ZENECA

ZENECA Agrochemicals
Jealott's Hill Research Station
Bracknell,
Berkshire RG42 6EY
UK

Telephone (01344) 42470
Telex 847556
Fax (01344) 55623

RAM 260: EPA ENFORCEMENT METHOD

RESIDUE ANALYTICAL METHOD FOR THE ANALYSIS OF ICIA5504 AND R230310 IN PEANUTS AND PECANS.

Authors: S R Burke & W Gentle Issuing Section: Residue Chemistry

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1 SCOPE

The analytical procedures described are suitable for the determination of residues of the fungicide ICIA5504, Figure 1, and its geometrical isomer, R230310 (Figure 2) in peanuts (kernel and hull) and pecans (kernel).

To date, in these laboratories, the method has been applied to peanut (kernel and hull) and pecan (kernel) samples and the limit of determination of the method is 0.05 mg kg^{-1}.

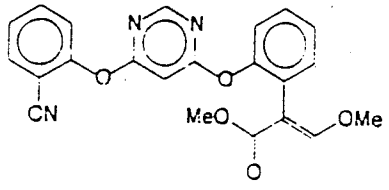

Figure 1: ICIA5504: Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate (IUPAC).

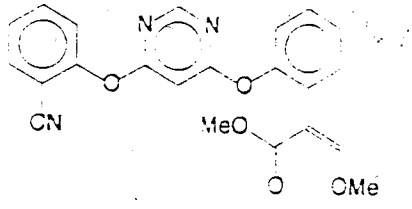

Figure 2: R230310: Methyl (Z)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate (IUPAC).

2 SUMMARY

ICIA5504 and R230310 residues in peanut (hull and kernel) and pecan (kernel) samples are extracted in 90:10/acetonitrile:water. An aliquot of all extracts is cleaned up by passing through a C18 Sep Pak cartridge and partitioning into dichloromethane (peanut hulls and pecan kernels only). The eluate is evaporated to dryness and redissolved in 75:25/ethyl acetate:toluene for further clean up by gel permeation chromatography (GPC) eluting through alumina and Florisil solid phase extraction cartridges. The eluate is evaporated to dryness and taken up in a known volume of acetone for analysis by gas chromatography with nitrogen-phosphorus detection (GC-NPD).

3 PROCEDURE

3.1 Extraction

a) Thoroughly mix the sample and weigh a representative aliquot (peanut or pecan kernel, 20 g, peanut hull 10 g,) into a centrifuge bottle.

b) Peanut (hull) and pecan (kernel) : Pre-wet samples with acetonitrile (10-20 cm^3) and soak for 15 minutes prior to fortification.

c) Fortify two control samples with an accurately known amount of ICIA5504 and R230310 as recovery checks. Macerate for three minutes in 90:10/acetonitrile:water (60 cm^3)

d) Filter the extract under vacuum through a Whatman No. 5 beneath a No 1 filter paper into a 250 cm^3 round bottomed flask. Rinse the centrifuge bottle and residuum with further extraction solvent and filter into the round bottomed flask. Adjust the filtrate to a suitable known volume (eg. 80-120 cm^3) with acetonitrile.

e) Peanut (hull) and pecan (kernel) - Attach a C18 Sep Pak to a 10 cm^3 glass syringe, pre- wet with 90:10/acetonitrile:water (~5 cm^3), discard. Push a 2g aliquot through the cartridge into a 100 cm^3 separatory funnel and partition with equivalent volumes, i.e = 2 g volume, of 5% sodium chloride and dichloromethane for approximately 2 minutes. Take a funnel, plug with glass wool and add anhydrous sodium sulphate (~10 g) and pre-wet with dichloromethane (~5 cm^3). Collect the dichloromethane layer through the anhydrous sodium sulphate into a round bottom flask and rinse with further dichloromethane (~5 cm^3), collecting in the same flask.

f) Peanut (kernel) - Take a funnel, plug with glass wool, add anhydrous sodium sulphate (~10g) and pre- wet with ethyl acetate (~5 cm^3. Attach a C18 Sep Pak cartridge to a 10 cm^3 glass syringe, pre-wet with 90:10/acetonitrile:water (~5 cm^3), discard. Push a 2g aliquot through the cartridge and sodium sulphate followed by 90:10/acetonitrile:water (~5 cm^3). Rinse the sodium sulphate with ethyl acetate (20 cm^3), collecting all in a 100 cm^3 round bottom flask.

g) All samples: Evaporate the aliquots to dryness on a rotary evaporator at ≤40°C and redissolve in 75:25/ethyl acetate:toluene (10 cm^3), for GPC clean-up, and ultrasonicate until deposit is dissolved.

3.2 GEL PERMEATION CHROMATOGRAPHY (GPC)

The conditions for sample clean-up by GPC will depend upon the equipment available. The operating manuals for the instruments should always be consulted to ensure safe and optimum use. The following conditions have been found to be satisfactory using a GPC Autoprep 1001 instrument available from Analytical Biochemistry Laboratories Inc.

Prior to use, each GPC column should be calibrated, as described in the instrument manual, in order to determine the dump and collect times for the compounds to be analysed.

(i)	Column:	Bio-Beads SX-3 (50 g) packed in a 60 cm x 25 mm glass column.
(ii)	Solvent System:	75:25 Ethyl Acetate:Toluene
(iii)	Flow Rate:	5 cm^3 min^{-1}.
(iv)	Sample Loop Volume:	5 cm^3

Note: On this instrument, at least 7.5 cm^3 of solvent will be required for each sample injection to fill the sample loop with 5 cm^3 (≡ 1g).

a) Inject each sample from 3.1(g), into an individual sample loop, via a 0.45µm Millex-HV filter unit using a 10 cm^3 glass syringe with metal luer lock fitting.

Gloves, lab coat and safety glasses must be worn in case of solvent spillage.

3.3 SOLID PHASE EXTRACTION CLEAN-UP (Alumina-N, Florisil)

a) The collected fractions from the GPC are placed inside 25 cm^3 glass syringe barrels connected in series with alumina-n (1 g) followed by Florisil (0.5 g) solid phase extraction cartridges.

b) Elute the collected fractions from the GPC through the solid phase extraction cartridges into 100 cm^3 round bottom flasks, pushing through any solvent remaining in the syringe.

c) Evaporate the eluate to dryness at ≤40°C and redissolve in an appropriate volume of acetone for analysis by GC-NPD.

Prior to use, each fresh batch of solid phase extraction columns should be calibrated for the crop to be analysed.

4 GAS CHROMATOGRAPHY WITH NITROGEN PHOSPHORUS DETECTION (GC-NPD)

The conditions for the analysis by GC-NPD 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 a Varian 3400 series gas chromatograph fitted with a Varian 8100 series autosampler. Conditions may need to be adjusted to compensate for the age of the column.

4.1 GC-NPD Conditions

(i) Column:	Rt_x - 200 (trifluoropropylmethyl polysiloxane), fused silica wall coated open tubular capillary 15 metres x 0.32 mm (0.5 µm film thickness).
(ii) Oven temperature:	70°C (hold 1 minute); program at 15°C minute^{-1} to 220°C; program at 10°C minute^{-1} to 320°C (hold 5 minutes). These conditions may need to be adjusted according to crop type.
(iii) Injector:	Septum programmable injector (SPI): 40°C (hold 0.1 minute); program at 300°C minute^{-1} to 250°C (hold 25 minutes). Injection volume = 2 µl.
(iv) Gas Flow Rates:	Helium (carrier): 2.5 cm^3 min^{-1} Helium (makeup): 28 cm^3 min^{-1} Air: 175 cm^3 min^{-1} Hydrogen: 4.5 cm^3 min^{-1}
(v) Detector:	Temperature at 320°C. Bead setting: 3.0 - 3.3 amps (depending on condition of bead). Attenuation: 4. Range: 12

Under these conditions, the retention times of ICIA5504 and R230310 were approximately 16.1 and 16.4 minutes respectively.

Note: These conditions should be adhered to as closely as possible. Conversion of the isomers (R230310 -> ICIA5504) has been seen to occur under lower carrier gas flow rates at high temperatures.

4.2 Calculation of ICIA5504 and R230310 Residue Results

a) Make repeated injections of 2 µl of a standard solution containing a mixture of ICIA5504 and R230310 each at 0.1 µg cm^{-3} into the GC operated under conditions described in 4.1 above. When a consistent response is obtained measure the peak heights or areas obtained for the standard.

b) Make a 2 µl injection of each sample solution and measure the peak heights or areas of the peaks corresponding to ICIA5504 and R230310.

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 g^{-1}, by proportionation of the ICIA5504 or R230310 peak height or peak area measured for the sample against that for the analytical standard solution.

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

APPENDIX 1

Typical Gas Chromatograms for ICIA5504 and RZ00010
Residue Determination in Peanuts (kernel)

Figure 1: 0.1 µg cm^{-3} CIA5504 and RZ00010 standard.

Figure 2: Untreated peanut (kernel) sample at 1.0 µg cm^{-3}.

Figure 3: Untreated peanut (kernel) sample at 1.0 µg cm^{-3} fortified at 0.1 µg [illegible]

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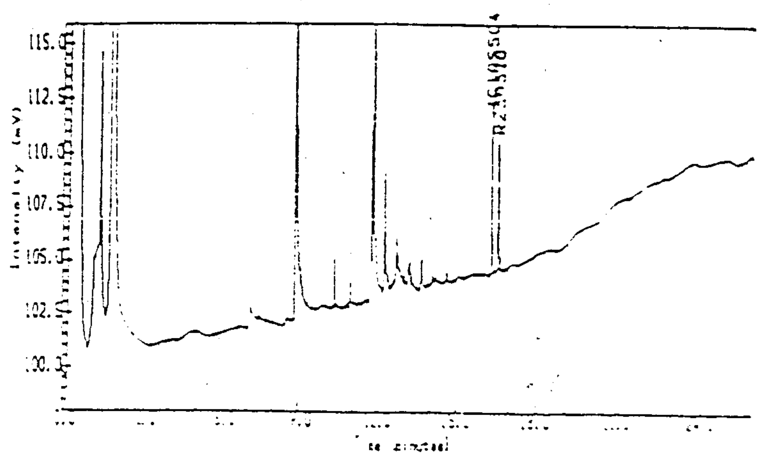
Figure 1

[RESIDUE] 15 AS6189A,1,1
Reported on 11-OCT-1995 at 16:26

Injection Report

Acquired on 14-DEC-1994 at 10:34



Sample Name : D9579/11C
Sample Id : 6189/94.1
Sample Type : Standard Amount=1.00000
Bottle No : 1

PEAK INFORMATION

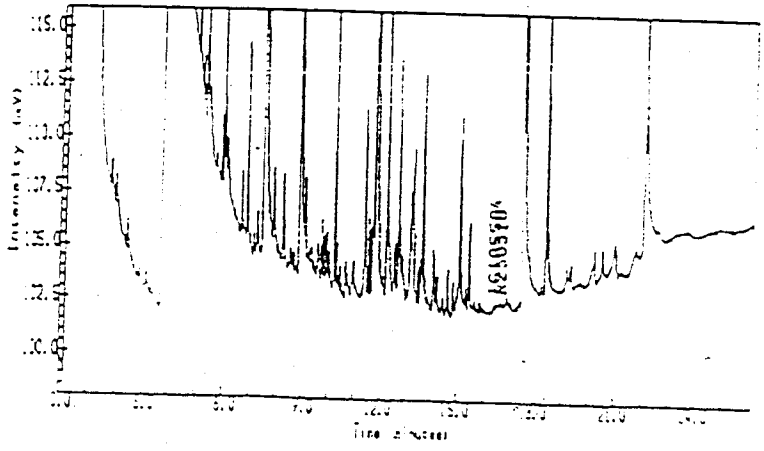
Peak	RT [illegible]	[illegible]	Area [illegible]	[illegible]	Peak name	[illegible]
1	[illegible]	[illegible]	30522	0.1000	[illegible]S504	[illegible]
2	[illegible]	[illegible]	14574	0.3355	P230310	[illegible]

Figure 2

[RESIDUE] 16 AS6189A,2,1
Reported on 11-OCT-1995 at 16:28

Injection Report

Acquired on 14-DEC-1994 at 11:06



Sample Name : D9915/57A/2
Sample Id : 6189/94/2
Sample Type : Control Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	Ret time	Height uV	Area uVs		Peak name	
1	16.347	270	844	0.0000		
2	16.517	164	374	0.0000		

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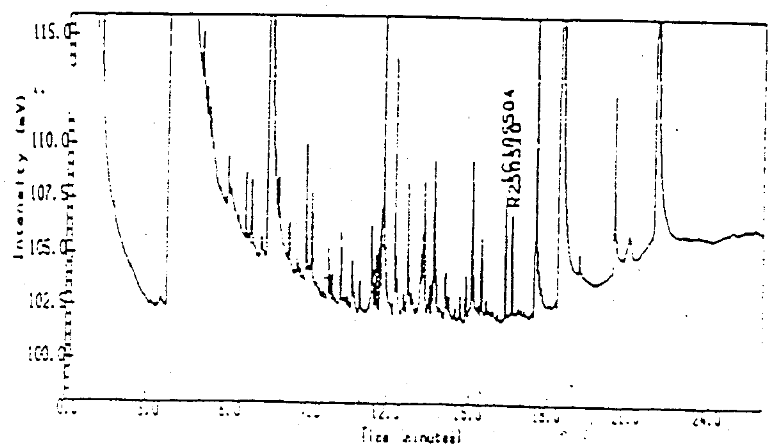
Figure 3

[RESIDUE] 16 AS6189A,8,1
Reported on 11-OCT-1995 at 16:29

Injection Report

Acquired on 14-DEC-199: at 14:21



Sample Name : D9915/57G/1
Sample Id : 6189/94/6
Sample Type : Recovery Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Hght uV	Area uVs	mg/kg	Peak name	Width
1	15.347	5771	1032	0.0980	[illegible]6504	[illegible]
2	15.608	4535	1109	0.1221	R230[illegible]	[illegible]

APPENDIX 2

Materials/Safety

1 Apparatus

a) Glass centrifuge bottles (250 cm^3 capacity) for sample extraction.

b) High speed homogeniser, eg. Sorval Omni-Mixer.

c) Filtration apparatus: Büchner funnel, adapter, filter paper (Whatman No.1 & No. 5, 9 cm).

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

e) Small glass funnels for filtration through sodium sulphate.

f) Rotary evaporator e.g Büchi

g) Vials for GC analysis.

h) Glass syringes (10 and 25 cm^3 capacity) available from Orme Scientific Limited, PO Box 3, Stakehill Industrial Park, Milddleton, Manchester M24 2RH.

i) Ultrasonic bath e.g Sonicor.

j) A gas chromatograph fitted with a nitrogen-phosphorus detector eg. Varian 3400 series, autosampler and integrator or data handling system.

k) A gel permeation chromatography instrument eg. GPC Autoprep 1001, available from Analytical Biochemistry Laboratories Inc. , P.O Box 1097, Colombia, Missouri 65205, USA.

2 Reagents

a) Solvents: acetone, acetonitrile, ethyl acetate, hexane, toluene and dichloromethane (distilled in glass).

b) Solid phase extraction sorbents, Alumina-N (1 g) and Florisil (0.5 g), available from International Sorbent Technology, Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan, UK.

c) Bio-Beads SX-3, 200-400 mesh, available from Bio-Rad Laboratories Ltd., Maylands Avenue, Hemel Hempstead Hertfordshire, UK.

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

e) Sodium chloride (Analar grade). Fison Scientific Equipment, Bishop Meadow Road, Loughborough, UK.

f) RtX-200 (trifluoropropylmethyl polysiloxane) fused silica wall coated open tubular capillary 30 metres x 0.32 mm internal diameter (0.5 µm film thickness) available from Thames Chromatography, Maidenhead, Berkshire, England, UK.

g) C18 sep-pak cartridges and Millipore filter units (0.45 µm, diameter 13 mm.) available from Waters UK Limited, The Quadrant, Blackmoor Lane, Watford, Hertfordshire, WD1 8YW.

h) Glass wool - contaminants are removed by treatment of the glass wool in a Soxhlet extraction with refluxing n-hexane (redistilled) for 2 hours.

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. Zeneca 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

	Acetone	Ethyl acetate	Acetonitrile	Hexane	Toluene	Dichloromethane
Harmful vapour	Yes	Yes	Yes	Yes	Yes	Yes
Highly flammable	Yes	Yes	Yes	Yes	Yes	No
Harmful by skin absorption	No	Yes	Yes	No	Yes	No
TLV mg/m^3	2400	1400	70	180	375	350

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 and R230310 solid to allow dilution in acetone to give 1000 µg cm^{-3} stock solutions in volumetric flasks. Make serial dilutions from the stocks to give 100 µg cm^{-3} standard solutions. Prepare 10 µg cm^{-3}, 1.0 µg cm^{-3} and 0.1 µg cm^{-3} mixed standard solutions of ICIA5504 and R230310 in acetone to be used for fortification of recovery samples. When not in use, always store the standard solutions, securely stoppered, in a refrigerator at ≤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.

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

5 CONTROL AND RECOVERY EXPERIMENTS

At least one untreated sample must be analysed 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 and RZ30310 should be analysed 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 0.02-0.05 $\mu g\ g^{-1}$

6 LIMIT OF DETERMINATION (QUANTITATION)

The limit of determination of the method can be assessed by carrying out recovery experiments at low levels of fortification (0.01 $\mu g\ g^{-1}$). In these laboratories the limit of determination have been set at 0.01 $\mu g\ g^{-1}$. Care must be taken when working at the limit of determination to minimise the risk of contamination.

7 LIMIT OF DETECTION

The limit of detection is set at 4 x baseline noise

8 EXAMPLES OF TYPICAL CHROMATOGRAMS - see Appendix