1. Introduction

Fenazaquin was extracted from soil by reflux using an acetonitrile/water solvent mix. The extract was evaporated and saline solution added. Fenazaquin was partitioned into hexane and the extracts further purified by use of solid phase extraction cartridges ('Florisil', followed by Aminopropyl).

Fenazaquin was determined by coupled gas chromatography-mass spectrometry.

Copies of the study protocol and protocol amendments are presented in Appendix 2. All processes were conducted as per the study protocol and protocol amendments.

2. Instrumentation and Apparatus

Gas Chromatograph	:	Hewlett Packard 5890 Series II Gas Chromatograph fitted with an HP 7673B automatic sampler.
Detector	:	HP 5989A Mass Spectrometer (MS Engine)
Heating Mantle	:	Electromantle ME
Solid Phase Extraction (SPE) Unit	:	Vac Master
Helical Evaporator Turbovap II	:	Zymark 46371/A
Reciprocating Shaker	:	Gerhardt LS5
Sample concentrator	:	Techne Dri-Block DB-3 with Techne SC-3 Sample Concentrator
Ultrasonic Bath	:	Decon FS 100b
Analytical Balance	:	Sartorius R200D
Laboratory Balance	:	Mettler PE 160
Laboratory Balance	:	Mettler PJ 6000
Laboratory Balance	:	Ohaus CT 600-S
Centrifuge	:	Beckman Model GP

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3. Reagents and Materials

Fenazaquin Standard	:	Batch: AGR 289213 Restec Reference: RESTD 030895 Purity: 99.3% Expiry: 10/95
1,4-Dibromonaphthalene	:	Kodak Laboratory Chemicals
Dichloromethane (Super Purity Solvent)	:	Romil
Acetonitrile (Super Purity Solvent)	:	Romil
Deionised Water	:	Prepared using an Elgastat deionising cartridge
Ethyl Acetate (Super Purity Solvent)	:	Romil
Hexane (Super Purity Solvent)	:	Romil
Trimethylpentane (TMP) (Super Purity Solvent)	:	Romil
Acetone (Super Purity Solvent)	:	Romil
Sodium Sulphate (Anhydrous)	:	Fisons
Sodium Bicarbonate	:	Fisons
'Florisil' (SPE)	:	Waters 'Sep-Pak' Solid phase extraction cartridges, Part No. 51960
AminoPropyl 'Bond-Elut' (SPE)	:	500mg large reservoir solid phase extraction cartridges, Cat. No. LR11304
Solvent Mix 1	:	Acetonitrile/deionised water (90/10, v/v)
Solvent Mix 2	:	Dichloromethane/ethyl acetate (99/1, v/v)
Solvent Mix 3	:	Dichloromethane/ethyl acetate (90/10, v/v)

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3. Reagents and Materials Continued

Solvent Mix 4	:	Hexane/Acetone (98/2, v/v) (Note: made on the day of use)
Solvent Mix 5	:	1,4-Dibromonaphthalene in trimethylpentane (1.0 µg/ml)
Salt Solution 1	:	5% Sodium Bicarbonate solution (aq)

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4. Method of Analysis

4.1 Extraction

A sub-sample of matrix (10 g) was weighed into a 250 ml round-bottomed flask, Solvent Mix 1 (100 ml) was added and the mixture refluxed for 60 minutes. The round-bottomed flask was removed from the heat and allowed to attain room temperature. The extract was transferred to an 8 oz jar. This was centrifuged (2000 rpm, 10 minutes) and the supernatant decanted into a turbovap tube. The round-bottomed flask was then rinsed with Solvent Mix 1 (50 ml) which was then transferred to the 8 oz jar, re-centrifuged and the supernatant combined with the original supernatant. The extract was then evaporated using the turbovap (air, 50°C) to approximately 15 ml.

4.2 Extract Purification

4.2.1 Liquid/Liquid Partition - Aqueous/Hexane

The extract from 4.1 was transferred to a 4 oz jar. Salt Solution 1 (75 ml) and hexane (20 ml) were added, first rinsing the turbovap tube. The extract was shaken using a reciprocating shaker (160 rpm, 15 minutes) and the mixture centrifuged (2000 rpm, 2 minutes). Anhydrous sodium sulphate (approx. 5 g) was weighed into an 8 dram vial. The hexane extract was added and shaken manually to remove any residual water. The dried hexane was then transferred to a clean 8 dram vial and evaporated to approximately 5 ml (air, 40°C).

The remaining aqueous extract was re-extracted with hexane (20 ml) and the partition repeated. The second hexane extract was transferred to the vial containing sodium sulphate and shaken, with addition of further sodium sulphate if required to remove residual water.

The dried hexane extracts were combined and evaporated until just dry (air, 40°C).

The residue was redissolved in dichloromethane (5 ml) and ultrasonicated for 1 minute.

4.2.2 'Florisil' Sep-Pak

A 'Florisil' Sep-Pak cartridge was conditioned with dichloromethane (10 ml). The extract from 4.2.1 was transferred to the Sep-Pak cartridge and allowed to elute. The vial was rinsed with dichloromethane (5 ml), transferred to the Sep-Pak and allowed to elute. All eluates were discarded. Dichloromethane (15 ml) was added to the Sep-Pak and the eluate discarded. Solvent Mix 2 (8 ml) was added and the eluate discarded. Solvent Mix 3 (12 ml) was added and the eluate collected. The extract was evaporated to dryness (air, 40°C) and the residue redissolved in hexane (3 ml) with the aid of an ultrasonic bath (1 minute).

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4.2.3 Aminopropyl 'Bond-Elut'

An aminopropyl 'Bond-Elut' was conditioned with hexane (6 ml). The extract from 4.2.2 was transferred onto the column and allowed to elute. The vial was rinsed with hexane (3 ml), added to the column and the eluate discarded. Solvent Mix 4 (9 ml) was added to the column and the eluate collected.

The extract was evaporated to dryness (air, 40°C) and the residue redissolved in Solvent Mix 5 (1 ml) with the aid of an ultrasonic bath (1 minute). Extracts were retained for quantitation of fenazaquin.

4.3 Chromatographic Conditions

Instrument	:	Hewlett Packard 5890 Series II Gas Chromatograph fitted with an HP 7673B automatic sampler.
Detector	:	HP 5989A Mass Spectrometer
Column	:	Retention Gap (5 m x 0.53 mm i.d. uncoated deactivated column). Analytical Column (30 m x 0.25 mm i.d.) coated with DB5-MS 0.25 µm film.
Ions Monitored	:	Fenazaquin: 145.1 amu (quantitation mass) 160.1 amu (confirmatory mass) 1,4-Dibromonaphthalene: 285.9 amu (internal standard) Dwell time 100ms
Temperatures:		
Transfer Line Temperature	:	280°C
Oven Temperature	:	300°C (5 mins) 20°C/min 40°C (2 mins)
Equilibration Time	:	3 minutes
Injector (on column)	:	150°C (5 mins) 30°C/min 40°C (2 mins)

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4.3 Chromatographic Conditions Continued

Mass Spectrometer Temperatures	:	Source 300°C
		Quad 100°C
Gases	:	Helium (carrier) 1.0 ml/min
Injection Volume	:	2.0 µl

Under these conditions fenazaquin had an approximate retention time of 15.2 minutes and 1,4-dibromonaphthalene had an approximate retention time of 11.9 minutes..

5. Preparation of Fortification and Calibration Standards

5.1 Fortification Standards

100mg of fenazaquin was dissolved in acetonitrile (100 ml) to give a 1000 µg/ml stock solution. This was diluted using acetonitrile to give fortification solutions as follows:

Parent Standard Concentration µg/ml	Aliquot Taken ml	Final Volume ml	Diluted Standard Concentration µg/ml
1000	5	25	200
1000	5	50	100
1000	5	100	50
100	5	20	25
100	5	50	10
50	5	50	5.0
25	5	50	2.5
10	5	50	1.0
5.0	5	50	0.5
0.5	5	50	0.05
0.5	5	100	0.025

5.2 Internal Standard Solution

100mg of 1,4-dibromonaphthalene was dissolved in trimethylpentane (100 ml) to give a 1000 µg/ml stock solution. This was diluted using trimethylpentane as follows:

Parent Standard Concentration µg/ml	Aliquot Taken ml	Final Volume ml	Diluted Standard Concentration µg/ml
1000	10	100	100
100	10	100	10
10	10	100	1.0

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5.3 Calibration Standards

The fenazaquin stock solution was diluted using trimethylpentane to give calibration standards in the range 0.01 - 2.0 µg/ml. Each contained 1,4-dibromonaphthalene as an internal standard and were prepared as follows:

Initial Analyte Standard Conc. (µg/ml)	Analyte Aliquot Taken (ml)	Initial Internal Standard Conc. (µg/ml)	Int. Std. Aliquot Taken (ml)	Final Volume (ml)	Final Analyte Conc. (µg/ml)	Final Internal Standard Conc. (µg/ml)
1.0	1	10	10	100	0.01	1.0
2.5	1	10	10	100	0.025	1.0
5.0	1	10	10	100	0.05	1.0
10	1	10	10	100	0.1	1.0
25	1	10	10	100	0.25	1.0
50	1	10	10	100	0.5	1.0
100	1	10	10	100	1.0	1.0
200	1	10	10	100	2.0	1.0

6. Fortification Levels

The analytical procedure was validated at fortification levels of 0.005 mg/kg (x 6), 0.01 mg/kg (x 2), 0.1 mg/kg (x 2) and 1.0 mg/kg (x 2) fenazaquin in soil.

Untreated matrix was fortified using standard solutions in acetonitrile as follows:

Weight taken for Analysis (g)	Concentration and Volume Added	Fenazaquin Added (µg)	Fortification Level (mg/kg)
10	5.0 µg/ml, 2 ml 0.5 µg/ml, 2 ml 0.05 µg/ml, 2 ml 0.025 µg/ml, 2 ml	10 1.0 0.1 0.05	1.0 0.1 0.01 0.005

In addition, three untreated soil samples were analysed in duplicate to confirm freedom from coextracted interferences.

7. Linearity of Response

The linearity of the detector response was established for each chromatographic run. Linearity was assessed by plotting peak area versus concentration for fenazaquin over the calibration range and establishing an acceptable correlation coefficient (>0.9950).

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8. Limit of Detection

The limit of detection was determined to be 20 pg of fenazaquin under the conditions given in section 4.3. This was performed by injection of 2.0 µl of a 0.01 µg/ml standard. This was observed to produce a peak response of at least three times the baseline noise.

9. Limit of Determination

The limit of determination of fenazaquin in soil was determined to be 0.005 mg/kg. This corresponded to a peak response of at least three times the baseline noise and was the lowest fortification level where accuracy and precision of individual recovery values gave acceptable results according to the quality criteria specified (ie. recovery from fortified matrices of 70-120%).

10. Calculations

Residues of fenazaquin found in the soil samples were calculated in the following manner:

$$F = \frac{A \times B \times C \times D}{E}$$

where A = Concentration of bracketing fenazaquin standard = 0.1 µg/ml

B = Final extract volume = 1 ml

C = Procedural dilution factor (not applicable)

D = Additional dilution factor (if applicable)

E = Initial sample weight = 10 g

During a chromatographic analysis each two sample extracts were bracketed by a mixed fenazaquin /1,4-dibromonaphthalene standard (0.1 µg/ml fenazaquin/1.0 µg/ml 1,4-dibromonaphthalene).

To account for the response of the internal standard, the following calculation was used:

Reference	Detector Response		Ratio	Mean	Residue found (mg/kg)
	Internal Standard	Fenazaquin			
Mixed Standard	x_1	x_2	$x_2/x_1 = X$		
Sample 1	k_1	k_2	$k_2/k_1 = K$	$\frac{X+Y}{2} = Z$	K/Z x F
Sample 2					
Mixed Standard	y_1	y_2	$y_2/y_1 = Y$		

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10. Calculations Continued

Fenazaquin residues (mg/kg), corrected for internal standard response, were determined using the following equation:

$$\text{Fenazaquin residue (mg/kg)} = K/Z \times F$$

11. Expression of Residue Results

For untreated samples, values which were >2% of the lowest validated level were calculated and used to correct residues in fortified samples.

Residues in untreated samples which were <20% of the limit of determination (0.005 mg/kg) were expressed as ND (Not Detected).

Residues below the limit of determination but ≥20% of the limit of determination were reported as <0.005 mg/kg.

12. Conclusions and Remarks

Using the method as specified, fenazaquin can be accurately and precisely quantified in soil between 0.005 mg/kg and 1.0 mg/kg.

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