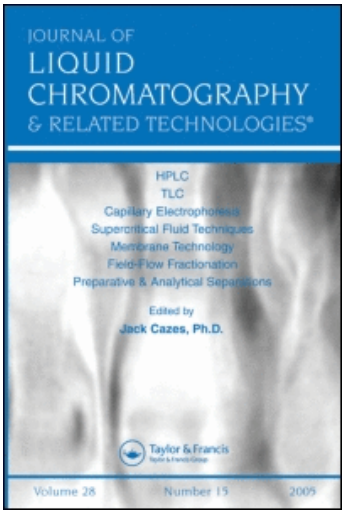

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Sensitivity Enhancement by Using an HPLC Flow-Through Sensor for Determination of Pesticide Mixtures

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SENSITIVITY ENHANCEMENT BY USING AN HPLC FLOW-THROUGH SENSOR FOR DETERMINATION OF PESTICIDE MIXTURES

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ABSTRACT

The coupling of a liquid chromatograph to a flow-through sensor based on the use of a cell packed with a support to retain trantiently the eluted analytes and located in an ordinary photometer is proposed. The performance of the arrangement has been tested by applying it to the determination of ternary mixtures of carbamate pesticides. The sensitivity of the method surpasses to other previously proposed as an in situ concentration of the monitored products at the detection point is performed. Thus, an enhancement of sensitivity of up to 50-times with respecto to the use of the same flow-cell without packed material is achieved.

INTRODUCTION

Flow-through (bio)chemical sensors are one of the simpler ways to overcome shortcomings from irreversible but regenerable sensors as the regeneration step can be easily automated [1-3]. These devices consist of conventional or special flow-cells packed with inert (C_{18} bonded silica gel, alumina) or active materials (ion-exchangers or chromogenic ligands bonded to different inert materials) placed in an ordinary photometer of fluorimeter. Any of the components of a (bio)chemical reaction can be

retained on the packed material in a temporary manner if the analyte [4,5] or product [6,7] are the retained species; or permanently if the reagent [8,9] or catalyst [10] are immobilized. The reaction and/or retention at the detection point are integrated in this way. Simultaneous determinations can also be accomplished based on small differences of the anlytes behaviour if a enough powerful detector is used to enlarge these differences. Such is the case with the determination of ternary mixtures of aromatic amines [4] and carbamate pesticides [11] by placing the packed flow-cell in a diode array spectrophotometer, or that of B_6 vimaters by using derivative synchronous fluorescence [7]. One of the more interesting features of this type of (bio)sensors is the enhanced sensitivity as a consequence of the in situ concentration of the monitored species at the detection point, which allows a wide manipulation of the determination range by changing the concentration interval.

The coupling of a flow-through sensor to a liquid chromatograph is proposed in this paper for the first time. The aim of this arrangement was to improve the features of the chromatographic method for separation/determination of carbamate pesticides by enhancing sensitivity, thus showing the usefulness of the proposed coupling. The pesticides selected for this purpose were carbofuran, propoxur and carbaryl, and the overall method is based on the retention of these carbamates on an ultrabase C_{18} chromatographic column, and derivatization of the eluted analytes based on their basic hydrolysis and subsequent coupling with diazotized sulphanilic acid, the products of which are monitored photometrically at 400 nm. This chemical system was previously used for the determination of these compounds by flow injection analysis (FIA) [11] and also for their joint and individual determination by use of an integrated flow-injection/HPLC system [12].

MATERIALS

Reagents

The chromatographic system consists of a high-pressure dual piston pump (Knauer, mod. 64), a high-pressure rotary injection valve (Knauer), and an ultrabase C_{18} chromatographic column, all of which are connected through stainless steel tubing of 0.5 mm ID. The continuous-flow, post-column derivatizing system consists of two low-pressure peristaltic pumps (Gilson Minipuls-2 and Ismatec S-840, both with four channels), a Rheodyne 5041 low-pressure injection valve which acts as a switching valve,

and a UV-visible spectrophotometer (Pye Unicam SP6-500) furnished with Hellma flow-cells (mod. 178.12QS, 10 mm optical path; 178.52QS, 1.5 mm optical path; and 138 OS, 1 and 2 mm optical path), and connected to a recorder (Radiometer, mod. REC 80) and integrator (Hitachi D-2500). All tubing of the continouos flow system was of 0.7 mm ID. The temperature of the continuous system was controlled by a bath thermostat (Abson BH-1).

Chemicals and Materials

Aqueous solutions containing 2 M NaOH, 2 g/l $NaNO_2$ and 2 g/l sulphanilic acid in 30% acetic acid were used.

The mobile chromatographic phase was prepared from bidistilled water and HPLC/degree acetonitrile (ACN from Romil Chemicals) in a 70:30 ratio, filtered through a 66 Nylon filter (0.45 μ pore size) and degassed with an ultrasonic bath and then with helium.

Stock standard solutions containing 1 g/l of the analytes in 1,4-dioxane were prepared from solid samples supplied by Chem Service, and used to make more dilute solutions by adding bidistilled water. The stock solutions were stored at 4 °C.

The solid support in the flow-cell was C_{18} bonded silica of 60-100 μm from Sep-Pak cartridges supplied by Waters.

Methods

Figure 1 shows the overall arrangement used, in which the sample containing the three pesticides (carbofuran -CBF-, propoxur -PPX- and carbaryl -CYL) was injected through the high-pressure valve of the chromatograph into the acetonitrile/water mobile phase. After separation, the eluted analytes merged with a basic stream, which hydrolyzed them along reactor R_1. The diazotization step of HNO_2 with sulphanilic acid takes place along R_2; on merging R_1 and R_2 the dyes form along R_3 by coupling of the phenols resulting from hydrolysis of the analytes and diazotized sulphanilic acid. Finally, a water stream merged with R_3 and decreased the organic content of the stream which attained the flow-cell, thus favouring the retention of the reaction products on the C_{18} bonded silica packed in the cell. Reactor R_4 ensured a complete mixture of the merged stream. A selecting valve, SV, placed after R_4, was switched after the data (peak area and peak height) of the three analytes were collected to re-establish the baseline, thus the system was ready for introduction of the next sample.

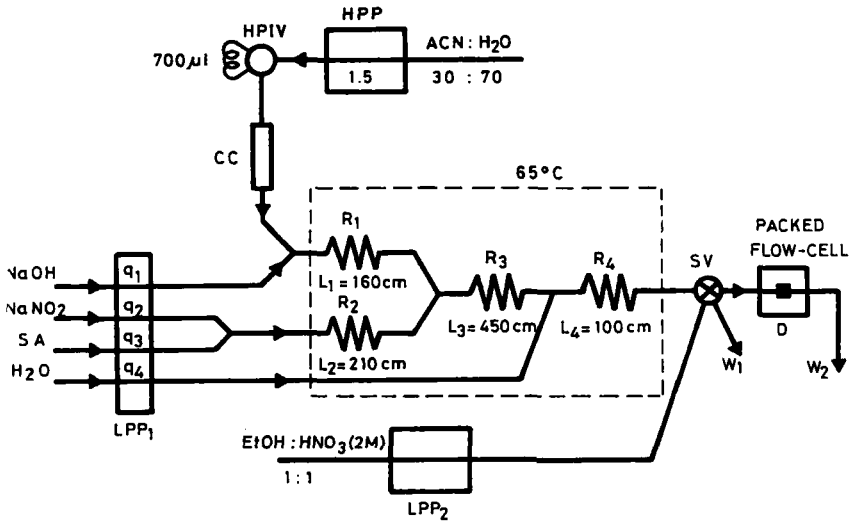

Fig. 1. HPLC/post-column derivatizing system/flow-through sensor arrangement for the determination of carbamate pesticides. HPP and LPP denote high and low pressure pumps, respectively, SA denotes sulphanilic acid, HPIV high pressure injection valve, CC chromatographic column, R reactor, SV selecting valve, D detector and W waste.

RESULTS AND DISCUSSION

The aim of the optimization was to achieve maximum retention of each monitored product on the support packed in the flow-cell, followed by its fast elution to avoid overlapping with the next product which attains the flow-cell.

Features of the Packed Cell

Several flow-cells of different optical path (10.0, 2.0, 1.5 and 1.0 mm) were assayed after packing them with the support. The 10 mm optical path cell was not useful as the absorbance of C_{18} bonded silica saturated the capacity of the detector. An optical path of 1.0 mm provided the best results as longer lengths gave rise to higher dispersion of the eluted plugs with appreciable overlapping of the reaction products of PPX and CBF.

The packing level of the support in the flow-cell was a key variable because if the solid phase did not reach the optical path, product measurements were actually made on the solution, and when the packing top was above the light beam, the support area with the highest product concentration (that closest to the surface, i.e. the area on which the incoming flow impinged) fell outside the sensed area. The packing level was studied between 14.5 and 19.0 mm, measured from the bottong of the cell. The signal of each product was maximal for a level of 16.0 mm. This level must be optimized for each detector, whose light source must iluminate all the surface in which the concentration occurs, but without lost of radiation towards other zones of the cell.

Optimization of Variables

Table 1 summarizes the ranges in which the different variables were optimized and the optimal values found by using the univariate method.

The optimal values of chemical variables were very similar or identical to those of the FIA/HPLC system previously described [12], but other variables as flow-rate and mobile phase of the chromatographic system and flow-rate of the continuous-post-column system were systematically optimized in order to achieve maximum retention of the monitored products on the flow-through sensor. This effect was favoured by increasing the water percentage in the stream which attains the flow-cell, which, in turn, was achieved by decreasing the organic percentage of the mobile phase and its flow-rate.

The composition of the mobile phase (acetonitrile/water 30:70) provided the best resolution in the minimum time. A decrease of the chromatographic system flow-rate from 2.0 ml/min to 1.5 ml/min resulted in an increase of the peak height between 23 and 30%, depending on the analyte, but also increased the time of the chromatogram from 18 to 25 min.

Despite the increase of dispersion, the increase of the flow-rate of the water *stream* of the post-column system increased the signals up to a value of 1.3 ml/min, above which the baseline became more and more irreproducible.

The increase of the injected sample volume increased the signals, but also the peak-witdh; so a sample volume of 700 μl (unusual in HPLC) was chosen as compromise.

An 1:1 ethanol-HNO_3 (2M) solution was found in a previous work [11] to be the most appropriate for eluting the product from the support. The flow-rate of this stream was not critical as the action of the eluent was highly efficient.

Table 1. OPTIMIZATION OF VARIABLES

Variable	Range estudied	Optimum value
NaOH, M	1 - 4	2
$NaNO_2$, g/l	1 - 4	2
Sulphanilic acid, g/l	1 - 4	2
Acetic acid, %	15 - 60	30
Temperature, °C	45 - 75	65
Flow-rate, ml/min, q_{HPLC}	10 - 2.0	1.5
q_1 , q_2	0.49 - 1.0	0.7
q_3	0.27 - 0.54	0.4
q_4	0.96 - 1.75	1.3
R_1, cm	80 - 360	160
R_2, cm	210 - 410	210
R_3, cm	200 - 650	450
R_4, cm		
V_i, µl	200 - 700	700
Optical path, mm	1 - 10	1
Packing level, mm	14 - 19	16

The passage of the mobile phase-reagents mixture through the support resulted in a continuous increase of the baseline, dipper when the percentage of the aqueous phase increased. There was impossible to obtain a constant baseline without a dramatic lost of sensitivity.

Features of the Proposed Method

Figure 2A show a chromatogram obtained with the optimal working conditions for which the drift of the baseline did not hinder to propose a useful method for the

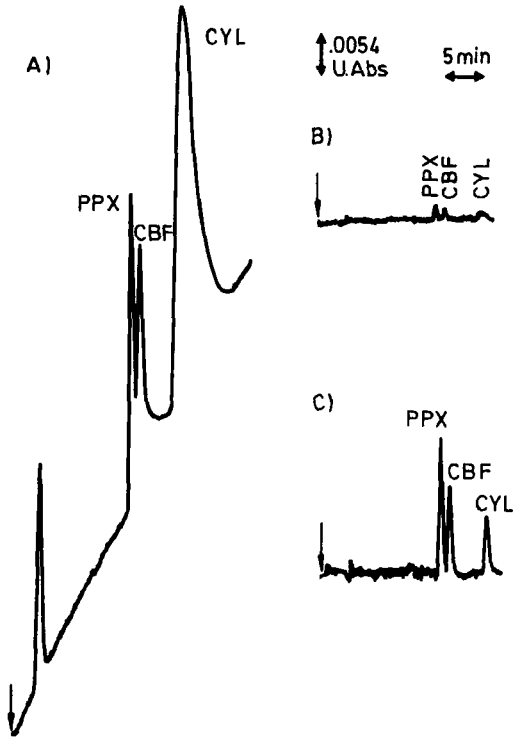

Fig. 2. Comparison of the sensitivity of the proposed method (A), with that afforded by the use of the same flow-cell without packed material (B) and with an unpacked cell with an optical path ten-times longer (C). CBF denotes carbofuran, PPX propoxur, and CYL carbaryl. The working conditions for all the three experiments were: Concentration of PPX and CBF 0.5 μg/ml, concentration of CYL 0.25 μg/ml, flow-rate of the chromatographic system 1.5 ml/min, overall flow-rate of the post-column system 3.2 ml/min, injection volume 700 μl.

Table 2. FEATURES OF THE PROPOSED METHOD FOR DETERMINATION OF CARBAMATE PESTICIDES

Analyte	t'_R (min)		Peak height				Peak area			
	average	r.s.d. %	Linear range[1]	Equation[2]	r	r.s.d. %[3]	Linear range[1]	Equation[2]	r	r.s.d. %[3]
PPK	11.93	±0.84	0.04 - 1.4	$Y=2.182\cdot10^{-3}$ + 0.0981 X	0.99981	±3.02	0.04 - 0.8	Y=15413 + 2542662·X	0.9986	8.05
CBF	13.11	±1.00	0.04 - 1.4	$Y=1.037\cdot10^{-3}$ + 0.0778 X	0.99998	±2.59	0.04 - 0.8	Y=-48552 + 2320613·X	0.9987	17.46
CYL	18.43	±1.19	0.025 - 0.7	$Y=6.485\cdot10^{-3}$ + 0.2384 X	0.9989	±2.04	0.03 - 0.4	Y=-424005 + 26957545·X	0.9969	7.52

[1] µg/ml

[2] X = concentration, µg/ml, Y = absorbance units

[3] n = 7; 0.2 µg/ml of PPX, CBF and CYL

[4] X = concentration, µg/ml, Y = µvolt.min

Table 3. RESOLUTION OF TERNARY MIXTURES OF PROPOXUR (PPX), CARBOFURAN (CBF) AND CARBARYL (CYL)

Added, μg/ml			Peak-height measurements						Peack-area measurements					
			Found			Recovery, %			Found			Recovery, %		
PPX	CBF	CYL	PPX	CBF	CYL	PPX	CBF	CYL	PPX	CBF	CYL	PPX	CBF	CYL
0.05	0.1	0.1	0.0510	0.1004	0.1093	102.9	100.4	109.3	0.0646	0.1048	0.1159	129.1	104.8	115.9
0.1	0.1	0.05	0.101	0.1004	0.0498	101.3	100.4	99.6	0.0988	0.1017	0.0432	98.8	101.7	86.3
0.2	0.4	0.2	0.199	0.3514	0.1973	99.6	87.9	98.7	0.1848	0.2753	0.1828	92.4	68.8	91.4
0.2	0.2	0.4	0.196	0.1973	0.3940	97.9	98.6	98.5	0.1955	0.1804	0.3858	97.7	90.2	96.4
0.1	0.2	0.3	0.0996	0.1753	0.3047	99.6	87.7	101.6	0.0913	0.1415	0.2984	91.3	70.8	99.6
0.1	0.3	0.2	0.0979	0.2700	0.2051	97.9	90.0	102.5	0.0747	0.2218	0.2066	74.7	73.9	103.3
0.2	0.3	0.1	0.2164	0.2942	0.1093	108.2	98.1	109.3	0.2046	0.2266	0.1129	102.3	75.5	112.9
0.3	0.2	0.1	0.3057	0.2193	0.1016	101.9	109.7	101.6	0.3038	0.1671	0.1028	101.3	83.5	102.8

determination of these pesticides, whose features appear in Table 2, in which a better values of the features based on peak height measurements rather than in peak area can be observed: wider linear interval, better regression coefficient and smaller relative standard deviation.

Sensitivity of the Proposed Approach

As shown in Fig. 2, the retention of the reaction products on the support packed in the flow-cell dramatically increased the sensitivity with respect to the use of the same cell but without support. The peak height increased 19.3, 20.0 and 50.0-times for PPX, CBF and CYL, respectively, by the in situ concentration of the reaction products of these analytes (Fig. 2A and B). Even the sensitivity was higher for the in situ concentration than for a non-packed flow-cell with an optical path ten-times longer (Fig. 2A and C). The improvement in this case was 2.3, 2.7 and 6.7-times for PPX, CBF and CYL, respectively.

Resolution of Pesticide Mixtures

The performance of the proposed method was tested by applying it to the resolution of ternary mixtures of the analytes. Different ratios of carbofuran, propoxur and carbaryl mixtures were assayed. Table 3 lists the added and found concentrations of these carbamates and the percent recoveries achieved both by using peak height and peak area measurements. The recoveries were between 87.7 and 109.7% for peak height measurements, while those based on area measurements provided worse values. Errors by excess were obtained for PPX/CBF concentration ratios upper than two owing to overlapping of the peaks.

CONCLUSIONS

The proposed method surpasses to other recent methods based on HPLC [12-14], and also to that based on the use of a flow-through sensor located in a diode array spectrophotometer [11] in several respects as determination limit, linear range of the calibration curve, precision and simplicity of the instrumental system. Thus, it must be concluded that the coupling of a flow-through sensor to a liquid chromatograph can improve the features of a method by increasing sensitivity as required through the in situ concentration of the analyte at the detection point.

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