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Short Communication

Specific gas chromatography–mass spectrometry analytical method for the determination of cyhexatin in animal feed

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ABSTRACT

The acaricide tricyclohexyltin hydroxide (cyhexatin) was determined in animal feed samples, using gas chromatographic–mass spectrometry in the electron impact mode. Sample extraction and derivatization (converting the analyte to an alkylated derivative) were performed using a tricyclopentyl analogue of this acaricide as internal standard to obtain a better analytical precision.

INTRODUCTION

Tricyclohexyltin hydroxide (cyhexatin; Fig. 1) is an acaricide effective against mites, used in a wide range of vegetables [1]. Methods of analysing this compound in commercial insecticide samples [2] and environmental samples [3,4] have been developed. Müller and co-workers [3,4] measured cyhexatin in water, sediments [3] and soil [4] samples, using ethyl magnesium bromide as a derivatizing reagent. Our goal was to develop a specific analytical method for cyhexatin to test animal feed samples for toxicological investigation. The method developed represents a modification of those described by Müller and co-workers. The resulting ethylated derivative was analysed by gas chromatography (GC)–mass-spectrometry (MS) operating in the selected ion recording mode. Tricyclopentyltin hy-

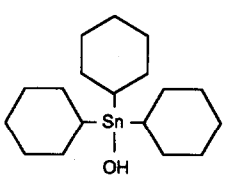

Fig. 1. The formula of cyhexatin.

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droxide was added as internal standard before extraction of the samples.

MATERIALS AND METHODS

Reagents

Cyhexatin and tricyclopentyltin hydroxide were from Oxon Italia (Pero, Milan, Italy); *n*-hexane (pesticide grade), hydrochloric acid, anhydrous calcium chloride and sodium sulphate were from Merck (Darmstadt, Germany); diethyl ether and 2-hydroxy-2,4,6-cycloheptatrien-1-one (tropolone) were from Fluka (Buchs, Switzerland); 2 *M* ethyl magnesium bromide in tetrahydrofuran was from Aldrich (Milwaukee, USA); Supelclean LC-Si silica gel columns were from Supelco (Bellefonte, USA).

TABLE I

INSTRUMENTAL CONDITIONS

Gas chromatograph	Varian 3400
Mass spectrometer	Finnigan MAT Incos 50
GC column	J&W DB-5, 30 m × 0.25 mm, film thickness 0.25 μm
Carrier gas	Helium, 0.5 m/s
GC injector	270°C, splitless mode (valve closed for 1 min, then open, split flow 10 ml/min)
GC programme	80°C for 2 min, 20°C/min gradient up to 270°C, final temperature maintained for 10 min
Ions monitored	315 *m/z* for cyhexatin 287 *m/z* for the internal standard
Scan time	50 ms

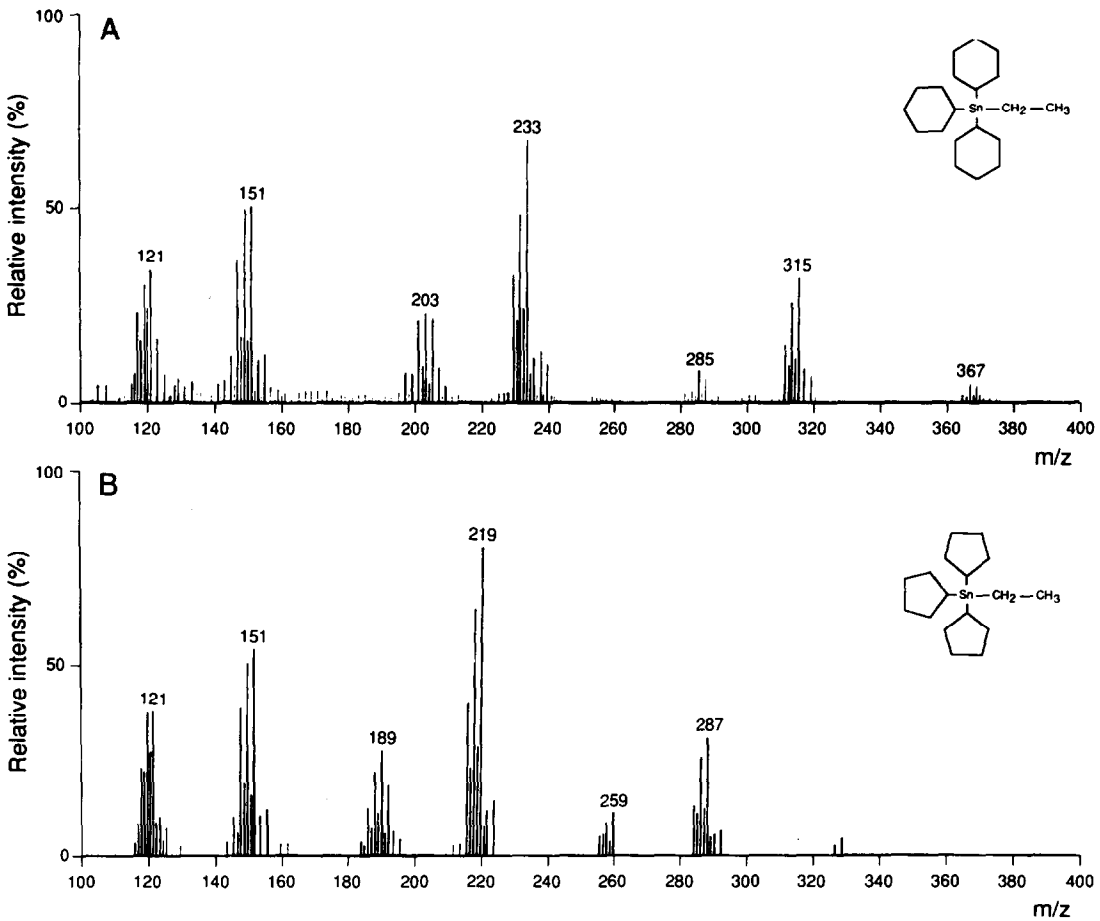

Fig. 2. (A) Mass spectrum of derivatized cyhexatin. (B) Mass spectrum of derivatized tricyclopentyltin hydroxide.

Standard solutions

Standard solutions of cyhexatin and tricyclopentyltin hydroxide were prepared in acetone.

Animal feed composition

The animal feed contained carbohydrates (56.3%, of which starch 38.6%), protein (17.7%), lipids (3.2%), cellulose (4.7%), water (12.9%) and ash (5.2%), including calcium (8400 mg/kg), sodium (2300 mg/kg), potassium (7300 mg/kg), phosphorus (5100 mg/kg) and magnesium (2130 mg/kg).

Sample extraction and derivatization

Analyses were done on standard animal feed with different amounts of cyhexatin added.

A 4-g aliquot of each sample was weighed. After addition of 80 μg of internal standard in acetone, the samples were acidified with 0.5 ml of 2 *M* hydrochloric acid then extracted twice with 10 ml of a 0.25% tropolone solution in diethyl ether. The organic phase was centrifuged and filtered on anhydrous calcium chloride. After concentration to 2 ml under a gentle stream of air, this extract was ready for derivatization according to Müller [3].

The derivatized extract was purified with a silica gel microcolumn (Supelclean LC-Si); the sample was first deposited on the column, then eluted with 10 ml of a diethyl ether–*n*-hexane (10:90) solution. A 1-μl aliquot of this solution was injected into the

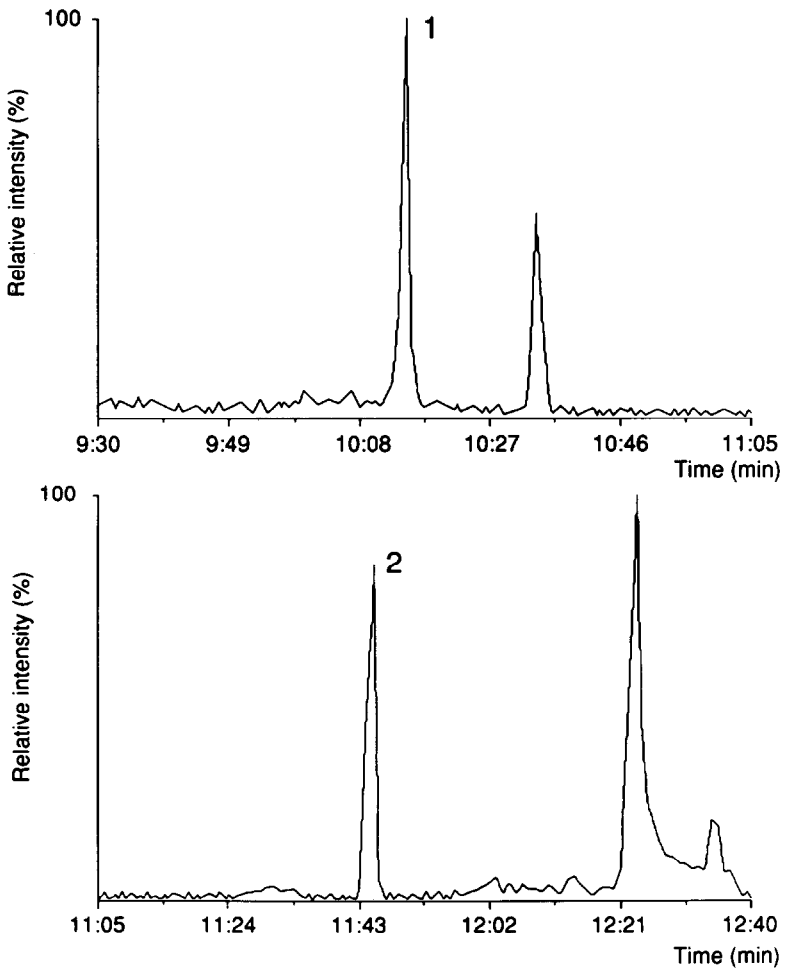

Fig. 3. Fragmentograms of *m/z* 315 (upper panel) and *m/z* 287 (lower panel), relative to a standard feed sample, showing the cyhexatin (1) and the internal standard (2) peaks.

GC–MS system for analysis. The GC–MS instrumental conditions are listed in Table I.

Calibration curve

Calculations were made on the basis of the analyte/internal standard peak area ratios in the samples with reference to a calibration curve obtained with standards of herbicide-free feed (4 g each), enriched with known amounts of cyhexatin (0, 10, 20 and 40 μg) and a constant amount of internal standard (80 μg).

RESULTS

Fig. 2 shows the mass spectra of cyhexatin (Fig. 2A) and of the internal standard (Fig. 2B) after derivatization.

The chromatogram of a calibration curve sample is shown in Fig. 3. The data of the calibration curves obtained on different days are reported in Table II as well as the standard deviation of these results. Average ratios and standard deviations of the 1-day replicates (four replicates for each concentration point) were: 0.22 ± 0.025 (for 2.5 μg/g), 0.493 ± 0.04 (for 5 μg/g) and 1.049 ± 0.133 (for 10 μg/g).

The calibration curve obtained using the internal standard in several cyhexatin-enriched feed samples at different concentrations is shown in Fig. 4. Linearity was good in the concentration range of interest. The blank feed samples and those spiked only with the internal standard made no detectable contribution and did not interfere with the cyhexatin peak.

Other organotin compounds similar to cyhexatin (triphenyl and tributyltin hydroxides) were tested as internal standards, together with the cyclopentyl analogue: this last gave the best results. Indeed, the other compounds gave a poor 1-day reproducibility in the fortified diet. Deuterium-labelled cyhexatin could be used as internal standard instead of the cyclopentyl analogue to achieve better accuracy.

The recoveries in feed samples varied from 46.1 to 77.4% with a mean of 56%; calculations were based on the internal standard peak areas in comparison with those found in pure samples of the ethylated compound. The relative standard deviation (R.S.D.) relative to eight replicates of a standard solution of cyhexatin plus the internal standard, derivatized as described, was 13.5%. The analysis of this same standard solution added to the diet (at a level of 5 μg/g) gave a R.S.D. of 15.0% for n (number of replicates) = 13. This suggests that the derivatization step is the major source of the variation found in the recoveries assay.

Considering the addition of cyhexatin in various amounts to samples of uncontaminated diet, the limit of detection of the method was about 20 ng/g. The limit of quantitation was about 45 ng/g.

TABLE II

RAW DATA OF EACH ASSAY CONCENTRATION DETERMINED ON DAY-TO-DAY REPLICATES

	Cyhexatin/internal standard response ratio		
	Concentration (μg/g)		
	2.5	5	10
	0.265	0.444	0.965
	0.172	0.380	1.055
	0.188	0.450	0.947
	0.240	0.442	1.040
	0.312	0.558	0.956
		0.494	1.035
		0.420	0.857
		0.466	1.035
		0.444	0.791
		0.365	0.714
		0.338	1.107
		0.555	0.999
		0.522	
Mean	0.235	0.452	0.958
S.D.	0.057	0.068	0.117

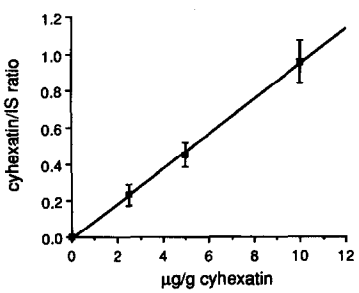

Fig. 4. Calibration curve of the method: each point is the mean ± S.D. of n samples: 0 μg/g (n = 1), 2.5 μg/g (n = 5), 5 μg/g (n = 13), 10 μg/g (n = 12). $y = -0.0072 + 0.0956x$. IS = Internal standard.

CONCLUSIONS

MS was used successfully to analyse an organotin compound, cyhexatin, and the method was more specific than using conventional GC detectors.

The use of an analogue of cyhexatin as internal standard for quantification ensures good precision in the desired concentration range.

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