Study Number : 93JH010

Report Title : ICIA5504 : Validation of a Method for the Determination of Residues in Drinking Water

SUMMARY

A series of procedural recovery experiments were carried out in which drinking water samples were fortified at various levels with ICIA5504. The samples were analyzed using the method described in notebook D7837/158.

The limit of determination was 0.1 μg litre^{-1}.

Fortification levels used ranged from 0.1 μg litre^{-1} to 2.0 μg litre^{-1} and satisfactory recovery levels (70 - 130%) were obtained for all of the analyses with the exception of one. This particular recovery, at 0.1 μg litre^{-1}, could not be quantified since the ICIA5504 peak was obscured by a contamination peak.

The detector response was linear in the range of 0.01 to 0.5 μg cm^{-3} for ICIA5504 when analyzed by high performance liquid chromatography using ultra violet detection.

Therefore, it was concluded that method D7837/158, (to be issued as SOP RAM/235/01, see Appendix A) was successfully validated for the analysis of ICIA5504 residues in drinking water using external standardisation.

1 INTRODUCTION

ICIA5504 is an ICI proprietary highly active fungicide providing a broad spectrum of disease control.

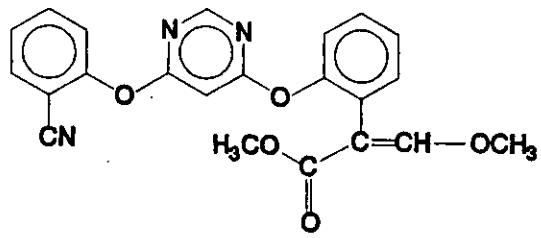

Figure 1: Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-3-methoxyacrylate (IUPAC).

A method was developed to determine residues of ICIA5504 in drinking water (ref: D book 7837/158). It utilises Empore™ extraction disks to adsorb the compound followed by quantitative determination of ICIA5504 residues using high performance liquid chromatography with ultra violet detection (HPLC-UV).

This report describes the analytical procedure and presents ICIA5504 validation data obtained when using the method described for the analysis of drinking water samples. This study was carried out between 3rd February and 12th May 1993 at ICI Agrochemicals' Residue Chemistry Section, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK.

2 MATERIALS AND METHODS

2.1 Test Material

ICIA5504 analytical standard ref: ASJ10008-01S with a purity of 99% was used for the method validation. It was obtained from ICI Agrochemicals' Chemical Development and Characterisation Section, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK. Standard solutions in acetone were prepared and 0.1 or 1.0 $\mu g\ cm^{-3}$ concentrations used for fortification of the samples. The stability of ICIA5504 in acetone has been investigated under study 93JH005 (not yet reported) and showed that it is stable up to six months.

2.2 Test System

The samples used for this study were collected from the tap water available at Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY. The samples were collected between 3rd February - 12th May 1993 and were analyzed immediately on the dates of sampling.

2.3 Analytical Procedure

The analytical procedure was validated by fortifying untreated drinking water samples with ICIA5504. The samples were then subjected to the full analytical procedure (Appendix A) and were analyzed by high performance liquid chromatography.

The results for the analyses were then evaluated to check that the ICIA5504 recoveries were within acceptable ranges (70 - 130%) and that the required limit of determination could be achieved.

The detector linearity of ICIA5504 was also confirmed by plotting a graph of detector response versus fortification level.

Summary of samples analyzed :

Control	5 replicates
Recovery 0.1 μg litre^{-1}	4 replicates
Recovery 0.2 μg litre^{-1}	4 replicates
Recovery 0.5 μg litre^{-1}	4 replicates
Recovery 1.0 μg litre^{-1}	4 replicates
Recovery 2.0 μg litre^{-1}	4 replicates

2.3.1 Precision

In addition, the precision of the analytical method was demonstrated by investigating reproducibility and repeatability. The reproducibility of the method was demonstrated by analysing water samples fortified at the limit of determination. This was carried out by two analysts, one of whom was unfamiliar with the procedure. The repeatability of the method was demonstrated by similarly analysing water samples fortified at the limit of determination. These samples were obtained from the same source and analyzed by the same operator, using the same apparatus, laboratory and within short intervals of time.

2.3.2 Extraction

Samples of tap water (200 cm^3) were measured out into round bottomed flasks and fortified with known amounts of ICIA5504 using a glass pipette. The Empore^{tm} extraction disks were placed in Millipore all-glass filter units. The disks were washed with ethyl acetate (10 cm^3) and allowed to dry, followed by methanol (10 cm^3). The disks were not allowed to dry at this stage, leaving a meniscus of methanol above the disks. The water samples (200 cm^3) were then filtered under vacuum through the disks into the base flasks, followed by clean dry air for five minutes to dry the disks.

Collection tubes were inserted into the base flasks and ICIA5504 residues were eluted with ethyl acetate (10 cm^3) drawing half the solvent through the disk and allowing to stand for approximately 1 minute before drawing the remainder through the disk. This was then repeated with a second 10 cm^3 of elution solvent. **Note**: Sodium sulphate was added to each collection tube before elution, to remove any remaining moisture before transferring the sample to round bottomed flasks (100 cm^3).

The eluates were evaporated to dryness at $\leq$30°C using a rotary evaporator and re-dissolved in HPLC mobile phase (2 cm^3) prior to analysis.

2.3.3 Analysis by HPLC - UV

The conditions for the analysis by HPLC will depend upon the equipment available. The following conditions were found to be satisfactory for the quantitative determination of ICIA5504 using a Waters 590 pump coupled to a Waters Intelligent Sample Processor (WISP) with a Waters 484 UV detector.

(i)	Column	:	15 cm x 4.6 mm internal diameter
(ii)	Column Packing	:	Hichrom Spherisorb 5 ODS2
(iii)	Mobile Phase	:	50:50/acetonitrile:water
(iv)	Flow Rate	:	1.5 cm^3 min^{-1}
(v)	Wavelength	:	220 nm

Under these conditions, the retention time of ICIA5504 was approximately five minutes.

1 SCOPE

The analytical procedures described are suitable for the determination of residues of the fungicide ICIA5504, Figure 1, in drinking water.

To date, in these laboratories, the method has been applied to tap water samples and the limit of determination of the method is 0.1 μg litre^{-1}.

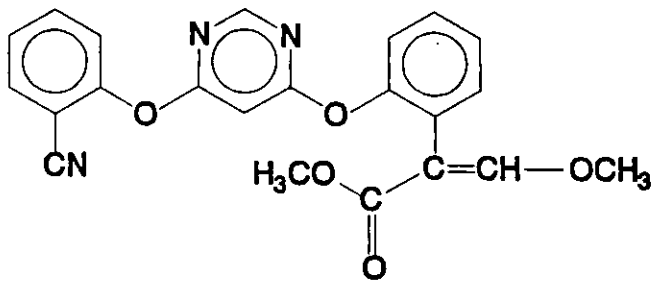

Figure 1: Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate (IUPAC).

2 SUMMARY

ICIA5504 is extracted by adsorption onto Empore™ extraction disks. After elution, the organic extract is evaporated to dryness and dissolved in mobile phase. Final quantitative determination is by high performance liquid chromatography using ultra-violet detection.

3 PROCEDURE

3.1 Extraction

a) Place an Empore™ filter disk in a Millipore all-glass filter unit.

b) Fortify an untreated water sample (200 cm^3) with an accurately known amount of ICIA5504 as a recovery check.

c) Wash the disk and filter unit with ethyl acetate (10 cm^3), allowing the disk to dry, followed by methanol (10 cm^3). Do not allow the disk to dry out at this stage, leaving a meniscus of methanol above the disk.

d) Filter the water sample (200 cm^3) under vacuum through the disk into the base flask.

e) Pass through clean dry air for five minutes to dry the disk.

f) Add sodium sulphate (~ 1g) to the collection tube before elution to remove any remaining moisture. Insert the collection tube into the filter unit and elute ICIA5504 with ethyl acetate (10 cm^3) drawing half the solvent through the disk and allowing to stand for approximately 1 minute before drawing the remainder through the disk. Repeat with a second 10 cm^3 of elution solvent.

g) Transfer the sample to a round bottomed flask and evaporate to dryness at $\leq 30°C$.

h) Re-dissolve the residue in mobile phase (2 cm^3) to give a 100 $cm^3\ cm^{-3}$ concentration solution.

4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The conditions for the analysis by HPLC will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe optimum use. The following conditions have been found to be satisfactory using the instruments detailed below.

4.1 Liquid Chromatography Conditions

a) Column — Spherisorb 5 ODS2 column 15 cm x 4.6 mm internal diameter.

b) Mobile Phase : 50:50/acetonitrile:water flowing at 1.5 cm^3 $minute^{-1}$ using a Waters 590 pump.

c) Injector : Waters Intelligent Sample Processor (WISP), 100μl injection volume.

d) Detector : Waters 484 ultra-violet detector at 220 nm, 0.01 aufs.

Under these conditions the retention time of ICIA5504 was approximately 5 minutes.

4.2 **Calculation of ICIA5504 Residue Results**

a) Make repeated injections of 100μl of a standard solution containing ICIA5504 at 0.1 $\mu g\ cm^{-3}$ into the HPLC operated under conditions described in Section 4. When a consistent response is obtained measure the peak height or area obtained for ICIA5504 in the standard.

b) Make an injection of each sample solution and measure the peak height or area of the peak corresponding to ICIA5504.

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

d) Calculate the residue in the sample, expressed as μg $litre^{-1}$, by proportionation of the ICIA5504 peak height or peak area measured for the sample against that for the analytical standard solution.

$$\text{Residue} = \frac{\text{peak height / area in sample}}{\text{peak height / area in standard}} \times \frac{\text{conc}^{n} \text{ of ICIA5504 standard}}{\text{conc}^{n} \text{ of sample solution}} \times \frac{\text{volume injected (std)}}{\text{volume injected (sample)}}$$

$$= \frac{\text{response}}{\text{response}} \times \frac{\mu g\ cm^{-3}}{L\ cm^{-3}} \times \frac{\mu L}{\mu L} = \mu g\ L^{-1}$$

5 CONTROL AND RECOVERY EXPERIMENTS

At least one untreated sample must be analyzed alongside any set of samples, using exactly the same method. This ensures that no unobserved contamination of the samples occurred prior to, or during, the analysis. At least two control samples, accurately fortified with a suitable known amount of ICIA5504, should be analyzed alongside every batch of treated samples. Fortification amounts should be based on anticipated residue levels. When no residues are expected, the recoveries should be fortified at low levels, typically 0.1-0.5 μg $litre^{-1}$. The percentage recovery of ICIA5504 should be used to correct the residue found in the treated samples for the analytical efficiency.

e.g For an 80% recovery

$$\text{Corrected residue} = \frac{\text{measured residue x 100}}{80}$$

6 LIMIT OF DETERMINATION

The limit of determination of the method can be assessed by carrying out recovery experiments at low levels of fortification (0.1 - 0.5 μg litre^{-1}). In these laboratories the limit of determination has been set at 0.1 μg litre^{-1}. Care must be taken when working at the limit of determination to minimise the risk of contamination.

1 **Apparatus**

a) Filtration apparatus, all glass filter holder, funnel and receiving flask available from Millipore (UK) Ltd., Watford, Hert.

b) Round bottom flasks (250, 100 cm^3 capacity).

c) Rotary evaporator e.g Buchi

d) Glass test tubes (25cm^3 capacity).

e) Waters 4cm^3 HPLC vials.

f) High performance liquid chromatograph fitted with an ultra-violet detector, autosampler and integrator or data handling system.

2 **Reagents**

a) Solvents: ethyl acetate and methanol (distilled in glass).

b) Extraction disks (Empore^{tm} C_{18}, 47 mm disks) available from Analytichem International

c) Granular anhydrous sodium sulphate (Analar grade). BDH Chemicals Ltd., Poole, UK.

d) Spherisorb 5 micron ODS2 column 15cm x 4.6 mm, available from Hichrom Ltd., Reading, Berks, UK.

e) A sample of ICIA5504 of known purity.

3 **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. ICI Laboratory Safety Manual) which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by G D Muir, The Chemical Society, London.

a) Solvent Hazards

	ethyl acetate	methanol
Harmful vapour	x	x
Harmful by skin absorption	x	x
Highly flammable	x	x
TLV (mg m^{-3})	1400	260

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

b) ICIA5504 has a divisional toxicity class of 4. ICIA5504 has a mammalian toxicity (acute oral LD_{50}) in rat greater than 5000 mg kg^{-1}.

4 **Preparation of Analytical Standards**

Weigh out accurately using a five figure balance, sufficient of ICIA5504 solid to allow dilution in acetone to give a 1000 μg cm^{-3} stock solution in a volumetric flask. Make serial dilutions of this stock to give 100 μg cm^{-3}, 10 μg cm^{-3}, 1.0 μg cm^{-3} and 0.1 μg cm^{-3} standard solutions in acetone to be used for fortification of samples. When not in use, always store the standard solutions, securely stoppered, in a refrigerator at $\leq 7°C$ to prevent decomposition and/or concentration of the solvent strength. Analytical standards should be freshly prepared from the solid material after six months of use.