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Determination of thiazopyr residues in soil and plants by gas chromatography with nitrogen–phosphorus detection and confirmation by gas chromatography–mass spectrometry

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Abstract

Thiazopyr residues have been determined in soil, grass weeds and seedlings of woody plants by gas chromatography. The herbicide was extracted from soil and plant samples with ethyl acetate and plant extracts were cleaned-up on a Florisil column. Thiazopyr levels were determined by gas chromatography with nitrogen–phosphorus detection and confirmed by gas chromatography with mass spectrometry, ion trap detection. The average recoveries were always higher than 97% with a relative standard deviation between 1 and 6%. The limit of detection for the herbicide was, at least, 0.01 μg/g in soil and 0.02 μg/g in plant with both gas chromatographic methods. Samples of soil and *Pinus pinea* L. plants, treated with thiazopyr, were also analyzed. © 1997 Elsevier Science B.V.

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1. Introduction

Thiazopyr [methyl-2-difluoromethyl-5-(4,5-dihydro-1,3-thiazol-2yl)-4-isobutyl-6-trifluoromethyl-nicotinate, Fig. 1] is a new pre-emergence herbicide of the pyridine family. It has been described as a herbicide with broad spectrum weed control activity [1], selective in perennial crops [2–4] and used in forestry where weed control is important to ensure survival and growth of young plantations [5,6].

An analytical procedure for the determination of thiazopyr residues was not found in the literature. The aim of this work was to develop a method of determination of thiazopyr in soil and plant samples by gas chromatography with nitrogen–phosphorus detection (GC–NPD) and confirmation of residues by gas chromatography–mass spectrometry (GC–MS). The plants studied were grass weeds from conifer plantations and three species of woody plants, *Pinus pinea* L., *Quercus suber* and *Quercus ilex*, typical Mediterranean species which are very abundant in Spanish forests [7].

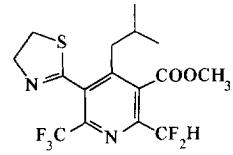

Fig. 1. Structure of thiazopyr.

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2. Experimental

2.1. Chemicals

Thiazopyr, 99.4% purity, was supplied from Monsanto (St. Louis, MO, USA). All the organic solvents used were analytical-reagent grade from Panreac (Spain). Florisil (60–100 mesh) was obtained from Fluka (Switzerland). The Florisil adsorbent was heated for 12 h at 120°C and 2% of Milli-Q grade water was then added to the chromatographic adsorbent for deactivation. Anhydrous sodium sulphate was purchased from Merck (Germany).

2.2. Equipment

GC–NPD analysis was performed with a Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen–phosphorus detector and automatic injector. A fused-silica capillary column, HP-1 (12.5 m×0.20 mm I.D.) and 0.33 μm film thickness was employed, with helium as carrier gas at a flow-rate of 1 ml/min. The injection port and detector temperatures were 270 and 300°C, respectively. The oven temperature was held at 100°C for 1 min and then programmed at 25°C/min to 250°C, held for 1 min.

A Pelkin-Elmer 8500 gas chromatograph equipped with an ion trap detection Finnigan (ITD) system was used for the GC–MS analysis. A fused-silica capillary column, BP-1 (12 m×0.22 mm I.D.) and 0.25 μm film thickness was employed with helium as carrier gas at 10 p.s.i. (1 p.s.i.=6894.76 Pa). Injection port and detector temperatures were maintained at 270 and 250°C, respectively. The oven temperature was held at 100°C for 1 min, programmed to 250°C at 25°C/min and then held for 1 min. A 2 μl injection in splitless mode, with the split valve closed for 1 min was utilized. Mass spectrometric acquisition parameters were the following: mass range 100–550; scan rate 1 s/scan, 4-μscans; radio frequency (r.f.) and voltage, 1.1 MHz and 0–7.5 kV; automatic gain control from 78 μs to 25 ms; solvent delay, 3 min.

2.3. Soil and plant samples

The soil used in this work was a sandy loam soil with the following characteristics: clay 11.53%, silt 23.09%, sand 64.81%, organic matter 1.75% and pH 6.68. Soil samples were taken from the top layer (0–10 cm) and homogenized by passing through a 2 mm mesh sieve.

Plants selected for this study were grass weeds taken from a conifer plantation and seedlings of *Pinus pinea* L., *Quercus suber* and *Quercus ilex*. The aerial part of plants was sampled for the analytical determinations.

Samples were fortified at 0.2, 0.5 and 1 μg/g of thiazopyr before extraction. In addition, a pinus plot was treated at 4 l/ha with Visor, a commercial formulation of this herbicide (24%, w/v) and plant and soil samples were taken for analysis one week after treatment.

2.4. Procedure

Soil (20 g) was extracted with 100 ml of ethyl acetate on an orbital shaker for 45 min. Solvent was decanted and soil extracted again with other 100 ml of ethyl acetate. Sample extracts were filtered under suction through Whatman No. 1 filter paper and the filter cake washed with ethyl acetate (2×25 ml). Solvent was evaporated to dryness under vacuum and the residue transferred to a tube with ethyl acetate and concentrated to a suitable volume before GC analysis.

Plant samples (2–2.5 g) were extracted with ethyl acetate (2×25 ml) in a Sorvall homogenizer. The extract was filtered under suction and the filter cake was washed twice with 10 ml of ethyl acetate. The organic extract was passed through anhydrous sodium sulphate and evaporated to dryness under vacuum. The residue was dissolved in 2 ml of ethyl acetate–hexane (1:1, v/v) for clean-up on a Florisil column.

2.4.1. Column clean-up

A glass column was packed with 9 g of deactivated Florisil (2% water) and with 2 g anhydrous sodium sulphate on top. The extract (2 ml) was added at the top of the column and eluted with 60 ml of a mixture of ethyl acetate–hexane (3:1, v/v). The eluate was evaporated to dryness on a rotary evaporator. The residue was dissolved in an appropriate volume of ethyl acetate and an aliquot analyzed by GC.

2.5. Quantitation

The concentration of thiazopyr was calculated by comparing the peak area obtained in the samples with that found for a known concentration of the herbicide, in the range of 0.2 to 1 μg/ml.

GC–MS detection was carried out by selecting the base peak (*m/z* 327) of thiazopyr mass spectrum, after acquisition of the total ion chromatogram of the sample.

3. Results and discussion

Extraction of thiazopyr from soil and plant samples has been carried out with ethyl acetate. This procedure produced clean soil extracts, while the plant extracts needed an additional clean-up on a Florisil column. Methanol was also used for herbicide extraction and good results were obtained with soil, grass weeds and pine plants, but somewhat low recoveries were obtained with *Quercus* seedlings.

3.1. Recovery

The proposed method has been validated by recovering thiazopyr from soil and plant samples fortified at 1, 0.5 and 0.2 μg/g before extraction.

The average herbicide recoveries obtained through the method were equal or higher than 97% in soil and plant samples. The relative standard deviation ranged between 1% and 6% (Table 1).

Table 1
Recovery of thiazopyr added to soil and plant samples

Sample	Added (mg/kg)	Recovery (%) (mean±S.D., n=3–5)
Soil	1	97.0±1.1
	0.5	99.2±3.1
	0.2	103.4±1.9
Grass weeds	1	98.7±5.0
	0.5	99.4±4.8
	0.2	103.3±6.0
Pinus pinea	1	100.8±4.9
	0.5	101.0±2.7
	0.2	104.3±1.0
Quercus suber	1	98.3±2.8
	0.5	97.9±3.0
	0.2	98.6±4.8
Quercus ilex	1	100.3±5.5
	0.5	99.4±3.5
	0.2	101.6±4.5

3.2. Linearity

A linear relationship between the peak area of thiazopyr and its concentration in samples was obtained for the range of concentrations tested, 0.2 to 1 μg/g. The equation of the regression line was $A=37\,993C+6720.9$, where A is the peak area and C the concentration. The correlation coefficient obtained was 0.995.

3.3. GC–MS

Table 2 gives the main ions (*m/z*) found in the mass spectrum of thiazopyr, their relative abundance and the proposed peak identity.

The base peak of the mass spectrum was found at *m/z* 327, which probably corresponds to the loss of $-F_3C$, and was used for quantitation of the herbicide. Other main peaks are observed at *m/z* 363, *m/z* 349 and *m/z* 381. The protonated molecular ion, *m/z* 397 [M+1], was also observed with a medium abundance.

Fig. 2 shows the mass spectrum of thiazopyr obtained with a 2 ng injection.

Table 2
Main ions and their relative abundance found in the mass spectra of thiazopyr

m/z	Relative abundance (%)	Proposed peak identity
397	37	$C_{16}H_{18}F_5N_2O_2S^+$
381	32	$C_{15}H_{14}F_5N_2O_2S^+$
363	98	$C_{16}H_{16}F_5N_2O_2^+$
349	42	$C_{15}H_{14}F_5N_2O_2^+$
327	100	$C_{15}H_{17}F_2N_2O_2S^+$

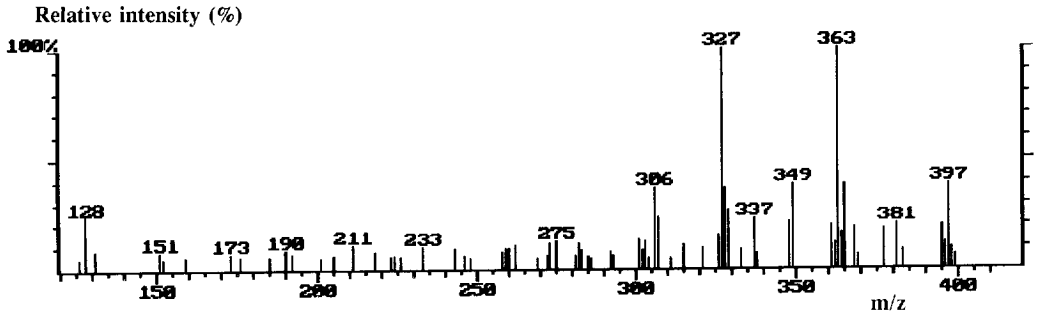

Fig. 2. Mass spectra of thiazopyr.

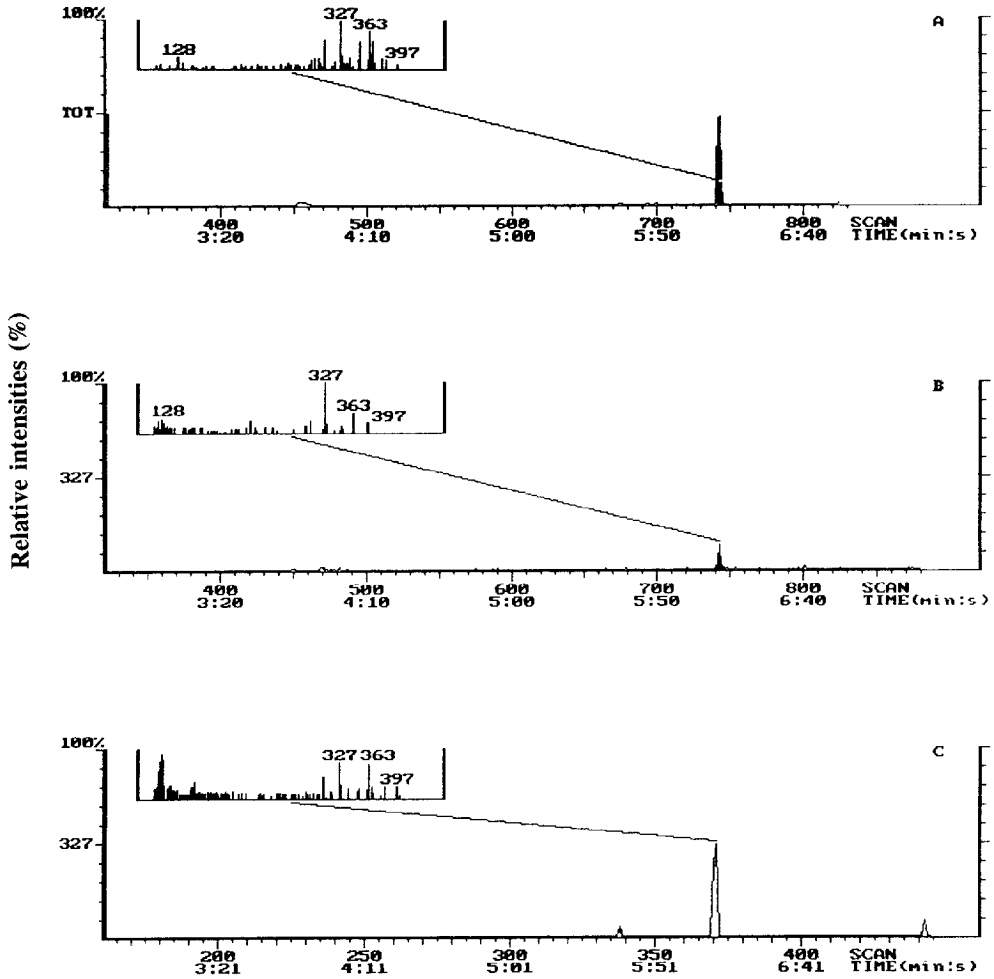

Fig. 3. GC–ITD chromatograms of thiazopyr with its mass spectrum. (A) Standard, 1 μg/ml; (B) detection limit in soil (0.01 μg/g); (C) detection limit in plant (0.02 μg/g).

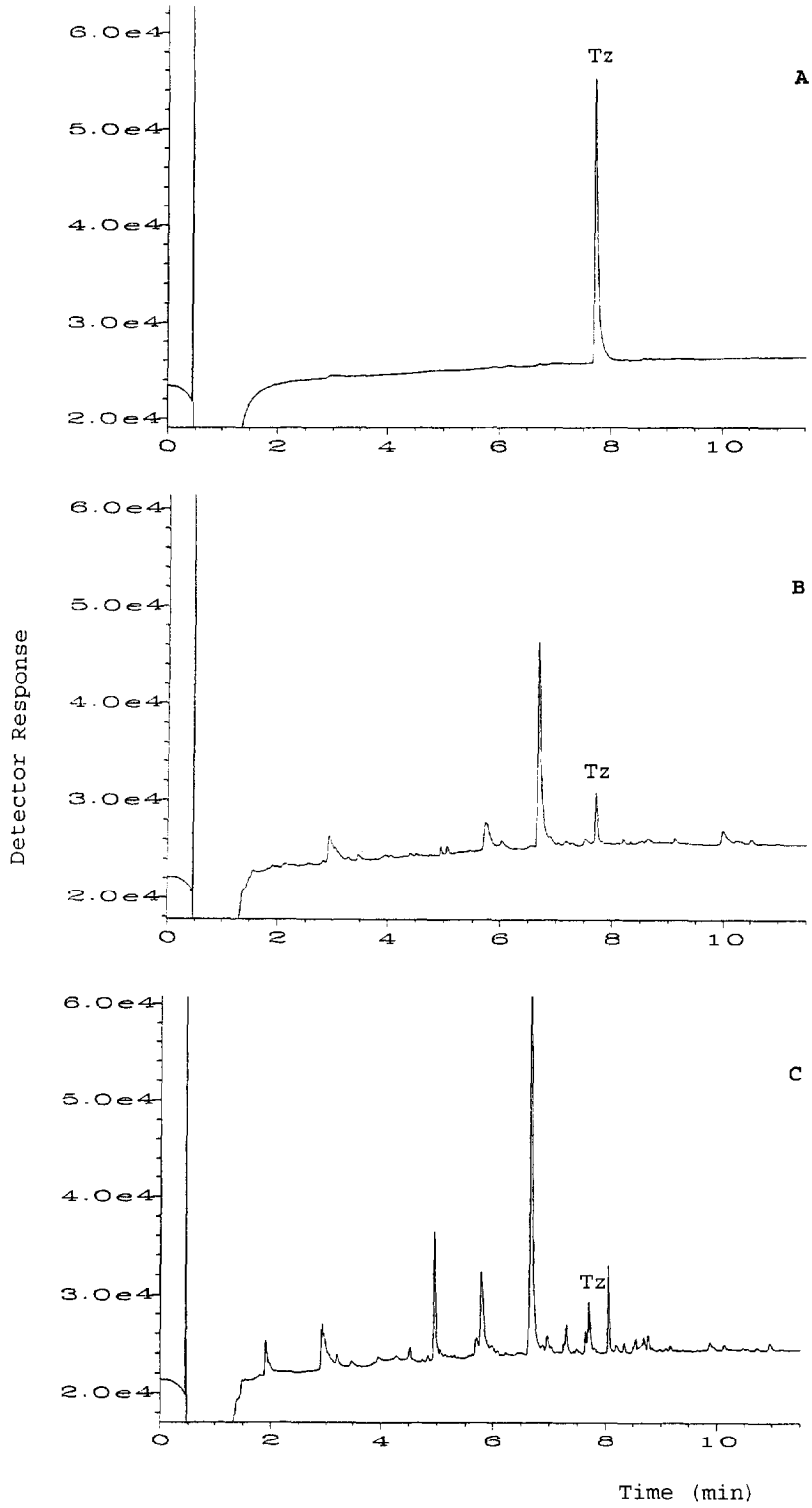

Fig. 4. GC–NPD chromatograms of thiazopyr (Tz). (A) Standard, 1 μg/ml; (B) detection limit in soil (0.01 μg/g); (C) detection limit in plant (0.02 μg/g).

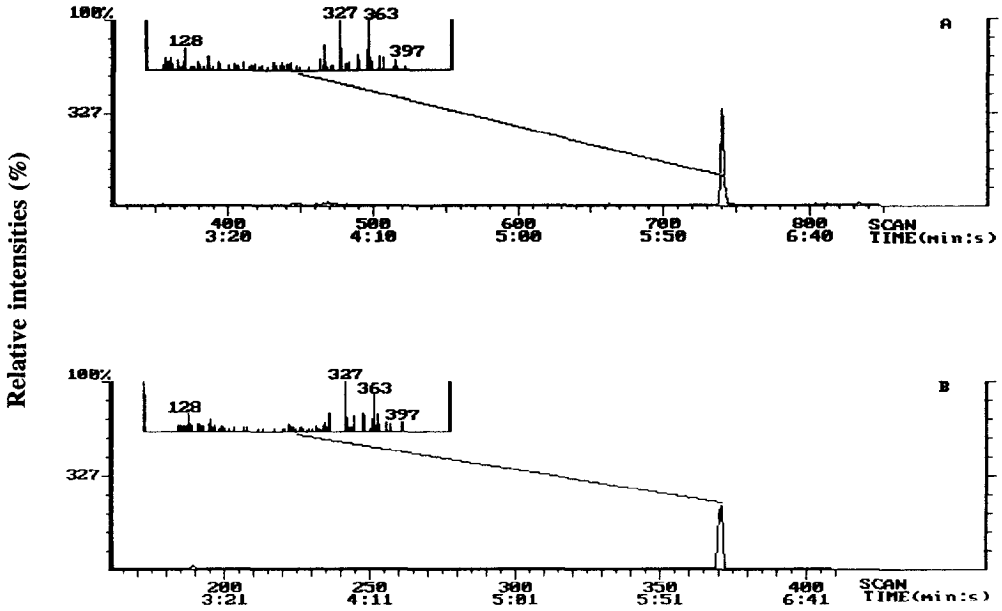

Fig. 5. GC–ITD chromatograms of treated soil and plant extracts. (A) Soil sample (0.64 μg/g of thiazopyr), (B) plant sample (0.98 μg/g of thiazopyr).

3.4. Detection limit and precision of method

The detection limit of the proposed method was determined considering a value equal or higher than three times the background noise [8]. It was lower than 0.01 μg/g of thiazopyr in soil and 0.02 μg/g in plant samples, Figs. 3 and 4, for both techniques GC–ITD with selected-ion monitoring (SIM) and GC–NPD. Table 3 gives the precision of the determination expressed as the relative standard deviation at the detection limit, which was acceptable and always lower than 7%.

Table 3
Retention times (t_R), detection limits (DL) and precision of the assay at the DL

Detector	Column[a]	t_R (min)	Detection limit (μg/g)		R.S.D. (%)[b]	
			Soil	Plant	Soil	Plant
NPD	HP-1	7.7	0.01	0.02	3.8	5.1
ITD	BP-1	6.2	0.01	0.02	4.1	6.7

[a] HP-1: crosslinked methyl silicone gum, 0.33 μm film, 12.5 m×0.20 mm I.D.. BP-1: crosslinked dimethylsiloxane, 0.25 μm film, 12 m×0.22 mm I.D..
[b] Relative standard deviation at the detection limit (n=5).

3.5. Real samples

The proposed methods were applied to the determination of the herbicide level in treated soil and plant samples. Fig. 5 shows the chromatograms of a soil sample containing 0.64 μg/g of thiazopyr and a *Pinus pinea* sample with a level of 0.98 μg/g of herbicide in the aerial part. These concentrations were determined by GC–NPD and confirmed by GC–ITD.

4. Conclusion

The results presented in this paper showed that the proposed methods are reproducible and sensitive enough to measure thiazopyr at residue level, in soil and plant samples, by GC–NPD and to confirm the identity of residues by their mass spectra with GC–ITD.

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