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Comparative study of the determination of tebufenozide in formulated products by gas chromatographic and liquid chromatographic methods

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Abstract

Thirteen formulated products (formulation concentrates and spray mixes) containing tebufenozide [N'-*tert.*-butyl-N'-(3,5-dimethylbenzoyl)-N-(4-ethylbenzoyl) hydrazine, also known as RH-5992; trade name MIMIC], were analyzed, after solvent dissolution by agitation, using direct gas chromatography (GC) and reversed-phase high-performance liquid chromatography (HPLC). The responses of the analyte to three GC detection methods (flame ionization detection, FID; nitrogen–phosphorus detection, NPD and electron-capture detection, ECD) using three fused-silica capillary columns of varying internal diameters were compared. The mini-bore (0.25 mm I.D.) DB-5 [(5% phenyl)-methylpolysiloxane] column, attached to the ECD system was better suited to quantify the analyte in formulated products than the mega-bore DB-1 (dimethylpolysiloxane) and DB-17 [(50% phenyl)-methylpolysiloxane] columns linked to the FID, NPD or ECD systems. Analysis by GC–FID and a reversed-phase HPLC method using an RP-8 column (10 μm particle size) with a mobile phase containing acetonitrile–dioxane–water and a diode-array UV detector set at 236 nm also gave values similar to the GC–ECD method. However, due to the rapidity and sensitivity of sample analysis, GC–ECD is the technique of choice for the quantification of MIMIC in formulated products.

1. Introduction

The hydrazine derivative tebufenozide (trade name: MIMIC), also known as RH-5992 [N'-*tert.*-butyl-N'-(3,5-dimethylbenzoyl)-N-(4-ethylbenzoyl) hydrazine] (Rohm & Haas, USA [1]) is a novel type of insect growth regulator interfering with the moulting process of lepidopteran insects. It acts as an agonist or mimic of insect moulting hormone, 20-hydroxyecdysone, by inducing premature, incomplete ecdysis resulting in death of the exposed insects. The material is lepidopteran specific and has no effect on crustacea, arachnida, or most other insect orders (beetles, aphids, flies etc.) and mites. It is essentially non-toxic to bees [2]. These desirable properties make the material a choice insecticide to suppress the lepidopteran insect populations in forests.

Aerial field trials conducted recently by the Forest Pest Management Institute, Natural Resources Canada–Canadian Forest Service, have

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shown that MIMIC is effective in controlling the spruce budworm, *Choristoneura fumiferana* (Clem.), a destructive pest that causes considerable damage to the spruce-fir forests of eastern North America and is threatening the wood supply from large areas of forest in eastern Canada [3]. In aerial spray trials, the technical MIMIC is formulated by adding different adjuvants (commonly referred to as formulants or inerts) such as solvents, wetting agents, stickers, spreaders, penetrants and emulsifying agents to produce a "formulation concentrate". The formulation concentrate is then diluted with water in the field to form the "spray mix" or end-use product which is then applied by aircraft.

No method has been reported in the open literature, until now, to quantify the MIMIC present in either formulation concentrates or spray mixes. A high-performance liquid chromatographic (HPLC) method with diode-array UV detection (DAD) to analyze the residues of MIMIC in various forestry matrices, after extraction and sample cleanup, has been published recently [4]. Also, confidential gas chromatographic (GC) methods developed by the manufacturer, to quantify the analyte after necessary extraction, cleanup and derivatization from some agricultural commodities and without derivatization for some formulated products are on record and will be made available, presumably, as and when the registration protocol of the material is completed [5]. Unfortunately none of these methods specifically address the analysis of MIMIC from formulation concentrates and spray mixes because of the assumption that some of the additives in them with similar chromatographic characteristics could cause interference resulting in irreproducibility of results.

We have developed a reliable and sensitive GC method to quantify MIMIC present in the formulation concentrates and spray mixes without any derivatization step for the analyte. We also examined the applicability of our HPLC method reported earlier [3] for this purpose and made a comparison of the two instrumental techniques. The response of MIMIC to three different GC detection methods, viz., flame ionization detection (FID), nitrogen–phosphorus detection (NPD) and electron-capture detection (ECD) using three different GC fused-silica capillary columns (DB-1, DB-5 and DB-17) with varying internal diameters was also examined and compared to the response of the analyte in HPLC–DAD. Our findings are reported in this paper.

2. Experimental

2.1. Chemical standards

Analytical grade MIMIC (99.6% purity, lot No. AMB 9-40B, m.p. 186–188°C) was supplied courtesy of Rohm & Haas (Philadelphia, PA, USA). A stock solution containing 25 mg/25 ml of the analyte was prepared in an amber-coloured volumetric flask using methanol as solvent and stored at 0°C in darkness to prevent potential photodegradation. The stock solution was stable for 10 weeks at these conditions and fresh samples were prepared afterwards if necessary. Working solutions were prepared by serial dilution of the stock solution in ethyl acetate for GC analysis using NPD and in acetonitrile for HPLC and GC analyses using FID and ECD. All samples were diluted to levels within the predetermined linear range of the detectors.

2.2. Formulation concentrates and spray mixes

Four formulation concentrates and nine spray mixes were used in the present study. Three of the formulation concentrates were supplied courtesy of Rohm & Haas and the fourth one was received from the scientist in charge of the Formulation Project at the Institute. The nine spray mixes used in the laboratory and field microcosm studies during 1992/93 were prepared in the laboratory using the formulation concentrates. Table 1 lists all the formulation concentrates and spray mixes studied along with the active ingredient present in each.

2.3. Reagents

Acetonitrile, dioxane, methanol and ethyl acetate were HPLC grade (Optima, Fisher Sci-

Table 1
List of formulations used in the study

Sample	Description	Formulation type	AI (%, w/v)
FC-1	RH-5992 2F (concentrate); lot No. L-0914	Emulsion	24.0
FC-2	RH-5992 2F (XF-87024); lot No. AL-1534-1	Emulsion	24.0
FC-3	RH-5992 2F (XF-93011); lot No. CDP-1293-C	Oil	24.0
FC-4	RH-5992 flowable concentrate (in the laboratory)	Emulsion	25.0
SM-1	Spray mix; 35 g AI/2 l (prepared from FC-1)	Emulsion	1.75
SM-2	Spray mix; 70 g AI/2 l (prepared from FC-1)	Emulsion	3.5
SM-3	Spray mix; 140 g AI/2 l (prepared from FC-1)	Emulsion	7.0
SM-4	Spray mix; 35 g AI/2 l (prepared from FC-2)	Emulsion	1.75
SM-5	Spray mix; 70 g AI/2 l (prepared from FC-2)	Emulsion	3.5
SM-6	Spray mix; 140 g AI/2 l (prepared from FC-2)	Emulsion	7.0
SM-7	Spray mix; 35 g AI/2 l (prepared from FC-3)	Emulsion–suspension	1.75
SM-8	Spray mix; 70 g AI/2 l (prepared from FC-3)	Emulsion–suspension	3.5
SM-9	Spray mix; 140 g AI/2 l (prepared from FC-3)	Emulsion–suspension	7.0

AI = Active ingredient.

entific) filtered through a Nylaflo nylon membrane filter, 0.2 μm pore size (No. 66602, Gelman Sciences). Water was deionized, glass-distilled by a Mega-Pure System (Model No. MP-6A, Corning) and filtered as above.

2.4. Apparatus

The HPLC system used in the study was a Hewlett-Packard (HP) (Analytical Division, Palo Alto, CA, USA) Model 1090M incorporating an HP diode-array detector (λ range 190 to 600 nm); an HP 9000 Series 300 (Model 310) computer work station (HP 79995R Series M operating software, Rev. 3.21); an automatic sampler; and a variable-volume auto-injector fitted with a 250-μl syringe. The instrument also had a binary solvent-delivery system with a helium-purge degassing system and two dual-syringe metering pumps that gave stable and reproducible flows. The instrument parameters used for the analysis of environmental substrates [3] were nearly the same for this study. The analytical column was a LiChrosorb RP-8, 10 μm, 200 × 4.6 mm I.D. (No. 79915MO-174, HP) which was preceded by a MOS-Hypersil (C_8), 5 μm, 20 × 4.0 mm I.D. guard column (No. 79916KT-121, HP). The mobile phase composition consisted of 50% water and 50% acetonitrile–dioxane (4:1, v/v) with a flow-rate of 0.8 ml/min maintained at an oven temperature of 40°C. The sample DAD wavelength was 236 ± 4 nm with a reference λ of 430 ± 50 nm. The auto-injector volume was set at 40 μl.

Two HP Model 5890A gas chromatographs, one fitted with FID and NPD systems, and the other fitted with an ECD system, were used in the study. Both gas chromatographs accommodated an HP 7673A autosampler and an HP 3392A computerized integrator for area and height measurements of the peaks. The fused-silica capillary columns tested in this study were (1) DB-1 (dimethylpolysiloxane), 15 m × 0.53 mm I.D. with 1.5 μm film thickness (d_f) (No. J1251012); (2) DB-5 [(5% phenyl)-methylpolysiloxane], 15 m × 0.25 mm I.D. with d_f 0.25 μm (No. J1225012); and (3) DB-17 [(50% phenyl)-methylpolysiloxane], 15 m × 0.53 mm I.D. with d_f 1.0 μm (No. J1251712), all from Chromatographic Specialties. A 1 m × 0.53 mm I.D. deactivated guard column (No. J1602535, Chromatographic Specialties) was joined to each column with a glass capillary column connector. Helium was used as the carrier gas throughout the study. Air and hydrogen were used as the detector gases, with helium make-up in the FID and NPD systems. We used 5% methane in argon as the make-up gas in the ECD system. The injection volume was 2 μl in the splitless mode of operation. The different gas flow-rates

and temperatures used form part of the discussion and are therefore listed in the Results and discussion section.

2.5. Sample preparation

To evaluate the analytical method using HPLC and GC, the formulation concentrates and spray mixes were agitated in a Magni Whirl mechanical shaker (Blue M Electric Company) at room temperature for 2 h at 200 excitations/min. From each sample, 100 μl, in triplicate, were pipetted into 100-ml volumetric flasks and diluted to the mark using acetonitrile (HPLC grade). After thorough mixing, 1.0 ml of each spray mix dilution and 100 μl of each formulation concentrate solution were further diluted to 10 ml in separate volumetric flasks. Depending on the concentration, some of the additives present in the formulation concentrates and spray mixes coagulated and precipitated when diluted with acetonitrile. This did not adversely affect either the solubility or the content of the active ingredient. A 1-ml volume of the diluted sample was then passed through an Acrodisc 3CR PTFE 0.45-μm filter (No. 4472, Gelman Sciences) and directly analyzed by HPLC or GC without any solvent partition or column cleanup. The injection volumes varied according to the technique and detector used. At least three replicate injections were made of each analyzed sample.

2.6. Calculations

The percentage (w/v) of MIMIC in a formulation concentrate or spray mix was determined by the expression

$$\text{MIMIC}\,(\%) = (A_{\text{sam.}}/A_{\text{std.}}) \cdot (C_{\text{std.}}/V_{\text{sam.}}) \cdot F \cdot 100\%$$

where $A_{\text{sam.}}$ is the mean peak area of three successive injections of the sample, $A_{\text{std.}}$ is the mean peak area of the standard injected immediately prior to and after the sample injections, $C_{\text{std.}}$ is the concentration of the standard in μg/ml, $V_{\text{sam.}}$ is the volume (μl) of formulation concentrate or spray mix taken for analysis and F is the dilution factor (μl · ml/μg) ($F = 10$ for formulation concentrates; $F = 1$ for spray mixes).

3. Results and discussion

3.1. HPLC–DAD

The chromatography performance (repeatability and linearity) for the insecticide on the RP-8 column (10 μm, 200 × 4.6 mm I.D.) was optimized by multiple injections of the standard solutions at varying concentrations. Under the optimum HPLC parameters listed earlier under Apparatus, the DAD response to MIMIC was linear over the concentration range of 2 to > 1000 ng in the 40-μl injection volume with a retention time (t_R) of 18.2 min. A linear regression of the data points (amounts injected and corresponding area counts) throughout the range gave a correlation coefficient of 1.000. The regression equation data are presented in Table 2.

Quantification of the formulation concentrates and spray mixes was based upon the peak areas obtained from triplicate injections of each sample and comparing them with the peak areas obtained for the appropriate external standards injected immediately prior to and after the sample. The results of the analysis for MIMIC content in thirteen samples by HPLC are given in Table 3. The values of active ingredient obtained by measurement agreed with the expected value for each sample (absolute error ranged from 0.6 to 6.4%) and the deviation was minimal [relative standard deviation (R.S.D.) ranged from 0.1 to 4.1%]. A typical HPLC chromatogram is illustrated in Fig. 1a. Although the resolution of the analyte was satisfactory, the retention time was longer than noted with GC methods discussed below. The method detection limit (MDL) for a 99% confidence level was determined as thrice the standard deviation (σ) using repeated measurements of low-level standards [6]. Usually, the MDL is determined from the variability of repeated blank measurements but since no response was observed for any

Table 2
Linear regression analysis of MIMIC calibration standards for different detectors using the regression equation: $y = b + mx$ [where y is the detector response, b is the intercept, m is the slope and x is the amount (ng) of MIMIC injected]

Detector	Intercept (V s)	Slope	r^2	Linear range (ng)		MDL (ng)
				Lower limit	Upper limit[a]	
HPLC–DAD	0.611[b]	2.02322	1.000	2	>1000	0.8
GC–FID	−0.00090	0.00582	0.999	0.10	>50	0.04
GC–NPD	−0.00115	0.00506	0.999	0.50	>50	0.10
GC–ECD[c]	0.02264	0.39035	1.000	0.05	40	0.02
GC–ECD[d]	0.01759	0.29211	1.000	0.05	40	0.02

[a] The upper limit was only tested to the reported values and may be much higher where indicated.
[b] The units for the DAD response are mAU s.
[c] GC conditions were similar to those used for FID and NPD (see text).
[d] Performed under final optimized GC conditions (see text).

blank injection, a low-level standard (less than 10 times the MDL) was used. The MDL for the analyte at the lower limit of concentration was found to be 0.8 ng for the detector.

3.2. GC–FID

The GC part of the study was initiated first by using FID. A 1 m × 0.53 mm I.D. deactivated

Table 3
Comparison of results obtained by HPLC–DAD, GC–FID and GC–ECD analysis

Sample	Active ingredient (%, w/v)									
	Theoretical	HPLC–DAD			GC–FID			GC–ECD		
		Mean ± S.D.	R.S.D. (%)	Error[a] (%)	Mean ± S.D.	R.S.D. (%)	Error (%)	Mean ± S.D.	R.S.D. (%)	Error (%)
FC-1	24.0	24.3 ± 1.0	4.1	1.2	24.1 ± 0.3	1.2	0.4	23.3 ± 0.1	0.4	2.9
FC-2	24.0	22.9 ± 0.1	0.4	4.6	21.2 ± 0.2	0.9	11.7	21.6 ± 0.4	1.9	10.0
FC-3	24.0	23.8 ± 0.1	0.4	0.8	23.3 ± 0.3	1.3	2.9	24.3 ± 0.1	0.4	1.2
FC-4	25.0	23.8 ± 0.9	3.8	4.8	22.6 ± 0.5	2.2	9.6	23.1 ± 0.4	1.7	7.6
SM-1	1.75	1.81 ± 0.01	0.6	3.4	1.67 ± 0.04	2.4	4.6	1.71 ± 0.02	1.2	2.3
SM-2	3.50	3.43 ± 0.01	0.3	2.0	3.42 ± 0.05	1.5	2.3	3.42 ± 0.04	1.2	2.3
SM-3	7.00	6.77 ± 0.01	0.1	3.3	6.94 ± 0.04	0.6	0.9	6.82 ± 0.07	1.0	2.6
SM-4	1.75	1.65 ± 0.01	0.6	5.7	1.63 ± 0.01	0.6	6.9	1.53 ± 0.08	5.2	12.6
SM-5	3.50	3.30 ± 0.02	0.6	5.7	3.23 ± 0.11	3.4	7.7	3.26 ± 0.10	3.1	6.9
SM-6	7.00	6.55 ± 0.04	0.6	6.4	6.85 ± 0.13	1.9	2.1	6.71 ± 0.14	2.1	4.1
SM-7	1.75	1.72 ± 0.01	0.6	1.7	1.70 ± 0.05	2.9	2.9	1.62 ± 0.03	1.9	7.4
SM-8	3.50	3.52 ± 0.01	0.3	0.6	3.48 ± 0.08	2.3	0.6	3.46 ± 0.05	1.4	1.1
SM-9	7.00	7.10 ± 0.03	0.4	1.4	7.23 ± 0.31	4.3	3.3	7.20 ± 0.18	2.5	2.9
Average			1.0	3.2		2.0	4.3		1.8	4.9

[a] This value represents the absolute error which can be defined as the deviation of the calculated result from the theoretical value in absolute terms of percentage.

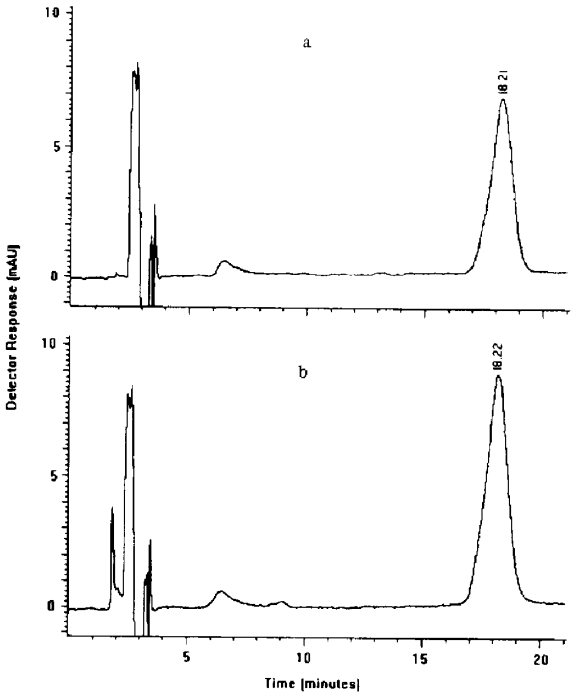

Fig. 1. Typical HPLC chromatograms of MIMIC (see text for HPLC parameters used). (a) DAD response to 5.0 μg/ml standard. (b) DAD response to representative MIMIC formulation.

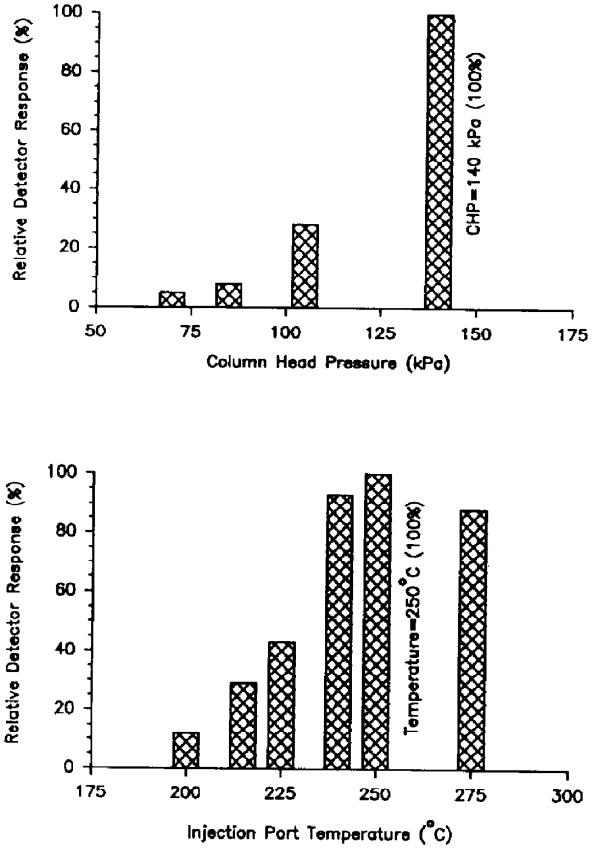

Fig. 2. Effects of (a) column head pressure and (b) injection port temperature on GC–FID response to MIMIC.

guard column was connected to the DB-5, 15 m × 0.25 mm I.D. fused-silica capillary column with a glass capillary column connector, installed and conditioned prior to attachment to the FID system. Subsequently, trials were carried out to get good detector response to the injected analyte by methodically selecting the appropriate GC parameters. Repeated injections of the standard significantly enhanced and stabilized the detector response producing a distinctive peak with area proportional to the concentration of the analyte. In addition to flow-rates of gases (detector), the column head pressure (CHP) and injection port temperature were optimized because they had significant effect on the sensitivity of FID to MIMIC. Fig. 2 shows the enormous variability in detector response to CHP and injection port temperatures.

The chromatographic performance for the detection of MIMIC was fine tuned and the suitable parameters established were: *Gases*: carrier (helium) CHP, 140 kPa (linear velocity, 55 cm/s); inlet purge flow, 60 ml/min (purge on at 0.5 min); septum purge flow, 3 ml/min; hydrogen (detector), 35 ml/min; air, 270 ml/min; make-up (helium), 30 ml/min. *Temperatures*: splitless injection port, 250°C; detector, 260°C. *Oven programme*: initial 60°C at 0.75 min; ramp rate A 40°/min; final A 210°C at 5 min; ramp rate B 10°/min; final B 240°C at 3.5 min. Using these operating conditions, peak area variations with similar concentrations of the analyte or the test sample was found to be less than 1%.

Under the above operating parameters, the t_R for the analyte was 12.5 min. The peak was sharp and well resolved with no chromatographic interference in the vicinity of the t_R of MIMIC. A typical chromatogram for MIMIC by FID is

presented in Fig. 3a. The detector response to MIMIC was found to be linear in the range 0.10 to >50 ng ($r^2 = 0.999$) in the 2-μl injection volume as shown in Table 2 and Fig. 4. The MDL of MIMIC using the FID was determined as 0.04 ng in the 2-μl injection volume. The analyte concentrations in the formulation concentrates and spray mixes obtained by the GC–FID using the DB-5 column are given in Table 3. The R.S.D. by this method ranged from 0.6 to 4.3% while the absolute error of the measurements varied from 0.4 to 11.7%. Fig. 3b illustrates a typical chromatogram obtained from the analysis of a MIMIC formulation.

Two chromatographic mega-bore columns, namely DB-1 and DB-17 were also tried to quantify the analyte in the formulation concentrates and spray mixes under similar operating parameters used for DB-5. Detector response and peak shape were inferior on these columns in comparison to the mini-bore DB-5 column.

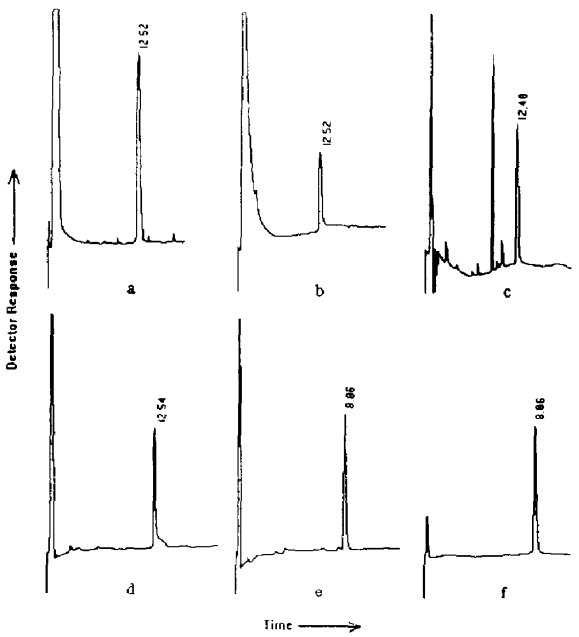

Fig. 3. Typical GC chromatograms of MIMIC (see text for various GC conditions used). (a) FID response to 25 μg/ml standard (integrator attenuation 2^5). (b) FID response to representative MIMIC formulation (attenuation 2^3). (c) NPD response to 25 μg/ml standard (attenuation 2^5). (d) ECD response to 1.0 μg/ml standard at GC conditions similar to FID and NPD (attenuation 2^7). (e) ECD response to 1.0 μg/ml standard at optimized conditions (attenuation 2^8). (f) ECD response to representative MIMIC formulation (attenuation 2^{11}). Numbers at peaks indicate retention times in min.

3.3. GC–NPD

To investigate the response of NPD to MIMIC, the DB-5 column used above was reconditioned and attached to the detector. After achieving required stabilization, the detector response was optimized (as discussed for FID) using the following parameters: *Gases*: carrier (helium) CHP, 140 kPa (linear velocity 55 cm/s); inlet purge flow, 60 ml/min (purge on at 0.5 min); septum purge flow, 3 ml/min; hydrogen (detector), 4 ml/min; air, 100 ml/min; make-up (helium), 35 ml/min. *Temperatures*: splitless injection port, 250°C; detector, 260°C. *Oven programme*: same as for GC–FID.

The NPD sensitivity was slightly lower than the FID one. Usually, the NPD provides greater sensitivity for nitrogen-containing compounds, however this was not observed in this particular study: further investigation is necessary. The NPD response was linear over the range of 0.50 to >50 ng ($r^2 = 0.999$) injected (Table 2, Fig. 4) versus 0.10 to >50 ng in FID with a MDL of 0.10 ng compared to the value of 0.04 ng in the FID. One major drawback of NPD was that column temperature had a significant effect on the sensitivity of the detector. Due to the temperature gradient, the baseline rose dramatically, therefore column compensation was done by subtracting the signal from a blank run from the signal of the sample run. However, even with column compensation, considerable baseline instability was observed at lower concentration levels especially when the attenuation was decreased. This hampered quantification of the analyte at low concentration levels compared to FID (see Table 2). A typical chromatogram for the NPD response of MIMIC is shown in Fig. 3c. The impurity peaks in the vicinity of the analyte peak did not in any way influence its resolution. These extraneous peaks, on further investigation, were due to the contaminations found in the septa of the injection vials. Replacement of

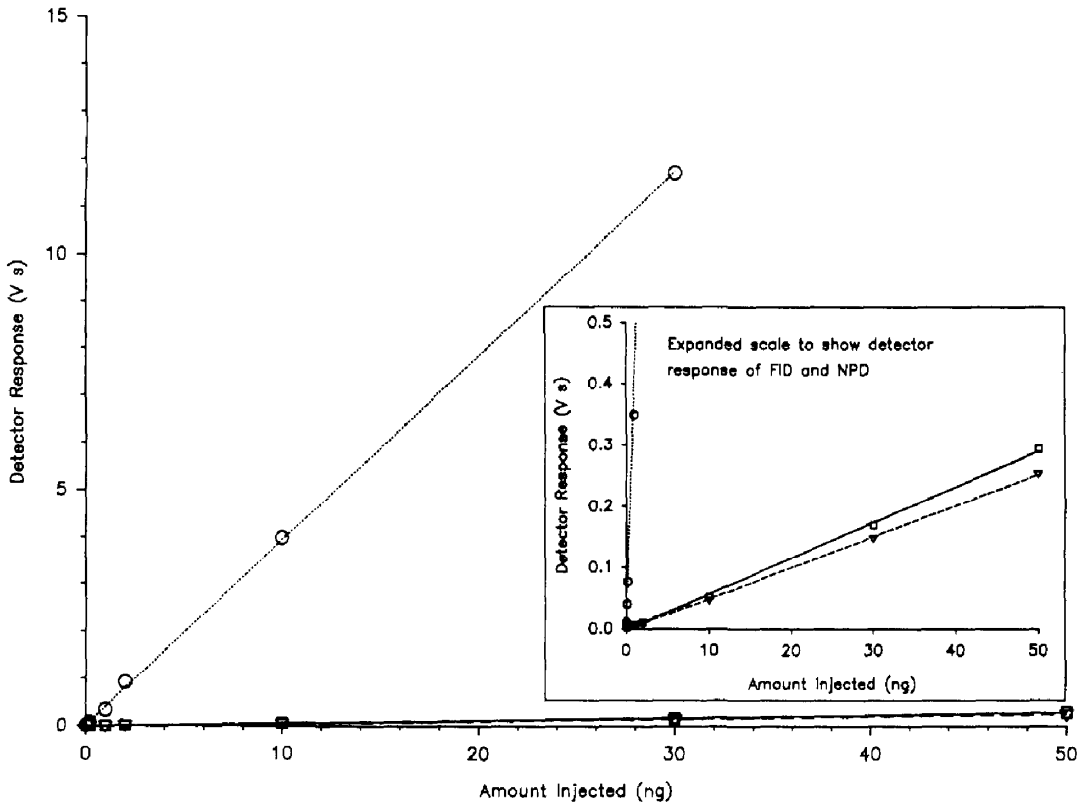

Fig. 4. A comparison of FID, NPD and ECD responses to MIMIC under identical GC conditions. ○ = ECD (V s = 0.02264 + 0.39035 ng, $r^2 = 1.000$); □ = FID (V s = −0.00090 + 0.00582 ng, $r^2 = 0.999$); ▽ = NPD (V s = −0.00115 + 0.00506 ng, $r^2 = 0.999$).

the septa, HP part 5181-1210, used to seal the injection vials with PTFE-lined (both sides) silicone septa (HP part 5181-1211), eliminated the appearance of these ghost peaks. A higher MDL (0.10 ng) coupled with a narrower linear range (0.5 to >50 ng) compared to FID, made NPD a less favourable choice for quantification of MIMIC. Because of these inherent problems, further attempts to quantify the analyte from formulation concentrates and spray mixes were not attempted. However, if other GC conditions are studied, evaluated and eventually optimized, NPD could very likely be useful to analyze the MIMIC present in these materials.

Attempts to chromatograph MIMIC using the DB-1 and DB-17 mega-bore columns were unsatisfactory due to massive baseline instability in addition to poor resolution (broad, tailing peaks) and non-reproducible retention times. Compared to FID, the performance of NPD was less than satisfactory in the analysis of MIMIC.

3.4. GC–ECD

To study the response of GC–ECD to MIMIC using the DB-5 column, the temperature conditions of the oven were initially set the same as in GC–FID and the other operating parameters were optimized as follows: *Gases*: carrier (helium) CHP, 140 kPa (linear velocity 55 cm/s); inlet purge flow, 60 ml/min (purge on at 0.5 min); septum purge flow, 3 ml/min; detector make-up (5% methane in argon), 80 ml/min. *Temperatures*: splitless injection port, 250°C; detector, 310°C. *Oven programme*: as for GC–FID.

Generally, the ECD response to MIMIC was nearly 60 to 70 times higher than that observed for FID and NPD. The linear range of ECD was found to be 0.05 to 40 ng ($r^2 = 1.000$) of MIMIC injected (Table 2, Fig. 4) with a MDL of 0.02 ng. ECD gave a narrower linearity range due to the marked increase in sensitivity, and the MDL observed (0.02 ng) was lower than with FID (0.04 ng) and NPD (0.10 ng). Due to the increase in sensitivity at these conditions, some peak tailing was observed for MIMIC which was not present in chromatograms obtained by FID and NPD (Fig. 3a and c). Fig. 3d illustrates the tailing effects of the above conditions on the peak shape of MIMIC at the increased sensitivity by ECD. The cause of the peak tailing, symptomatic of asymmetric elution, was probably due to the final column temperature being too low since MIMIC is relatively non-volatile. An alternative oven temperature program introduced as follows: initial 60°C at 0.75 min; ramp rate 30°/min; final 280°C at 2 min.; eliminated the distortion resulting in a more symmetrical peak shape for the analyte. The retention time of MIMIC using these improved parameters was 8.0 min while the linear range, r^2 and MDL were not noticeably affected (Table 2). Fig. 3e shows the sharp, symmetrical peak obtained for the analyte using ECD. In Table 3 are shown the results of analysis of formulation concentrates and spray mixes of MIMIC. The R.S.D. values ranged from 0.4 to 5.2% and the absolute error of the measurements varied from 1.2 to 12.6% illustrating the smallest variance between the HPLC–DAD, GC–FID and GC–ECD methods.

The DB-1 mega-bore column (15 × 0.53 mm I.D.) was tested with ECD under the same GC conditions except that the carrier flow (helium) was maintained at 15 ml/min and the make-up gas flow was reduced to 65 ml/min. The results obtained from injections of the MIMIC standard were very comparable to those of the mini-bore DB-5 column under similar GC conditions. At a flow-rate of 15 ml/min, the retention time and peak areas obtained were nearly identical to the earlier observations. One major drawback to this mega-bore column was that the increase in carrier flow through the ECD system caused a rising baseline during temperature programming as observed with NPD.

The DB-17 mega-bore column (15 × 0.53 mm I.D.) was similarly tested but did not yield satisfactory results due to baseline drift and tailing peaks. Under identical conditions as for the DB-1 mega-bore column, the retention time of the analyte increased to 19.0 min giving a broad peak with appreciable reduction in sensitivity.

3.5. GC formulation analysis

Typical chromatograms obtained from injections of the formulations by HPLC–DAD, GC–FID and GC–ECD using the DB-5 column are shown in Figs. 1b, 3b and 3f, respectively. No interfering peaks were observed by either HPLC or GC analysis. The results obtained by these methods, as mentioned earlier, are quite similar and are given in Table 3. The results indicate that any method is equally suitable for the analysis of MIMIC in various types of formulations, whether they are oil or aqueous based. Overall, the HPLC method gave the lowest R.S.D. and absolute error values as compared to the GC methods. However, this difference was not at all significant. The methods provide a direct, time- and laboratory-saving approach to the analysis of MIMIC formulation concentrates and spray mixes. However, GC analysis using ECD with the mini-bore DB-5 column may be preferred because of its higher sensitivity than the other methods and the lower retention time for the analyte and less solvent consumption as compared to HPLC.

4. Conclusions

The results of this study show that the active ingredient MIMIC in various formulation concentrates and spray mixes can be easily analyzed by HPLC–DAD, GC–FID or GC–ECD techniques. The extraction requires no cleanup and the chromatograms are free from any extraneous peaks. The methods are rapid, precise and sensitive with excellent chromatographic resolu-

tion. The total chromatographic analysis and the total analysis time on average for the HPLC and GC methods were respectively 30 and 15 min and 4 and 2.5 h/sample. The mini-bore DB-5 fused-silica capillary column gave good separation and acceptable analysis time compared to the mega-bore columns. Current studies also show that GC–ECD methods have considerable potential to analyze the residues of MIMIC in forestry substrates such as conifer foliage, forest soils, natural water etc. at sub-μg to pg levels.

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