Macedonian Journal of Chemistry and Chemical Engineering, Vol. **31**, No. 1, pp. 39–46 (2012)
ISSN 1857-5552
UDC: 543.544.5.068.7:632.95

MJCCA9 – 590
Received: March 21, 2011
Accepted: November 4, 2011

Original scientific paper

RP-HPLC-DAD METHOD FOR SIMULTANEOUS DETERMINATION OF DESMEDIPHAM, PHENMEDIPHAM AND ETHOFUMESATE IN A PESTICIDE FORMULATION

Lenče Velkoska-Markovska*, Biljana Petanovska-Ilievska, Lila Vodeb

Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Blvd. Aleksandar Makedonski bb, P.O. Box 297, 1000 Skopje, Republic of Macedonia
levemar@gmail.com; lencevm@zf.ukim.edu.mk

A fast, simple, precise and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) method with UV-DAD for simultaneous determination of desmedipham, phenmedipham and ethofumesate in the pesticide formulation "Inter OF" has been developed. The analysis was performed on a LiChrospher 60 RP-select B (25 cm × 0.4 cm, 5 μm, Merck) analytical column, with mobile phase of methanol/water (60/40, *V/V*), flow rate of 1 ml/min, UV-detection at 230 nm and constant column temperature at 25 ºC. The following parameters were determined for the developed method: retention factor, separation factor, limit of detection (LOD), limit of quantification (LOQ), precision of obtained results for peak area, linearity, recovery of analyte and active ingredients quantity in a pesticide formulation.

Keywords: desmedipham; phenmedipham; ethofumesate; RP-HPLC; UV-DAD

RP-HPLC-DAD МЕТОД ЗА ИСТОВРЕМЕНО ОПРЕДЕЛУВАЊЕ НА ДЕСМЕДИФАМ, ФЕНМЕДИФАМ И ЕТОФУМЕСАТ ВО ПЕСТИЦИДНА ФОРМУЛАЦИЈА

Разработен е брз, едноставен, прецизен и точен метод со помош на реверзно-фазна високоефикасна течна хроматографија (RP-HPLC) и ултравиолетов детектор со низа од диоди (UV-DAD) за истовремено определување на десмедифам, фенмедифам и етофумесат во пестицидната формулација "Inter OF". Анализата е изведена на аналитичката колона од типот LiChrospher 60 RP-select B (25 cm × 0,4 cm, 5 μm, Merck) со мобилна фаза составена од метанол/вода (60/40, *V/V*), проток 1 ml/min, UV-детекција на 230 nm и константна температура на колоната од 25 ºC. За разработениот метод се определени следниве параметри: ретенциски фактор, сепарациски фактор, линеарност, граница на детекција и квантификација, прецизност за површината под пиковите, аналитички принос и содржина на активните компоненти во пестицидната формулација.

Клучни зборови: десмедифам; фенмедифам; етофумесат; RP-HPLC; UV-DAD.

1. INTRODUCTION

Desmedipham, phenmedipham and ethofumesate are some of the most popular post-emergence herbicides [1], which control a wide range of grass and broadleaf weeds and virtually kill everything except the sugar beet [2]. These three substances represent active ingredients in the pesticide formulation "Inter OF" which appears in the form of liquid emulsifiable concentrate (EC) and is used for mortification of annual broadleaf weeds in sugar beet.

Desmedipham (Figure 1a), ethyl 3′-phenylcarbamoyloxycarbanilate or ethyl 3-phenylcarbamoyloxyphenylcarbamate (IUPAC) [3–6] and phenmedipham (Figure 1b), me-

thyl 3-(3-methylcarbaniloyloxy)carbanilate or 3-methoxycarbonylaminophenyl 3′-methylcarbanilate (IUPAC) [3, 4, 7, 8] belong to the group of bis-carbamate herbicides. They have similar structure and mode of action in plants and they are selective systemic herbicides that are absorbed through the leaves and inhibit the photosynthetic electron transport. Desmedipham is used to control a broad spectrum of broadleaf weeds in beet crops, in particular sugar beet. It is usually sprayed in combination with phenmedipham and ethofumesate. Phenmedipham is a broadleaf herbicide used in beet crops, especially sugar beet, spinach, garden (table) beets and strawberries.

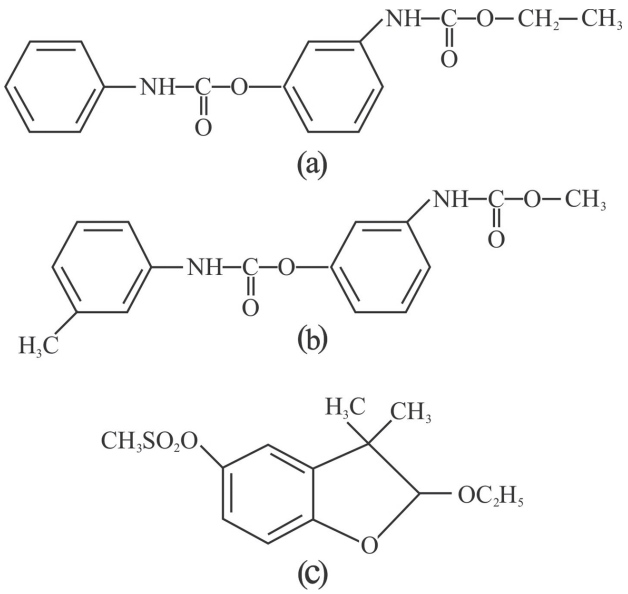

Fig. 1. Chemical structures of the active ingredients: desmedipham (a), phenmedipham (b) and ethofumesate (c)

Ethofumesate (Figure 1c), (±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate (IUPAC) is a benzofuranyl alkanesulfonate herbicide [3, 4, 9, 10]. It is a selective systemic herbicide that is absorbed by the roots and emerging shoots (grasses). Inhibits the growth of meristems, retards the cellular division, and limits formation of waxy cuticle. Ethofumesate is registered for preplant, preemergence and postemergence use to control broadleaf and grass weeds in sugar beets, garden beets, table beets, carrots, and turf.

Quantitative determination of these active ingredients has been accomplished by HPLC or colorimetry (for desmedipham), HPLC or titration (for phenmedipham) or by GLC (for ethofumesate) [3].

Analysis for determination of phenmedipham in technical and EC formulations has been performed by RP-HPLC, using butyl benzoate as an internal standard and UV-detection at 238 nm, according to the CIPAC-handbook reference method [11]. Also, the titrimetric method is suitable for the determination of the active ingredient phenmedipham in technical formulation [11]. The actual CIPAC-handbook referee method for determination of ethofumesate in technical, EC and SC (suspension concentrate) pesticide formulations is by RP-HPLC, using UV-detection at 225 nm with ethyl benzoate as an internal standard [12]. For determination of desmedipham in pesticide formulation, CIPAC referee method has not been found.

Velkoska-Markovska *et al*. developed normal-phase high-performance liquid chromatography (NP-HPLC) methods with UV-detection for simultaneous determination of ethofumesate, phenmedipham and desmedipham in EC herbicide formulation "Inter OF" [13, 14]. Krongaard *et al*. used RP-HPLC method for simultaneous determination of ethofumesate, phenmedipham and desmedipham in SC herbicide formulations available on the Danish market, such as "Betanal Optima SC" and "Kemifam Pro SC" [4]. Analysis has been performed on Prodigy ODS (250 mm × 4.6 mm, 5 μm) column with methanol/water (65/35, *V*/*V*) as a mobile phase and UV-detection at 225 nm.

However, no standard reference CIPAC or (provide definition) AOAC analytical method for simultaneous determination of desmedipham, phenmedipham and ethofuesate has been found. On the other hand, the dichloromethane is highly volatile, and the work with this solvent is difficult. Hence, the aim of this paper is to investigate the possibility of developing a new RP-HPLC method for simultaneous determination of desmedipham, phenmedipham and ethofumesate in the EC pesticide formulation "Inter OF".

2. EXPERIMENTAL

2.1. *Reagents and chemicals*

HPLC-grade methanol and acetic acid were purchased by Sigma-Aldrich (Deisenhofen, Germany). Water was deionized and then distilled from glass apparatus. The pure analytical standards of desmedipham (99.7 %), phenmedipham (99.6 %) and ethofumesate (99.9 %) were from Bayer (Germany). Pesticide formulation "Inter OF" was procured free of charge from Herbos (Croatia). It was declared as containing 71 ± 7 g/l of desmedipham, 91 g/l ± 9 g/l of phenmedipham and 112 g/l ± 7 g/l of ethofumesate. The density of the pesticide formulation "Inter OF" was 1.011 g/ml. For determination the column's dead-time sodium nitrate, supplied from Alkaloid (Macedonia), was used.

2.2. *Equipment*

The HPLC analysis was performed with a Perkin Elmer liquid chromatography system consisting of a binary LC Pump (model 250), an injection valve with 20 μl sample loop (manual injection), and UV-Diode Array Detector (model LC 235). Data processing and integration were performed with Omega software [15]. All solvents and solutions for HPLC analysis were degassed in an ultrasonic bath before use. Constant column temperature was maintained with Column thermostat Spark Holland "Mistral" (type 880). The investigations were carried out on a Hypersil ODS (25 cm × 0.46 cm, 5 μm, Sigma-Aldrich) and LiChrospher 60 RP-select B (25 cm × 0.4 cm, 5 μm, Merck) analytical columns.

2.3. *Preparation of standard solutions*

Stock solutions of desmedipham, phenmedipham and ethofumesate were prepared by dissolving 0.0195 g, 0.0202 g and 0.1015 g of the pure analytical standards in methanol in a 25 ml volumetric flask. All solutions were stored in a refrigerator at 4 ºC. Stock solutions were used to prepare standard mixtures with different herbicide concentrations (15.55–186.64 μg/ml for desmedipham, 16.10–193.15 μg/ml for phenmedipham and 81.12–973.44 μg/ml for ethofumesate). These standard mixtures were prepared from 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml of each stock solution in 10 ml volumetric flask and dissolved with the mixture of methanol/water (50/50, *V*/*V*).

The calibration curves of desmedipham, phenmedipham and ethofumesate were obtained with triplicate injections (10 μl each) of standard mixtures. The area and height of individual peaks and the corresponding amount of desmedipham, phenmedipham and ethofumesate were used to construct the standard curves using the least-squares method.

2.4. *Preparation of sample solutions*

Sample solutions of pesticide formulation "Inter OF" were prepared in a 25 ml volumetric flask by dissolving the weighed amounts of 0.2608 g and 0.4030 g in methanol. The samples were degassed for 20 min in an ultrasonic bath, and from each sample solution 1 ml was transferred in a 10 ml volumetric flask and dissolved with the mixture of equal volumes of methanol and water. These solutions were filtered through 0.45 μm Spartan-T syringe filters and four injections were performed with 10 μl each.

The solutions for recovery experiment were prepared by dissolving 1 ml of each sample solution in a 10 ml volumetric flask. In each solution a known amount of each analyte was added (31.2 μg/ml and 62.4 μg/ml for desmedipham, 32.4 μg/ml and 64.8 μg/ml for phenmedipham, and 162.4 μg/ml and 324.8 μg/ml for ethofumesate). All the sample solutions were filtered through 0.45 μm Spartan-T syringe filters and the four injections were performed with 10 μl each for all cases.

3. RESULTS AND DISCUSSION

HPLC analysis for simultaneous determination of active ingredients in the pesticide formulation "Inter OF" was performed at 230 nm, because the UV spectra of these active components in methanol/water mixture (60/40, *V*/*V*), show that they have a band with absorption maximum at 235 nm for desmedipham and phenmedipham, and 225 nm for ethofumesate (Figure 2). In addition, to confirm the specificity of the developed method, UV-diode array detection was used to check the peak purity and analyte peak identity [16]. The overlaid spectra obtained by comparing the absorption spectra of a pure analytical standard of active ingredient and absorption spectra of the same analyte in the pesticide formulation are illustrated in Figure 2a for desmedipham (with a purity index of 1.1), in Figure 2b for phenmedipham (purity index = 1.1), and in Figure 2c for ethofumesate (purity index = 1.2) and confirmed the identity of the analytes.

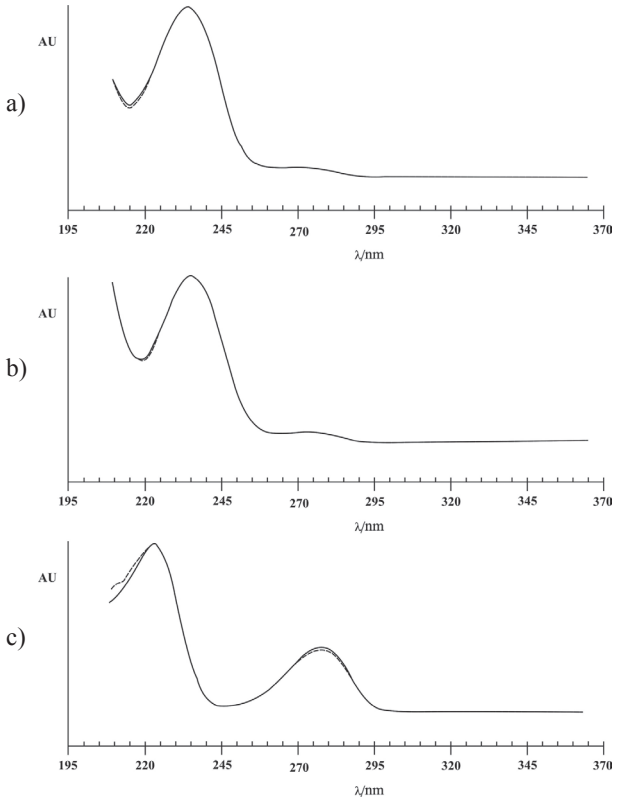

Fig. 2. Overlaid UV spectra obtained by comparing the absorption spectra of a pure analytical standard of active ingredient and absorption spectra of the same analyte in the pesticide formulation for (a) desmedipham (1.548 μg), (b) phenmedipham (1.986 μg) and (c) ethofumesate (2.491 μg) in methanol/water (60/40, V/V)

For simultaneous determination of active ingredients in the pesticide formulation "Inter OF" similar chromatographic conditions were used as those described by Krongaard *et al.* [4]. Namely, Hypersil ODS (25 cm × 0.46 cm, 5 μm) analytical column, mobile phase consisted of methanol/water (60/40, V/V), flow rate of 1 ml/min, UV-detection at 230 nm were used at constant column temperature at 20 ºC. Under these experimental conditions the obtained chromatographic peaks of all three analytes were asymmetric, i.e. with tailing (Figure 3 a,b,c). On the other hand, the peaks of desmedipham (I) and phenmedipham (II) from their standard mixture were not completely separated (Figure 3d). Because of these reasons, the possibility for making mistakes in calculation of concentrations of analytes were increased. There are many reasons for tailing phenomenon, such as unsuitable choice of mobile or stationary phases, which can be remedied by changing the mobile and/or stationary phases, changing the pH (for example, with addition of acetic or formic acid), or using a different method [17]. To achieve the good separations with symmetric peaks shape different volume of 0.05 mol/L acetic acid were added to the standard mixtures of three active ingredients, but no significant changes were noticed.

Further investigations were performed on LiChrospher 60 RP-select B (25 cm × 0.46 cm, 5 μm) analytical column with mobile phase consisted of methanol/water with different volume ratio (30–60 % water), flow rate of 1 ml/min, at constant column temperature at 25 ºC, and UV-detection at 230 nm. The best separation with symmetrical peaks shape and good index purity (1.1 for desmedipham and phenmedipham and 1.2 for ethofumesate) was achieved with mobile phase of methanol/water (60/40, *V*/*V*) (Figure 4). Under these chromatographic conditions the obtained value for column dead time was 1.55 min, and the mean value for the retention times were 7.89 min for desmedipham, 8.58 min for phenmedipham and 9.75 min for ethofumesate. Hence, the calculated values for retention factors (k') were 4.09 (for desmedipham), 4.53 (for

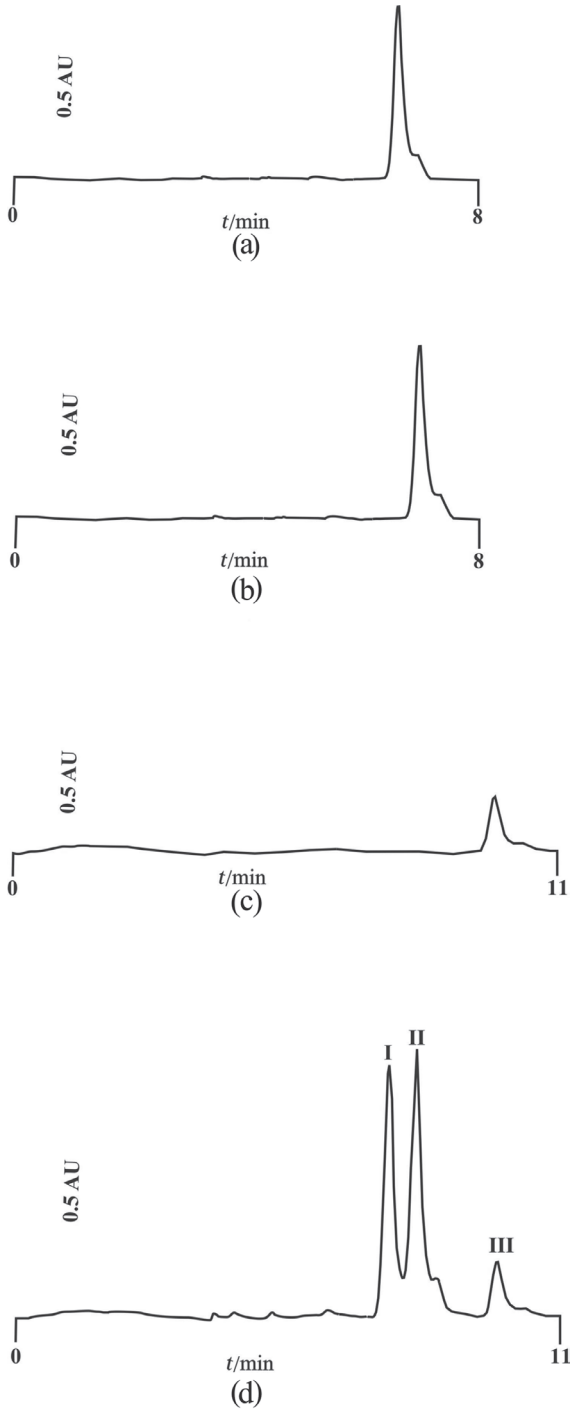

Fig. 3. Chromatograms obtained from analytical standard (0.5 μg) of desmedipham (a), phenmedipham (b) and ethofumesate (c) and their standard mixture (d) on the Hypersil ODS column with 60:40 (*V/V*) methanol-water as mobile phase

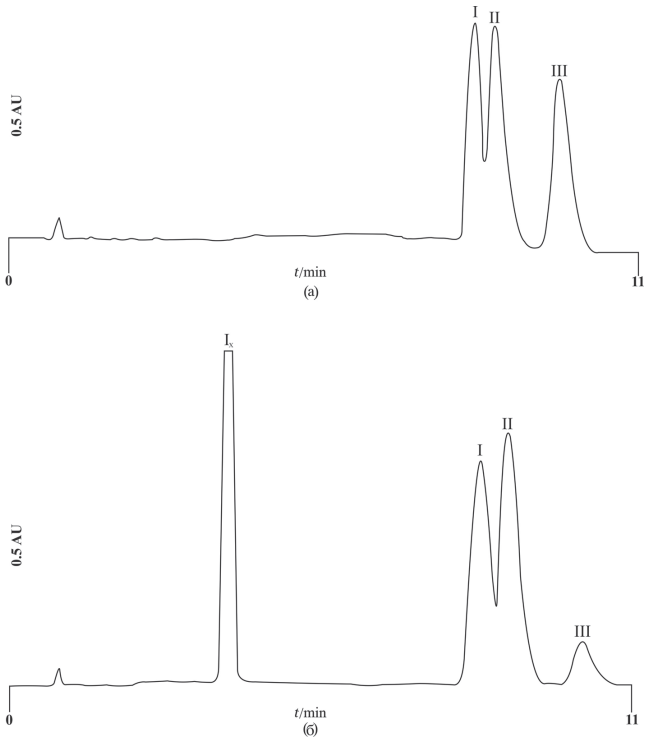

Fig. 4. Chromatograms obtained from desmedipham (I), phenmedipham (II) and ethofumesate (III) on the LiChrospher 60 RP-select B column with 60:40 (*V/V*) methanol-water as mobile phase: (a) standard mixture (1.248 μg desmedipham, 1.296 μg phenmedipham, 6.496 μg ethofumesate); (b) pesticide formulation "Inter OF" (1.548 μg desmedipham, 1.986 μg phenmedipham, 2.491 μg ethofumesate)

phenmedipham) and 5.29 (for ethofumesate). The estimated values for the separation factors (α) between the adjacent peaks were: $\alpha_{I,II} = 1.11$ and $\alpha_{II,III} = 1.17$.

Calibration graphs were constructed by plotting the injected amount of the standard of active ingredient as a function of the peak area and height. The curves followed Beer's law in the range of 0.156–1.872 μg (or 15.6–187.2 μg/ml) for desmedipham, 0.162–1.944 μg (or 16.2–194.4 μg/ml) for phenmedipham, and 0.812–9.744 μg (or 81.2–974.4 μg/ml) for ethofumesate. The obtained results for multiple correlation coefficients (R^2) indicated, preferably the use of peak area as a variable. The method revealed good linearity, because the obtained values for $R^2 \geq 0.9990$. The results are given in Table 1.

The limits of detection (LOD) was defined as the amount of analyte for which the signal-to-noise ratio (*S*/*N*) was 3, whereas the limits of quantification (LOQ) was defined as

T a b l e 1

Linearity and sensitivity data for determination of investigated compounds

Compound	Linearity range (μg/ml)	Regression equation	R^2	LOD (μg/ml)	LOQ (μg/ml)
Desmedipham	15.6-187.2	$y = 3{\cdot}107x + 96171$	0.9990	0.179	0.593
Phenmedipham	16.2-194.4	$y = 4{\cdot}107x - 87737$	0.9995	0.186	0.616
Ethofumesate	81.2-974.4	$y = 6{\cdot}106x - 273307$	0.9998	1.845	6.09

T a b l e 2

Statistical data for repeatability

	Compound	Mean ± SD	RSD (%)	Proposed acceptable RSD (%)
Peak area	Desmedipham	41579921 ± 703326.2	1.69	2.0
	Phenmedipham	49161163 ± 717672.9	1.46	1.92
	Ethofumesate	39617922 ± 370729.6	0.94	1.87

the amount of analyte for which S/N =10 at 0.05 (define) AUFS sensitivity level. The LOD and LOQ for each compound are listed in Table 1.

The precision was expressed as repeatability of obtained results [17, 18] which was evaluated for peak areas of the analytes from eight successive injections of each analytical standard with concentration 124.8 μg/ml (for desmedipham), 129.6 μg/ml (for phenmedipham), and 649.6 μg/ml (for ethofumesate) within 3 days (Table 2). Testing of the

T a b l e 3

Results from recovery experiments (n = 4)

	Mass of analyte (μg)	Pure analyte added (μg)	Total analyte found (μg) (±SD)	Recovery (%)	RSD (%)
Desmedipham	0.713	0.312	1.031 ± 0.009	101.92	0.85
	0.713	0.624	1.326 ± 0.012	98.24	0.88
	1.141	0.312	1.448 ± 0.011	98.40	0.78
	1.141	0.624	1.780 ± 0.017	102.40	0.98
Phenmedipham	0.933	0.324	1.254 ± 0.005	99.07	0.39
	0.933	0.648	1.574 ± 0.013	98.92	0.83
	1.440	0.324	1.762 ± 0.024	99.38	1.37
	1.440	0.648	2.077 ± 0.028	98.30	1.33
Ethofumesate	1.188	1.624	2.805 ± 0.046	99.57	1.63
	1.188	3.248	4.375 ± 0.075	98.12	1.72
	1.787	1.624	3.379 ± 0.033	98.03	0.99
	1.787	3.248	4.975 ± 0.082	98.15	1.66

results according to the criteria laid down in CIPAC Document 3807 [19] indicated a very good precision of peak area under the conditions used in the tested method.

The accuracy of the method was confirmed by standard additions [19, 20]. Accuracy of the method was expressed as the deviation between the calculated mean value obtained by examination and the true value of the spiked amounts of the analyte into a sample matrix that already contains some quantity of the analyte (Table 3). As it is shown in Table 3, the obtained values for recovery are within the following ranges (97.0 – 103.0 % for desmedipham and phenmedipham, and 98.0 – 102.0 % for ethofumesate) which are according to CIPAC criteria [19]. Consequently, it was concluded that the proposed method is accurate enough for determination of active ingredients (desmedipham, phenmedipham and ethofumesate) in the pesticide formulation "Inter OF".

The obtained mean concentrations of active ingredients in the pesticide formulation "Inter OF" were 70.34 g/l (n = 8, RSD = 1.73 %) for desmedipham, 90.22 g/l (n = 8, RSD = 0.28 %) for phenmedipham and 113.65 g/l (n = 8, RSD = 1.35 %) for ethofumesate. These values corresponded to the values declared by the manufacturer.

This study shows the new possibility for simultaneous determination of desmedipham, phenmedipham and ethofumesate in the pesticide formulation "Inter OF" by the RP-HPLC-DAD using LiChrospher 60 RP-select B column. The developed method showed high value of multiple correlation coefficients for calibration equations and repeatability of peak area. The run time of assay obtained from this chromatography condition was about 10 min. The proposed method is fast, simple, precise, accurate and suitable for a routine analysis of active ingredients (desmedipham, phenmedipham and ethofumesate) in the pesticide formulation "Inter OF" according to CIPAC rules.

REFERENCES

[1] I. Deveikyte, V. Seibutis, Broadleaf weeds and sugar beet response to phenmedipham, desmedipham, ethofumesate and triflusulfuron-methyl, *Agronomy Research* **4** (Special issue), 159–162 (2006).

[2] B. R. Weeden, Potential of Sugar Beet on the Atherton Tableland. A Report for the Rural Industries Research and Development Corporation, Project No DAQ-211A, 2000.

[3] C. Tomlin, *The Pesticide Manual Incorporating the Agrochemicals Handbook*, 11th Ed., Crop Protection Publications, 1997, pp. 291–292, 410–411,787–788.

[4] T. Krongaard, K. K. Petersen, C. Christoffersen, *Control of Pesticides 2002*, National Environmental Research Institute, Ministry of the Environment, 2003.

[5] European Commission, Health & Consumer Protection Directorate-General (Desmedipham), Appendix I, 2004.

[6] EPA, Reregistration Eligibility Decision for Desmedipham, 1996.

[7] European Commission, Health & Consumer Protection Directorate-General (Phenmedipham), Appendix I, 2004.

[8] EPA, Reregistration Eligibility Decision for Phenmedipham, 2005.

[9] European Commission, Health & Consumer Protection Directorate-General (Ethofumesate), Appendix I, 2002.

[10] EPA, Reregistration Eligibility Decision for Ethofumesate, 2005.

[11] *CIPAC Method Handbook C (Phenmedipham),* 77/TC/M/3.2, 1985, pp. 2181.

[12] *CIPAC Method Handbook J (Ethofumesate),* 233/TC/M/3, 2000, pp. 43.

[13] L. Velkoska-Markovska, B. Petanovska-Ilievska, L. Vodeb, Simultaneous determination of phenmedipham, desmedipham and ethofumesate in pesticide formulation Inter OF by normal phase high performance liquid chromatography, *Acta Chromatogr*. **20**, 109–118 (2008).

[14] L. Velkoska-Markovska, B. Petanovska-Ilievska, L. Vodeb, HPLC method development for simultaneous determination of phenmedipham, desmedipham and ethofumesate in the pesticide "Inter OF" using LiChrosorb CN columns, *Plant Protection*, XX (20), 137–141 (2009).

[15] Omega, Perkin – Elmer Corp., Norwalk, USA, 1989.

[16] D. R. Jenkie, Chromatographic Method Validation: A Review of Current Practices and Procedures. I: General Concepts and Guidelines, *J. Liq. Chromatogr. Related Technol*. **19**, 737–757 (1996).

[17] V. R. Meyer, *Practical High-Performance Liquid Chromatography*, 2nd Ed., John Wiley & Sons, Chichester, England, 1994, pp. 1–267.

[18] W. J. Lough, I. W. Wainer, *High Performance Liquid Chromatography, Fundamental Principles and Practice*, Blackie Academic & Professional, Chapman & Hall, Glasgow, UK, 1996, pp. 1–167.

[19] Guidelines on method validation to be performed in support of analytical methods for agrochemical formulations, CIPAC Document 3807, http://www.cipac.org (01.10.11).

[20] R. L. Snyder, J. J. Kirkland, L. J. Glajch, *Practical HPLC Method Development*, 2nd Ed., John Wiley & Sons, Inc., New York, 1997, pp. 688–689.