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High-performance liquid chromatographic determination of flumetsulam, a newly developed sulfonamide herbicide in soil

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Abstract

An analytical method is described for measuring residues of flumetsulam, [N-(2,6-difluorophenyl)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide] in soil using high-performance liquid chromatography (HPLC). The soil is extracted with aqueous $NaHCO_3$, and the extract solution acidified then passed through a C_{18} solid-phase extraction (SPE) disc. The concentrated extract obtained is then cleaned up by gel permeation chromatography (GPC) on Bio-Beads SX-3 prior to analysis by reversed-phase HPLC with UV detection. A detection limit of 4 μg/kg is obtained by this method which is suitable for routine residue decay trials.

Keywords: Soil; Sample preparation; Environmental analysis; Flumetsulam; Pesticides; Sulfonamide

1. Introduction

Flumetsulam [N-(2,6-difluorophenyl)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide] (Fig. 1), is a sulfonamide herbicide recently developed by DowElanco. It has a broad spectrum activity on many broad-leaf weeds and good crop selectivity [1]. It has a high herbicidal activity at low application rates of 10–50 g/ha. In New Zealand, flumetsulam is being investigated for control of some broad-leaf weeds in crops. Recently it has been shown that flumetsulam was quite persistent in acid soils and that its degradation rate increased with degree of sorption [2] and soil temperature [3]. The estimated half-life of flumetsulam ranges from 2 weeks to 4 months across diverse soils of varied pH and organic carbon content [2].

The typically low application rates used for sulfonamide herbicides makes their chemical analysis difficult. Direct determination of flumetsulam by gas chromatography (GC) has not been possible due to its thermal instability. However, a method for measuring flumetsulam residues in soil extracts has been reported [3,4] which uses derivatization with

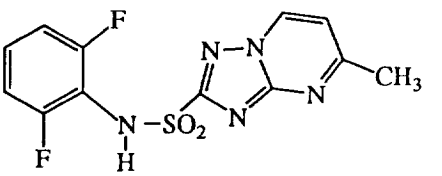

Fig. 1. Structure of flumetsulam.

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methyl iodide to form the N-methyl derivative followed by GC–MS using selected ion monitoring. HPLC has been used to separate radiolabelled flumetsulam for soil sorption and degradation studies [2]. Recently Rahman et al. [5] reported a bioassay method for determination of flumetsulam in soil. While such bioassays can reach very low detection limits and provide semi-quantitative data on residues, they are non-specific and there is a delay time of 2–4 weeks before effects can be measured. As part of studies into the activity and degradation of flumetsulam in soil we required a direct chemical analytical method. This paper describes an extraction and clean-up method suitable for routine measurement of flumetsulam in soil using HPLC.

2. Experimental

2.1. Instrumentation

GPC clean-up was performed using a Gilson 432 sample processor with a Shimadzu LC-6A pump and a Pharmacia 10 mm I.D. glass column packed with Bio-Beads SX-3, bed length 50 cm. HPLC was performed using Spectra-Physics 740B pumps, a Rheodyne 7120 manual injector fitted with a 1 ml loop and a Shimadzu SPD-2A variable-wavelength detector. Data acquisition was by peak height using a Spectra-Physics SP4270 integrator. All analyses were performed on a Zorbax SB-C_{18} (15 cm×4.6 mm I.D., 5 μm particle size) reversed-phase column which was held in a Micromeritics column oven compartment.

2.2. Chemicals

All solvents were of pesticide grade (Mallinckrodt, Paris, KY, USA). Sodium sulfate (Mallinckrodt) was heated at 600°C for 6 h and was stored at 50°C before use. Flumetsulam, CAS number 098967-40-9, purity 99.6% was obtained from DowElanco.

2.3. Properties of flumetsulam

Flumetsulam has a molecular mass of 325.3 g/mol, a pK_a of 4.6 and water solubilities of 0.049 g/l at pH 2.5 and 5.65 g/l at pH 7.0. It has an octanol–water partition coefficient of 0.21 and a soil sorption coefficient (K_{oc}) between 5 and 182 l/kg. It is essentially non-volatile, with a vapour pressure of $3 \cdot 10^{-7}$ mPa at 25°C. It has also been shown that no hydrolysis of flumetsulam occurs after 2 months in aqueous solutions buffered to pH 5, 7 and 9 and that no hydrolysis occurs after 6 months under acidic conditions (DowElanco, unpublished data).

2.4. Standard solutions

A primary standard solution at a concentration of 100 μg/ml was prepared by dissolving flumetsulam (99.6%, 5.0 mg) in 50 ml of ethyl acetate. Dilution (1:10) of this stock standard solution was made with ethyl acetate to obtain working standard solution (10 μg/ml). Analytical standards (1–10 μg/ml) for HPLC calibration were prepared from portions of the working standard solution by evaporating the solvent to dryness with a stream of nitrogen and dissolving the residue in methanol–0.5% acetic acid in water (10:90).

2.5. Soil sample fortification

The soil used for this experiment was a Horotiu sandy loam (pH 5.9, organic carbon 6.7%, sand 76%, silt 25%, and clay 8%), sieved to pass 2 mm. A known amount of the primary standard solution (100 μl) or working standard solution (250, 125 and 50 μl) was added to ca. 62.5 g sub-samples of sieved field-moist soil (50 g on oven-dry basis) and the samples thoroughly mixed and stood for 1 h. These additions resulted in flumetsulam concentrations in soil of 200, 50, 25 and 10 μg/kg dry mass, respectively. Three replicates at each fortification level, including unspiked controls, were extracted, cleaned-up and analysed by HPLC as described below.

2.6. Soil extraction

Moist soil sub-samples (62.5 g, ca. 50 g dry mass) were extracted with aqueous sodium hydrogencarbonate solution (0.1 *M*, pH 8.2; 100 ml). The suspension was stirred and sonicated for 3 min. Following centrifugation (10 min, 3000 rpm), the aqueous solution was decanted and the extraction

procedure was repeated twice. The combined extracts were adjusted to pH 2 with 5 *M* HCl (ca. 6–7 ml) and methanol (2 ml) was added. An Empore C_{18} disc (47 mm diameter) with a glass fibre filter (1 μm) on top was conditioned with methanol (10 ml) followed by deionized water (2×10 ml), leaving some water on the disc. The extract solution was passed through the conditioned disc and the eluate discarded. The discs were then dried by air suction for 15 min, and a sample tube (25 ml) was placed under the filter outlet. The adsorbed material was eluted with ethyl acetate (2×10 ml). The first 10 ml was allowed to pre-wet the disc without vacuum for 1 min before being sucked through and then the second 10 ml of ethyl acetate was applied and sucked through. The combined eluates were dried over anhydrous sodium sulfate and rotary evaporated to near dryness.

2.7. GPC Clean-up

The samples were redissolved in 1.6 ml ethyl acetate–cyclohexane (1:1) for GPC clean-up [6]. A volume (1 ml) was injected onto the GPC column and the column was eluted with ethyl acetate–cyclohexane (1:1) at 1 ml/min. The first fraction (21 ml) containing lipids and pigments was discarded and then the pesticide fraction (next 14 ml) was collected and evaporated to dryness with a stream of nitrogen. The residue was redissolved in methanol–0.5% acetic acid in water (10:90) for HPLC analysis.

2.8. Soil extraction using acidified acetone–water

An extraction method using a stronger solvent system [3,4] was also compared in this study. Briefly, flumetsulam residue in soil was extracted using acetone–0.1 *M* HCl (90:10). Following evaporation of the acetone, the samples were diluted with 0.005 *M* HCl and passed through an Alltech (Deerfield, IL, USA) Extract-Clean C_{18} column (500 mg, 2.8 ml). The eluent from the column was evaporated to dryness. Since the sample at this stage was still highly coloured, the residue was redissolved in 1.6 ml ethyl acetate–cyclohexane (1:1) for GPC clean-up as described above before analysis by HPLC.

2.9. HPLC condition for flumetsulam analysis

All analysis were performed on a Zorbax SB-C_{18} reversed-phase column held at 35°C and using a mobile phase of methanol–0.5% acetic acid in water (25:75) run isocratically at a flow-rate of 1 ml/min. The detection was performed at 250 nm and 0.02 a.u.f.s. Sample injection volume was 100 μl.

3. Results and discussion

Flumetsulam eluted from the HPLC as a narrow peak at 13.1 min (see Fig. 2a). The proposed optimum wavelength (250 nm) for measurement of flumetsulam in soil extracts was determined by examining both a standard solution and a blank soil extract using different UV detector wavelengths. Although the response for the flumetsulam standard was greater at lower wavelengths (response at 220–230 nm ca. 4–5 times that at 250 nm), and showed a secondary maximum near 278 nm, the HPLC chromatograms of a blank extract of Horotiu soil showed that interferences from coextractives were comparatively less at 250 nm. There is the potential that other wavelengths may provide optimum sensitivity for extracts from different soil types with different coextractive profiles. Alternatively a diode-array type detector could be used.

A UV detector response curve at 250 nm was obtained by injecting duplicate standard solutions (0.1–100 μg/ml). The response of flumetsulam was linear in the range studied and the correlation coefficient determined was 0.990. Under the conditions used the minimum concentration detectable was about 0.1 μg/ml. This is lower than recently reported by Galletti et al. [7] for HPLC with UV detection of the structurally similar sulfonylurea herbicides.

3.1. Reproducibility

The inter-day reproducibility of the retention time and peak height were examined by using a 2 μg/ml standard and 200 μg/kg spiked soil extracts throughout the course of the experiment; a total of 45 injections of each over 15 days. The results obtained showed that the flumetsulam peak height variabilities

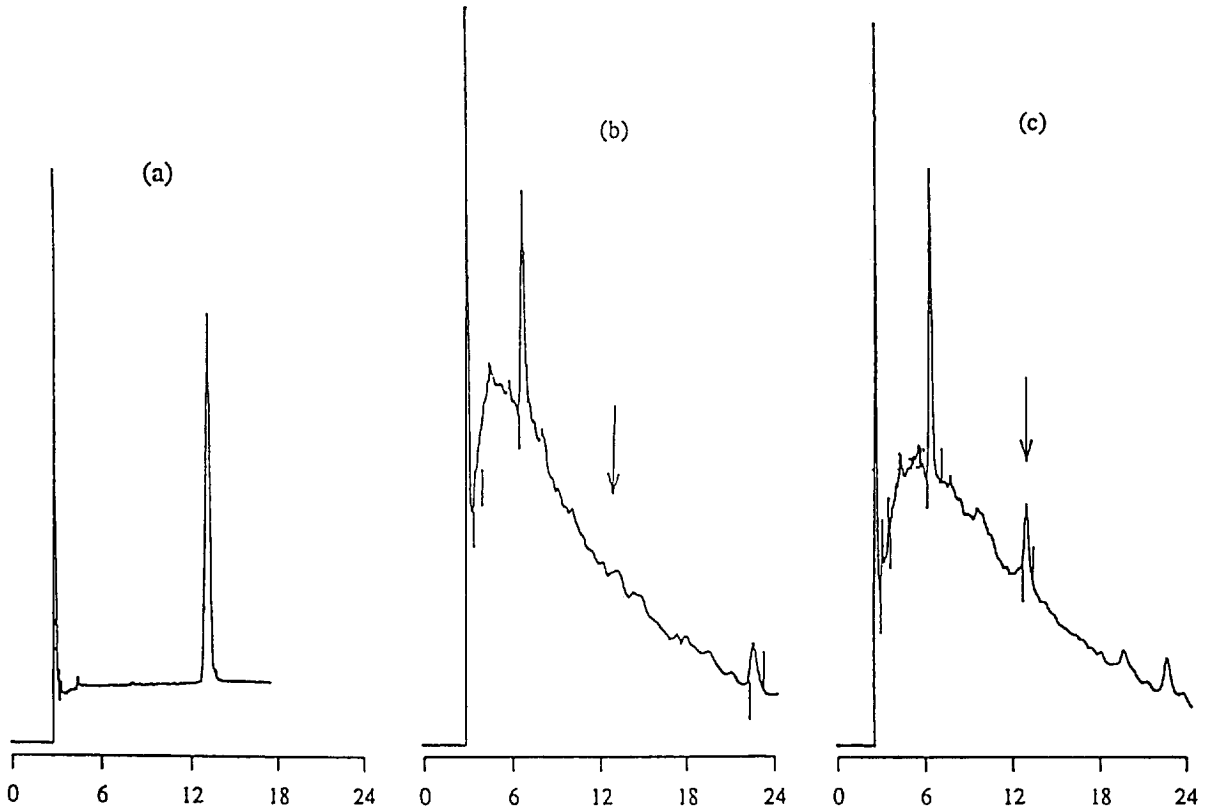

Fig. 2. HPLC chromatograms of (a) flumetsulam standard, (b) untreated soil extract and (c) soil spiked with 10 μg/kg of flumetsulam. The arrow indicates the retention time of the herbicide in either (b) untreated soil, or (c) spiked soil.

for both the 2 μg/ml standard and the spiked soil extract were within 4.6% R.S.D. Retention time fluctuation measured in the same way showed the maximum R.S.D. value of 3.8%.

3.2. Recovery

Fig. 2b and c shows HPLC chromatograms for unspiked soil and for soil spiked at 10 μg/kg, respectively. The chromatograms for unspiked soil showed lack of interference in the retention region for flumetsulam. The recovery data for flumetsulam spiked into soil is presented in Table 1. The recoveries were 66.4 to 69.1% over the spiked range (10 to 200 μg/kg) with good reproducibility, even at low levels (mean R.S.D. 4.8%). The detection limit was 4 μg/kg, limited principally by analyte signal to detector noise ratio, and could be lowered by use of larger injection volumes.

3.3. Extraction

The present extraction method follows one recently published for the analysis of a range of sulfonylurea herbicides in soil [6] using 0.1 *M* $NaHCO_3$ as extractant and previously reported for chlorsulfuron in soil [8,9]. We also compared the extraction and clean-up steps for the measurement of flumet-

Table 1
Recovery of flumetsulam from spiked soil as determined by HPLC

Amount spiked (μg/kg)	Mean recovery (%)[a]	R.S.D (%)
200	67.0	4.4
50	67.5	4.6
25	69.1	3.7
10	66.4	6.6
Mean	67.5	4.8

[a] $n=15$ at 200 μg/kg; $n=3$ at 50, 25 and 10 μg/kg.

sulam in soil using acetone–0.1 *M* HCl and C_{18} SPE column cleanup [4]. Although slightly higher recoveries (79% at 200 μg/kg) of flumetsulam were obtained when using this extraction method, the HPLC background from coextractives was also much greater. The detection limit for flumetsulam in the high organic matter Horotiu soil was 25 μg/kg which was six times higher than that for the aqueous bicarbonate extraction method. We therefore preferred to use the present method for our degradation studies which were designed to allow correlation of extractable residues with plant bioassay data. It was felt that a milder extractant was more appropriate for this.

The workup of the crude extracts was straightforward using the SPE method described. The SPE disc system applied in this method to concentrate the extracts provided enhanced speed, lower solvent use and more reproducible recoveries than liquid–liquid extraction procedure published for other herbicides [8,9]. SPE has also been shown to be more reproducible than liquid–liquid extraction for residues of sulfonylurea herbicides in soil and water [7]. With this method, more samples can be extracted in a day with less glassware and solvent compared to the extraction method using acidified acetone–water [4]. The recoveries, while not complete, were very reproducible in the residue range 10–50 μg/kg and were suitable for studies on degradation of flumetsulam in soil.

3.4. Reversed-phase C_{18} HPLC column

This is the first example of the use of a sterically protected deactivated reversed-phase column for analysis of sulfonamide and sulfonylurea herbicides. Such columns are claimed to provide improved peak symmetry for polar and non-polar compounds as well as prolonged column lifetime with mobile phases of low pH. The HPLC separation conditions for flumetsulam were chosen after testing several options, including the use of internal standards, manipulating methanol concentration in the mobile phase and concentration of acetic acid in the aqueous acetic acid–methanol mixtures. A number of sulfonylurea herbicides were examined as possible internal standards, including thifensulfuron and triasulfuron which eluted closest to flumetsulam. Both isocratic and gradient conditions were tested. Isocratic conditions were found to give the best results for flumetsulam with least interferences, but both thifensulfuron and triasulfuron gave long retention times (79 and 86 min, respectively) which made them unsuitable for use as internal standards. Under gradient conditions, these sulfonylureas eluted earlier. However, interference from untreated soil made quantitation difficult. It was therefore decided to measure absolute recoveries of flumetsulam by the external standard method. Flumetsulam retention time was not affected by changing the concentration of acetic acid in the mobile phase in the range 0–1%.

3.5. Clean-up

Complete recovery of flumetsulam standard sample passed through the Bio-Beads SX-3 column showed that the herbicide was not adsorbed by the gel or affected by the mobile phase (cyclohexane–ethyl acetate). The optimum interval for collection of the flumetsulam fraction from the GPC column with high recovery (>98%) was 22–35 min. GPC provided a reproducible clean-up which removed a great deal of interfering organic matter which otherwise obscured the flumetsulam peak during HPLC determination.

4. Conclusion

A method has been developed for analysis of residues of the sulfonamide herbicide flumetsulam in soil which uses isocratic reversed-phase HPLC conditions with UV detection, without derivatization. Consistent recovery data for flumetsulam from the high organic matter test soil in the concentration range 10–200 μg/kg soil showed the method is adequate for measurement of the residues in soil during degradation studies on the herbicide. A detection limit of 4 μg/kg flumetsulam in soil was established.

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