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Simultaneous and Simple Determination of Four Active Ingredients of a Pesticide Formulation Using Gas Chromatography-Flame Ionization Detector

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Abstract: A study was conducted to develop method for the separation and quantification of four active ingredients of a pesticide formulation. The sample was commercial insecticide spray, containing the ingredients: cypermethrin, di-allethrin, tetramethrin and piperonyl butoxide (PBO). The liquid-liquid extraction was done with dichloromethane. Analysis of samples was carried out by capillary gas chromatography and flame ionization detection. 4-aminoantipyrine served as an internal standard. The method demonstrated acceptable linearity, specificity, precision and accuracy. This method is recommended for using in standard and quality control centers for the purity determination of pesticide preparations formulations.

Key words: Pesticides, cypermethrin, di-allethrin, tetramethrin, piperonyl butoxide, GC-FID

INTRODUCTION

Analysis of pesticide formulation has been of interest in many areas, especially in the insecticidal and quality control laboratories. Pyrethrins and pyrethroids comprise an important group of insecticides. They have very low rate of use (10 g active ingredients/ha) and excellent efficacy (Chen and Wang, 1996). These features of pyrethroids and pyrethrins combined with their desirable environment properties of short persistence and no toxicity to mammals (Heudorf and Angerer, 2001; Diel *et al.*, 1999; Tsuji *et al.*, 1996; Miyamoto *et al.*, 1995), have made them among the most frequently used pesticides today. They are often properly formulated and combined with a suitable antioxidant, such as PBO, to develop the synergistic effects (Chen and Wang, 1996; Wang *et al.*, 2003).

Pyrethrins and pyrethroids pesticides are nonpolar compounds and they extensively metabolized by the cleavage of the ester linkage, oxidation and conjugation and detoxified metabolites renally eliminated (Heudorf and Angerer, 2001; Leng *et al.*, 1997; Aprea *et al.*, 1997). The pesticide formulation considered to study in this work was claimed to contain the following active ingredients: cypermethrin, di-allethrin, tetramethrin and PBO: the structure of these compounds are shown in Fig. 1.

Almost all of the analytical methods for pyrethrins and pyrethroids are based on chromatography techniques, mainly Gas Chromatography (GC) and some High Performance Liquid Chromatography (HPLC). An

extensive review of the chromatographic methods employed for the determination of these in food, corps and environmental media has been published by Chen and Wang (1996). Several analytical methods have been established for identification and quantitation of the mentioned chemicals and their metabolites, either solely or in combination with other compounds, in plasma and urine samples. These methods used Gas Chromatography-Electron Capture Detector (GC-ECD) (Miyamoto *et al.*, 1995; Leng *et al.*, 1997; Cai *et al.*, 2002; Abu-Qare and Abou-Donia, 2001; Colume *et al.*, 2001), GC-Mass Spectroscopy (GC-MS) (Heudorf and Angerer, 2001; Hardt and Angerer, 2003; Angerer and Ritter, 1997), chiral analysis by GC-ECD (Liu and Gan, 2004) and High Performance Liquid Chromatography (HPLC) (Aprea *et al.*, 1997; Cai *et al.*, 2002; Wang *et al.*, 1999).

Wang *et al.* (2003) developed an isocratic reverse phase (RP)-HPLC for simultaneous determination of (S)-methoprene, MGK264, PBO and pyrethrins in pesticide formulation. The identification of the peaks was done using LC-ESI-MS. Otterbach and Wenclawiak (1999) compared three different extraction methods for pyrethrins from powdered chrysanthemic flowers as commercial insecticide and allethrin from paper strips by Supercritical Fluid Chromatography and Flame Ionization Detector (SFC-FID).

GC is still the method of first choice for the analysis of pyrethrins and pyrethroids (Chen and Wang, 1996). Due to the selectivity, reliability, easy to maintenance and operation of FID, GLC-FID methods is preferred over the

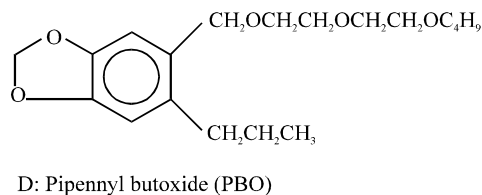
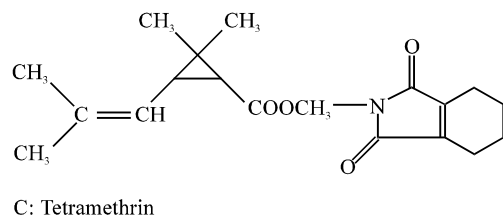
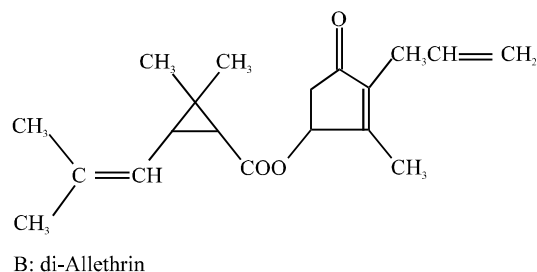
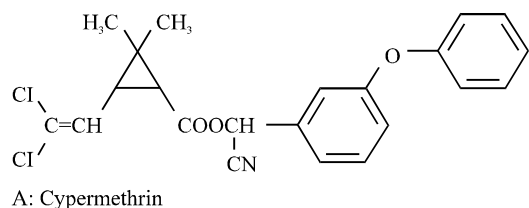


Fig. 1: Molecular structures of cypermethrin (A), di-allethrin (B), tetramethrin (C) and PBO (D) in the pesticide formulation.

other detectors of GC for analyzing the intended formulation of pesticides. There is little mentioned in the literature for the determination of these compounds by the GLC-FID methodology.

The objective of this study was to develop a GLC-FID method for simultaneous determination of four active ingredients cypermethrin, di-allethrin, tetramethrin and PBO in a commercial product of pesticide formulation. Then the developed method was validated for specificity, linearity, precision and accuracy.

MATERIALS AND METHODS

Chemical and solvents: Analytical standards of cypermethrin, di-allethrin, tetramethrin, PBO and the Internal Standard (IS), 4-animoantipyrine (4-AAP), were

obtained from Sigma. Analytical-reagent grade solvent dichloromethane and anhydrous sodium sulfate were obtained from Merck (Germany).

Pesticide formulation came from Rangin Teif-e-Sheemi Inc. Iran, Tehran. Standard 4-animoantipyrine solution at a concentration of 40 mg mL⁻¹ was prepared by dissolving appropriate quantities of standard in dichloromethane (DCM).

Sample preparation: Sample solutions for the precision test were prepared by dissolving 5 mL of pesticide formulation samples, which had been dehydrate by adding anhydrous sodium, in DCM that contained 2 mL of the 4-AAP (IS) solution. The volume of solution were attained to 10 mL with DCM. The final concentration of IS was 8 mg mL⁻¹ in all samples.

Calibration procedure: To prepare stock standard solution for the linearity and accuracy test, a mixture of cypermethrin, di-allethrin, tetramethrin and PBO according to the appropriate proportion of each compound in the sample, was accurately weighted and dissolved in DCM. Subsequently, the solution was diluted with DCM to obtain five working solutions that cover the expected range of target values in samples. Each standard solution was added 2 mL of the IS solution (40 mg mL⁻¹) and the final volume reaches to 10 mL by DCM. The final concentration of IS was 8 mg mL⁻¹ in all standard solutions. The accuracy test was carried out by running four different samples with five different standard solutions in triplicate. Inter- and intra-day accuracy was expressed as percentage deviation from the spiked value using the following equation:

$$\text{Accuracy: Error\%} = [(C_{\text{mean cal.}} - C_{\text{spiked}}) / C_{\text{spiked}}] * 100$$

Where, $C_{\text{mean cal.}}$ is the mean calculated concentration for each solution and C_{spiked} is the spiked theoretical concentration. Inter and intra-day precision of the method was expressed as the Relative Standard Deviation (RSD%) of the mean calculated concentration for each solution.

Linear calibration curves were obtained by plotting the ration of peak areas: IS area of individual chemical against the corresponding concentration. In the case of mixture of isomers for cypermethrin, the global analytical signal was obtained by summing the peak areas of all isomers.

Instruments and apparatus: Experiments were carried out by using a Hewlett-Packard HP 6890 gas chromatograph equipped with a Flame Ionization Detector (FID) system. Chromatographic separation was achieved by using a fused-silica capillary column (30 m×0.23 mm ID) coated with 5% phenylmethylpolysiloxane (film

Table 1: Retention times, linear equations and regression coefficient of quantification for the four ingredients of insecticide formulation

Pesticides or I.S	t _R (min)	Linear equation	Regression coefficient (R ²)
4-Aminoantipyrine	7.40	-	-
Di-allethrin	7.90	Y = 0.1922X-0.0233 Y = 0.19087X-0.001	0.99966 0.99670
PBO	11.00	Y = 0.1387 X-0.106 Y = 0.1317X-0.012	0.99940 0.99850
Tetramethrin	11.35	Y = 0.17204X-0.012 Y = 0.1808922X-0.006	0.99800 0.99930
Cypermethrin	13.46- 13.67	Y = 0.18761X-0.0095 Y = 0.1762X-0.0052	0.99850 0.99870

The global analytical signal was obtained by summing the peak areas of all isomers.

thickness 0.25 μm). The injector was in the mode of split (10:1) and its temperature was maintained at 270°C throughout. The column temperature was raised from 180°C (hold 1 min) to 220°C (hold 0.00 min) at 5°C min⁻¹ and then to 280°C (hold 30 min) at 30°C min⁻¹. Nitrogen (Varian gas, Tehran, Iran) at a flow-rate of 1.5 mL min⁻¹ was used as carrier gas. The instrument control and data processing utilities included the use of Hewlett-Packard application software GC CHEMSTATION (Agilent technology).

The detection was carried out by a flame ionization detector; with the temperature of 300°C and the ratio of H₂/air: 35/350. The H₂ for FID was provided by a Hydrogen generator (PACKARD).

RESULTS AND DISCUSSION

GC analysis: Optimal condition for the determination of pyrethroids and PBO was investigated. According to the condition mentioned in experimental, gas chromatograms of standard solution (Fig. 2A) and sample (Fig. 2B) are shown in Fig. 2. Under the described condition (Stationary phase: fused-silica capillary column [30 m×0.23 mm ID] coated with 5% phenylmethylpolysiloxane [film thickness 0.25 μm]; Injector: split (10:1), 270°C; Column temperature program: 180°C (hold 1 min) to 220°C (hold 0.00 min) at 5°C min⁻¹ and then to 280°C (hold 30 min) at 30°C min⁻¹, Carrier gas: Nitrogen, 1.5 mL min⁻¹) the four active ingredients and IS were well resolved. Retention times of pesticides investigated here (mean of three trials) are indicated in Table 1.

GLC-FID method validation: Chromatographic method validation consisting of method specificity, linearity, precision and accuracy was undertaken in order to demonstrate the suitability of the analytical method for the determination of four active ingredients in this formulation (Shah *et al.*, 2000).

Specificity: Specificity is a measurement of the degree of interference in the analysis of complex pesticide formulation. The samples and standard solutions were stored in an oven at 78°C for two weeks before GLC-FID

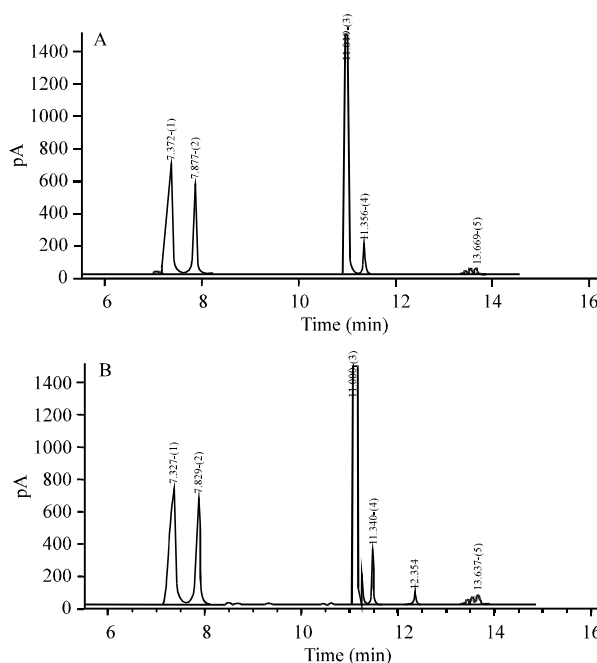


Fig. 2: Typical chromatograms of cypermethrin, di-allethrin, tetramethrin and PBO, (A) standard solution and (B) sample solution

analysis. The results showed that chromatograms presented the absence of interference from degradation products which are crucial to the targeted analysis of analytical method (Fig. 2).

Response linearity: As shown in Table 1, a linear relationship between the ratio of cypermethrin, di-allethrin, tetramethrin and PBO area to IS area versus the corresponding concentration (ppm) was obtained over the range of 50-200% of the target values. Data were collected from triplicate injections of each solution. A linear regression was obtained for each ingredient. Regress analysis showed a good linear relationship ($r^2 > 0.99$) for all components (Fig. 2 and Table 1).

Accuracy: Inter and intra-day accuracy was expressed as percentage deviation from the spiked value. As shown in Table 2, the Intra-day accuracy are acceptable for all four

Table 2: Intra-day accuracy and precision for the analysis of di-allethrin, tetramethrin, PBO and cypermethrin in pesticide formulation

	Concentration		Error (%) (Accuracy)	RSD (%) (Precision)
	added (mg mL ⁻¹)	Mean concentration determined (mg mL ⁻¹)		
di-Allethrin	1.25	1.280	2.40	1.30
	1.66	1.645	-0.90	2.30
	2.50	2.460	1.06	2.20
	3.50	3.500	0.00	1.10
PBO	5.00	5.010	0.20	1.40
	6.25	6.500	4.00	1.30
	8.33	8.230	-1.20	2.20
	12.50	12.300	-1.60	0.00
	17.50	17.400	-0.57	2.80
Tetramethrin	25.00	25.100	0.40	3.00
	0.25	0.260	4.00	0.80
	0.33	0.325	-1.50	10.60
	0.50	0.476	-4.80	0.00
	0.70	0.710	1.40	11.90
Cypermethrin	1.00	1.000	0.00	3.40
	0.25	0.260	4.00	10.80
	0.33	0.328	0.60	0.66
	0.50	0.490	-2.00	11.00
	0.70	0.692	-1.10	4.20
	1.00	1.010	1.00	3.70

Table 3: Inter-day accuracy and precision for the analysis of di-allethrin, tetramethrin, PBO and cypermethrin, in pesticide formulation

	Concentration		Error (%) (Accuracy)	RSD (%) (Precision)
	added (mg mL ⁻¹)	Mean concentration determined (mg mL ⁻¹)		
di-Allethrin	1.250	1.340	7.20	3.80
	1.660	1.680	1.20	1.90
	2.500	2.430	-2.80	5.00
	3.500	3.660	4.50	4.30
	5.000	5.020	0.40	1.20
PBO	6.250	6.450	3.20	7.10
	8.330	8.200	-1.50	2.40
	12.500	12.150	-2.80	2.50
	17.500	17.900	2.30	1.70
	25.000	24.900	-0.40	1.60
Tetramethrin	0.250	0.260	4.00	3.80
	0.330	0.324	-1.50	1.90
	0.500	0.497	-0.60	5.00
	0.700	0.697	-0.43	4.30
	1.000	1.000	0.00	1.20
Cypermethrin	0.250	0.255	2.00	2.50
	0.330	0.325	-1.50	0.22
	0.500	0.490	-2.00	3.60
	0.700	0.720	2.80	2.20
	1.000	1.050	5.00	3.30

compounds (<2.4% for di-allethrin, <4% for PBO, <4.8 for tetramethrin and <4% for cypermethrine). Inter-day accuracy was also acceptable for di-allethrin (<7.2%), PBO (<3.2%), tetramethrin (<0.43%) and cypermethrin (<2.8%) (Table 3).

Inter and intra-day precision of the method was expressed as the relative standard deviation (RSD) of the mean calculated concentration for each standard. Intra-day precision was acceptable for all four compounds (<2.3% for di-allethrin, <3% for PBO, <10.6 for tetramethrin and <10.8% for cypermethrin) (Table 2). Inter-day precision was also acceptable for di-allethrin (<5%), PBO (<7.1%), tetramethrin (<4.3%) and cypermethrin (<3.6%) (Table 3).

Pyrethrins were developed as pesticides from extracts of dried and powdered flower heads of *Chrysanthemum cinerariaefolium*. The active principles of these are esters of cyclopropane carboxylic acids with cyclopentanone alcohols, giving six possible structures. Once the basic structure of the pyrethrins had been discovered, synthetic analogues, pyrethroids, were developed and tested. Initially esters were produced using the same cyclopropane carboxylic acids, with variations in the alcohol portion of the compounds. The first commercial synthetic pyrethroid, allethrin (Fig. 1B), was produced in 1949, followed in the 1960s by dimethrin, resmethrin, prothrin, proparthrin and tetramethrine (Fig. 1C). Synthetic pyrethroids with this basic cyclopropane carboxylic ester structure (and no cyano group substitution) are known as type I pyrethroids. The insecticidal activity of synthetic pyrethroids was enhanced further by the addition of a cyano group at the benzylic carbon atom to give alpha-cyano (type II) pyrethroids. Examples of type II pyrethroids include cyphenothrin and cypermethrin (Fig. 1A). Piperonyl butoxide (3,4-methylenedioxy-6-propylbenzyl n-butyl diethyleneglycol) ether is a derivative of piperic acid. This compound is an antioxidant and formulated by pyrethroids to develop the synergistic effects (Chen and Wang, 1996; Wang *et al.*, 2003).

In conclusion, a GLC-FID method for the analysis of cypermethrin, di-allethrin, tetramethrin and PBO in a pesticide formulation has successfully been developed. This new method is simple, rapid and precise considering the presence of four chemical in formulation. This method doesn't need extracting the sample. The method achieved good separation of the four ingredients of formulation. Furthermore, all the four active ingredients in the formulation could be separated and determined in less than 15 min. According to the amount of pesticides ingredients in commercial formulations, this method can fulfill the requirements of the insecticide industry and quality control labs for analyzing this formulation. In addition, from the analytical point of view, this method would be used to separate the other pyrethroid compounds with the similar structure to those analyzed in this study such as cyfluthrin, bioallethrin, cyhalothrin, permethrin, d-phenothrin and other pyrethroids.

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REFERENCES

- Abu-Qare, A.W and M.B. Abou-Donia, 2001. Simultaneous determination of malathion, permethrin, DEET (N,N-diethyl-m-toluamide) and their metabolites in rat plasma and urine using high performance liquid chromatography. *J. Pharm. Biomed. Anal.*, 26: 291-299.
- Angerer, J and A. Ritter, 1997. Determination of metabolites of pyrethroids in human urine using solid-phase extraction and gas chromatography-mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.*, 695: 217-226.
- Apra, C., A. Stridori and G. Sciarra, 1997. Analytical method for the determination of urinary 3-phenoxybenzoic acid in subjects occupationally exposed to pyrethroid insecticides. *J. Chromatogr. B Biomed. Sci. Appl.*, 695: 227-236.
- Cai, J., B. Liu, X. Zhu and Q. Su, 2002. Determination of pyrethroid residues in tobacco and cigarette smoke by capillary gas chromatography. *J. Chromatogr. A.*, 964: 205-211.
- Chen, Z.M. and Y.H. Wang, 1996. Chromatographic methods for the determination of pyrethrin and pyrethroid pesticide residues in crops, foods and environmental samples. *J. Chromatogr. A.*, 754: 367-395.
- Columbe, A., S. Cardenas, M. Gallego and M. Valcarcel, 2001. Selective enrichment of 17 pyrethroids from lyophilised agricultural samples. *J. Chromatogr. A.*, 912: 83-90.
- Diel, F., B. Horr, H. Borck, H. Savtchenko, T. Mitsche and E. Diel, 1999. Pyrethroids and piperonyl-butoxide affect human T-lymphocytes *in vitro*. *Toxicol. Lett.*, 107 :65-74.
- Hardt, J and J. Angerer, 2003. Biological monitoring of workers after the application of insecticidal pyrethroids. *Int. Arch. Occup. Environ. Health*, 6: 492-498.
- Heudorf, U. and J. Angerer, 2001. Metabolites of pyrethroid insecticides in urine specimens: Current exposure in an urban population in Germany. *Environ. Health Perspect.*, 109: 213-217.
- Leng, G., A. Leng, K.H. Kuhn, J. Lewalter and J. Pauluhn, 1997. Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: Urinary metabolite profile following inhalation. *Xenobiotica*. 27: 1273-1283.
- Liu, W and J.J. Gan, 2004. Determination of enantiomers of synthetic pyrethroids in water by solid phase microextraction-enantioselective gas chromatography. *J. Agric. Food Chem.*, 52: 736-741.
- Miyamoto, J., H. Kaneko, R. Tsuji and Y. Okuno, 1995. Pyrethroids, nerve poisons: how their risks to human health should be assessed. *Toxicol. Lett.*, pp: 82-83, 933-940.
- Otterbach, A. and W. Wenclawiak, 1999. Ultrasonic/soxhlet/supercritical fluid extraction kinetics of pyrethrins from flowers and allethrin from paper strips. *Fresenius J. Anal. Chem.*, 365: 472-474.
- Shah, V.P., K.K. Midha, J.W. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilverary, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan and A. Yacobi, 2000. Bioanalytical method validation- a revisit with a decade of progress. *Pharm. Res.*, 17: 1551-1557.
- Tsuji, R., N. Isobe and H. Kawasaki, 1996. Effect of pyrethroids on pentobarbital-induced sleeping time in relation to the chemical structure. *Toxicol.*, 106: 131-137.
- Wang, I.H., V. Subramanian, R. Moorman, J. Burleson and J. Ko, 1999. Chiral high-performance liquid chromatography of N-octyl bicycloheptene dicarboximide and confirmatory studies using liquid chromatography-tandem mass spectrometry and two-dimensional nuclear magnetic resonance spectroscopy. *J. Chromatogr. A.*, 864: 271-281.
- Wang, I.H., R. Moorman and J. Burleson, 2003. Isocratic reversed-phase liquid chromatographic method for the simultaneous determination of (S)-methoprene, MGK264, piperonyl butoxide, sumithrin and permethrin in pesticide formulation. *J. Chromatogr. A.*, 983: 145-152.