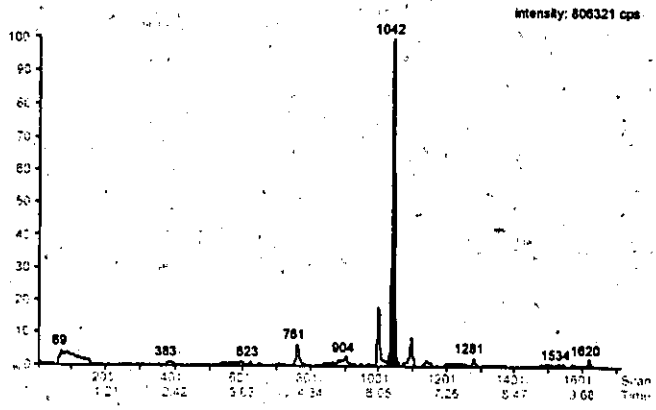


Figure 7 : Untreated Drinking Water Sample, Sample Concentration Factor 500. (SIM)

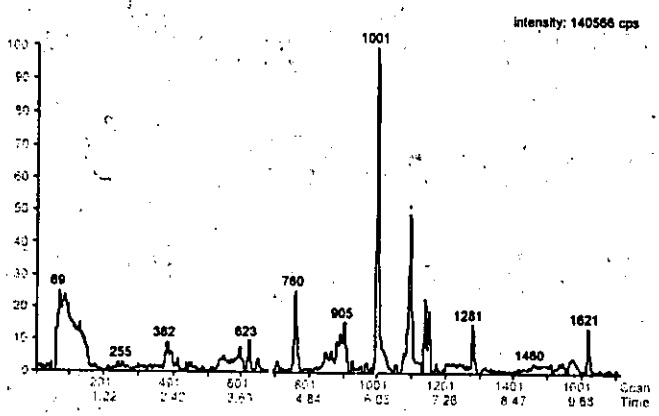
MacQuan, version 1.6
Printed: Thu, Mar 16, 2000 12:16
Calibration File: CalibFile Path: Solex Data 2000.00/201-250.00/227 PP804 SH 28/2/00:
Comments: No comments

Page 1 of 1

00/227 - 002 J5765/34B Mon, Feb 28, 2000 17:06
J5765/34

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.35 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.30
Area 0
Height 0
Start Time 0.00
End Time 0.00
Integration Width 0.00
Retention Time 0.00
Integration Type M



REFERENCE COPY

Figure 8 : Untreated Drinking Water Sample Fortified at 0.05 µg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 91% (SIM)

MacQuan, version 1.6
Printed: Thu, Mar 16, 2000 12:17
Calibration File: CalibFile Path: Solex Data 2000:00:201-250:00:227 PP604 SH 29/2/00
Comments: No comments

Page 1 of 1

00/227 - 004 J5765/34D Mon, Feb 28, 2000 17:31
J5765/34

10.53 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.52 Q1 Scan, 1714 scans
TIC

Noise Thres.	5.0
Quant Thres.	1.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	2
Expected RT	6.30
Area	1246910
Height	392976
Start Time	6.25
End Time	6.44
Integration Width	0.19
Retention Time	6.30
Integration Type	M

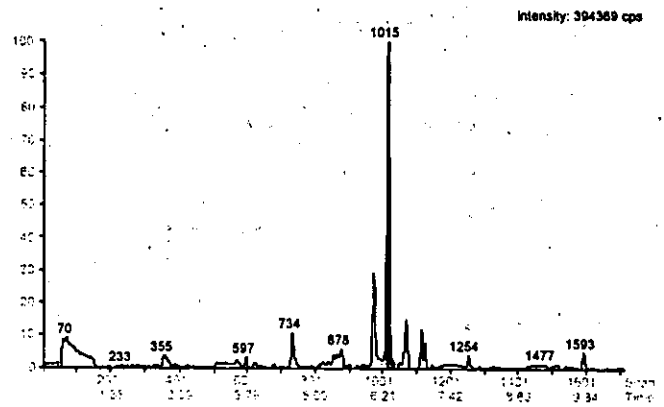


Figure 9 : Untreated Drinking Water Sample Fortified at 0.5 µg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 105%. (SIM)

MacQuan, version 1.6
Printed: Thu, Mar 16, 2000 12:18
Calibration File: CalibFile Path: Solex Data 2000:00:201-250:00:231 PP604 SH 29/2/00
Comments: No comments

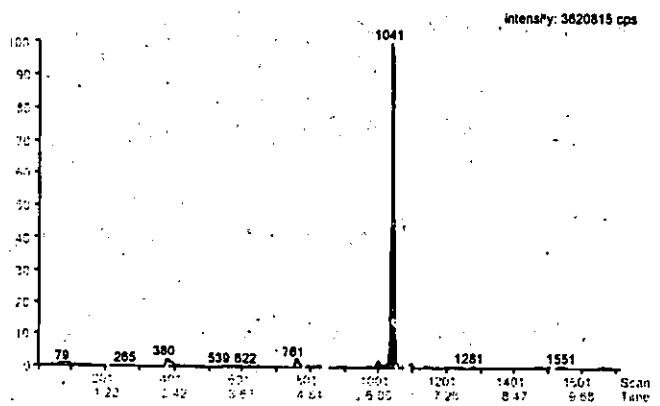
Page 1 of 1

00/231 - 003 J5765/35C Tue, Feb 29, 2000 19:04
J5765/35

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC

Noise Thres.	5.0
Quant Thres.	1.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	2
Expected RT	6.29
Area	11762589
Height	3620659
Start Time	6.11
End Time	6.41
Integration Width	0.30
Retention Time	6.29
Integration Type	A - VB



REFERENCE COPY

Figure 10 : Tralkoxydim Sea Water Matrix Standard, 0.1 μg ml^{-1}. Sample Concentration Factor 500. (SIM)

MacQuan, version 1.8 — Page 1 of 1
Printed: Thu, Mar 16, 2000 12:29
Calibration File: CalibFile Path: Scex Data 2000:00/268 804 sh 9/3/00:
Comments: No comments

00/268-005 J5765/46A Thu, Mar 9, 2000 21:36
J5765/46

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 5326644
Height 1649224
Start Time 6.10
End Time 6.39
Integration Width 0.28
Retention Time 6.28
Integration Type A - VB

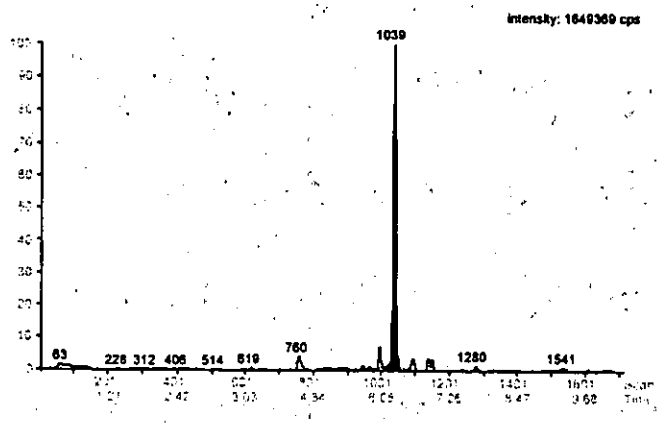


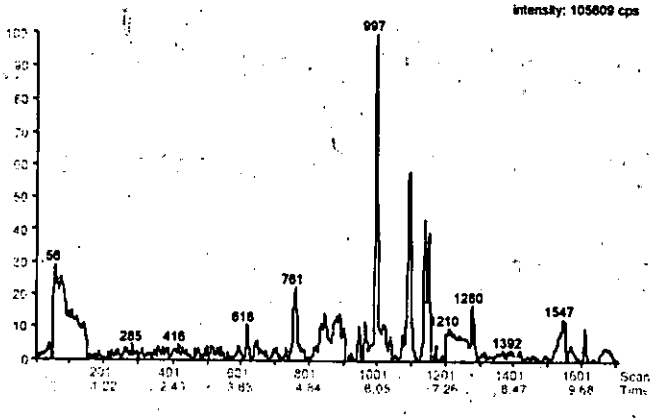
Figure 11 : Untreated Sea Water Sample, Sample Concentration Factor 500. (SIM)

MacQuan, version 1.8 — Page 1 of 1
Printed: Thu, Mar 16, 2000 12:27
Calibration File: CalibFile Path: Scex Data 2000:00/268 804 sh 9/3/00:
Comments: No comments

00/268-002 J5765/44B Thu, Mar 9, 2000 20:59
J5765/46

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 0
Height 0
Start Time 0.00
End Time 0.00
Integration Width 0.00
Retention Time 0.00
Integration Type M



REFERENCE COPY

Figure 12 : Untreated Sea Water Sample Fortified at 0.05 µg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 105%. (SIM).

MacQuan, version 1.6 Page 1 of 1
Printed: Thu, Mar 16, 2000 12:28
Calibration File: CalibFile Path: Sciex Data 2000:00/268 604 sh 9/3/00:
Comments: No comments

00/268-003 J5765/46C Thu, Mar 9, 2000 21:11
J5765/46

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 1458610
Height 466131
Start Time 6.21
End Time 6.39
Integration Width 0.18
Retention Time 6.28
Integration Type A - VB

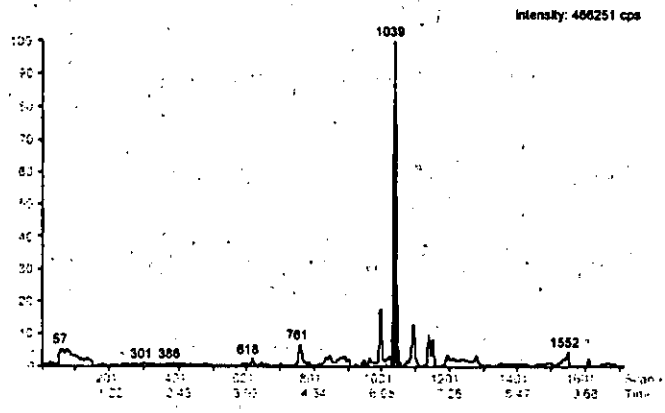


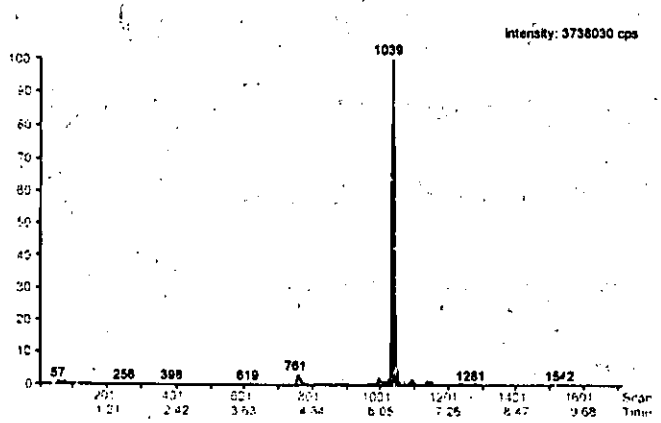
Figure 13 : Untreated Sea Water Sample Fortified at 0.5 µg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 78%. (SIM).

MacQuan, version 1.6 Page 1 of 1
Printed: Thu, Mar 16, 2000 12:29
Calibration File: CalibFile Path: Sciex Data 2000:00/268 604 sh 9/3/00:
Comments: No comments

00/268-006 J5765/46E Thu, Mar 9, 2000 21:49
J5765/46

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.36 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.28
Area 12154475
Height 3737881
Start Time 6.09
End Time 6.40
Integration Width 0.31
Retention Time 6.28
Integration Type A - VB



REFERENCE COPY

Figure 14 : Tralkoxydim River Water Matrix Standard, 0.1 μg ml^{-1}. Sample Concentration Factor 500. (SIM).

MacQuan, version 1.6 — Page 1 of 1
Printed: Thu, Mar 16, 2000 12:22
Calibration File: CalibFile Path: Scan Data 2000:00:255 604 sh 6/3/00:
Comments: No comments

00/255-005 J5765/40A Mon, Mar 6, 2000 18:29
J5765/40

10.38 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.37 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.29
Area 3528285
Height 1062898
Start Time 6.25
End Time 6.40
Integration Width 0.15
Retention Time 6.28
Integration Type M

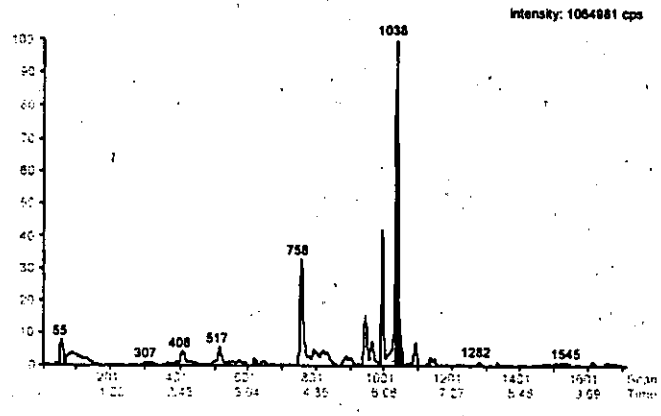


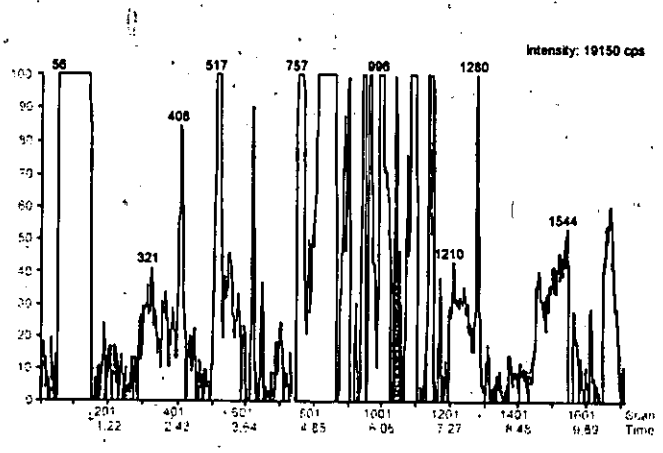
Figure 15 : Untreated River Water Sample , Sample Concentration Factor 500. (SIM).

MacQuan, version 1.6 — Page 1 of 1
Printed: Thu, Mar 16, 2000 12:20
Calibration File: CalibFile Path: Scan Data 2000:00:255 604 sh 6/3/00:
Comments: No comments

00/255-002 J5765/40B Mon, Mar 6, 2000 17:51
J5765/40

10.37 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 10.37 Q1 Scan, 1714 scans
TIC
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.29
Area 84050
Height 19077
Start Time 6.25
End Time 6.46
Integration Width 0.21
Retention Time 6.30
Integration Type M



REFERENCE COPY

Figure 16 : Untreated River Water Sample Fortified at 0.05 μg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 106%. (SIM).

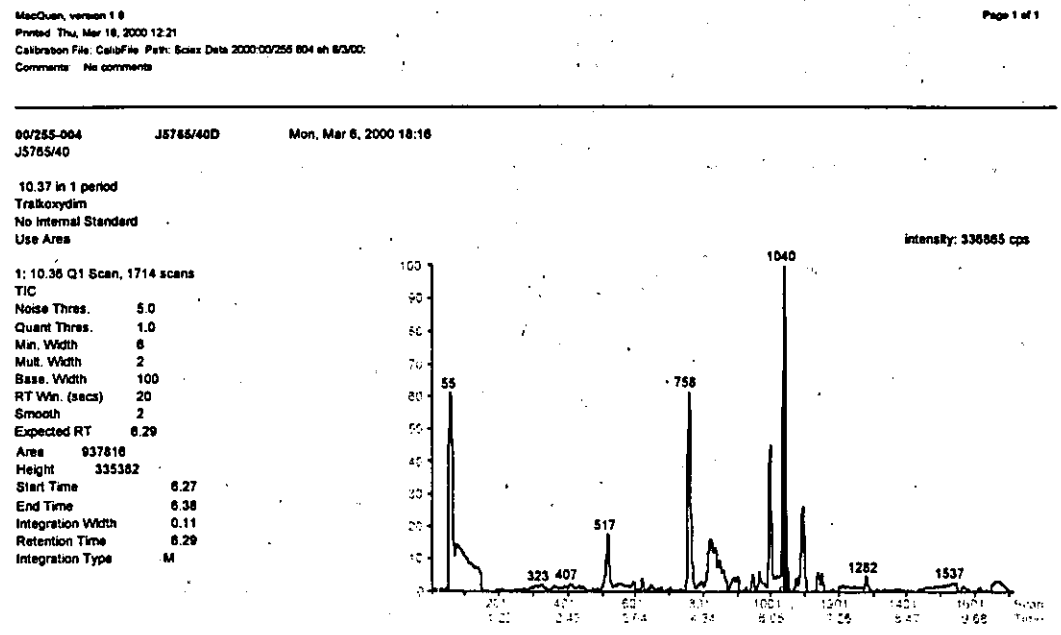
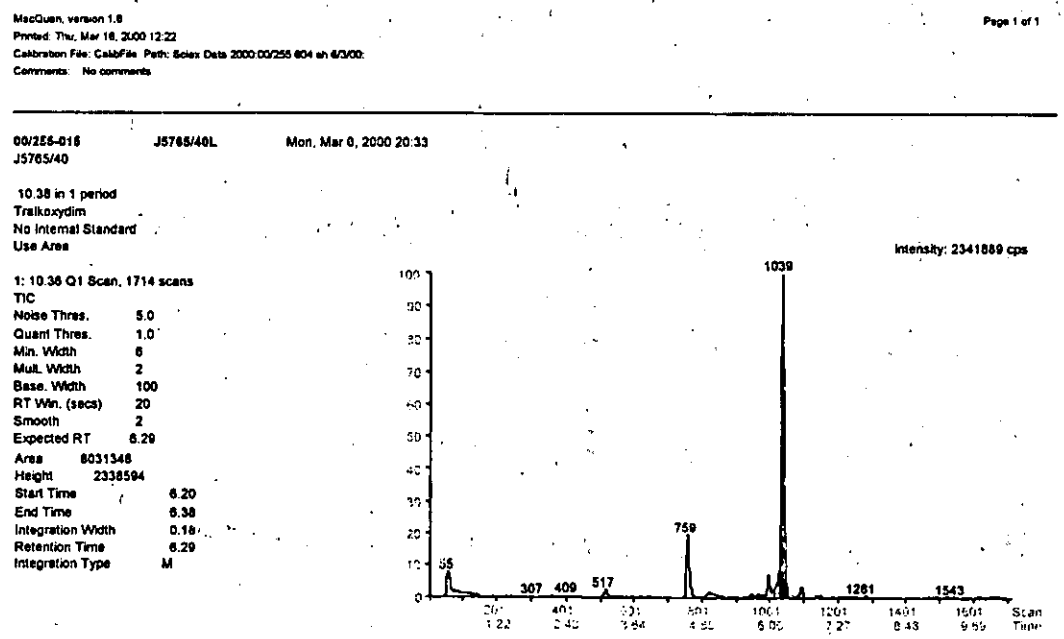


Figure 17 : Untreated River Water Sample Fortified at 0.5 μg l^{-1} with Tralkoxydim. Sample Concentration Factor 500. Recovery = 99%. (SIM).



REFERENCE COPY

Figure 18 : Tralkoxydim River Water Matrix Standard, 0.05 μg ml^{-1}. Sample Concentration Factor 100. (SRM).

MacQuan, version 1.6
Printed: Thu, Mar 16, 2000 12:11
Calibration File: CalibFileTRALKOXYDIM Path: Soex Data 2000:00/051-100:00/078 PP804 SH 25/1/00:
Comments: No comments

Page 1 of 1

00/078 -001 J5765/13A Tue, Jan 25, 2000 21:14
J5765/13B

10.00 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 9.98 MRM, 1169 scans
330.0->136.0
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.37
Area 2422
Height 601
Start Time 6.11
End Time 6.38
Integration Width 0.27
Retention Time 6.27
Integration Type M

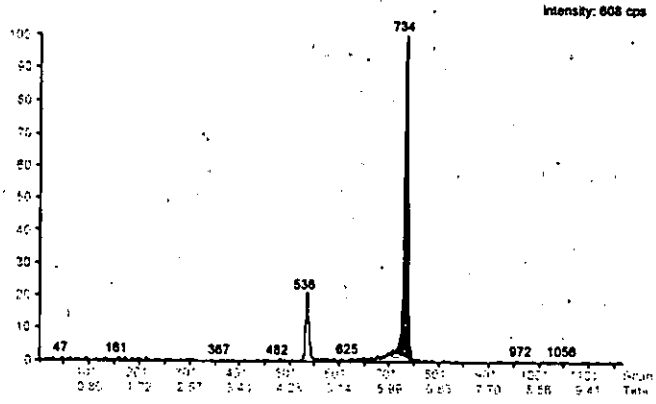


Figure 19 : Untreated River Water Sample , Sample Concentration Factor 100. (SRM).

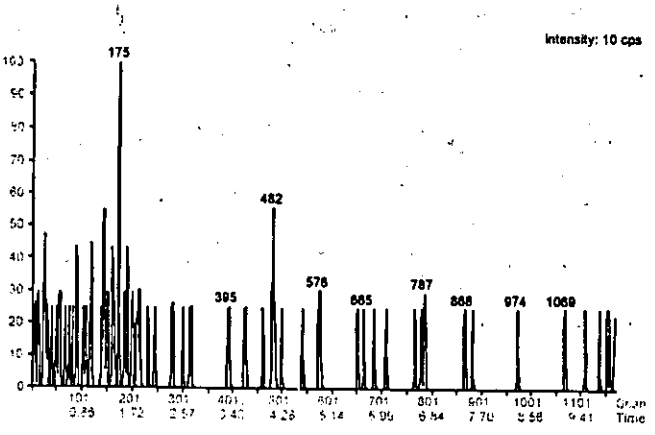
MacQuan, version 1.6
Printed: Thu, Mar 16, 2000 12:12
Calibration File: CalibFileTRALKOXYDIM Path: Soex Data 2000:00/051-100:00/078 PP804 SH 25/1/00:
Comments: No comments

Page 1 of 1

00/078 -002 J5765/13B Tue, Jan 25, 2000 21:26
J5765/13B

10.00 in 1 period
Tralkoxydim
No Internal Standard
Use Area

1: 9.99 MRM, 1170 scans
330.0->136.0
Noise Thres. 5.0
Quant Thres. 1.0
Min. Width 6
Mult. Width 2
Base. Width 100
RT Win. (secs) 20
Smooth 2
Expected RT 6.37
Area 0
Height 0
Start Time 0.00
End Time 0.00
Integration Width 0.00
Retention Time 0.00
Integration Type



REFERENCE COPY

Figure 20 : Untreated River Water Sample Fortified at 0.05 μg l^{-1} with Tralkoxydim. Sample Concentration Factor 100. Recovery = 74%. (SRM).

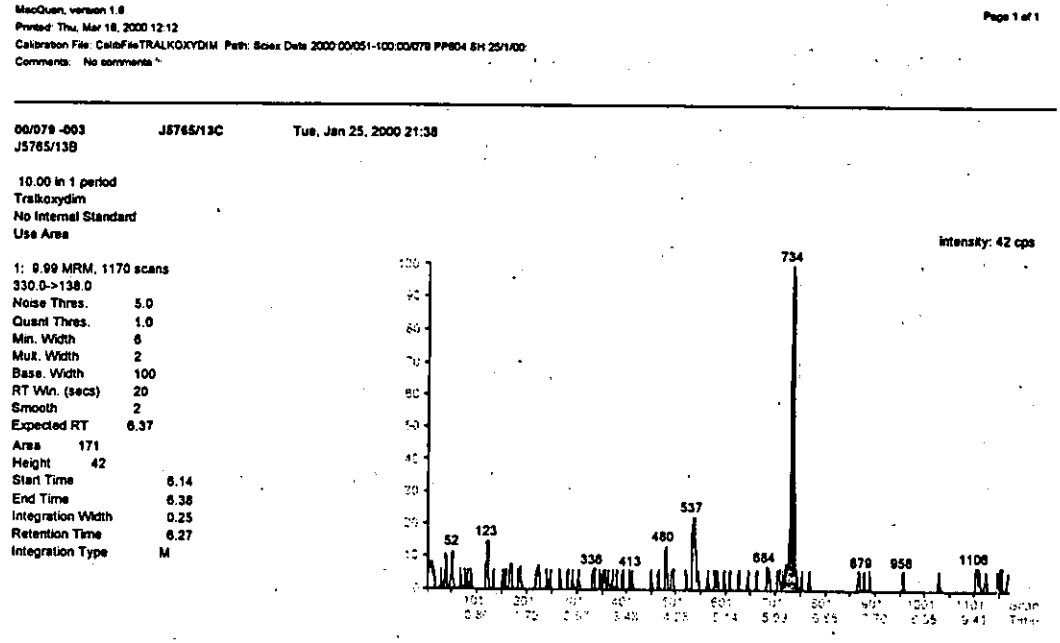
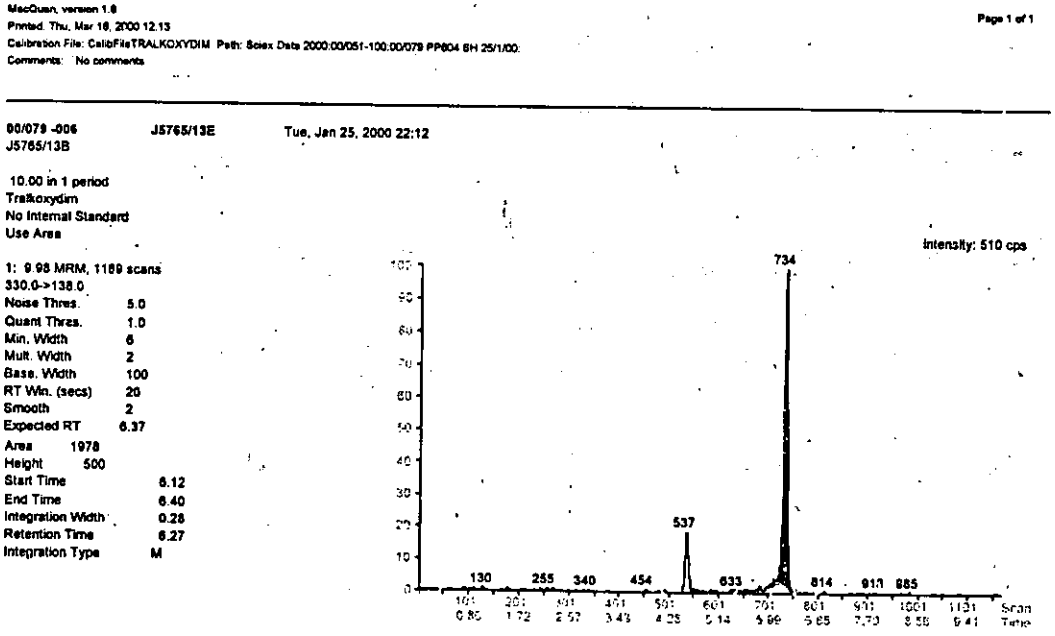


Figure 21 : Untreated River Water Sample Fortified at 0.5 μg l^{-1} with Tralkoxydim. Sample Concentration Factor 100. Recovery = 92%. (SRM).



REFERENCE COPY

Appendix 5 : Detector Linearity Graphs

Figure 22 : HPLC-MS (SIM) Detector Calibration Graph for Tralkoxydim. Plot Not Forced Through Zero.

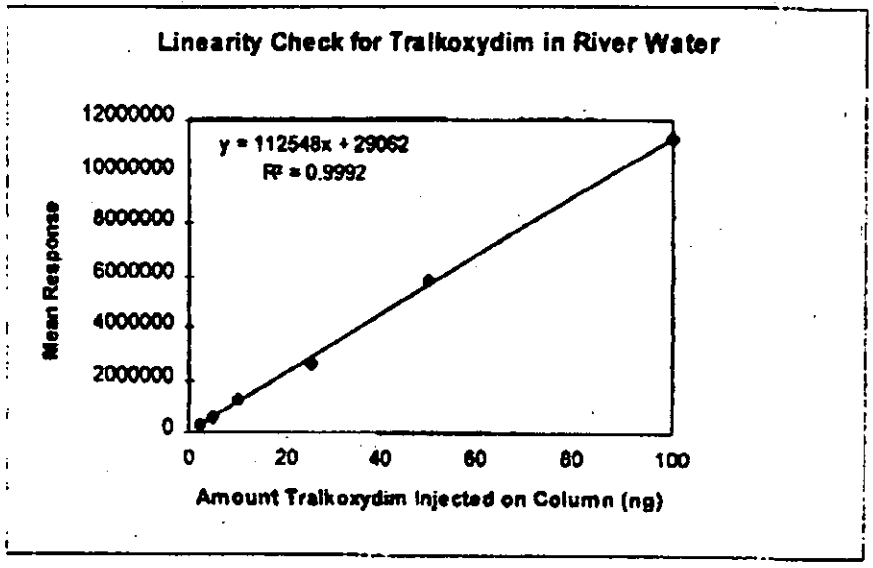
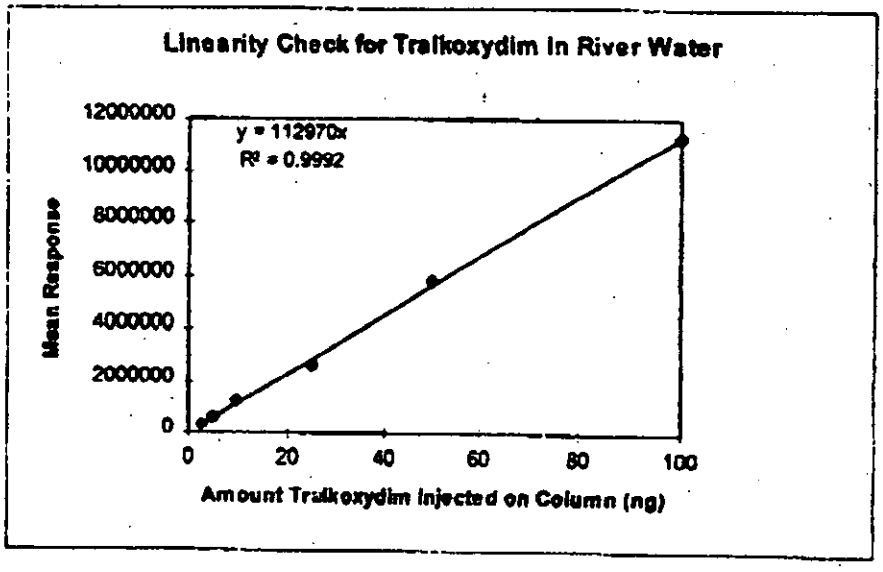


Figure 23 : HPLC-MS (SIM) Detector Calibration Graph for Tralkoxydim. Plot Forced Through Zero.



REFERENCE COPY