Acta Chromatographica 20(2008)1, 109–118
DOI: 10.1556/AChrom.20.2008.1.9

Simultaneous Determination of Phenmedipham, Desmedipham, and Ethofumesate in a Pesticide Formulation by Normal-Phase High-Performance Liquid Chromatography

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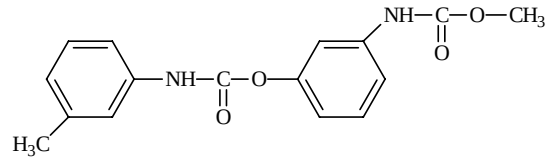
Summary. A normal-phase high-performance liquid-chromatographic method has been developed for simultaneous quantitative analysis of phenmedipham, desmedipham, and ethofumesate in a pesticide formulation. Analysis was performed on a 25 cm × 0.4 cm, 5-µm particle, CN column with *n*-hexane–dichloromethane 40:60 (*v*/*v*) as mobile phase at a flow rate of 1 mL min^{-1}. UV detection was performed at 270 nm; the constant column temperature was 25°C. The run time under these chromatographic conditions was less than 8 min. Calibration plots were linear in the concentration range 76–380 µg mL^{-1} for phenmedipham, 72–360 µg mL^{-1} for desmedipham, and 52–260 µg mL^{-1} for ethofumesate. Statistical evaluation by analysis of variance showed the intra-day repeatability (n = 8) and inter-day precision (n = 3) of the assay were satisfactory. The sensitivity of the method, as the limits of detection (*LOD*) and quantification (*LOQ*) for each active ingredient, was also determined.

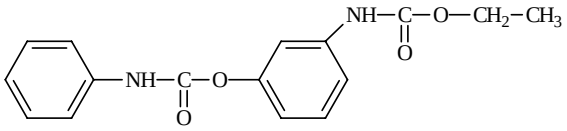
Introduction

The control of weeds is an essential aspect of productive agriculture. Uncontrolled weeds that emerge with the crop typically cause from 50 to 100% loss of yield [1]. Weeds compete with crops for moisture, light, and nutrients. They may also interfere with harvesting, making the process less efficient. Herbicides, or chemical weed killers, are the primary tools used to manage weeds. Phenmedipham, desmedipham, and ethofumesate are among the most popular post-emergence herbicides [1]. The pesticide formulations Betanal, active ingredient phenmedipham, and Tramat, active ingredient ethofumesate, are specific sugar beet chemicals which are control a wide range of grass and broad-leaved weeds and kill virtually everything except the sugar beet [2].

Phenmedipham (ISO), methyl 3-(3-methylcarbaniloyloxy)carbanilate or 3-methoxycarbonylaminophenyl 3′-methylcarbanilate (IUPAC), is a bis-carbamate herbicide (*Fig. 1a*) [3–6]. It is a selective systemic herbicide that is absorbed through the leaves and inhibits photosynthetic electron transport. Phenmedipham is a broadleaf herbicide used on beet crops, especially sugar beet, spinach, garden (table) beet, and strawberries.



(a)



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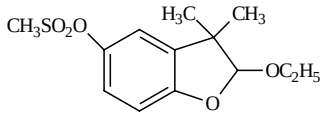

(c)

Fig. 1. The chemical structures of (a) phenmedipham (b), desmedipham, and (c) ethofumesate

Ethyl 3′-phenylcarbamoyloxycarbanilate, or ethyl 3-phenylcarbamoyloxyphenylcarbamate (IUPAC), common name desmedipham (ISO) (*Fig. 1b*), is another bis-carbamate herbicide [3, 4, 7, 8]. Its structure and mode of action on plants are similar to those of phenmedipham. It is used to control a wide range of broad-leaved weeds in beet crops, especially sugar beet. It is usually sprayed in combination with phenmedipham and ethofumesate. This active component is a selective systemic herbicide which is absorbed through the leaves and inhibits photosynthetic electron transport.

Ethofumesate (ISO), (±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulphonate (IUPAC) (*Fig. 1c*), is a selective benzofuranyl alkanesulphonate herbicide [3, 4, 9, 10] that is absorbed by roots and emerging shoots (grasses). It inhibits the growth of meristems, retards cell division, and limits the formation of waxy cuticle. Ethofumesate is registered

for preplant, pre-emergence, and post-emergence use to control broad-leaved and grass weeds in sugar beet, garden beet, table beet, carrots, and turf.

Analytical determination of phenmedipham is by titration or HPLC [3]. Residues of the parent compound have been analysed by GLC, in soil by HPLC, or by hydrolysis to *m*-toluidine, derivatives which were determined by GLC with ECD or colorimetry [3]. Analysis of desmedipham has been performed by HPLC or colorimetry [3] and desmedipham residues have been hydrolysed to aniline and determined by GLC or HPLC [3]. GLC with FPD has been used for determination of ethofumesate and its residues [3].

The CIPAC (Collaborative International Pesticides Analytical Council) handbook [11] reference method for determination of phenmedipham in technical and emulsifiable concentrates is reversed-phase HPLC with UV detection at 238 nm and butyl benzoate as internal standard. The titrimetric method is suitable for the determination of the active ingredient in technical phenmedipham [11]. Analysis for determination of ethofumesate in technical, EC, and SC pesticide formulations has been performed by reversed-phase HPLC with ethyl benzoate as internal standard and UV detection at 225 nm, in accordance with the CIPAC handbook reference method [12]. A CIPAC reference method for determination of desmedipham in pesticide formulations has not been found.

An LC method using on-line trace-enrichment, gradient elution, and diode-array detection has been described for trace-level determination of several phenylcarbamate herbicides, including phenmedipham and desmedipham, in environmental water samples [13]. Tatarkovicova and Stransky used RP-HPLC with UV detection for determination of carbamate pesticides in soil [14]. Pesticides, including carbamates, in water samples have been concentrated then analyzed by RP-HPLC on an Aquapore RP-300 column with a methanol–water mixture as mobile phase [15]. A simple quantitative TLC method for analysis of residues of herbicides inhibiting photosynthesis has been compared with a capillary GLC for analysis of atrazine, chloridazone, lenacil, phenmedipham, and desmedipham in sugar beet and sugar [16]. Perret et al. developed a reversed-phase LC–MS method for simultaneous determination of two carbamate residues (phenmedipham and desmedipham) and related metabolites (*m*-aminophenol, aniline, and *m*-toluidine) in soil [17]. Trace determination of carbamate pesticides, including desmedipham, in ground and surface waters has been achieved by LC on a C_{18} silica column with post-column fluorescence detection or LC diode-array UV detection coupled on-line to a solid-phase-extraction system [18]. Krongaard et al. used reversed-phase high-performance liquid chromatography on a 250 mm × 4.6 mm, 5-µm particle,

Prodigy ODS column, with methanol–water as mobile phase, and UV detection at 225 nm, for simultaneous determination of ethofumesate, phenmedipham, and desmedipham in herbicide formulations [4].

There is, however, no standard reference CIPAC or AOAC (Association of Official Analytical Chemists) analytical method for simultaneous determination of phenmedipham, desmedipham, and ethofumesate. Because the solubility of these three herbicides in nonpolar solvents, for example hexane (0.5 g L^{-1} for phenmedipham and desmedipham, 4 g L^{-1} for ethofumesate) and dichloromethane (16.7 g L^{-1} for phenmedipham, 19.8 g L^{-1} for desmedipham, and >600 g L^{-1} for ethofumesate) is better than in water (0.0047, 0.007, and 0.05 g L^{-1} for phenmedipham, desmedipham, and ethofumesate, respectively), the objective of the work discussed in this paper was to investigate the possibility of developing a normal-phase HPLC method for simultaneous determination of phenmedipham, desmedipham, and ethofumesate in the pesticide formulation Inter Of.

Experimental

Equipment and Materials

HPLC analysis was performed with a Perkin–Elmer liquid chromatograph comprising a binary LC Pump (model 250), an injection valve with 20-µL sample loop (manual injection), and a model LC 235 UV diode-array detector. Constant column temperature was maintained with a Spark Holland 'Mistral' (type 880) column thermostat. Compounds were separated on three analytical columns:

25 cm × 0.4 cm, 5-µm particle, LiChrosorb Si 60 (Merck, Germany); 3.3 cm × 0.46 cm, 3-µm particle, HS Pecospher 3×3 Silica (Perkin–Elmer); and 25 cm × 0.4 cm, 5-µm particle, LiChrosorb CN (Merck).

HPLC-grade *n*-hexane and dichloromethane were purchased from Merck. All solvents and solutions for HPLC analysis were degassed in an ultrasonic bath before use. Pure analytical standards of phenmedipham (99.6%), desmedipham (99.7%) and ethofumesate (99.9%) were from Bayer (Germany). The pesticide formulation Inter Of, as a liquid emulsifiable concentrate (EC), was procured free of charge from Herbos (Croatia). The declared content was 91 ± 9 g L^{-1} phenmedipham, 71 ± 7 g L^{-1} desmedipham, and 112 ± 7 g L^{-1} ethofumesate. The density of the pesticide formulation Inter Of is 1.011 g mL^{-1}.

Preparation of Standard Solutions

Stock solutions of phenmedipham, desmedipham, and ethofumesate were prepared by dissolving 0.0475, 0.0450, and 0.0325 g, respectively, of the pure analytical standards in 1:1 (*v*/*v*) *n*-hexane–dichloromethane in a 25-mL volumetric flask. All solutions were stored under refrigeration at 4°C.
The stock solutions were used to prepare standard mixtures of different concentrations by dilution of 0.4, 0.8, 1.2, 1.6, and 2 mL of each stock solution with the same solvent mixture in 10-mL volumetric flasks.

Calibration plots for phenmedipham, desmedipham, and ethofumesate were constructed after triplicate analysis (10 µL each) of the standard mixtures. The area and height of individual peaks and the corresponding amounts of phenmedipham, desmedipham, and ethofumesate were used to construct the plots by least-squares regression, using Omega statistical software [19], with external standard multilevel calibration by linear fit.

Preparation of Sample Solutions

Sample solutions of pesticide formulation Inter Of were prepared by dissolving 0.3147 g and 0.6382 g in 1:1 (*v*/*v*) *n*-hexane–dichloromethane in a 25-mL volumetric flask. The samples were degassed for 15 min in an ultrasonic bath then 1 mL of each solution was transferred to a 10-mL volumetric flask and diluted to volume with the same solvent mixture. Each of these solutions was injected in triplicate (10-µL injections).

The recovery of the method was determined by transferring 1 mL from each sample solution to a 10 mL volumetric flask and diluting to volume with 1:1 (*v*/*v*) *n*-hexane–dichloromethane. A known amount of each analyte was added to each solution (82 and 164 µg mL^{-1} for phenmedipham, 74 and 148 µg mL^{-1} for desmedipham, and 44 and 88 µg mL^{-1} for ethofumesate) and each of the spiked solutions obtained was analysed in triplicate (10-µL injections). Because the sample solutions were clear, filtering was not necessary.

Results and Discussion

The UV spectra of phenmedipham, desmedipham, and ethofumesate in 40:60 (*v*/*v*) *n*-hexane–dichloromethane show they have absorption maxima at 274, 272, and 280 nm, respectively. The chromatographic peaks obtained for phenmedipham and desmedipham at 270 nm are more intense than those obtained at 280 nm. Because an intense peak is obtained for etho-

fumesate at both wavelengths, HPLC analysis for simultaneous determination of the active components of the pesticide formulation Inter Of was performed at 270 nm. In addition, to confirm the specificity of the method, UV diode-array detection was used to check peak purity and analyte peak identity [20]. Overlaid spectra obtained from a pure analytical standard and from the same compound in the pesticide formulation confirmed the identity of the analytes.

In preliminary experiments, several analytical columns were tested. Investigation of the LiChrosorb Si 60 column was performed with mobile phases containing *n*-hexane–dichloromethane in different volume ratios (0–100% dichloromethane) at flow rates from 1–2 mL min^{-1}. It is known that when retention times are too long the mobile phase must be strengthened by increasing the concentration of the stronger solvent [21]. When 100% dichloromethane and a flow rate of 2 mL min^{-1} were used, however, only ethofumesate was eluted in a 25-min run, i.e. under these conditions phenmedipham and desmedipham were retained.

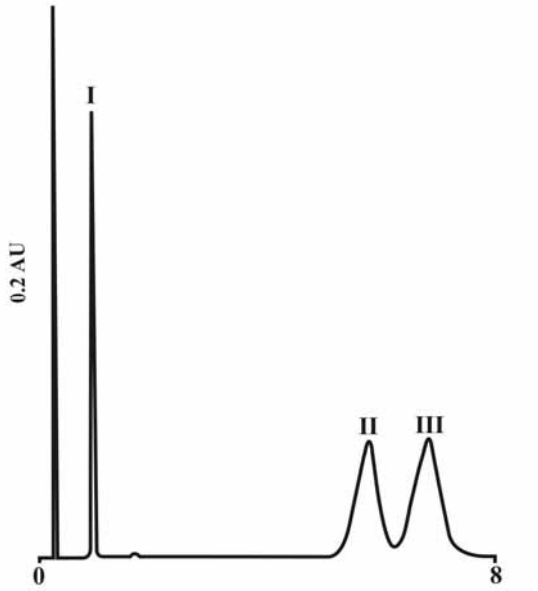

Fig. 2. Chromatogram obtained from a standard mixture of ethofumesate (I), desmedipham (II), and phenmedipham (III) on the HS Pecospher 3×3 Silica column with 100% dichloromethane as mobile phase

The HS Pecospher 3×3 Silica column was investigated under the same set of conditions. When 100% dichloromethane was used as mobile phase at a flow rate of 2 mL min^{-1} retention times of approximately 0.91, 5.53, and 6.53 min were obtained for ethofumesate, desmedipham, and phenmedi-

pham, respectively (*Fig. 2*). The dead time for this column under these conditions was 0.26 min, so the retention factor for ethofumesate was 2.5. For the other two active ingredients, however, the capacity factors were very high (20.27 for desmedipham and 24.11 for phenmedipham). Because of the high volatility of dichloromethane, work with this solvent is difficult, so further quantitative investigation was not performed.

For further investigation the LiChrosorb CN column was used. The best separation, with symmetrical peak shapes and good purity indexes (1.1 for ethofumesate and 1.2 for desmedipham and phenmedipham), was achieved with 40:60 (*v*/*v*) *n*-hexane–dichloromethane as mobile phase at a flow rate of 1 mL min^{-1} (*Fig. 3*). Under these conditions the mean retention times were 5.77, 6.56, and 6.86 min for ethofumesate, desmedipham, and phenmedipham, respectively. The estimated column dead time was 2.57 min, so the calculated retention factors (*k*) were 1.24 for ethofumesate, 1.55 for desmedipham, and 1.67 for phenmedipham. The separation factors (*a*) between adjacent peaks were 1.24 and 1.08.

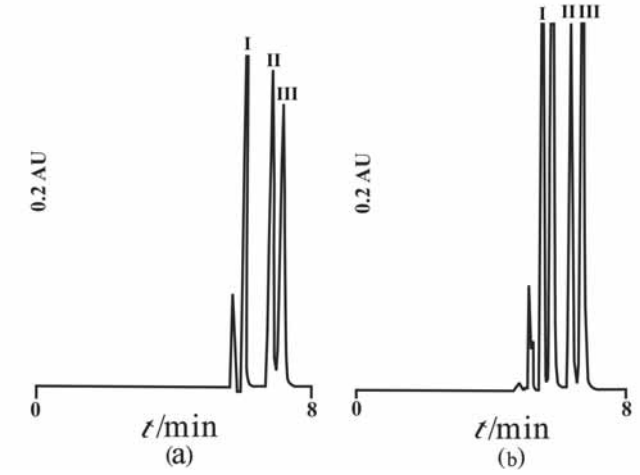

Fig. 3. Chromatograms obtained from ethofumesate (I), desmedipham (II), and phenmedipham (III) on the LiChrosorb CN column with 40:60 (*v*/*v*) *n*-hexane–dichloromethane as mobile phase: (a) standard mixture; (b) pesticide formulation

Inter Of

The day-to-day ($n = 3$) and within-day ($n = 8$) repeatability [21, 22] were evaluated for the retention times, peak areas, and peak heights of phenmedipham, desmedipham, and ethofumesate by replicate ($n = 8$) successive injections of analytical standards at concentrations of 304, 288, and 208 μg mL^{-1} for phenmedipham, desmedipham and ethofumesate, respect-

ively. Testing of the results by ANOVA revealed that *F* values calculated for retention times, peak areas, and peak heights were smaller than the table values for all the compounds. It was therefore concluded that there were no significant differences between the assays within and between days. The relative standard deviations of peak heights (from 1.79 to 2.42%) were smaller than those of peak areas for each active ingredient (2.50 to 3.53%); this is not unexpected because the peaks were not separated to baseline (*Fig. 3*).

Calibration graphs were constructed by plotting the amount of standard injected as functions of peak area and peak height. The peak areas and heights were used as dependent variables and their values were treated with the Omega statistical software [19] with external standard multilevel calibration by linear fit. The curves followed Beer's law in the range 0.76–3.8 μg (or 76–380 μg mL^{-1}) for phenmedipham, 0.72–3.6 μg (72–360 μg mL^{-1}) for desmedipham, and 0.52–2.6 μg (or 52–260 μg mL^{-1}) for ethofumesate (*Table I*).

Table I. Linear range and limits of detection and quantitation for phenmedipham, desmedipham, and ethofumesate in the pesticide formulation Inter Of

	Phenmedipham	Desmedipham	Ethofumesate
Linear range (μg mL^{-1})	76–380	72–360	52–260
Limit of detection (μg mL^{-1})	41.2	41.1	32.5
Limit of quantitation (μg mL^{-1})	137.4	137.1	108.3

The results obtained for the correlation coefficients (R^2) indicated use of peak height as variable was preferable. The linearity of the method was good; the values obtained for the correlation coefficients were 0.9983 for phenmedipham, 0.9988 for desmedipham, and 0.9982 for ethofumesate.

Limits of detection (*LOD*) and quantification (*LOQ*) for the compounds were determined by construction of calibration plots in the low-concentration ranges (19–380 ng or 1.9–38 μg mL^{-1} for phenmedipham and desmedipham and 15–300 ng or 1.5–30 μg mL^{-1} for ethofumesate). The limit of detection (*LOD*) was calculated as three times the ratio of the *SD* to the slope of the low-concentration calibration plot ($LOD = 3 \times SD/\text{slope}$), and the limit of quantification (*LOQ*) was calculated as ten times the same ratio ($LOQ = 10 \times SD/\text{slope}$) [23]. The limits of detection (*LOD*) were 0.412 μg (41.2 μg mL^{-1}) for phenmedipham, 0.411 μg (41.1 μg mL^{-1}) for desmedi-

pham, and 0.325 μg (32.5 μg mL^{-1}) for ethofumesate at 0.02 AUFS sensitivity. The limits of quantification (*LOQ*) were 1.374 μg (137.4 μg mL^{-1}) for phenmedipham, 1.371 μg (137.1 μg mL^{-1}) for desmedipham, and 1.083 μg (108.3 μg mL^{-1}) for ethofumesate. Under these chromatographic conditions sensitivity was best for ethofumesate.

The accuracy of the method was confirmed by the method of standard additions. It was expressed as the deviation between the calculated mean value obtained by examination and the true amount of analyte added to sample matrix already containing some of the analyte. The mean concentrations of the active ingredients in the pesticide formulation tested were in agreement with the values declared by the manufacturer (*Table II*).

Table II. Results from recovery experiments ($n = 3$)

		Mass of analyte (μg)			Recovery (%)	*RSD* (%)
		Before addition	After addition	Added		
Sample solution I	Phenmedipham	1.243	2.070	0.82	100.85	0.62
			2.887	1.64	100.24	0.65
	Desmedipham	0.890	1.628	0.74	99.73	0.10
			2.296	1.48	95.00	0.22
	Ethofumesate	1.360	1.804	0.44	100.91	1.04
			2.202	0.88	95.68	0.22
Sample solution II	Phenmedipham	2.515	3.341	0.82	100.73	0.86
			4.176	1.64	101.28	0.26
	Desmedipham	1.767	2.500	0.74	99.05	0.84
			3.174	1.48	95.07	0.14
	Ethofumesate	2.736	3.183	0.44	101.59	0.28
			3.613	0.88	99.66	0.33

Conclusion

A convenient normal-phase HPLC method with a LiChrosorb CN column has been developed for simultaneous determination of phenmedipham, desmedipham, and ethofumesate in the pesticide formulation Inter Of. High correlation coefficients were obtained for calibration equations, reproducibility of retention time, peak area, and peak height was good, and analytical run time was short (8 min). The proposed method is simple, relatively rapid, and sufficiently precise for routine analysis of the active ingredients (phenmedipham, desmedipham and ethofumesate) in the pesticide formulation Inter Of.

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