Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 64 (2004) 742–749
Talanta
www.elsevier.com/locate/talanta

# Multiwavelength fluorescence based optosensor for simultaneous determination of fuberidazole, carbaryl and benomyl

J.F. García Reyes, E.J. Llorent Martínez, P. Ortega Barrales, A. Molina Díaz*

*Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Paraje Las Lagunillas, E-23071 Jaén, Spain*

Received 18 January 2004; received in revised form 23 March 2004; accepted 26 March 2004
Available online 25 May 2004

**Abstract**

In the present work, a novel approach is proposed for the simultaneous determination of three widely used pesticides (namely, fuberidazole (FBZ), carbaryl (CBL) and benomyl (BNM)). The proposed method is based on a single continuous-flow solid surface fluorimetric multi-optosensor implemented with the use of a minicolumn placed just before the flow-through cell and filled with $C_{18}$ silica gel. The three pesticides are determined from an only injection (simultaneous determination): the minicolumn strongly retains two of them while the third develops a transitory signal when passing through the sensing solid microzone. Then, two alternate eluting solutions appropriately selected perform the sequential elution of the two pesticides from the minicolumn, achieving the detection zone and developing their transitory signals. The proposed optosensor works under optimal sensitivity conditions for all the three analytes because of the use of multi-wavelength fluorescence detection mode, so recording three different signals corresponding at three pairs of optima excitation/emission wavelengths. Using a sample volume of 2100 μl, the system was calibrated in the range 0.5–15, 40–800 and 50–1000 $\mu g\,l^{-1}$ with detection limits of 0.09, 6 and 9 $\mu g\,l^{-1}$ for FBZ, CBL and BNM, respectively. The R.S.D values ($n = 10$) were lower than 2% in all cases. The proposed methodology was applied satisfactorily to water samples. Recovery percentages ranging from 97.8 to 101.1%, 97.9 to 103% and from 97 to 105% for FBZ, CBL and BNM, respectively, were obtained.


*Keywords:* Flow-injection; Fluorescence; Pesticides; Environmental analysis

## 1. Introduction

Due to environmental considerations, one of the most important tasks in analytical science nowadays is the development of novel analytical methodologies capable of determining trace levels of organic pollutants, present in the environment. Therefore, the development of sensitive, selective and cost-effective methods of analysis is being pursued with great interest by many researchers.

Immunoanalytical techniques (viz. immunosensors, immunoassays, ...) have proven its feasibility for environmental purposes in recent years, providing methods which involve minimal sample clean-up, high selectivity, relatively simple handling and low detection limits [1,2].

Fluorescence spectroscopy has also been widely exploited in recent years for environmental screening purposes, because of its inherent sensitivity [3,4]. Nevertheless, fluorimetric measurements based only on selection of excitation/emission wavelengths, generally lack of selectivity because of the wide profile of both excitation and emission spectra. A remarkable increase of selectivity on fluorescence measurements has been obtained recently by the implementation of strategies such as polarization fluorescence, synchronous fluorescence, selective quenching or variable angle scanning fluorescence. In these sense, these strategies are usually accompanied by signal processing by means of mathematical algorithms such as partial least-squares regression (PLS), principal components regression (PCR) or multivariate linear regression (MLR). Several fluorimetric multi-residue methods has been developed in recent years, based on afore mentioned methodologies [5–8].

Solid-phase spectroscopy (SPS) is a methodology based on the retention and preconcentration of the target species

* Corresponding author. Tel.: +34-953012147; fax: +34-953012141.
*E-mail address:* amolina@ujaen.es (A. Molina Díaz).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.03.049

on an active solid support in combination with direct measurement on the solid phase by the use of a molecular spectroscopic technique. SPS increases both sensitivity and selectivity, because of the retention/preconcentration performed on the solid support [9–11]. Solid phase spectrofluorimetry has been applied to the determination of several pesticides in water [10,11]. Nevertheless, this technique is high time-consuming and not appropriated for multicomponent analysis.

In this sense, the implementation of SPS and unsegmented flow analysis systems has been widely used in recent years, in order to develop continuous-flow methods originating the so called flow-through optosensors [12–21]. Separation, preconcentration and detection steps, are carried out automatically and simultaneously without sample manipulation during the measurement process. Flow-through optosensors involve the use of a solid support placed in the flow-cell of a non-destructive spectroscopic detector, where the analyte is immobilized temporarily for its detection, and then, appropriately eluted [14].

Despite the field of continuous-flow optosensors has been widely exploited, a scarce amount of multiparameter flow-through sensors (i.e. sensors for multicomponent analysis) has so far been described. There are two main strategies in the development of multi-parameter sensor: discrimination by means of signal processing [15,16], or by implementation of a separation step prior to detection [21].

The implementation of a separation unit just prior to detection can foster the development of straightforward, selective and sensitive multi-analyte flow-through optosensors. A very simple approach to achieve the simultaneous determination of two/three analytes is the use on-line of a minicolumn filled with the appropriate active solid support in order to achieve a single and complete separation of the analytes just before they reach the sensing zone. The separation is employed to make possible the sequential arrival of the analystes to the sensing zone, that is their temporal discrimination. This broads the potential of flow-through optosensors towards to multidetermination (multisensors). The simultaneous determination of more than one analyte can be achieved by the retention on-line of one (or more) of them in the minicolumn while another one passes to the sensing zone. The sequential and selective elution of (an)other analyte(s), by using the appropriate eluting solution(s) allows to determine simultaneously two/three analytes by simply performing a sequential arrival of them to the detection zone.

In this paper, a multiparameter-responding flow-through fluorescence based optosensor is described for the simultaneous determination of a mixture of three widely used pesticides (namely fuberidazole (FBZ), carbaryl (CBL) and benomyl (BNM)). Because of the strong spectral overlapping of these analytes, a separation previous to the detection in the flow-through sensing zone is performed by using a minicolumn coupled on-line to the flow-injection system. This work contributes to extend the field of multiparameter flow-through optosensors, providing a novel and straightforward methodology for the resolution of mixtures of pesticides with overlapped spectra without performing any derivatization reaction. To the best of our knowledge this is the first fluorimetric flow-through optosensor capable of analysing simultaneously three analytes, by means of a temporary sequentiation in their arrival to the sensing zone.

## 2. Experimental

### 2.1. Chemicals and solutions

All standard solutions were prepared from analytical reagent-grade chemicals by using pure solvents and doubly distilled water.

FBZ [2-(2′-furyl)benzimidazole)] (Riedel de Haën) and CBL [1-naphthyl-*N*-methylcarbamate] (Riedel de Haën) stock solutions of 200 $\mu g\,ml^{-1}$ were prepared by dissolution of the appropriate amount in absolute ethanol (Panreac). These solutions remained stable for at least one month when stored under refrigeration at 4 °C. BNM [methyl 1-(butylcarbamoyl)benzimidazol-2-yl carbamate] (Riedel de Haën) stock solution (200 $\mu g\,ml^{-1}$) were prepared by dissolution of the appropriate amount in 5 M HCl solution. This solution remained stable for at least two weeks at the same conditions described above.

Working standard solutions containing BNM, CBL and FBZ were prepared by suitable dilution of the stock solution with a 1.5 M HCl (Panreac) solution.

Methanol (Panreac) was used, as carrier solution (30% MeOH (v/v)), as eluting solution 1 (55% MeOH (v/v)) and also as eluting solution 2 (75% MeOH (v/v)). $C_{18}$ bonded phase silica gel beads (Waters, Milford, USA) with average particle sizes of 55–105 μm, was used as an active solid support.

### 2.2. Apparatus and instruments

Relative fluorescence intensity measurements were performed in a Cary-Eclipse Luminescence Spectrometer (Varian Inc., Mulgrave (Australia)), equipped with a Hellma flow cell 176.052-QS (25 μL of inner volume and a light path length of 1.5 mm) (Jamaica, NY, USA). The spectrofluorimeter, which operated in multi-wavelength detection mode, was connected to a computer with a Cary Eclipse (Varian) software package for data collection and treatment. The flow cell was filled with $C_{18}$ silica gel microbeads, as a slurry suspension in methanol, with the aid of a syringe. The flow-through cell was blocked at the outlet with glass wool, to avoid displacements of the $C_{18}$ gel beads.

The flow-injection manifold used is depicted in Fig. 1. It was built using a four-channel Gilson Minipuls-3 peristaltic pump (Villiers le Bel, France) fitted with a rate selector, three Rheodhyne type 5041 injection valves (Cotati, CA, USA), two of them used as a selecting valves. A 50 mm length minicolumn (1.5 mm i.d). packed with 55 mg of $C_{18}$ silica

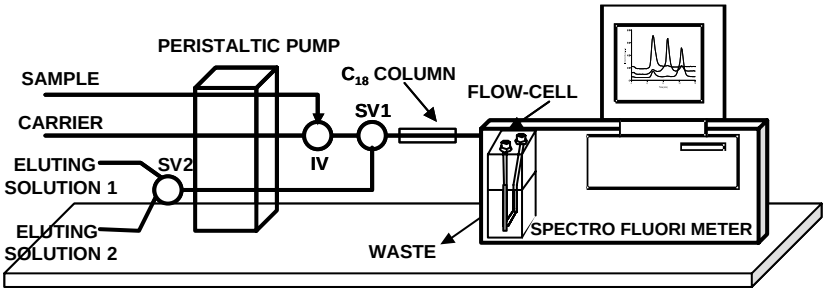


Fig. 1. Flow-injection assembly. IV: injection valve; SV1: selection valve 1; SV2: selection valve 2.

gel beads was inserted in the manifold just before the flow-cell. The outlet of the minicolumn was blocked with glass wool to avoid displacements of the solid support. 0.8 mm i.d. PTFE tubing was also used.

### 2.3. General procedure

The continuous-flow configuration is outlined in Fig. 1. Sample solutions (600 or 2100 μl) containing the analytes, were inserted into the carrier stream (MeOH 30% (v/v)) by means of a six-port rotary injection valve, and were pumped through the flow-assembly at a flow-rate of 1.0 ml $min^{-1}$.

The continuous-flow methodology for the simultaneous resolution of FBZ, CBL and BNM mixtures comprises three simple and comprehensive steps:

(1) The sample plug is brought into contact with the mini-column packed with $C_{18}$ gel beads, analyte 1(FBZ) passing through it reaching the sensing microzone and developing its transient fluorescence signal (continuously recorded at 314/356 nm, $\lambda_{exc}/\lambda_{em}$). The other two analytes were retained in the minicolumn.

(2) Then, by turning the selection valve SV1, analyte 2 (CBL), is carried out by an appropriate carrier/eluting solution (eluting solution 1: MeOH 55%, v/v) to the solid-phase detection zone, where its transient signal is monitored at 281/336 nm ($\lambda_{exc}/\lambda_{em}$).

(3) Finally, BNM, is eluted from the minicolumn by means of another appropriate eluting solution (eluting solution 2: MeOH 75%, v/v), developing its transient signal monitored at 293/398 nm ($\lambda_{exc}/\lambda_{em}$). A fiagram is shown in Fig. 2.

## 3. Results and discussion

### 3.1. Preliminary studies

#### 3.1.1. Spectral characteristics

The spectral features of FBZ, CBL and BNM were recorded in both gel-phase and aqueous solution. The gel-phase spectra was recorded in "stopped-flow" mode, with the optimized carrier and eluting solutions. The three

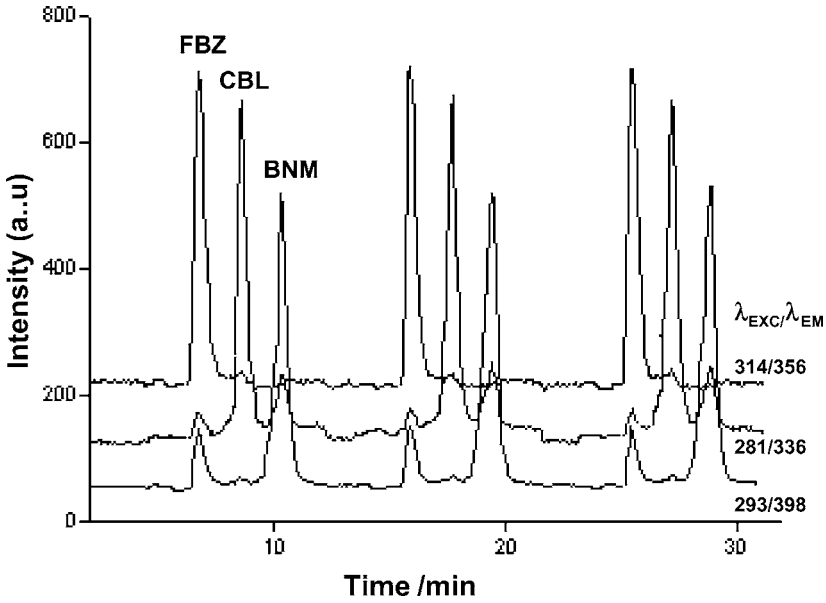


Fig. 2. Recorded signals in the determination of 10, 600 and 1000 μg $L^{-1}$ of fuberidazole (FBZ), carbaryl (CBL) and benomyl (BNM) respectively using a sample volume of 2100 μL.

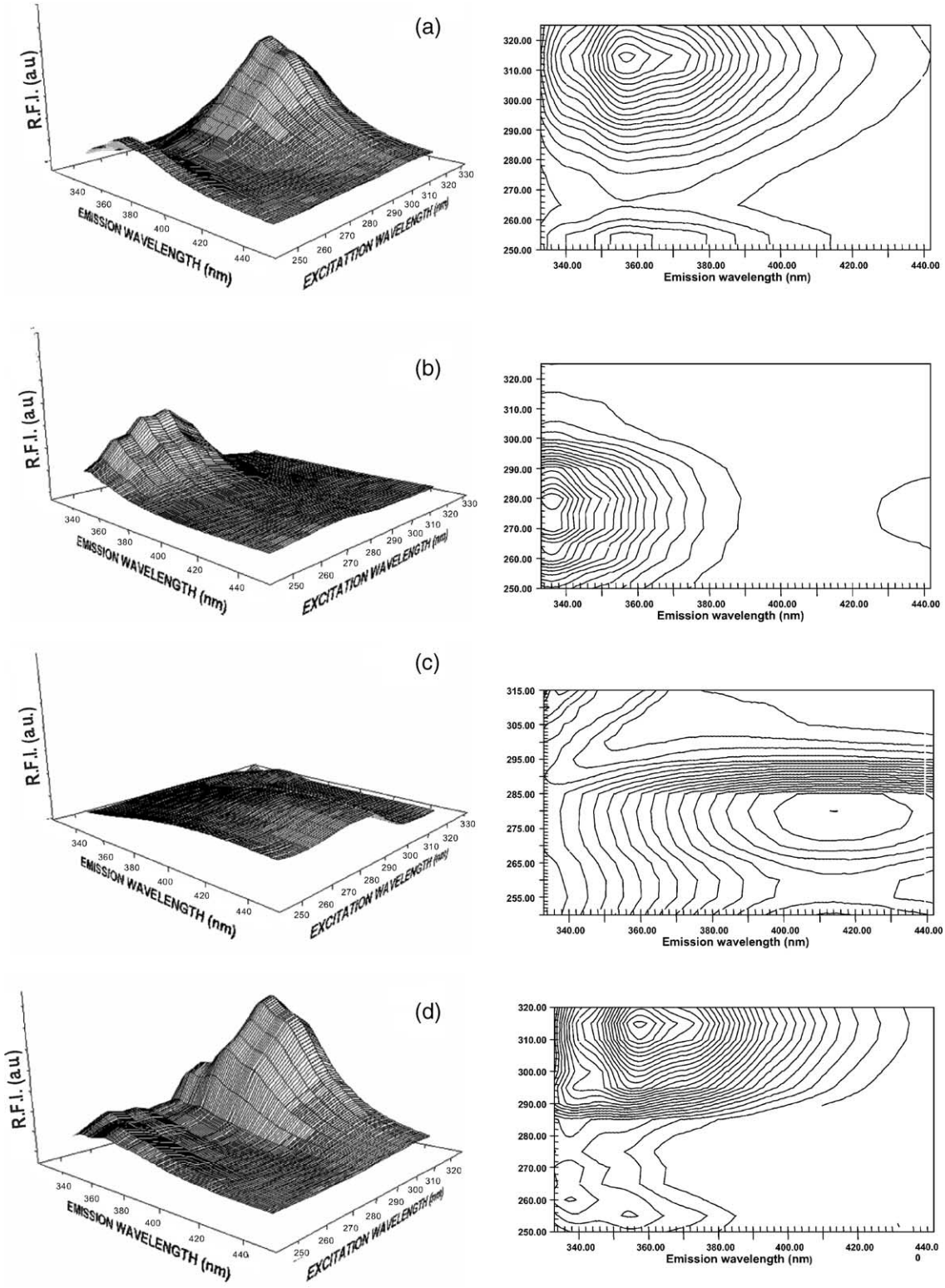


Fig. 3. (Left) Three-dimensional fluorescence spectra plots in aqueous solution of fuberidazole (a), carbaryl (b), benomyl (c) and the mixture (d). (Right) Respective contour maps.

dimensional intrinsecal fluorescence spectra, collected in aqueous solution, showed maxima excitation/emission wavelengths at 312/356, 282/335 and 283/406 nm (plateau) for FBZ, CBL and BNM, respectively. The spectra recorded in gel-phase showed maximum excitation/emission wavelengths at 314/356, 281/336 and 293/398 for FBZ, CBL and BNM, respectively. The differences in the optima wavelengths of the analytes in aqueous solution and gel-phase media can be attributed to changes of the environment surrounding the analyte on the solid phase in relation to that in homogeneous solution.

Taking into account the wide profiles of excitation and emission fluorescence spectra of these fluorophores, significant errors in the individual measurement of one analyte in the presence of the others were expected. Nevertheless, the discrimination obtained by the sequential immobilization and further individual determination, made possible the simultaneous determination (that is, only one injection is performed) without needing discrimination by means of signal or spectra processing (Fig. 3).

### 3.1.2. Selection of the solid support

Taking into account the structure of the selected pesticides (see Fig. 4) (both fuberidazole and benomyl, show a benzimidazolic group with basic nitrogen atoms) three types of suitable active solid phases were studied for the proposed method: a cation exchanger on dextran (Sephadex SP C-25), a non polar sorbent ($C_{18}$ silica gel) and a dextran type without exchangeable groups (Sephadex G-15) were tested. $C_{18}$ bonded silica gel beads proved to be the most suitable solid support since it was the unique material that interacted with all the three pesticides in a wide variety of experimental conditions.

### 3.1.3. Instrumental variables

Relative fluorescence measurements carried out in gel-phase media are usually affected by background signal level higher than those obtained in homogeneous solution, due to the presence of solid support in the zone irradiated by the excitation light beam. For this reason, instrumental conditions were carefully optimised in order to achieve the best possible signal to background ratio.

A study of the optimum photomultiplier tube voltage was undertaken within the range from 400 to 800 V. A value of 650 V. was chosen as a compromise value between high analytical signal and low background signal. The instrument excitation and emission slits widths were studied in the range from 1 to 20 nm. 5 and 20 nm width values were chosen for excitation and emission slit width, respectively, in order to obtain an optimum value of the ratio analyte/$C_{18}$ background signals, at the selected pairs of excitation/emission wavelengths for each pesticide.

The selected excitation and emission wavelengths to perform the determination of FBZ, CBL and BNM were 314/356, 281/336 and 293/398 nm. These values correspond to the maximum excitation and emission wavelengths for each analyte. The use of multi-wavelength fluorescence detection mode allowed that the three pairs of excitation/emission wavelengths could be simultaneously recorded. So simultaneous monitoring at different pairs of wavelengths could be performed in this apparatus configuration, allowing to work in optimum sensitivity conditions for the three analytes simultaneously.

### 3.2. Optimisation of separation: amount of solid support in the precolumn

The solid-phase selected in the minicolumn for using was the same as that used in the flow-cell, because it was the unique solid support which interacted with all the three analytes. BNM was retained on the $C_{18}$ silica gel beads much strongly than the other two analytes. Therefore, the separation study was carried out by varying the amount of $C_{18}$ silica gel beads in the minicolumn. The optimum amount of solid support used in the precolumn was determined as the amount enough to obtain a complete resolution between FBZ and CBL, because the complete separation between CBL and BNM was easily achieved, due to the strong retention of BNM in the silica gel minicolumn. In this study, the nature of the carrier and eluting solutions were taken into

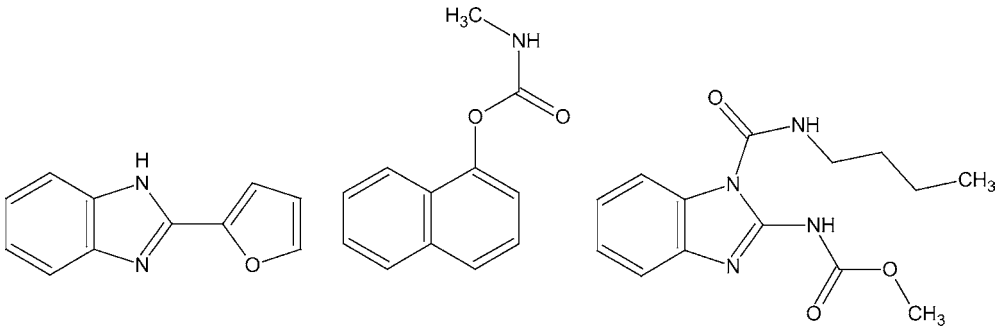


Fig. 4. Structure of the selected pesticides.

account, as these variables had a significant influence in the separation of the analytes. Therefore, the study of the separation was carried out with the optimal composition of the carrier and eluting solution. The optimum separation was achieved by means of a column (1.5 mm, i.d.) filled with 55 mg of $C_{18}$ (50 mm, length), because it gave a satisfactory and complete separation in the minimum possible time, avoiding overpressure system problems.

### 3.3. Chemical variables

Taking into account the instability of BNM in aqueous solutions, and its possible degradation to its major degradation product carbendazim, sample solutions were prepared in acid media, conditions in which BNM remained stable during the analysis, without partial degradation to carbendazim. In this sense, in order to asses the stability of benomyl during the analysis, both standard solutions and samples were acidified at 1.5 M HCl, conditions in which BNM remained stable during 24 h at room temperature [22].

#### 3.3.1. Optimisation of carrier and eluting solutions

Bearing in mind the non-polar nature of $C_{18}$ silica gel beads, several carrier solutions were prepared containing mixture of methanol and water in different proportions. Optimisation of the carrier and eluting solution were performed individually for each analyte, with the optimum amount of solid support packed in the precolumn. The effect of the composition of the carrier and eluting solutions in the separation was taken into account, because the separation of the analytes with the optimum amount of packed material in the precolumn only was achieved in certain composition values of the carrier and eluting solutions.

Carrier solution (for FBZ) was studied with aqueous methanol solutions in the range from 15 to 35% MeOH (v/v). It was observed that low proportions of methanol did not perform the appropriate elution of FBZ. On the other hand, with methanol percentages higher than 30%, the separation between FBZ and CBL could not be obtained satisfactorily. Eluting solution 1 was studied with aqueous methanol solutions with proportions ranging from 45 to 60% MeOH (v/v). With methanol percentages higher than 60% the separation between CBL and BNM could not be obtained. Eluting solution 2 was studied with methanol solutions with proportions in the range from 60 to 75% MeOH (v/v). Therefore, 30, 55 and 75% MeOH (v/v) solutions were chosen respectively as a compromise between sensitivity and low elution time.

### 3.4. Study of flow-injection variables

The flow-injection variables studied were the sample volume and the effect of the flow-rate. The sample volume study was carried out in the range from 200 to 3000 μl (by varying the sample loop length of the injection valve). The higher the sample volume injected, the higher the amount of the analyte sorbed on the solid support, and, hence, the higher the sensitivity showed by the solid phase flow-through sensing system. Analytical signal increased linearly up to about 3000 μl for CBL and FBZ, and up to 2000 μl for BNM. 600 and 2100 μl were chosen, taking into account both sensitivity and sampling frequency. We have to bear in mind that by using a higher sample volume (i.e., 4000 μl), a remarkable increase in sensitivity could be obtained (about 50% in relation to 2100 μl). However, it should be noted that a drastical decrease in sampling frequency would be obtained in these conditions.

Table 1
Figures of merit

| Parameter | FBZ | CBL | BNM |
|---|---|---|---|
| Linear dynamic range ($\mu g\,l^{-1}$) | 0.5–15 | 40–800 | 50–1000 |
| Calibration graph | | | |
| Intercept | −2.0 | −2.7 | 4.1 |
| Slope ($l\,\mu g^{-1}$) | 46.85 | 0.8570 | 0.4016 |
| Correlation coefficient | 0.9999 | 0.9994 | 0.9994 |
| Detection limit ($\mu g\,l^{-1}$) | 0.09[a] | 6.0[a] | 9[a] |
| Quantification limit ($\mu g\,l^{-1}$) | 0.30[b] | 20[b] | 30[b] |
| R.S.D. (%) ($n = 10$) | 1.33 (10)[c] | 1.54 (600)[c] | 1.80 (1000)[c] |
| Sampling frequency ($h^{-1}$) | 7[d] | | |

[a] $3\sigma$ criterion ($n = 10$).
[b] $10\sigma$ criterion ($n = 10$).
[c] Concentration level ($\mu g\,l^{-1}$).
[d] Sampling rate for the simultaneous determination.

Table 2
Interference study

| Foreign species | Tolerated[a] interferent/analyte (w/w) ratio | | |
|---|---|---|---|
| | FBZ | CBL | BNM |
| $Cl^-$, $CO_3^{2-}$, $PO_4^{3-}$, $Na^+$, $SO_4^{2-}$, $AcO^-$, $K^+$ | 10000[b] | 2000[b] | 500[b] |
| Aldicarb[c] | 1000[b] | 20[b] | 5[b] |
| Aminocarb[c] | 1000[b] | 20[b] | 5[b] |
| Bendiocarb[c] | 1000[b] | 20[b] | 5[b] |
| Carbendazim[c] | 1000[b] | 20[b] | 5[b] |
| Imazalil | 1000[b] | 20[b] | 5[b] |
| Methiocarb[c] | 1000[b] | 20[b] | 5[b] |
| Morestan[c] | 1000[b] | 20[b] | 5[b] |
| Simazine | 1000[b] | 20[b] | 5[b] |
| Carbofuran[c] | 100 | 2 | 5[b] |
| *O*-phenylphenol[c] | 100 | 2 | 5[b] |
| Naphthylacetic acid[c] | 50 | 0.4 | 5[b] |
| Thiabendazole[c] | 5 | 20[b] | 5[b] |

[a] Tolerance level was defined as the amount of foreign specie that produced an error not exceeding ± 5% in the determination of each analyte.
[b] Maximum ratio tested.
[c] Fluorophore.

Table 3
Recovery study

| | FBZ | | CBL | | BNM | |
|---|---|---|---|---|---|---|
| | Added ($\mu g\,l^{-1}$) | Recovery ± R.S.D. (%)[a] | Added ($\mu g\,l^{-1}$) | Recovery ± R.S.D. (%)[a] | Added ($\mu g\,l^{-1}$) | Recovery ± R.S.D. (%)[a] |
| Well water | 1 | 100.1 ± 0.9 | 40 | 100 ± 2 | 100 | 99 ± 2 |
| | 2 | 100 ± 2 | 80 | 99.1 ± 0.6 | 200 | 97 ± 1 |
| | 4 | 97.8 ± 0.7 | 120 | 97.9 ± 0.5 | 400 | 99.1 ± 0.9 |
| River water | 1 | 101 ± 1 | 40 | 103 ± 1 | 100 | 105 ± 2 |
| | 2 | 100.2 ± 0.9 | 80 | 103.0 ± 0.8 | 200 | 103 ± 1 |
| | 4 | 99.4 ± 0.7 | 120 | 100.4 ± 0.5 | 400 | 101 ± 1 |
| Dam water | 1 | 100.0 ± 0.8 | 40 | 98 ± 1 | 100 | 99 ± 2 |
| | 2 | 100.4 ± 0.7 | 80 | 101.0 ± 0.8 | 200 | 98 ± 1 |
| | 4 | 98.8 ± 0.6 | 120 | 102.3 ± 0.5 | 400 | 98.4 ± 0.9 |
| Irrigation water | 1 | 101 ± 1 | 40 | 98 ± 1 | 100 | 100 ± 1 |
| | 2 | 100.1 ± 0.8 | 80 | 101.2 ± 0.8 | 200 | 99 ± 2 |
| | 4 | 100 ± 1 | 120 | 102.2 ± 0.7 | 400 | 97.9 ± 0.8 |

[a] $n = 3$.

The effect of the flow-rate was also investigated. The flow-rate was studied from 0.50 to 1.10 ml $min^{-1}$. At higher values, overpressure problems occurred in the system. By increasing the flow-rate, the analytical signal and the elution time progressively decreased for all the three analytes, due to their lower retention and consequently the sampling frequency increased. As a compromise between sensitivity and total signal time, 1.00 ml $min^{-1}$ flow-rate was chosen. Higher values involved a substantial loss of sensitivity with a scarcely increase in sampling frequency.

### 3.5. Figures of merit

In the optimised conditions, the analytical parameters of the proposed method were studied. Calibration graphs were obtained simultaneously for the three analytes according to the procedure above described. The system was calibrated for two different sample volumes: 600 and 2100 μl. Results obtained for the higher sample volume are summarized in Table 1. R.S.D. and sampling frequency were also established.

### 3.6. Study of potentially interfering species

In this study, foreign species which are likely to be present in real samples, were added to solutions containing 0.01, 0.5 and 2.0 $\mu g\,ml^{-1}$ of FBZ, CBL and BNM respectively (using a sample volume of 2100 μl), and their influence on the analytical signal was investigated). A 1000 $\mu g\,ml^{-1}$ level of each ionic specie was tested first, and if any interference was observed, the ratio interference:analyte (w/w) was reduced progressively until the interference ceased.

The study of potential interferences, resulting from other common pesticides was also undertaken. Results obtained are summarised in Table 2. It can be concluded that FBZ, CBL and BNM can be analysed without significant errors by the proposed method, in the presence of higher concentration levels of potentially interfering compounds, which are likely to be present in real samples.

### 3.7. Analytical applications

In order to illustrate the usefulness of the proposed method, it was applied to the simultaneous determination of FBZ, CBL and BNM in different types of environmental water samples. These water samples were found to be free from BNM, CBL and FBZ. Therefore, spiked samples were prepared by adding known amounts of the pesticides at different concentration levels within the range 1–4, 40–120 and 100–400 $\mu g\,l^{-1}$ for FBZ, CBL and BNM, respectively.

As can be seen in Table 3, the results obtained showed good agreement with the amount added in each sample, with good mean recovery percentages for all the analytes. Moreover, a standard deviation lower than 2% was obtained, in most cases.

## 4. Conclusions

For the first time, an on-line solid-phase separation has been implemented with a continuous-flow fluorimetric optosensor to perform the resolution of a three-analyte mixture (FBZ, CBL and BNM). Although the proposed methodology obviously does not compete with the analytical potential of chromatographic techniques, it is rapid, simple and cost-effective, so it can be used to resolve mixtures of a reduced number of analytes (two/three), being in this case, an interesting alternative to chromatographic methods. Furthermore, minor sample treatment is required and no prior extraction is needed. The proposed methodology represents an interesting contribution to the field of flow-through optosensors by means of the use of a new strategy which combines on-line separation/retention with solid-phase fluorimetric multi-

wavelength transduction. New studies are being carried out in our laboratory in order to expand the potential of fluorimetric flow-through multi-optosensors field.

## Acknowledgements

The authors acknowledge the financial support from MCYT (Project no. BQU 2002-02872), partially co-financed by FEDER funds. J. F. G. R. also acknowledges an FPU program scholarship from MECD.

## References

[1] U. Schobel, C. Barzen, G. Gauglitz, Fresenius J. Anal. Chem. 366 (2000) 646.
[2] M.C. Hennion, D. Barceló, Anal. Chim. Acta 362 (1998) 3.
[3] A. Coly, J.J. Aaron, Talanta 46 (1998) 815.
[4] R.A. Agbaria, P.B. Oldham, M. McCarroll, L.B. McGrown, I.M. Warner, Anal. Chem. 74 (2002) 3952.
[5] A. Navas Díaz, F. García Sánchez, M.M. López Guerrero, Talanta 60 (2003) 629.
[6] D. Patra, A.K. Mishra, Trends Anal. Chem. 21 (2002) 787.
[7] G. De Armas, M. Miró, J.M. Estela, V. Cerdá, Anal. Chim. Acta 471 (2002) 173.
[8] M. Martínez Galera, D. Picón Zamora, J.L. Martínez Vidal, A. Garrido Frenich, A. Espinosa-Mansilla, A. Muñoz de la Peña, F. Salinas López, Talanta 59 (2003) 1107.
[9] P. Ortega Barrales, M.L. Fernández de Córdova, A. Molina Díaz, Anal. Chem. 70 (1998) 271.
[10] F. Capitán, E. Alonso, R. Avidad, L.F. Capitán Vallvey, J.L. Vilchez, Anal. Chem. 65 (1993) 1336.
[11] J.L. Vilchez, L.F. Capitán Vallvey, J. Rohand, A. Navalón, R. Avidad, Analyst 120 (1995) 1609.
[12] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 173 (1985) 3.
[13] A. Sanz-Medel, Anal. Chim. Acta 283 (1993) 367.
[14] M. Valcárcel, M.D. Luque de Castro, Flow-Through (Bio)chemical Sensors, Elsevier, Amsterdam, 1994.
[15] M.J. Ayora Cañada, M.I. Pascual Reguera, A. Molina Díaz, L.F. Capitán Vallvey, Talanta 49 (1999) 691.
[16] S. Ortega Algar, N. Ramos Martos, A. Molina-Díaz, Talanta 60 (2003) 313.
[17] P. Ortega Barrales, M.J. Ayora Cañada, A. Molina Díaz, S. Garrigues, M. de la Guardia, Analyst 124 (1999) 579.
[18] J.F. Fernández Sánchez, A. Segura Carretero, C. Cruces Blanco, A. Fernández Gutiérrez, Talanta 60 (2003) 287.
[19] J. Díaz-García, J.M. Costa Fernández, N. Bordel, R. Pereiro, A. Sanz-Medel, Anal. Chim. Acta 486 (2003) 1.
[20] A. Domínguez Vidal, P. Ortega Barrales, A. Molina Díaz, Talanta 56 (2002) 1005.
[21] J.F. García Reyes, P. Ortega Barrales, A. Molina Díaz, Anal. Chim. Acta 493 (2003) 35.
[22] R.P. Singh, C.H. Marvin, I.D. Brindle, J. Agric. Food Chem. 40 (1992) 1303.