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Determination of dithiocarbamate pesticides in occupational hygiene sampling devices using the isooctane method and comparison with an automatic thermal desorption (ATD) method

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Abstract

Two new methods for the determination of dithiocarbamate pesticides in occupational hygiene sampling devices are described. Dithiocarbamate spiked occupational hygiene sampling devices, consisting of glass fibre (GF/A) filters, cotton pads, cotton gloves and disposable overalls, were reduced under acidic conditions and the CS_2 evolved as a decomposition product was extracted into isooctane. The isooctane was then analysed using gas chromatography with mass spectrometry, for CS_2, which provided a quantitative result for dithiocarbamates. Recoveries obtained were generally within a 70–110% range and reproducibilities better than 15% RSD were typically achieved. The method has been successfully applied to samples collected during occupational exposure surveys. A second method employing automatic thermal desorption–gas chromatography–mass spectrometry (ATD–GC–MS) has also been developed and applied to the direct analysis of GF/A (airborne) samples. The method relies on the thermal degradation of dithiocarbamates to release CS_2, which is used to quantify the analytes. Thiram spiked GF/A filters gave an average recovery of 107% with an RSD of 4%. The performance of the two analytical methods were directly compared by analysing sub-portions of GF/A filters collected during a survey to evaluate occupational exposures to thiram during seed treatment operations. Both methods performed well for the analysis of airborne (GF/A) samples and produced results in good agreement. ATD–GC–MS is the preferred method for studies involving GF/A (airborne) samples only. Because of the wider applicability of the isooctane method for other sampling devices, it is the preferred choice when carrying out surveys which require a dermal as well as respirable exposure assessment.

Keywords: Thermal desorption; Air analysis; Dithiocarbamates; Carbamates; Pesticides

1. Introduction

Dithiocarbamates (DTCs) are widely used as fungicides in agriculture because of their high chemical and biological activity and low production costs. DTCs are also used as accelerators in rubber vulcanisation and as lubricants [1]. Generally the DTCs are not considered to be highly toxic, however, toxicity is increased with the presence of a heavy metal ion in the molecule, such as iron in ferbam [2]. Short-term exposure to DTCs can cause eye, respiratory

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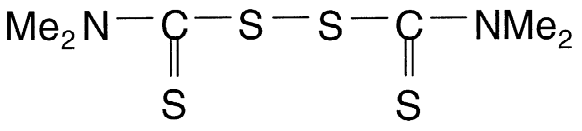
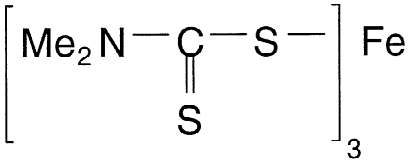
and skin irritation whilst long-term exposures may cause dermatitis and skin sensitisation. The structures of three common DTCs—ferbam, thiram and ziram which were used in this study—are shown in Fig. 1.

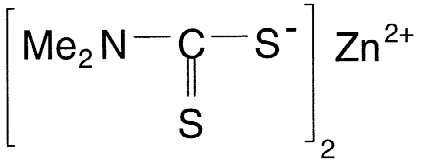
The Health and Safety Executive (HSE) conducts occupational exposure surveys to determine the potential risks to operators when pesticides are applied [3–6]. Recently this has involved assessments of exposure to pesticides during seed treatment applications carried out at farms and at larger scale treatments sites. Thiram, a DTC, was identified as one of the pesticides commonly used in these seed treatments. For the purposes of the survey, respirable exposure was determined by collecting pumped air samples onto glass fibre (GF/A) filters, while dermal exposure was determined from depositions on a series of cotton gauze pads, lightweight cotton gloves and disposable overalls worn by the worker(s) [7–9].



THIRAM



FERBAM



ZIRAM

Fig. 1. Structures of ferbam, thiram and ziram.

Analysis of DTCs has been the subject of numerous papers [10–20]. Many analysis techniques have been applied to DTC residues on foodstuffs, the most widely used being spectrophotometric [16,17] and head space gas chromatography (GC) analysis of carbon disulphide (CS_2) evolved during acidic treatment of DTC residues [18,19].

Jongen et al. [20] used high-performance liquid chromatography (HPLC) and headspace GC to determine occupational exposure (as inhalatory and dermal exposure) to the ethylenebis dithiocarbamates maneb and zineb. HPLC analysis allows speciation of the DTCs, whereas GC provides a more sensitive determination.

The GC method for the analysis of thiram in foodstuffs involves the addition of acidic tin(II) chloride to the substrate, heating and then headspace analysis for CS_2, a decomposition product of DTCs. The main drawback of this method is that it requires manual injection which is labour intensive and has the potential to increase errors. Additionally, it precludes overnight and weekend analysis which may reduce sample throughput.

To overcome these limitations, we developed an automatic thermal desorption–gas chromatography–mass spectrometry (ATD–GC–MS) analytical method. GF/A filters rolled into glass Perkin-Elmer ATD tubes were analysed for the CS_2 evolved by thermal decomposition of the analyte during the thermal desorption process. Quantification was carried out by comparison with a set of dithiocarbamate standards analysed using the same method. Acceptable recovery data were achieved (i.e within 70–110% [21]).

The ATD–GC–MS method was also applied to the analysis of dermal sampling devices. The spiked devices were extracted using an appropriate solvent and 25-μl aliquots of the extracts were impregnated onto GF/A filters contained within ATD tubes then analysed by ATD–GC–MS for CS_2. However, recoveries were found to be low and variable. We therefore decided to investigate an alternative method for dermal devices to provide improved recoveries.

Recently there has been much interest in the so-called isooctane method [22–24] for the analysis of

DTCs in foodstuffs where the CS_2 evolved, following heating under acidic conditions, is extracted into a layer of isooctane which is then analysed for CS_2 content by GC employing MS or flame-photometric detection (FPD). Our laboratory has applied the isooctane method to the analysis of DTCs on a range of spiked occupational hygiene sampling devices and devices collected from surveys assessing exposures to thiram. Recoveries and reproducibilities have been investigated and found to be within acceptable limits [21]. In addition three surveys were carried out monitoring for thiram as airborne (GF/A) exposure only, specifically to allow the two analytical methods to be compared on field samples.

The lack of specificity associated with the two methods described does not present a problem for our work because DTCs are usually applied individually in seed treatments.

Full details of the development and assessment of the isooctane method (for all sampling devices) and ATD–GC–MS (GF/A only) are presented in this paper.

2. Experimental

2.1. Chemicals and solutions

Ferbam (purity 84.5%), thiram (purity 99%) and ziram (purity 99.5%) were obtained from Qmx Labs, Great Yeldham, Halsted, UK. Carbon disulphide (purity 99.9%) was obtained from Sigma–Aldrich, Poole, UK. The dichlorodimethylsilane (99%, Aldrich) for the silanisation of glassware was diluted with cyclohexane to give a 5% solution. All solvents were Distol grade (Fisher).

DTC stock solutions (50–300 μg/ml) were prepared in acetone (ferbam), ethyl acetate (thiram and ziram); CS_2 standards were prepared in isooctane. Calibration standards were prepared gravimetrically from the stock solutions. All stock and standard solutions were stored in the dark at 4 ± 2 °C.

2.2. Glassware and bottles

To prevent the possibility of sample adsorption, all glassware was deactivated by rinsing thoroughly with a 5% solution of dichlorodimethylsilane in cyclohexane. The glassware was then rinsed three times in cyclohexane and washed (end-capped) with methanol.

Glass ATD tubes were obtained from Perkin-Elmer (Beaconsfield, UK). PTFE bottles of sizes between 30 and 1000 ml, and glass bottles with polypropylene screw caps and pouring rings of sizes between 100 and 500 ml, were obtained from Fisher Scientific UK. React-Vials (10 ml) with PTFE–silicone backed seals and screw caps were supplied by Pierce and Warriner, UK.

2.3. Sampling devices

Glass fibre (GF/A) filters (Whatman, Maidstone, UK), lightweight cotton gloves (RS Electrical Components, Corby, UK), cotton filmated swabs (Philip Harris Medical, Birmingham, UK) and Tyvek Protech® disposable overalls (DuPont, UK) were used.

2.4. Instrumentation and apparatus

The ATD–GC–MS system consisted of a Perkin-Elmer ATD 400 automatic thermal desorption system with Perkin-Elmer ATD Control software (version 1.0A). The ATD was interfaced with a Hewlett-Packard 5890A gas chromatograph fitted with a Hewlett-Packard HP-1 column (cross-linked polydimethylsiloxane, 60 m×0.25 mm, 0.25 μm film thickness) and a Hewlett-Packard 5970 series mass-selective detector with Hewlett-Packard G1034C MS ChemStation software. ATD was performed in a two-stage desorption process with a transfer of desorbed vapours to the gas chromatograph by a regulated flow of helium via a transfer line heated at a constant 225 °C. Primary desorption was for 10 min at 400 °C with vapours being trapped onto the cold trap (fitted with Perkin-Elmer air monitoring trap) set at −30 °C followed by secondary desorption from the cold trap at 350 °C for 5 min. The ATD 400 was set up to give a split ratio of ~10% (single split; desorb flow 20 ml/min; outlet flow 10 ml/min). The transfer line to mass spectrometer was set at 280 °C and the oven temperature programme was 40 °C for 5 min, ramping at 2 °C/min to 50 °C; total run time is 10 min. Helium (>99.996%) was used as the carrier gas and electronic pressure control in constant

flow mode delivered 0.98 ml/min. Selected ion monitoring (m/z 76 and 78, for CS_2) data were collected throughout the 10-min run.

The GC–MS system used for the isooctane analysis consisted of a Hewlett-Packard 5890 series II gas chromatograph fitted with a Hewlett-Packard HP5-MS column (5% phenyl and 95% dimethylsiloxane, 30 m×0.25 mm internal diameter, 0.25 μm film thickness) and a Hewlett-Packard 5972 Series mass selective detector with Hewlett-Packard G1701 BA ChemStation software. The GC system was operated in split mode with a split ratio of ~20.4:1. The transfer line to the mass spectrometer was set at 280 °C and the oven temperature programme was 40 °C for 2 min, ramping at 20 °C/min to 100 °C; total run time is 5 min. Helium (>99.996%) was used as the carrier gas and electronic pressure control in constant flow mode delivered 0.98 ml/min. Selected ion monitoring (m/z 76 and 78 for CS_2) data were collected between 0 and 2 min.

A Decon F5400b Ultrasonic Bath (Decon Labs, Hove, UK) was used for sample extraction.

2.5. Procedures

2.5.1. Spiking devices

Occupational hygiene sampling devices were spiked by syringe, with either ferbam, thiram or ziram. Spike levels were: 4.8–5.7 μg on GF/A filters, 5–100 μg on cotton pads, 15–100 μg on cotton gloves, 145–187 μg on socks and 225 μg (thiram only) on overalls. Following spiking, samples were left for 1 h to allow the solvent to evaporate.

2.5.2. Field samples

Field samples were exposed during various operations in the seed treatment industry, including treating and bagging of seed and also cleaning down of equipment and machinery. On the three sampling visits, where only air (GF/A) samples were collected, two pumps were used per sampling point. All samples were collected according to standard occupational exposure survey protocol [7–9] and analysis for thiram was performed.

2.5.3. Extraction/work-up

2.5.3.1. ATD–GC–MS method

GF/A samples were rolled up and inserted into a glass ATD tube which was then capped at both ends using PTFE caps and analysed for CS_2 by ATD–GC–MS. Calibration standards were prepared by spiking strips of GF/A filter, contained in a glass ATD tube, with known amounts of DTC and removing the solvent under a gentle stream of nitrogen.

2.5.3.2. Isooctane method

The contaminated device is placed in the appropriate silanised vessel: 10 ml Reacti-Vial for GF/A filters, 100-ml bottle for cotton pads, 250 ml bottle for cotton gloves and 500 ml bottle for socks. Because of their bulk, disposable overalls were solvent desorbed (750 ml ethyl acetate) and a 5-ml portion gently reduced to dryness under nitrogen in a 10-ml Reacti-Vial. Tin(II) chloride (1.5%) in 5 *M* HCl; 4 ml for GF/A, 70 ml for pads, 175 ml for gloves and 400 ml for socks and isooctane; 1 ml for GF/A, 15 ml for pads, 25 ml for gloves and 50 ml for socks were added and then the vessels were sealed with appropriate caps. Samples were incubated in a water bath at 75–80 °C for 90 min with occasional swirling and then allowed to cool to room temperature. Aliquots of the isooctane layers were then transferred to GC vials for analysis of CS_2 by GC–MS. Quantification was carried out by comparison with a set of CS_2 in isooctane standards.

If multi-residue analysis is required, samples can be extracted (sonication for 30 min) into ethyl acetate. After removal of a small quantity for standard residue analysis, a 5-ml portion of extract is transferred to a Reacti-Vial and reduced to dryness under a stream of nitrogen. The procedure for GF/A filters is then followed. A set of field samples was successfully analysed in this manner.

3. Results and discussion

All results are quoted as DTC, based on CS_2 content.

3.1. ATD–GC–MS method

Replicate spiking of thiram (8.634 μg) onto GF/A filters and subsequent ATD–GC–MS analysis of the whole filter gave an average recovery of 107±4% (±RSD %) for $n=10$.

Blank GF/A filters consistently exhibited a small CS_2 peak and so normal $3\times S/N$ criteria for the determination of limits of detection (LOD) could not be applied. The reliable LOD, based on a low calibration standard, was estimated to be ~0.05 μg per filter (Table 1). The split flow settings used throughout the work gave a quantification range of 0.05–15 μg per filter which is adequate for all loadings likely to be experienced in this type of survey.

Calibration graphs were linear with correlation coefficients of 0.997 or better for concentrations between 50 and 1500 ng of DTC per filter. For concentrations up to 50 ng per filter, calibration graphs were in a quadratic form.

3.2. Isooctane method

For the isooctane method the appropriate conversion factor is used to give a result in DTC from the CS_2 result. For example: 1 mol thiram decomposes to give 2 mol CS_2. RMMs: thiram=240, CS_2=76 (so 2 mol=152). Conversion factor therefore=240/152=1.58, and thiram result=CS_2 result×1.58.

Fig. 2 shows chromatograms from a 1.0-μg/ml CS_2 standard and a thiram exposed cotton pad field sample. The CS_2, which elutes at ~1.3 min, is well resolved from interferences.

Calibration graphs were linear (correlation coefficients of 1.000) over the standard range, i.e. 0.1–30 μg/ml using six calibration points.

Table 2 provides the recoveries obtained from a selection of thiram spiked sampling devices analysed using the isooctane method. Recoveries from all devices are generally very good, in particular the GF/A and cotton pad results, while lowest figures are from thiram spiked socks, gloves and overalls although given the likely loading values, these are still acceptable.

Estimated LODs ($3\times S/N$) are shown in Table 1. With the exception of disposable overalls, no blank interferences were observed, and in this case it was the equivalent of 12 μg per overall. This is many times lower than a typical loading from a survey, but it may be possible to improve on this if required, by concentrating a larger volume of the solvent extract to dryness.

Thiram results obtained using the isooctane method on samples collected from the occupational exposure surveys (Table 3) showed that the LODs were adequate for the loadings experienced. Furthermore, the results obtained from those samples on which multi-residue analysis was performed (Table 4) showed good correlation with other analytes in terms of levels found and the order of contamination, i.e. thiram concentrations were consistently the second or third highest of a total of seven analytes.

3.3. Comparison of ATD–GC–MS and isooctane methods for GF/A (airborne) samples

Once the isooctane method had been developed and recoveries shown to be good, a direct comparison between the two methods was performed. Three surveys were carried out monitoring for thiram on GF/A filters only. Each sampling point had two pumps and prior to analysis each filter was cut in half, with one half being analysed by the isooctane method and the other half being analysed by the ATD–GC–MS method. A set of sample results (recovery corrected) is shown in Table 5.

The result shows the good agreement between the two methods, with trends being similar. Any differences may be due to sampling variations occurring across the filter or as a result of handling the filter during cutting.

The ATD method is easier and more straight-

Table 1
Limits of detection for occupational hygiene sampling devices

Device	Estimated limit of detection (μg/device)			
	Ferbam	Thiram	Thiram (ATD)	Ziram
GF/A	0.09	0.05	0.05	0.06
Pad	0.82	0.7	–	0.9
Glove	1.4	1.2	–	1.5
Sock	2.7	2.4	–	3
Overall	14	12	–	

LODs are based on $3\times S/N$ with the exception of the ATD method which is quoted as a ''reliable'' LOD, necessary due to small blank interferences.

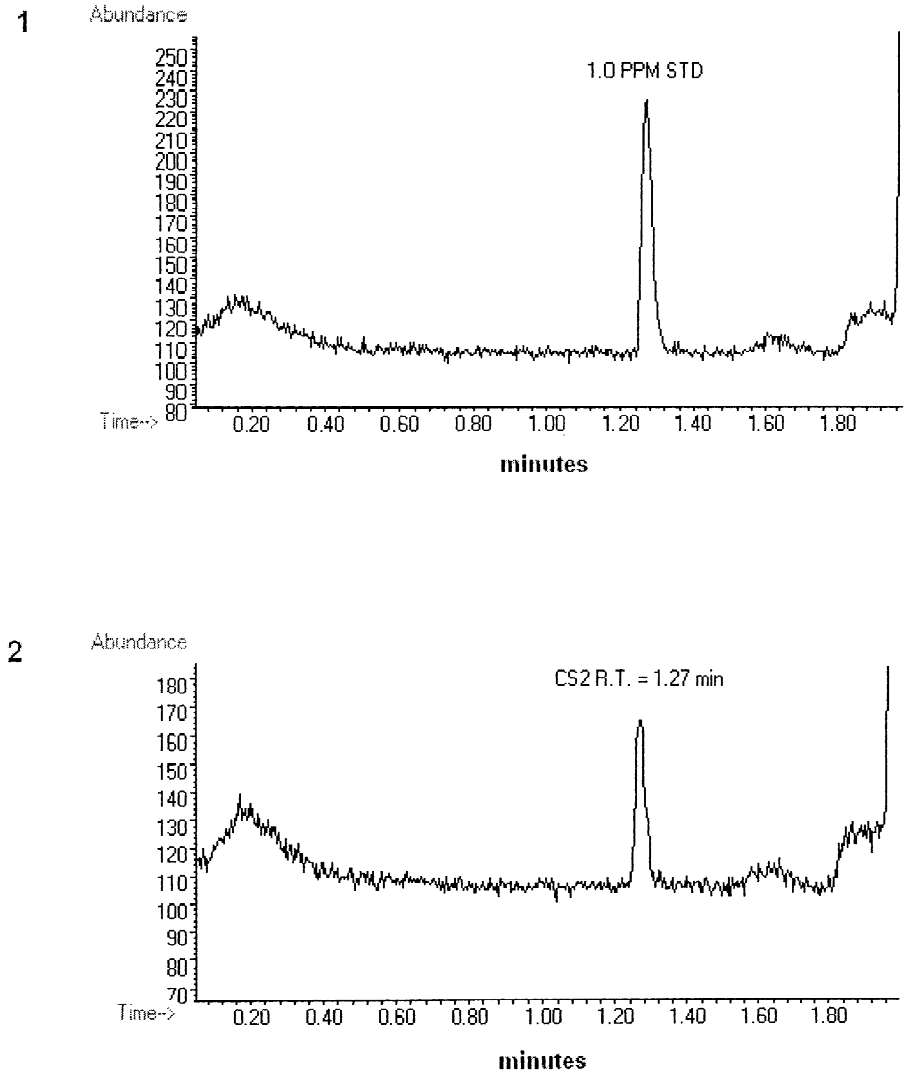


Fig. 2. Total ion chromatograms (combined m/z 76 and 78) for isooctane samples. Chromatogram 1 shows the trace obtained from a 1.0-μg/ml CS_2 standard and chromatogram 2 is from a thiram contaminated cotton pad collected as part of one of the field surveys. R.T., retention time.

forward to perform in that the filter is simply rolled up and inserted into the ATD tube. However, the method is a ''single-shot'' analysis, which carries the risk of data loss if any instrument problems are encountered.

4. Conclusions

The ATD–GC–MS method provides a rapid and sensitive screening method for the analysis of GF/A (air) filter samples, giving a quicker analysis time than the isooctane method. It is the method of choice when determining respirable exposure only. However, limitations of the method include the risks associated with a ''single-shot'' analysis and the restriction to GF/A (airborne) samples only.

The isooctane method described in this paper provides a simple route to the analysis of three dimethyldithiocarbamates, ferbam, thiram and ziram, in occupational hygiene sampling devices. The method is not selective for the different DTCs, however

Table 2
Recoveries from spiked sampling devices using the isooctane method

Sample type	DTC	Spike (μg)	Average recovery (%) (±RSD %)	*n*
Pad	Ferbam	84.6/89.6[a]	88±14	13
Pad	Thiram	72.6/86.4[a]	77±16	13
Pad	Ziram	86.1/87.9[a]	82±16	13
Glove	Ferbam	94	100	2
Glove	Thiram	96.8	71	2
Glove	Ziram	100.4	100	2
Sock	Ferbam	187.9	78	2
Sock	Thiram	145.2	67	1
Sock	Ziram	157.8	87	2
GF/A	Ferbam	5.6	105	3
GF/A	Thiram	4.8/5.7[a]	82±10	13
GF/A	Ziram	5.6	97	2
Overall[b]	Thiram	224.9	67	1

[a] Denotes two different spiking levels.
[b] Denotes sample was solvent desorbed prior to isooctane work-up.

Table 3
Summary of thiram results obtained from occupational exposure surveys (isooctane method)

Sampling device	Number of samples	Sample range (μg/device)	
		Low	High
GF/A filter	68	BDL	8
Cotton pads	24	BDL	4800
Cotton gloves	9	BDL	630
Disposable gloves	3	7500	20 600
Overall (per half)	7	BDL	67 100

BDL, below detection limit.

Table 5
Comparison of isooctane and ATD methods

Sample	Recovery corrected thiram result (μg)	
	Isooctane method	ATD method
1	0.211	ND
2	0.735	0.48
3	0.092	0.136
4	0.241	0.225
5	0.371	0.444
6	2.313	1.679
7	0.232	0.174
8	0.177	0.117
9	0.471	0.3
10	0.352	0.191
11	0.362	0.384
12	0.19	0.201
13	0.074	0.089
14	ND	(0.018)

Figure in brackets is semi-quantitative as it is below the reliable limit of detection. ND, not detected.

in most occupational hygiene surveys the identity of the analyte will already be known and, in the case of seed treatments, normally restricted to a single DTC. Recoveries and reproducibilities from the various spiked sampling devices are within acceptable limits. The method has also been used for analysis of several batches of samples taken as part of the recent occupational exposure survey on seed treatments, providing satisfactory results [25].

Because the isooctane method allows the full range of occupational hygiene sampling devices to be analysed using the same method and also permits multi-residue analysis, it is now the method of

Table 4
Recovery corrected data from multi-residue occupational exposure survey

Sample	Recovery corrected data (μg/device)		
	Thiram	Fluquinconazole	Carboxin
Pad 1	13	9	17
Pad 2	169	137	242
Pad 3	ND	<1	ND
Pad 4	512	106	779
Pad 5	1720	1223	2375
Pad 6	1176	153	820
Pad 7	5	8	7
Glove 1	460	173	298
Glove 2	1003	202	622
Overall top	51 524	23 624	65 521
Overall bottom	61 217	4112	7068

Data for the disposable overalls is reported uncorrected for recovery as recovery data for the other analytes were not available.

choice in our laboratory when assessing both dermal and respirable exposure. It is expected that further work will be carried out to look at the application of the method to other DTCs.

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