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Determination of pesticides fenoxycarb and permethrin by sequential injection chromatography using miniaturized monolithic column

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ABSTRACT

Sequential injection chromatography system equipped with miniaturized 10 mm monolithic column was used for fast simultaneous determination of two pesticides—fenoxycarb (FC) and permethrin (PM). The system was composed of a commercial sequential injection analysis (SIA) system (FIAlab® 3000, 6-port selection valve and 5.0 mL syringe pump), commercially available column Chromolith™ RP-18e (10 mm × 4.6 mm i.d.) (Merck®, Germany) and CCD UV–vis detector (USB 2000, Ocean-optics) with 1.0 cm Z-flow cell, absorbance was monitored at 225 nm. The mobile phase used for analysis was acetonitrile/water (60:40, v/v), flow rates were 0.6 mL min^{-1} for elution of fenoxycarb and 1.2 mL min^{-1} for elution of permethrin. For each analysis 4.8 mL of mobile phase was used. The chromatographic resolution between both compounds was >8 and analysis time was <6.5 min under the optimal conditions. Limits of detection were determined at 2.0 μg mL^{-1} for fenoxycarb and 1.0 μg mL^{-1} for permethrin. Samples were prepared by diluting with mobile phase and injected volume was 10 μL for each analysis. Developed method was applied to analysis of both pesticides in veterinary pharmaceutical foams and sprays ARPALIT® Neo (Aveflor, Czech Republic). SIC method was compared with validated method (HPLC, reverse phase 100 mm monolithic column, gradient elution).

1. Introduction

Chromatography is widely used in separation of mixtures in almost all branches of chemistry. Sequential injection chromatography (SIC) based on integration of very short monolithic column (10–50 mm) into flow manifold has been introduced as a new generation of flow methods analysis and extended the possibilities of sequential injection analysis (SIA). SIC technique enables determination of simple multi-compound samples by chromatography [1].

SIA, highly versatile technique based on a programmable flow [2] has been proposed by Ruzicka and Marshall as an efficient tool for automated liquid handling [3]. The "single-line" technique is based on forward and reverse movement of a piston of syringe pump, which together with a multi-position selection valve enables precise sampling of liquid chemicals into the system and propelling of the sequenced zones to the reactors and detector [4]. Automation, speed of the analysis and low consumption of sample and reagents are the most important features that favour the SIA technique for application in many fields of analysis, primarily by more complicated operations such as sample pre-treatment, derivatization reactions or monitoring of long lasting processes [5–7].

On the other hand, SIA technique itself has generally one important drawback—it generally cannot provide the separation procedure and analysis of multi-component samples. This weak point was first time solved by insertion of short monolithic column into SIA manifold, creating a technique called sequential injection chromatography [8]. The monolithic columns (formed by "sol–gel" technology from a single piece of porous silica gel) enable operation at high flow rates with lower-back pressure [9]. This feature is used for integrating these columns into a SIC manifold and for extending the possibilities of low pressure flow technique. SIC manifold was successfully applied to the analysis of relatively simple multi-component samples mainly in pharmaceutical area [1].

Coupling of monolithic columns with SIA/FIA manifolds show increasing trend in flow analysis area. Zacharis et al. have incorporated monolithic strong anion-exchanger disk (CIM®) into SIA manifold for on-line drug–protein interaction studies [10]. Multisyringe flow system with three solenoid valves coupled with Chromolith™ Flash RP-18e (25 mm × 4.6 mm i.d.) monolithic column created by González-San Miguel et al. have been used for determination of β-lactam antibiotics [11]. García-Jiménez et al. have employed Chromolith™ Guard Cartridge RP-18 endcapped (5 mm × 4.6 mm i.d.) monolithic pre-column in FIA for analysis of

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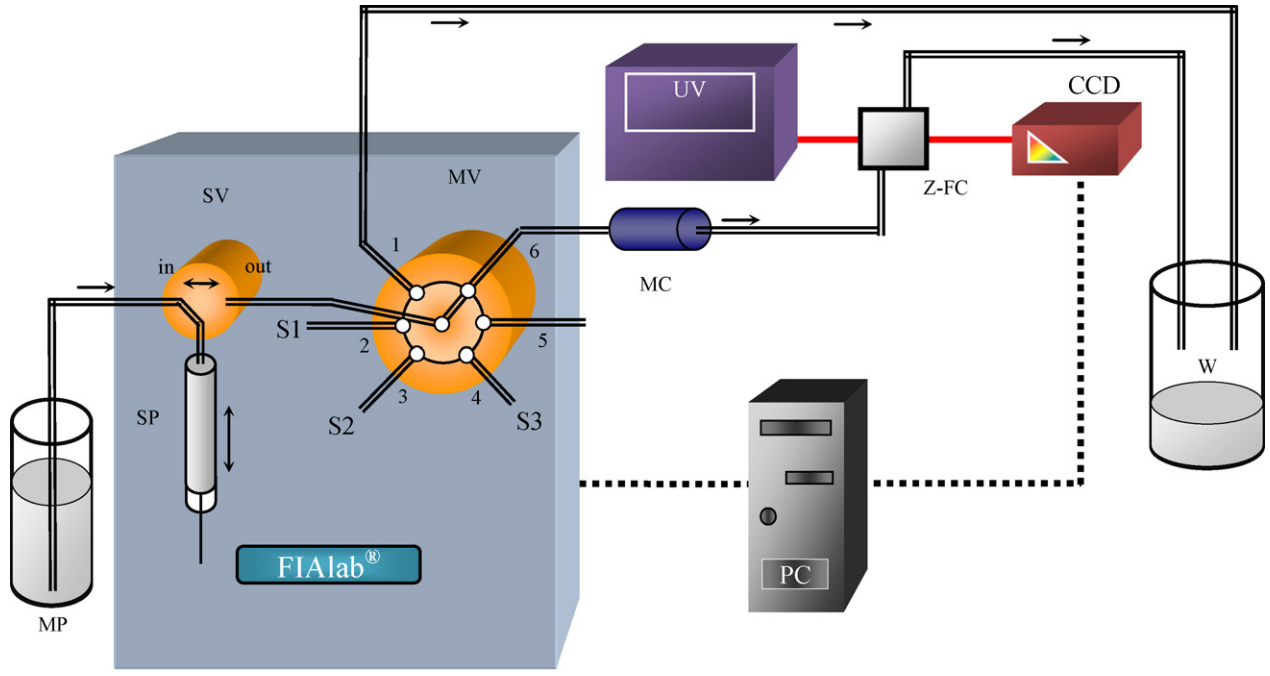

Fig. 1. Scheme of SIC set-up for determination of FC and PM. CCD: CCD UV–vis detector; MC: monolithic column; MP: mobile phase; MV: 6-port multi-position valve; PC: computer; SP: syringe pump; SV: solenoid valve; S1, S2, S3: Sample 1, 2, 3; UV: UV lamp, W: waste, Z-FC: Z-flow cell.

antioxidants, preservatives and sweeteners in food and cosmetics [12], while Claver et al. have used same system for determination of parabens in cosmetics [13]. Adcock et al. have incorporated monolithic column Chromolith™ Flash RP-18e (25 mm × 4.6 mm i.d.) with 5 mm pre-column into a hybrid FIA/HPLC manifold for determination of opiate alkaloids and biogenic amines (neurotransmitter metabolites) with chemiluminescence detection [14].

Permethrin (PM) [3-phenoxybenzyl (±) *cis*/*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate]—a pyrethroid insecticide, is widely used throughout the world as a wide-spectrum insecticide for numerous crops and also for indoor pest control in the public health sector and housing. There are four isomeric forms: two *cis* (PMC) and two *trans* (PMT) of technical PM (the *cis* and *trans* isomers differ in the spatial arrangement of the atoms). Product formulations of PM can vary greatly in isomeric content so the sum of all forms is usually declared.

Fenoxycarb (FC) [ethyl 2-(4-phenoxyphenoxy)ethylcarbamate] is a carbamate insect growth regulator used to control a wide variety of insect pests. It is used as a fire ant bait and for flea, mosquito, and cockroach control, and can also be used to control butterflies, moths, beetles, and scale and sucking insects on olives, vines, cotton, and fruit. It is also used to control these pests on stored products, and is often formulated as a grit or corncob bait. Compared to other carbamates, FC is one of the least toxic in this chemical class.

There have been recently a number of reports in literature dealing with various analytical methods for the determination of FC [15–18] and PM [19–24], mainly by HPLC and GC methods, but there is no analytical method in literature for simultaneous determination of FC in the presence of PM.

2. Experimental

2.1. Apparatus

2.1.1. Sequential injection chromatography system

A commercially available FIAlab® 3000 instrument (FIAlab® Instruments Inc., Bellevue, USA) with a syringe pump (syringe reservoir 5.0 mL) and a 6-port selection Cheminert® valve (Valco Instrument Co., Houston, USA) was used. The manifold was equipped with fiber-optic CCD UV–vis detector USB 2000 (Ocean Optics Inc., Dunedin, USA), 10 mm Z-flow cell (Avantes, CO, USA) and UV light source D-1000-CE (Analytical Instrument Systems Inc., Flemington, USA). The whole SIC system was controlled by the program FIAlab®. Flow lines were made from 0.50 mm i.d. PTFE tubing. Samples were aspirated through the 6-port multi-position valve then delivered to the monolithic column and to the detector. Direct sample separation was performed on reversed phase C-18 monolithic column Chromolith™ RP-18e (10 mm × 4.6 mm i.d.) (Merck®, Germany). The monolithic column was placed between the multi-position valve and flow cell of the detector. Manifold set-up depicted in Fig. 1.

2.1.2. HPLC apparatus

Validated HPLC method for veterinary product samples was performed on HPLC system Shimadzu Prominence LC-20A series with DAD detector (set on 225 nm). System was equipped with the monolithic column Chromolith™ Performance RP-18e (100 mm × 4.6 mm i.d.) (Merck®, Germany). Gradient elution was under the following conditions: 0–4 min acetonitrile/water (60:40, v/v), 4–8 min acetonitrile/water (95/5, v/v) and flow rate 2.0 mL min^{-1}. The sample injection volume was 10.0 μL.

2.2. Reagents

Permethrin mixture of *cis* and *trans* isomers standard was obtained from Riedel-de-Haën (Germany, contend of 94.4% *cis*/*trans* isomers), FC standard was obtained from Riedel-de-Haën (Germany, contend of 99.9%). Acetonitrile (Chromasolv, for LC) was obtained from Sigma–Aldrich. All other used chemicals were of analytical grade quality. Millipore Milli-Q RG (Millipore s.r.o., Czech Republic) ultra pure water was used for preparing the solutions. Mobile phases were degassed by helium before use. Stock standards were dissolved in acetonitrile 80% (v/v) at concentration of 6.0 mg mL^{-1} (PM) and 1.5 mg mL^{-1} (FC), all were stored at 5 °C for

1 month. The final concentrations of the sample working standard solutions and reference standards for veterinary preparations analysis were prepared by diluting the stock solution in the mobile phase.

2.3. Method and sample preparation

2.3.1. Mobile phase

The optimal mobile phase for separation FC and PM was acetonitrile/water (60:40, v/v). Flow rates were 0.6 mL min^{-1} for elution of FC and 1.2 mL min^{-1} for elution of PM. Volume of mobile phase used for one analysis was 4.8 mL. All experiments were done at ambient temperature.

2.3.2. Solutions and sample preparation

The tested preparations were ARPALIT® Neo spray containing 470 mg of PM and 120 mg of FC in 100 g of solution, ARPALIT® Neo foam containing 480 mg of PM and 120 mg of FC in 100 g of foam and ARPALIT® Neo mechanical spray containing 470 mg of PM and 120 mg of FC in 100 g of solution (all preparations Aveflor, Czech Republic). Preparation of spray samples was done by the following procedure: 1.0 g of well-shake spray was squirt into 25.00 mL calibrated flask and filled to the mark with mobile phase, mixed and dissolved by 5 min sonication. Foam samples were prepared as follows: 2.0 g of well-shaken foam was added into small beaker with 20.0 mL of mobile phase, diluted and transferred into 50.0 mL calibrated flask and filled to the mark with mobile phase, mixed and dissolved by 5 min sonication. The comparative standard solution was same for all the analysis and it was prepared by diluting stock standard solution in 25.00 mL calibrated flask and the flask was filled to the mark with mobile phase and mixed. The final concentrations of the analytes in comparative standard solution were 240.0 μg mL^{-1} of PM and 60.0 μg mL^{-1} of FC. Standards and samples were measured in triplicate and the mean peak height values were used for data acquisition.

3. Results and discussion

3.1. Method development and optimization

In this work for the first time very short monolithic column (10 mm of length) has been used in the SIC method. The optimization of mobile phase was started with acetonitrile/water (50:50, v/v) mobile phase. The mobile phases containing acetonitrile as organic part have shown better separation of peaks and peak symmetry than methanol containing mobile phases. The optimization was focused on finding an appropriate acetonitrile/water composition to achieve a sufficient symmetry of the peaks of FC and PM together with a good separation of compounds and a short retention time. Both substances had still same chromatographic properties within changing of pH so the adjustment of mobile phase's pH was not necessary. There was not necessary to separate precisely PMC and PMT forms of PM (double-peak) because they were declared in preparations as a sum. The optimal mobile phase for the separation of FC and PM was found acetonitrile/water (60:40, v/v). Flow-rate was set to 0.6 mL min^{-1} for elution of FC; first volume of 1.2 mL and flow-rate 1.2 mL min^{-1} for elution of PM (rest volume of 3.6 mL). The sample injection volume was 10.0 μL. Detector was set into 225 nm to ensure good response of FC and PM and to prevent disturbances of additives. The proposed system enabled successful separation of target analytes in the time 6.7 min (1.6 min for aspiration of mobile phase, 0.0016 min for aspiration of sample and 2.0 plus 3.0 min for elution of analytes). Total volume of mobile phase for one analysis was 4.8 mL. The sequence of particular steps of the SIC control program for ARPALIT Neo® determination (a single cycle) is described in Table 1. Peak height evaluation was performed using the FIAlab® software.

Table 1
The sequence of particular steps of the SIC control program for ARPALIT Neo® determination (a single cycle)

Action	Unit	Parameter	
Mobile phase aspiration	Syringe pump	Flow rate (μL s^{-1}) Volume, aspiration (μL)	100 4800
Sample aspiration	Multi-position valve Syringe pump	Valve port Flow rate (μL s^{-1}) Volume (μL)	2 10 10
Elution of FC	Multi-position valve Syringe pump	Valve port Flow rate (μL s^{-1}) Volume, dispension (μL)	6 10 1200
Elution of PM	Syringe pump	Flow rate (μL s^{-1}) Volume, dispension (μL)	20 3600

3.2. Parameters of sequential injection chromatography process

The target compounds were successfully separated using the proposed procedure. Basic chromatographic parameters were calculated from experimental data, such as retention times, peak symmetry, number of theoretical plates and peak resolution; they are given in Table 2 in comparison to HPLC results. Since content of PM in preparations is calculated as a sum of all forms, it is not necessary to separate them completely.

3.3. Validation and analytical parameters of the method

The validation showed good results for all analytical parameters (linearity, sensitivity, repeatability, recovery, selectivity, precision and accuracy). Linearity was established with a series of working solutions prepared by diluting the stock solution with mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak height was used for the calibration curve. The calibration graphs involved five experimental points for FC (concentration range 7.6, 23.1, 69.1, 138.3 and 276.5 μg mL^{-1}) and it is described by the following equation: $A = (0.0029 \pm 0.0001)\,c + (0.0256 \pm 0.0211)$ (where A is the absorbance and c the analyte concentration), the correlation coefficient was 0.995; for PM six experimental points (concentration range 3.4, 11.3, 37.8, 126.0, 252.0 and 420.0 μg mL^{-1}) and they are described by following

Table 2
Characterization of SIC process and its comparison with validated HPLC method

	Fenoxycarb		*Cis*, permethrin		*Trans*, permethrin	
	SIC	HPLC	SIC	HPLC	SIC	HPLC
Retention time (min)	0.5	1.8	3.0	7.2	3.3	7.5
Peak symmetry	3.1	1.3	1.4	1.4	1.6	1.1
Number of theoretical plates	99	1009	1149	27,855	682	37,250
Peak resolution	$R_{FC/PMC}$ = 8.75	$R_{FC/PMC}$ = 36.30	$R_{PMC/PMT}$ = 0.90	$R_{PMC/PMT}$ = 2.25		

Table 3
SIC Analytical parameters and method validation results

	Fenoxycarb	Permethrine	
		Cis	*Trans*
Calibration range, (μg mL^{-1})	7.6–276.5	3.4–420.0	
Correlation coefficient	0.995	0.994	
Limit of detection, 3σ (μg mL^{-1})	2.00	1.00	
Limit of quantification, (μg mL^{-1})	6.00	3.00	
System precision, spray diluted 10× (%)[a]	1.22	0.60	0.56
System precision, spray diluted 20× (%)[a]	1.71	1.54	1.85
System precision, spray diluted 100× (%)[a]	1.59	2.54	1.80
Repeatability of time, t_R (%)[a]	1.50	0.90	1.03
Method precision (%)[b]	2.29	2.98	
Accuracy, spike recovery (%)[c]	98.9	97.5	

[a] Relative standard deviation (R.S.D.) values were calculated for repeated standard injections (n = 6).
[b] R.S.D. for repeated injections of multiple sample preparations (n = 6).
[c] Spiked sample solutions.

equations: $A = (0.0021 \pm 0.0001)\ c - (0.0365 \pm 0.0241)$, the correlation coefficient was 0.994. The limit of detection (LOD) was calculated by comparison of the threefold variation of signal to noise ratio (3 S/N) obtained from analysis of the standards, and the limit of quantification (LOQ) was defined as the lowest measured quantity above which the analyte can be quantified at a given statistical level of (3 LOQ). The system precision of the method was determined by preparing the standards of FC and PM at three concentration levels (spray diluted 10 times, spray diluted 20 times and spray diluted 100 times) and peak heights for each compound were determined after processing each six times. The method validation results obtained under the final conditions are shown in Table 3. To validate the precision of the method a number of six different veterinary insecticide sample solutions were used, which were prepared from the same batch and analyzed consecutively. This approach provides a means of covering the precision of the entire method, from sample preparation to data handling. The precision of the method calculated as R.S.D. of six-sample determination, including sample preparation, was 2.29% for FC and 2.98% for PM. The accuracy of the method was carried out measuring of the veterinary samples fortified with known quantity of the analytes (addition of 100% amount of FC and PM standards to veterinary preparation). Spiked sample solutions and un-spiked sample solutions were compared for recovery evaluation. The method accuracy results—mean values of the recoveries were found as 98.9% for FC and 97.5% for PM. Assay values of recoveries show that the method allows direct determination of FC and PM in commercial dosage forms in the presence of other adjuvants.

3.4. Determination in veterinary products

The novel method has been applied to the determination of FC and PM in ARPALIT® Neo spray, foam and mechanical spray. The veterinary preparations were commercially available on the Czech market. The interference effect of adjuvants (D-panthenol, tween 20, hydroxyethylcellulose, cetrimonium bromide, phenyl trimethicone) was not observed under the optimized analytical conditions. The samples were prepared just by 25-fold dilution with mobile phase. The mean values of found amount were 0.14% of FC and 0.60% of PM in foam, 0.14% of FC and 0.58% of PM in spray and 0.15% of FC and 0.59% of PM in mechanical spray (declared amount of FC 0.14–0.16% and of PM 0.57–0.63% both in spray and foam). Two packages (cans) of one batch of each kind of preparations were measured in our study. Representative sequential injection chromatogram showing successful separation of active substances of ARPALIT® Neo spray is shown in Fig. 2.

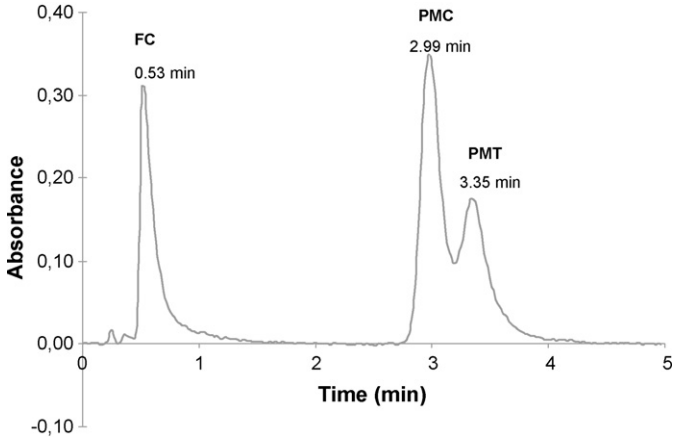

Fig. 2. SI chromatogram of the separation of active substances of ARPALIT® Neo. Mobile phase: acetonitrile/water (60:40, v/v), flow rate 0.6 mL min^{-1} for elution of FC (1.2 mL) and 1.2 mL min^{-1} for elution of PMC and PMT (*cis* and *trans* isomer) (3.6 mL); UV detection at 225 nm.

The SIC determination results were compared with validated method (HPLC)—(samples of foam: FC 0.14% and PM 0.60%; spray: FC 0.15% and PM 0.58%; mechanical spray: FC 0.15% and PM 0.59%).

4. Conclusion

Very short Chromolith™ RP-18e (10 mm × 4.6 mm i.d.) (Merck®) column was first time implemented into SIC manifold. This set-up was proved to be a convenient and efficient tool for the separation and determination of mixture of FC and PM in veterinary preparations. The assay showed good precision and accuracy and results were compared to established HPLC method. Advantage of SIC method were lower consumption of mobile phase, use of isocratic elution, shorter time of analysis, simple preparation and easy sample handling. All these features enable reduction of cost per analysis. Easy liquid manipulation not attainable by classical HPLC set-up, dimensions and portability of the SIC system provides the opportunity for possible analysis "on field".

Recent development and increasing trend of SIC practical applications resulted in commercially available SIChrom™ liquid chromatography analyzer by FIAlab® Instruments (Bellevue, WA, USA) [25]—equipped with more powerful pump (enables use of higher flow rates and longer columns) and chemical resistant Lab-On-Valve system for variable sample handling.

In summary, the SIC system provides a useful alternative to existing chromatographic methods and can be an important tool for the rapid separation and quantification of several compounds not only in analysis of pesticides in veterinary preparations.

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