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Simultaneous gas chromatographic–mass spectrometric quantitation of the alkylbenzene inert components, pesticide manufacturing by-products and active ingredient in two malathion formulations

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Abstract

A rapid, reliable and effective method for direct determination of the inert components, manufacturing by-products of the pesticide, and active ingredient in two malathion formulations has been established using capillary gas chromatography–mass spectrometry (GC–MS) with the internal standard method. The C_2-, C_3- and C_4-alkylbenzenes, the major pesticide manufacturing by-products (*O*,*O*,*S*-trimethylthionophosphate, diethyl maleate and *O*,*O*,*O*-trimethylthionophosphate), and malathion were resolved, and quantified in the same chromatogram. Structural identification was based on MS total ion current data, comparison of GC retention times with those of authentic standards, and retention indices. *O*,*O*,*S*-Trimethylthionophosphate was quantified at 3.57±0.31% (w/w) in one malathion formulation. While the malathion contents were within specifications for both formulations, the total alkylbenzene contents were not.

Keywords: Retention indices; Malathion; Alkylbenzenes; Pesticides; Organophosphorus compounds; Trimethylthionophosphates

1. Introduction

The major uses of malathion [*S*-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethylphosphorodithioate] are to control insects that spread malaria and to kill fruit flies [1]. Malathion is often applied as a diluted emulsion–concentrate formulation. The latter consists of the active pesticide, a surfactant, a petroleum fraction and other adjuvants [2]. The non-pesticidal ingredients are the ''inert components''. The ''xylene'' fraction of petroleum distillate [3,4] is often used [5]. Alkylbenzenes are used as markers of environmental pollution by petroleum products [6]. The analytical methods for alkylbenzenes in crude oils have been reported [7–9], but none for malathion formulations with ''xylene'' inert components, or simultaneous quantitation of manufacturing by-products.

Malathion is manufactured by the reaction of *O*,*O*-dimethylphosphorodithioic acid and diethyl maleate or fumarate [10] in an inert solvent at atmospheric pressure at 20–150°C for about 16–24 h [10,11]. Some solvents include the lower aliphatic monohydric alcohols, ketones, aliphatic esters, aromatic hydrocarbons or trialkyl phosphates [10]. After reaction, the cooled mixture is extracted with benzene,

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and the extract is washed with 10% sodium carbonate in water. The organic layer is dried, filtered and then concentrated in vacuo to give liquid malathion [11]. The *O,O,S*-trimethylthionophosphate has been found as a by-product [12], but not quantified.

The accurate, reliable analysis of all components in pesticide formulations is important to public health because of their many field applications, and the non-pesticidal components can facilitate pesticide dermal absorption, the major route of exposure, as well as permeation through protective materials [13,14]. The presence of the more toxic ''oxon'' by-products of phosphorodithoate pesticides may also be important. In the present study, the volatile alkylbenzene inert components and pesticide manufacturing by-products in two malathion emulsion concentrate formulations were simultaneously identified and quantified by gas chromatography–mass spectrometry (GC–MS) for the first time.

2. Experimental

2.1. Reagents

One formulation was Aqua Malathion 8 ''Aqua'' (FMC, Philadelphia, PA, USA) of 80.5% (w/w) malathion. The other one was Prentox Malathion Emulsifiable Insecticide ''Prent'' (Prentiss, Sanderville, GA, USA) with 50% (w/w) malathion. Malathion (>95%) was from Pfaltz and Bauer (Waterbury, CT, USA). *m*-Xylene (Spectrograde) and 1,3,5-trimethylbenzene (99%) were from Kodak (Rochester, NY, USA). The following alkylbenzenes were from Aldrich (Milwaukee, WI, USA): isopropylbenzene, 99%; *n*-propylbenzene, 98%; 3-ethyltoluene, 99%; 4-ethyltoluene, 99%; and 1,2,3-trimethylbenzene, 99%. 1,2,4-Trimethylbenzene (99%) was from Sigma (St. Louis, MO, USA). *m*-Diethylbenzene, 98%, diethyl maleate, 98% and diethyl fumarate, 99% were from Chem Service (Westchester, PA, USA). Internal standard *o*-diethylbenzene (95%) was from Fluka (Ronkonkoma, NY, USA). Optima grade hexanes and certified *o*-xylene were from Fisher Scientific (Fair Lawn, NJ, USA). Phosphorus pentasulfide, 99% (Aldrich), methanol (Optima grade, Fisher Scientific), sodium hydroxide (Fisher Scientific), and anhydrous magnesium sulfate, $MgSO_4$ (Fisher Scientific) were utilized for synthesis. Milli-Q (Bedford, MA, USA) deionized water was used for all aqueous solutions and washing.

2.2. GC–MS

A Hewlett-Packard 5890 gas chromatograph equipped with a 5988A quadrupole 70 eV positive ion electron impact mode mass spectrometer was used. System control and data acquisition were achieved with a HP 5970C ChemStation Version 3.1. The capillary column was a chemically-bonded fused-silica DB-1701 (J&W–Alltech Scientific, Deerfield, IL, USA) with 30 m×0.32 mm I.D. and 1-μm film thickness. The constant conditions were: carrier gas, 99.9999% purity helium (Alphagaz, Walnut Creek, CA, USA) at a flow-rate of 3.0 ml/min; injection mode, splitless; injector, GC–MS transfer line and ion source temperatures were 250°C.

2.3. Synthesis of O,O,S-trimethylthionophosphate

No commercial *O,O,S*-trimethylthionophosphate standard was available and hence it had to be synthesized in the laboratory. The synthesis utilized the following reaction [15]:

$$12CH_3OH + P_4S_{10} \rightarrow 4(CH_3O)_2(P=S)SCH_3 + \text{unidentified acid} + H_2S \quad (1)$$

Phosphorus pentasulfide (0.10 mol) was added slowly in small portions over a period of 2 h into refluxing methanol (0.6 mol) at 55–65°C. The mixture was refluxed for an additional 2 h. Excess methanol and hydrogen sulfide were removed by rotary evaporation at 50°C. The concentrated mixture was filtered under vacuum to remove residual sulfur. The filtrate was washed with excess 10% aqueous sodium hydroxide, and then extracted into hexane. The extracts were washed with Milli-Q water dried by anhydrous $MgSO_4$. The hexane was rotary evaporated at 90°C. The liquid product was weighed and kept in a vacuum desiccator until constant mass. The dried liquid was then diluted in hexane (1:10, v/v). A 1-μl aliquot was injected into the GC–MS system for identification. The net mass of the final product was 17.38 g, a 50.5% yield. The product was

identified by MS and compared with the spectrum in the NBS MS Library Version 3.2. The degree of fit was 99%.

2.4. GC–MS analysis of malathion formulations

Prent and Aqua were separately diluted 1:10 in hexane. A 1-μl aliquot was then injected to identify the major components in the total ion current (TIC) mode relative to authentic standards in hexane. The selected ion monitoring (SIM) mode was used for quantification (Table 1). The temperature program for the analysis of alkylbenzenes and malathion in Prent and Aqua was: 70°C held for 18 min then ramped at 25°C/min to 250°C; and held for 10 min. The temperature program used for analysis of the by-products in Aqua was: 55°C held for 0.5 min then ramped at 10°C/min to 150°C; and held at 150°C for 5 min; ramped at 20°C/min to 250°C and held for 20 min.

The standard solutions to define the linear range contained two C_2 side-chain-benzene compounds, eight C_3 side-chain-benzene compounds, and malathion (Table 1). The internal standard was *o*-diethylbenzene. A five-point calibration curve that demonstrated the linear range was established for each target compound. The relative response factors (RRFs) for each compound were calculated relative to the internal standard. Prent and Aqua were then diluted into the linear ranges with hexane. The dilution factor was 250. All analyses were performed at least in triplicate.

2.5. Retention indices

I values for alkylbenzenes were calculated using [16,17]:

$$I_A = 100N + 100\frac{\log t'_{R(A)} - \log t'_{R(N)}}{\log t'_{R(N+1)} - \log t'_{R(N)}} \quad (2)$$

where I_A is the retention index of component A; $t'_{R(A)}$ is the adjusted retention time of A; and $t'_{R(N)}$ and $t'_{R(N+1)}$ are the adjusted retention times of the *n*-paraffins with carbon numbers N and $N+1$, respectively.

3. Results and discussion

3.1. Identification and characterization of alkylbenzene homologs

The analytes in Table 1 detailing their linear ranges and response factors are listed in order of increasing retention time. The relative standard de-

Table 1
Linear ranges and relative response factors of formulation volatile components determined by the internal standard method

Compounds	M_r	Target ion (m/z)	Linear range (ng)	RRF (ng^{-1})	S.D. (ng^{-1})	R.S.D. (%)
o-Diethylbenzene[a]	134	105				
m-Xylene	106	91	0.51–18	0.27	0.015	5.6
o-Xylene	106	91	0.51–17	0.30	0.010	3.3
Cumene	120	105	0.52–18	0.37	0.015	4.1
n-Propylbenzene	120	91	0.52–18	0.48	0.017	3.5
3-Ethyltoluene	120	105	2.8–18	0.40	0.017	4.3
4-Ethyltoluene	120	105	2.8–18	0.36	0.023	6.4
Mesitylene	120	105	2.8–18	0.34	0.015	4.4
2-Ethyltoluene	120	105	0.51–12	0.38	0.015	3.9
1,2,4-Trimethylbenzene	120	105	0.51–17	0.32	0.010	3.1
1,2,3-Trimethylbenzene	120	105	0.52–18	0.39	0.0058	1.5
m-Diethylbenzene	134	105	0.52–15	0.22	0	0
Malathion	330	125	4.6–230	0.044	0.0020	4.5

[a] Internal standard.
M_r = Molecular mass.
Target ion = the most abundant m/z for each compound.
RRF = Relative response factor using 5 ng of internal standard.

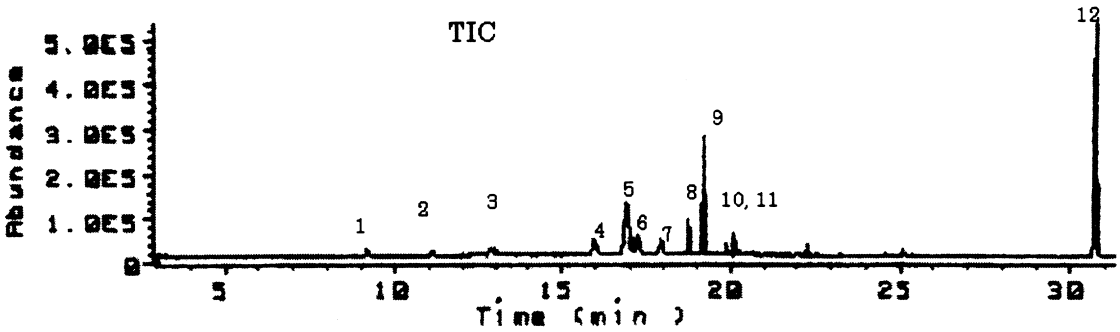

Fig. 1. Chromatogram of Prentox formulation. Peaks: 1=*m*-xylene; 2=*o*-xylene; 3=cumene; 4=*n*-propylbenzene; 5=3-ethyltoluene; 6=4-ethyltoluene; 7=mesitylene; 8=2-ethyltoluene; 9=1,2,4-trimethylbenzene; 10=1,2,3-trimethylbenzene; 11=*m*-diethylbenzene; 12=malathion.

viations (R.S.D.s) obtained from three determinations of RRF were below 5% except for the R.S.D.s for *m*-xylene and 4-ethyltoluene. All alkylbenzenes except 3- and 4-ethyltoluene and mesitylene had working linear ranges from 0.51 to 18 ng. The most sterically hindered positional isomer always had the longest retention time t_R.

Fig. 1 shows the TIC GC–MS chromatogram of Prent. No aromatic hydrocarbons eluted after 22 min. Peak 5, 3-ethyltoluene of t_R 11.5 min, and peak 9, 1,2,4-trimethylbenzene of t_R 15.3 min, appear more abundant than other alkylbenzene inert components without considering RRFs. The structural identification and characterization of alkylbenzenes in Prent were based on mass spectral data in both TIC and SIM modes and comparison of GC t_R data with reference standards. Peaks 5 and 6 were not well resolved. Comparison of t_R, *I* and mass spectra with those of standard compounds confirmed the presence of 3-ethyltoluene and 4-ethyltoluene, respectively. The same chromatogram of the alkylbenzenes was obtained for Aqua, except that peak areas were much lower than those of Prent at the same dilution ratios.

3.2. Retention indices

Eleven major alkyl-substituted compounds were identified in Prent (Table 2). These included two C_2

Table 2
Retention indices of the formulation alkylbenzene inert components

Compounds	t_R (min)[a]	Calculated *I*	Literature *I*	R.E. (%)
m-Xylene	9.1	852.6	872.4	−0.11
o-Xylene	11.2	892.1	892.8	−0.078
Cumene	12.9	923.4	923.8	−0.043
n-Propylbenzene	16.1	963.4	954.6	0.92
3-Ethyltoluene	16.9	979.5	962.4	1.8
4-Ethyltoluene	17.2	983.4	963.6	2.1
Mesitylene	17.9	990.8	968.8	2.3
2-Ethyltoluene	18.7	1010	979.8	3.1
1,2,4-Trimethylbenzene	19.1	1031	992.2	3.9
1,2,3-Trimethylbenzene	19.8	1064	1028.2[b]	3.5
m-Diethylbenzene	20.1	1069	–	–

t_R = Retention time.
[a] The GC temperature program was 70°C held for 18 min then ramped at 25°C/min to 250°C; and held for 10 min.
Calculated *I* = retention index calculated by Eq. (2).
Literature *I* = retention index from Refs. [7,16].
R.E. = Relative error.
[b] This *I* value was obtained from Ref. [7], which used a HP-5 column.

side-chain-benzene isomers, eight C_3 side-chain-benzene isomers and one C_4 side-chain-benzene compound. The *I* values of these alkylbenzenes and literature values [7,16] are provided in Table 2. No *I* values have been reported for this DB-1701 column. The literature *I* values were for a DB-5 and HP-5 fused-silica column. However, the two series of *I* values are still within ±5% agreement [18]. The *I* value of *m*-diethylbenzene is reported for the first time. The agreement of the other *I* values with the literature provides confidence in the accuracy of the new *I* values. The DB-1701 column therefore resolves these alkylbenzene isomers and homologs adequately.

3.3. Compositions of Prent and Aqua

Table 3 gives the composition of Prent. The major inert components were 3-ethyltoluene and 1,2,4-trimethylbenzene (peaks 5 and 9, respectively in Fig. 1), their sum being 26±2% (w/w) of the formulation and 57% (w/w) of the 11 alkylbenzenes. The latter were 45.4±1.5% (w/w) of the formulation, significantly different at $p \leq 0.05$ from the nominal 50%, assuming the same R.S.D. for the latter. The mass percentage of malathion was 52.0±2.0%, not statistically different at $p \leq 0.05$ [19] from the nominal 50%. The total mass balance was 97.3±4.4%, not significantly different from 100%.

For Aqua, the total mass percentage of aromatic alkylbenzenes was 0.0270±0.0030% (w/w). The malathion concentration was 999±42 mg/ml, which accounted for 88.4±3.7% (w/w) and was not significantly different from nominal at $p \leq 0.05$. The mass balance was thus 88.4±3.7%, significantly different from 100% at $p \leq 0.05$. The initial reactant diethyl maleate was present at 1.06±0.09%. The by-product *O,O,S*-trimethyl thionophosphate comprised 3.57±0.31%. The mass balance was then 93.1±4.1%, not significantly different from 100%. *O,O,O*-Trimethyl thionophosphate was also identified from its mass spectrum by comparison to the NBS library. Since the compound was not commercially available and there was no published synthetic method, quantitative data cannot be reported. Assuming this analyte has the same RRF as its *O,O,S*-trimethyl analog, it comprises 0.18% of the formulation.

4. Conclusions

The xylene range inert components of the tested malathion formulations contained C_2-, C_3- and C_4-side-chain-benzene congeners and isomers as confirmed by retention times, mass spectra and retention indices. While malathion contents were accurate, the alkylbenzene content on the label for one formulation was not. The *I* values of the alkylbenzenes for the DB-1701 capillary column were reported for the

Table 3
Composition of the Prentox formulation

Compound	Concentration (mg/ml)	Percentage (%, w/w)
m-Xylene	15±1.2	1.5±0.12
o-Xylene	9.1±0.66	0.93±0.067
Cumene	21±1.0	2.1±0.10
n-Propylbenzene	25±1.0	2.5±0.10
3-Ethyltoluene	130±7.2	13±0.73
4-Ethyltoluene	42±0.61	4.3±0.061
Mesitylene	32±1.5	3.3±0.15
2-Ethyltoluene	29±1.5	3.0±0.15
1,2,4-Trimethylbenzene	130±7.9	13±0.81
1,2,3-Trimethylbenzene	6.3±0.36	0.64±0.037
m-Diethylbenzene	9.8±0.31	1.0±0.031
Malathion	510±20	52±2.0
	Mass balance	97.3±4.4

first time and were comparable with the *I* values of a DB-5 capillary column. The *I* value for *m*-diethylbenzene is reported for the first time on any column. One formulation contained by-products (two trimethyl thionophosphates), and initial reactant ester from the malathion manufacturing process, these being quantified for the first time. It is recommended that pesticide manufacturers provide detailed composition data on their labels and material safety data sheets on components exceeding 1% (w/w). The analysis of each pesticide formulation should be performed before field application to facilitate interpretation of field results.

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