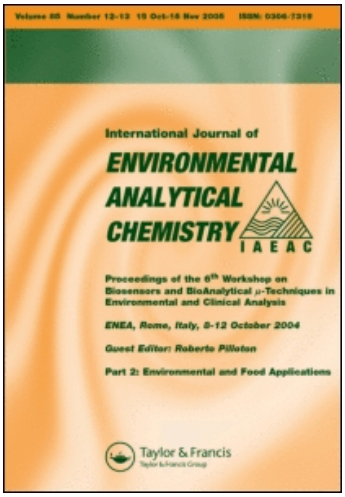

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ULTRA-TRACE ANALYSIS OF METOSULAM, A NEW TRIAZOLOPYRIMIDINE HERBICIDE, IN SOIL USING LC-UV AND TSP-LC-MS

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Metosulam (DE-511), N-(2,6-dichloro-m-tolyl)-5,7-dimethoxy-[1,2,4] triazolo [1,5a]pyrimidine-2-sulphonamide is a new selective herbicide developed for the control of broadleaf weeds in cereals and maize.

The application of new generation low use rate herbicides, such as metosulam, has in turn created the need for the development of ultra-trace residue methodologies. To help investigate the environmental impact of metosulam, levels at sub-part per billion (μg/kg) in soil were determined.

Application of capillary gas chromatography, with various injector systems, was unable to analyse metosulam methyl derivative due to the inherent thermal instability of the molecule. Liquid chromatography with ultraviolet detection (LC-UV) was used to quantify residues of the herbicide following a series of selective clean-up procedures. The chosen methodology used simple reagents plus standard equipment and although the molar absorbtivity of metosulam is poor, analysis of soils of various texture classes at the desired sensitivity level, was achieved.

Thermospray Liquid chromatography with mass spectrometry (TSP-LC-MS) was used as a confirmatory technique for the analysis of metosulam in the same soils. Excellent agreement between the two techniques was observed.

Details of the clean-up procedures used to produce desired selectivity and the relative merit of the two detection systems are discussed.

KEY WORDS: Metosulam, soil analysis, thermospray, liquid chromatography, ultra-trace analysis.

INTRODUCTION

Metosulam (Figure 1) is a new selective herbicide for post-emergence use in cereals and maize for the control of broad leaf weeds by the inhibition of the enzyme acetolactate synthase. It is based on new chemistry and is a member of the triazolopyrimidine family of herbicidal compounds[1].

During the development phase of metosulam its fate in the ecosphere was determined. Part of this process involved field studies to help understand the fate of this compound in soil under normal agricultural practice. These studies involved field dissipation trials and 'at harvest' soil sampling at sites where metosulam was applied to cereals. Application rates for metosulam are low (10–30g/ha) in line with industry commitment to develop lower application rate compounds. To enable meaningful kinetics to be derived from these

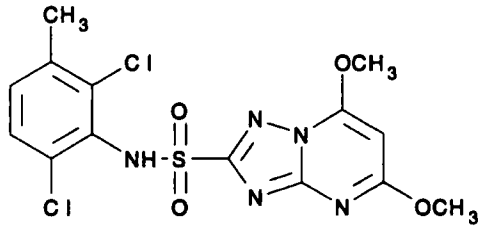

Figure 1 Metosulam

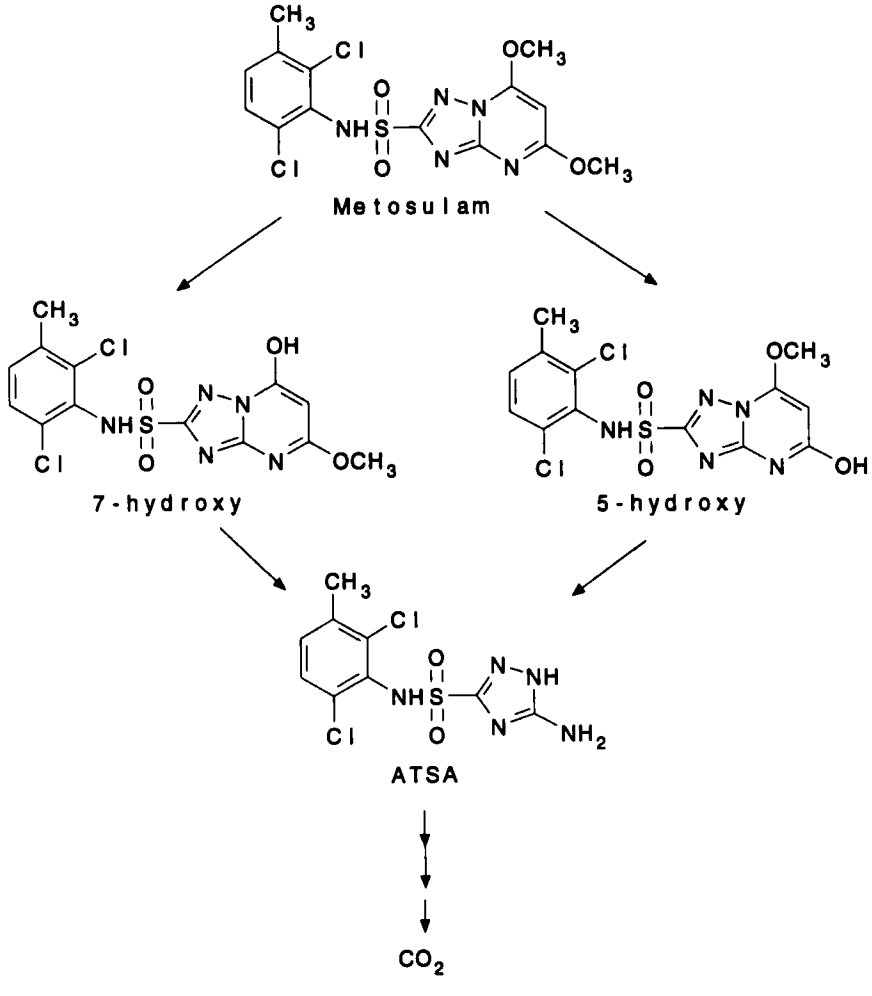

Figure 2 Proposed metabolic pathway for Metosulam in soil

dissipation trials and accurate assessment of soil concentrations in 'at harvest' soil samples, analytical methodology was required to determine at or below μg/kg concentrations of metosulam in soil. In addition, parent metosulam would need to have sufficient selectively to be determined from its metabolites. Laboratory studies show that metosulam degrades via the 5- and 7-hydroxy analogues to form 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulphonamide (ATSA) and eventual mineralisation (Figure 2) by demethylation and partial ring opening of the pyrimidine ring.

Metosulam is a weak acid (pKa 4.8) and has low vapour pressure which makes direct gas chromatography (GC) an impractical option. The virtual lack of available functional groups on the molecule makes derivatisation, with a suitable commercial GC or liquid chromatography (LC) derivatising reagent, difficult. Attempts to derivatise the sulphonamide bridge of the molecule with alkylating reagents were successful, however the resulting N-methyl derivative often produced multiple peaks by GC even with cold on column injection.

Metosulam shows poor sensitivity to liquid chromatography detectors ie. UV, electrochemical, fluorescence. Although the molar absorptivity of metosulam is poor it has been found that with careful selective clean-up procedures, including conversion to the N-methyl derivative, LC-UV can determine metosulam down to 1 μg/kg[2]. By careful adjustments in the analytical procedure it was felt this determination limit could be lowered down to ultra-trace levels.

Thermospray Liquid Chromatography Mass Spectrometry (TSP-LC-MS) has been used for the quantitative analysis of sulphonamide antibiotics in milk at the μg/kg level[3]. TSP-LC-MS could be considered as one of the most suitable mass spectrometry techniques for the determination of medium to high polarity compounds, and was thus considered ideal for both confirmatory and quantitative applications in the case of metosulam residues in soil extracts.

In this paper, we describe the use of LC-UV, following a selective clean-up, and TSP-LC-MS to assay fortified soils and field derived soils samples. The performance of the two techniques was evaluated with respect to each other and their ability to reproducibly assay down to sub-μg/kg levels.

EXPERIMENTAL

Materials

Metosulam analytical standard (>99.5%) was used to make all stock solutions which were stored at ≤4°C. Solvents were all 'distol' or 'HPLC' grade. Silica and aminopropyl Bond Elut cartridges (500mg) available from Jones Chromatography Ltd. All other apparatus were standard commercially available items.

Liquid chromatography with UV detection

Varian Star LC system: consisting of 9010 solvent delivery system, 9095 autosampler with 50μl sample loop and Spectromonitor 3100 ultra-violet detector—available from Varian Associates Ltd.

LC column: 250mm × 2.1mm i.d. Kromasil KR100-5C8 (5μm)—available from Hichrom Ltd.

LC separation of N-methyl derivative of metosulam was achieved using 50:50 acetonitrile/water isocratically at 0.30ml/minute.

Peak height or peak area information using Hewlett-Packard 3350A Laboratory Data System—available from Hewlett-Packard.

Liquid chromatography with MS detection

The liquid chromatography system consisted of a Varian 9010 ternary solvent delivery system and a Varian 9095 autosampler with a 50μl sample loop.

LC column: 35mm × 4.6mm i.d. Kromasil KR100-5C8 column (5μm)—available from Hichrom Ltd.

Mobile phase consisted of 50:50 acetonitrile/0.2% acetic acid. Isocratic program at a flow rate of 1.5ml/minute.

A Finnigan MAT (San Jose, CA, USA) TSQ 70 triple quadrupole mass spectrometer equipped with a Finnigan TSP ion source and interface was used. The column was connected directly to the interface via a 0.5μm in-line HPLC filter. The vaporiser temperature was set at *ca* 100°C and the source and manifold set at 150°C and 70°C respectively.

The mass spectrometer was operated in the multiple ion monitoring mode using chlorine isotope ions at m/z 432, m/z 434 and m/z 436, scan time 0.2s per ion.

The Varian autosampler was activated from the 'analysis' window of the TSQ70 mass spectrometer, with an analysis time of six minutes in total (one minute equilibration time).

Analytical procedure

Metosulam was doubly extracted from 50g soil by shaking with a 100ml mixture of 94:6 acetonitrile/0.2M hydrochloric acid initially for two hours followed by 15 minutes.

The extracts were then centrifuged, decanted and combined prior to evaporating to *ca.* 10ml. Metosulam was partitioned into 2 × 10ml methyl-tertiary-butyl ether prior to loading on a preconditioned aminopropyl Bond Elut cartridge. The cartridge was washed with 5ml dichloromethane and 5ml of 15:85 methanol/dichloromethane followed by elution with 5ml of 5:95 formic acid/dichloromethane.

The eluate was evaporated and reconstituted into 3ml of dichloromethane and loaded onto a silica Bond Elut cartridge. The sample vial was rinsed with 5ml of dichloromethane which was used to wash the cartridge. Metosulam was then eluted from the cartridge using 8ml of 2.5:97.5 methanol/dichloromethane. The eluate was evaporated to dryness and metosulam was purified by extractive methylation. This utilised the formation of an ion-pair with 500μl of 50:50 tetrabutyl ammonium hydroxide in ammonia solution followed by methylation with 50μl of methyl iodide in 1ml of toluene. The samples were agitated using a vortex mixer at room temperature for 90 minutes.

After methylation, 10ml of water was added and metosulam was extracted into 2 × 7ml of toluene. The toluene layers were then applied to a conditioned silica Bond Elut cartridge.

The cartridge was washed with 5ml of 5:95 acetone/toluene prior to elution with 5ml of 10:90 acetone/toluene.

The acetone/toluene mixture was evaporated to dryness and the residuum reconstituted in 250µl of mobile phase. Quantification was carried out by LC of metosulam methyl derivative using an ultraviolet detector at 240nm. A schematic overview of the procedure is shown in Figure 3.

RESULTS AND DISCUSSION

Although the inherent UV sensitivity of metosulam is poor 1.86×10^4 l/mol.cm, simple selective clean-up procedures incorporating scale-up factors such as increasing soil weight

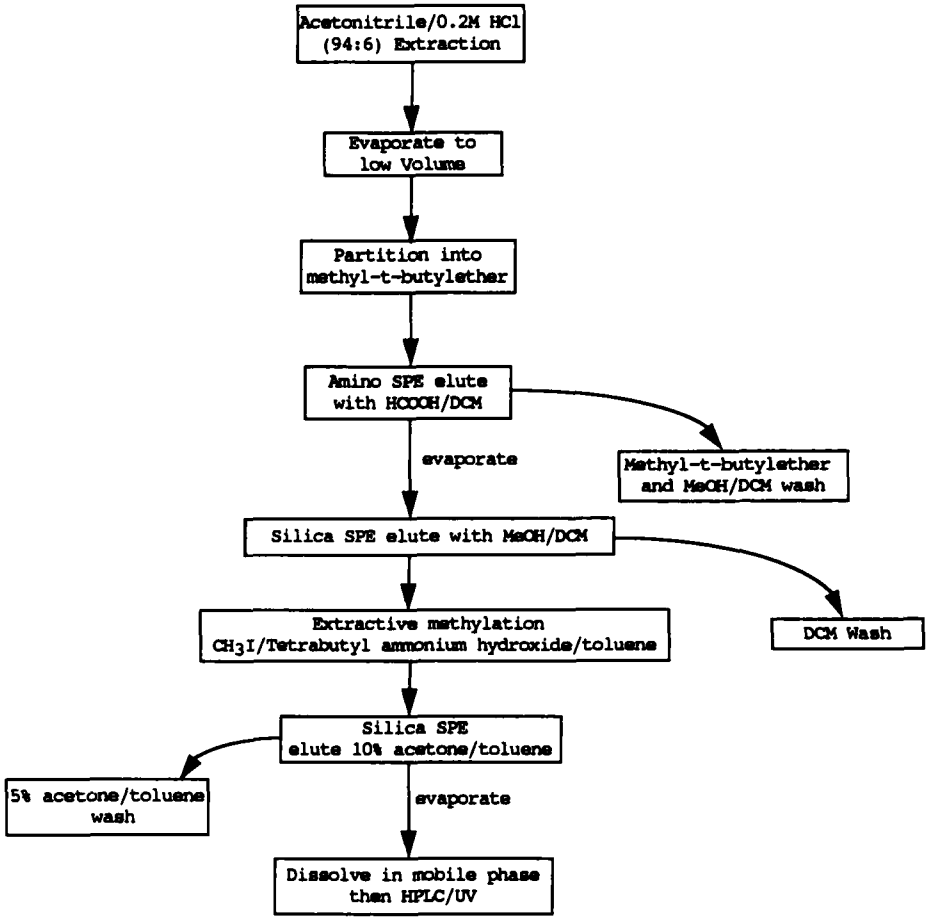

Figure 3 Schematic overview of residue methodology for determining Metosulam in soil

and decreasing final solution volume, enabled sub-μg/kg quantification of metosulam in soil. Excellent chromatographic selectivity was obtained between metosulam-methyl, its structurally related metabolites and soil co-extractives.

The clean-up stages utilised readily available, affordable equipment and reagents found in most laboratories. The amino solid phase cartridge was found to be critical in removing all 7-hydroxy and ATSA metabolites and the majority of the 5-hydroxy metabolite. The remainder of the 5-hydroxy metabolite was removed by the subsequent silica solid phase cartridge clean-up. It was vital to remove these metabolites, in particular the mono hydroxylated analogues, as these would form metosulam-methyl on methylation leading to inaccurate quantification. In addition the two solid phase clean-up steps removed substantial quantities of polar material and pigments. The use of extractive methylation, via ion-pair formation, provided further clean-up and conversion to the N-methyl derivative which altered favourably its chromatographic selectivity. The final silica solid phase cartridge provided a post methylation clean-up of the derivatised extract. The degree of clean-up is clearly demonstrated with quantification down to the desired ultra-trace range (Figure 4).

The soil residue method gave recoveries (Table 1) ranging from 81–117% (x = 97%). Procedural recoveries during the course of our investigations were typically in this range. UV detector linearity, in the range 0.02–2.0μg/ml, over the period of analysis was excellent (r = 0.9998 RSD = 0.018%).

TSP-LC-MS was used for the analysis of a range of fortified and trial samples taken over the experimental period often in a fully automated mode. The excellent selectivity of the mass spectrometer gave little or no contribution from the soil substrate (Figure 5). This

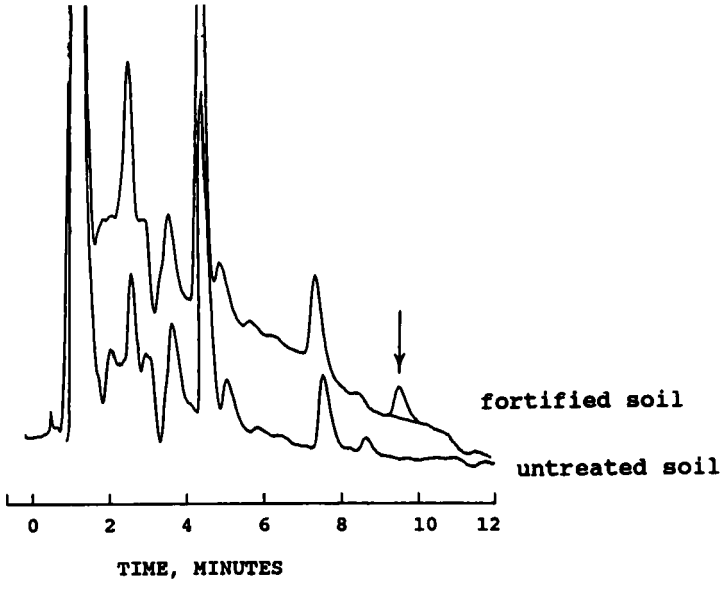

Figure 4 Chromatogram of 0.2μg/kg of Metosulam in soil determined by LC-UV

Table 1 Recovery data of Metosulam in soil quantified by LC-UV

Soil Type	*Fortification Rate (µg/kg)*	*% Recovery*
Loamy Sand	0.2	104, 104
Clay Loam	0.2	101, 117
Silt Loam	0.2	87, 81
Clay Loam	0.5	94, 96
Silt Loam	1.0	92, 97
		Mean $\overline{97 \pm 10}$

enabled the use of a shorter analysis time and rapid sample throughput. Approximately 70 injections can be performed over a working day.

Each day of operation the linearity of the TSQ as a detector was verified over a 6 batch experimental period. For a series of standards in the range 0.02µg/ml to 1.0µg/ml the correlation coefficient was 0.9918 (RSD = 0.623%). This compares favourably with that seen for LC-UV.

The instrument sensitivity and reproducibility was enhanced by injecting a number of 'soil matrix' extracts prior to analysis of a batch of samples. This conditioning appeared to give improved signal stability during the analysis period and will be vital to include in future analysis protocols with this instrument.

During the experimental period good agreement between the LC-UV and TSP-LC-MS was observed (Figure 6).

The correlation between these two systems was r = 0.9245. The data shown was derived from 81 results during this period. Eight of these results were not detected by TSP-LC-MS but had values quantified by LC-UV. These results were all between 0.2 and 0.35µg/kg just above the lowest validated level. Close inspection of these eight samples revealed the

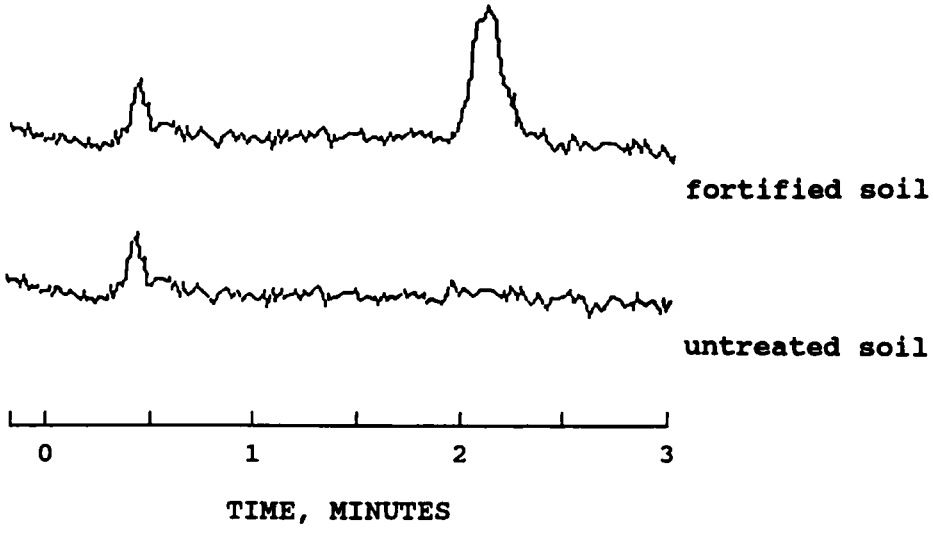

Figure 5 Chromatogram of 0.2µg/kg of Metosulam in soil determined by TSP-LC-MS

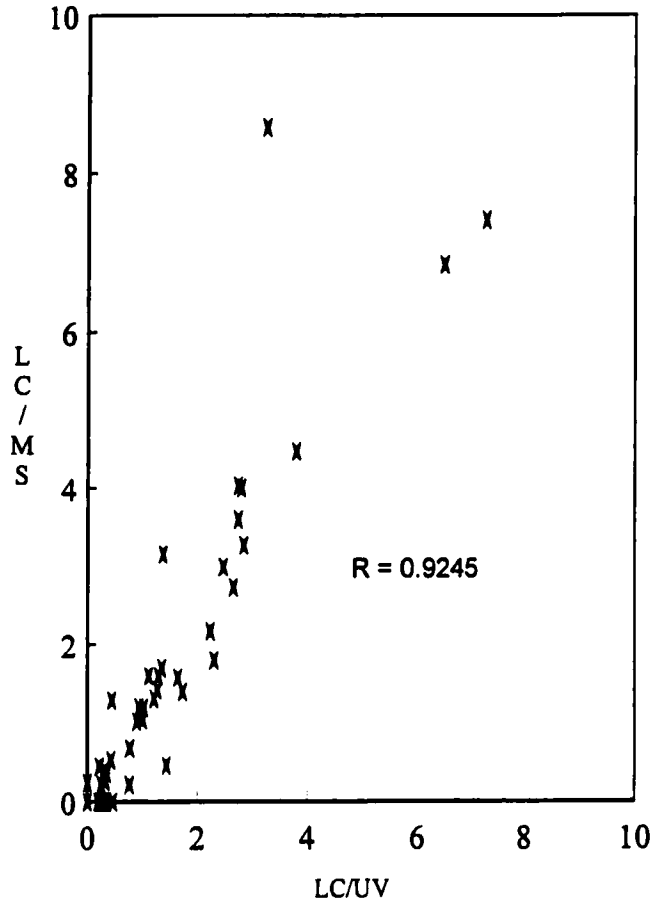

Figure 6 Correlation between results, in µg/kg, of Metosulam in soil by LC-UV and TSP-LC-MS

majority of these soils to be of high organic matter. Very few apparently erroneous results were obtained, however, it is interesting to note an apparent positive bias in favour of the TSP-LC-MS data. Although this bias is small future investigations will be carried out to help explain this effect.

CONCLUSIONS

The combination of LC-UV and TSP-LC-MS, with selective clean-up, provided reliable data which helped understand the fate of metosulam in soil. The combination of the techniques was especially valuable in determining metosulam in high organic matter soils at or near the lowest validated level. Both techniques are complimentary and if coupled

on-line (LC-UV-MS), with suitable adjustments in chromatographic conditions, would provide extra confidence in data generated at sub-μg/kg concentrations.

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