Talanta 115 (2013) 462–467

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Separation of a binary mixture of pesticides in fruits using a flow-through optosensor

E.J. Llorent-Martínez, I. Delgado-Blanca, A. Ruiz-Medina, P. Ortega-Barrales *

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

ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form
31 May 2013
Accepted 6 June 2013
Available online 14 June 2013

Keywords:
SIA
Automation
Plaguicide
MRL
On-line separation

ABSTRACT

A flow-through optosensor is here proposed for the determination of mixtures of two widely used pesticides, carbendazim and o-phenylphenol, in fruits. The pesticides are separated on-line using an additional amount of solid support, C_{18} silica gel, in the flow-through cell. The resolution is performed due to the different retention/desorption kinetics of the analytes when interacting with the C_{18} microbeads. Therefore, both separation and determination are integrated in the same cell, considerably simplifying the system. In addition, the use of Sequential Injection Analysis provides a high degree of automation and minimum wastes generation. After the analytes are separated, their native fluorescence is measured, obtaining linearity in the 2.0–30 and 1.1–20 mg kg^{-1} ranges for carbendazim and o-phenylphenol. The detection limits are 0.60 and 0.33 mg kg^{-1} for carbendazim and o-phenylphenol respectively. The proposed method fulfills the maximum residue limits (MRLs) established in Europe and USA for these pesticides in cherries, pineapple, and mango: 5–10 mg kg^{-1}. In order to demonstrate the suitability of the method, several samples have been analyzed and the obtained results compared with a chromatographic method.

1. Introduction

In the last years, the detection of environmental hazardous chemicals has been rising in demand. Pesticides (herbicides, fungicides or insecticides) are substances intended for preventing, destroying, repelling or mitigating any pest. Their worldwide use can lead to their accumulation in soil, water, atmosphere, and agricultural products, and they can therefore exist in harmful levels, posing an environmental threat. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species [1]. As a result, the development of new analytical methods for the determination of these compounds in a wide variety of samples is today a high-interest research area.

Carbendazim (CBZ) and o-phenylphenol (OPP) are two widely used fungicides, mainly used for the post-harvest treatment of fruits and vegetables. Hence many analytical methodologies have been described for their reliable determination in a wide range of matrices. Modern analytical methods for their quantitation usually involve liquid chromatography [2–8] or gas chromatography [9–11] coupled to mass spectrometry detection These methodologies present unique outstanding properties for multi-residue analysis in complex matrices, due to their high sensitivity and selectivity. In recent years, optical [12,13] and electrochemical [14,15] methods have also been utilized for the analysis of CBZ. Compared to chromatography, these methods focus in only a small number of analytes and specific applications. Among them, the design of flow-based analytical methods is a field of special interest due to their intrinsic favorable characteristics, especially their low-cost and low wastes generation [16,17]. In order to improve other features of flow methods, such as sensitivity and selectivity, a possible modification is the implementation of Solid-Phase Spectroscopy (SPS) in flow analysis, which is called flow-through optosensing. In these systems, the target compound is retained on an appropriate solid support, placed in the flow-through cell, and its detection is performed directly on these microbeads, therefore improving the selectivity and sensitivity [16,18,19].

A high percentage of the flow optosensors described in scientific literature have focused in the analysis of pharmaceuticals and water samples [20–22], although that tendency has changed in the last few years, observing applications in the alimentary field for both contaminants and natural compounds [23–25]. Although multi-parametric sensors have been previously described [26–29], they have been mainly applied to the determination of the target compounds in clinical or water samples, and very rarely in food products [24].

In this manuscript we present a bi-parameter flow-through optosensor for the determination of two widely used pesticides,

---

* Corresponding author. Tel.: +34 953 212757; fax: +34 953 212940.
E-mail address: portega@ujaen.es (P. Ortega-Barrales).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.06.007

carbendazim (CBZ) and o-phenylphenol (OPP), in fruits. The most common strategy for the on-line separation of the analytes in the flow-system consists in using a mini-column filled with the same solid support used in the flow-through cell and placed just before the cell [24,26,28,30]. In addition, a less used approach consists in using an extra amount of solid support in the flow-cell [27,31]. In both cases, the separation of the analytes is achieved due to their differences in the sorption/elution process on the solid support microbeads. However, performing the separation directly in the flow-cell clearly simplifies the flow manifold and has been the selected strategy in this work. This is the first time that a bi-parameter flow-through optosensor, separating the analytes without using a pre-column, is described with applications in the alimentary field.

Sequential Injection Analysis (SIA) has been chosen as the flow methodology, increasing the automation and robustness of the developed method. Using QuEChERS [32] as the extraction procedure, CBZ and OPP can be fluorimetrically detected after their on-line separation. The proposed method fulfills the maximum residue limits (MRLs) established in Europe and USA for these pesticides in cherries, pineapple and mango: 5–10 mg kg^{-1} [33,34]. In addition no matrix-effect has been observed, therefore avoiding the use of matrix-matched standards for the proposed applications.

2. Experimental

2.1. Reagents and solutions

CBZ and OPP (Sigma, Alcobendas, Madrid, Spain) stock solutions of 100 μg mL^{-1} were prepared by weighing the appropriate amount of analyte and dissolving in methanol (MeOH). The solutions remained stable for at least 1 month when kept away from light at about 4 °C. These stock solutions were used to prepare the working standard solutions by suitable dilution with deionized water.

MeOH, sodium chloride and anhydrous magnesium sulfate were obtained from Panreac (Barcelona, Spain); all of them were reagent grade. Ammonia and acetonitrile (HPLC grade) were obtained from Sigma. Primary-secondary amine (PSA) (Supelclean PSA SPE bulk packing) was obtained from Supelco (Bellefonte, PA, USA). C_{18} bonded phase silica gel beads (Waters, Milford, USA) with 55–105 μm of average particle size, were used as solid support. All the pesticides used in the interference study were obtained from Sigma.

2.2. Apparatus and instruments

A FIALab-3500 automatic analyzer (FIALab Instruments, Inc., the USA) was used to construct the manifold (Fig. 1). The analyzer includes two 5-mL syringe pumps, a holding coil, a peristaltic pump and an 8-port multiposition valve, being all the components computer-controlled by FIALab 5.0 software. The analyzer was connected to a Cary-Eclipse Luminescence Spectrometer (Varian Inc., Mulgrave, Australia) for luminescence measurements. A Hellma flow cell 176.052-QS (25 μL inner volume and 1.5 mm light path length) was used. The cell was filled with C_{18} solid support microbeads, as slurry suspension in methanol, with the aid of the peristaltic pump. The outlet of the cell was blocked with glass wool, avoiding the movement of the solid support but allowing the flow of solutions. An additional amount of solid support was used in the cell as a strategy to separate the analytes, just above the detection area. The manifold is shown in Fig. 1, representing only the components of the FIALab that have been used in the method, for simplicity's sake.

A T18-basic Ultra-Turrax from IKA (Spain) was used for the homogenization of the analyzed samples.

An HPLC method was selected as reference method. CBZ and OPP separation was carried out using an HPLC system (Agilent Series 1100, Agilent Technologies, Santa Clara, CA, USA), consisting of vacuum degasser, autosampler, and a binary pump. A reversed phase C_{18} analytical column of 50×2.1 mm^2 and 2.6 μm particle size (Kinetex, Phenomenex) was used for the separation, at ambient temperature. Mobile phases A and B were water with 0.1% formic acid (v/v) and acetonitrile, respectively. A linear gradient from 10% B to 70% B in 15 min was used, with a flow-rate of 0.35 mL min^{-1}. The HPLC system was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray interface. CBZ was determined in positive-ion mode, $[M+H]^+$ at m/z 192 with a fragment at m/z 160. OPP was determined in negative-ion mode, $[M–H]^-$ at m/z 169.

2.3. Sample treatment

The QuEChERS method [32] was selected for the extraction of CBZ and OPP from the selected samples (cherries, pineapple and mango), being all of them obtained from local markets.

An Ultra-Turrax was used for sample homogenization. Pineapples were peeled before being ground, stones were removed from mangos before grinding them, and cherries were directly ground.

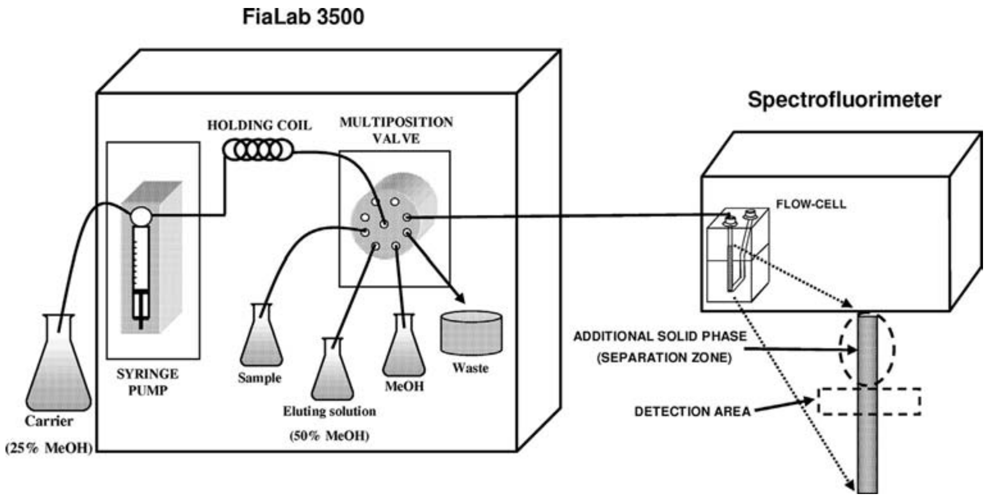

Fig. 1. SIA manifold.

For the extraction procedure, 15 g of previously homogenized sample were weighed in a 50 mL PTFE centrifuge tube and 15 mL acetonitrile were added. The screw cap was closed and the tube was vigorously shaken for 1 min by hand ensuring that the solvent interacted well with the entire sample. Then, 2.5 g of sodium chloride and 6 g of anhydrous $MgSO_4$ were added, repeating the shaking process for 1 min to prevent coagulation of $MgSO_4$. Then, the tube was centrifuged at 3700 rpm for 3 min. 5 mL of the supernatant (acetonitrile phase) were taken with a pipette and transferred to a 15 mL centrifuge tube. After adding 750 mg anhydrous $MgSO_4$ and 250 mg PSA sorbent, the tube was vigorously shaken for 30 s by hand and centrifuged again for 3 min. The obtained supernatant was further diluted 80-fold with deionized water prior to analysis by the proposed method.

2.4. General procedure

The flow rate of the syringe pump was set at 100 μL s^{-1} for aspiration and 22 μL s^{-1} for propulsion. The lower flow-rate for the propulsion of solutions was caused by the potential overpressures due to the solid support placed in the flow-cell; high flow-rates could produce leaking of solutions between connections.

Before starting the recording of the analytical signal, the solid support was conditioned with 4 mL of carrier solution (25% MeOH:H_2O, v-v). All experiments were carried out by triplicate and the analytical signal was expressed as mean values of peak height.

Once the solid support was conditioned, the procedure used for the analysis of each sample was as follows:

(a) 500 μL of carrier solution and 200 μL of sample solution were aspirated and pumped toward the waste in order to avoid contamination with the previous sample.

(b) 2000 μL of carrier solution and 800 μL of sample solution were aspirated again and pumped toward the flow-cell. The on-line separation of the analytes took place on the solid support placed inside the flow-cell (Fig. 1). OPP was strongly retained on the top part of the solid support (non-irradiated area), whereas CBZ passed through the flow-cell (due to its faster retention/desorption kinetic process), developing its analytical signal ($\lambda_{exc}/\lambda_{em}$=276/306 nm/nm) in the detection area (irradiated zone). CBZ was completely eluted from the solid phase by the carrier itself, whereas OPP remained retained in the upper part of the cell.

(c) After CBZ had developed its analytical signal, 1500 μL of eluting solution (50% MeOH:H_2O, v-v) were introduced in the system in order to elute OPP from the upper part of the solid support, recording its analytical signal ($\lambda_{exc}/\lambda_{em}$=250/345 nm/nm) in the detection area. 500 μL of carrier solution was also inserted in the system in order to prepare the solid support for the next sample solution.

3. Results and discussion

3.1. Spectral characteristics and instrumental parameters

The most common solid supports used in flow-through optosensing are the ion-exchangers Sephadex, the non-ionic C_{18} silica gel and the polymeric supports Amberlite XAD. Previous studied carried out in our research group showed favorable retention of CBZ and OPP in C_{18} silica gel, so it was the selected one to carry out the separation and detection of the pesticides.

The spectral characteristics of CBZ and OPP were recorded with each individual analyte retained on the C_{18} solid support placed in the flow-cell. Fluorescence spectra showed maxima excitation/emission wavelengths at 276/306 and 250/345 nm/nm for CBZ and OPP, respectively, therefore selecting these wavelenghts for further experiments.

Fluorescence measurements recorded in solid phase are affected by background signals higher than the ones found in homogeneous solutions. This effect is obviously due to the presence of the solid microbeads in the flow-cell. Therefore, instrumental parameters must be carefully studied in order to achieve the best possible signal-to-background ratio. For this reason, the voltage of the photomultiplier tube (400–1000 V) and the instrument excitation and emission slit widths (5–20 nm) had to be optimized. Taking into account that the MRLs established for CBZ and OPP are similar, but that CBZ presents lower sensitivity, the instrumental parameters were adjusted in order to improve the analytical response from CBZ. As a result, the photomultiplier tube voltage was set at 620 V and the excitation and emission slit widths were fixed at 5 and 10 nm, respectively.

3.2. Separation of CBZ and OPP and chemical variables

The resolution of CBZ and OPP was performed by a temporary sequentiation in the arrival of the analytes to the detection area. This was achieved due to the different sorption/elution kinetic process of the analytes on the solid support placed in the flow-cell (separation zone in Fig. 1). Therefore, the amount of solid support packed in the flow-cell had to be carefully examined in order to achieve the complete separation of the analytes. The flow-cell was filled with different amounts of C_{18} beads (40–70 mg), as slurry suspension in methanol, using the peristaltic pump. In all cases, the entire light-path of the cell was filled, covering the detection zone, and the required amount of solid support in the separation zone was studied. This study showed that low amounts of C_{18} beads could not prevent OPP from performing its analytical signal before CBZ was completed eluted (Fig. 2a and b). However, when the separation zone was filled with solid support microbeads (Fig. 2c), the separation was complete. Finally, 65 mg of C_{18} beads (26 mm height above the irradiated area) were required to resolve the mixture CBZ/OPP. The filling process became highly reproducible when the exact amount of solid support was weighed to fill the flow-cell (RSDs lower than 10%). More than 80–90 samples could be analyzed without replacing the solid support.

Then, the nature of carrier and eluting solutions was also optimized for both CBZ and OPP. Taking into account the non-polar nature of C_{18} silica gel beads, solutions containing MeOH and water in different proportions are usually used as carrier and eluting solutions. In the first place, we tested different solutions as carrier in the 10–40% range (MeOH:H_2O, v-v). The selected percentage of MeOH had to be high enough to elute CBZ from the solid support, but not too high, so OPP could remain retained on the separation zone. A 25% MeOH:H_2O solution was selected as the optimum carrier solution. Once the carrier had been fixed, the eluting solution for OPP was studied in the 30–70% range (MeOH:H_2O, v-v). In this case, it was observed that OPP could not be eluted with percentages of MeOH lower than 50%, and that the analytical signal decreased for higher percentages due to a lower retention of OPP on the solid support. Therefore, 50% MeOH was selected for further experiments.

Finally, although the solid support could be completely regenerated by the eluting solution when CBZ and OPP standards were analyzed, the baseline slightly increased after each insertion of real samples due to the complex matrix; this increase is due to the compactation of the solid support beads. In Fig. 3, it can be observed this increase in the baseline after each sample determination. Although the analytical signal remained constant (both baseline and peak height increased at the same time), 600 μL of MeOH was inserted after several sample insertions in order to

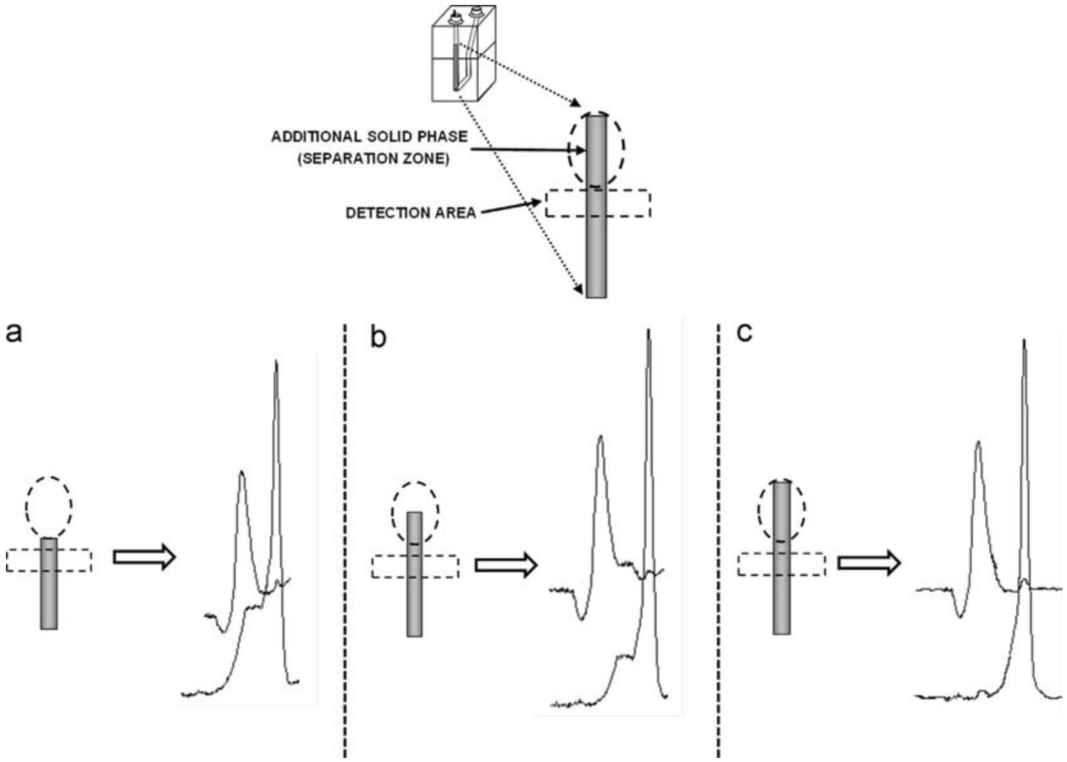

Fig. 2. Optimization of the amount of solid support in the flow-cell: (a) no separation area; (b) ½ separation area; and (c) full separation area.

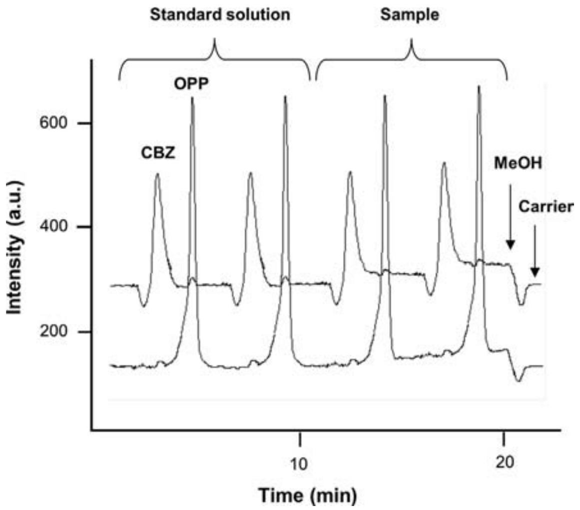

Fig. 3. SIA profile from a standard solutions and from a pineapple sample, both with 8 and 10 mg kg^{-1} of CBZ and OPP, respectively. It can be observed an increase in the baseline after the insertion of sample solution due to the solid support compactation, and its complete recovery after MeOH insertion.

recover the baseline and eliminate the high compactation of the solid microbeads. This effect can be observed in Fig. 3. In this same figure, the absence of baseline increase when standard solutions are injected can also be appreciated.

3.3. *SIA variables*

First of all, the sample volume was optimized. In flow-through optosensors, if the volume of sample inserted in the system increases, the amount of analyte retained on the beads also increases. Therefore, the analytical response and the sensitivity are also higher. However, the insertion of high volumes of sample also produces a decrease in sample throughput. As a result, a compromise between sensitivity and sampling frequency is usually adopted. In the proposed method, the sample volume was studied from 100 up to 1500 μL, using standard solutions corresponding to 10 and 8 mg kg^{-1} of CBZ and OPP, respectively. It was observed that the analytical signal increased linearly up to 1000 μL of sample solution for CBZ and OPP. The regression equations for the effect of the sample volume on the analytical signal were $I=1.9+293.5\,V$ ($r=0.997$; V in mL) and $I=-5.4+416.6\,V$ ($r=0.998$; V in mL) for CBZ and OPP, respectively. However, a sample volume of only 800 μL was selected, because the sensitivity was good enough for the analysis of real samples, obtaining a high sample throughput. 2000 and 1500 μL of carrier and eluting solutions were required for the elution of CBZ and OPP, respectively. The corresponding graph for this study is shown in Fig. 4.

After the volumes of solutions were optimized, the effect of the flow-rate was also studied. For the aspiration of solutions, a flow-rate of 100 μL s^{-1} was selected in all cases, in order to shorten the time of analysis. For the process of delivering solutions toward the flow-though cell, the flow-rate was investigated from 15 to 28 μL s^{-1}. By increasing it, the sampling frequency increased but over-pressure could appear in the system (leading to leaking of solutions in tubing connections). Finally 22 μL s^{-1}, the highest possible flow-rate without over-pressure problems, was selected.

3.4. *Analytical parameters*

The analytical parameters of the system were studied using the optimized conditions previously discussed. Table 1 contains the figures of merit of the proposed method using a sample volume of 800 μL. The calibration graph was constructed using 7 standard solutions, and the data were fitted by standard least-squares treatment. The proposed methodology was able to produce analytical fits with good linearity in the range 2.0–30 and 1.1–20 mg kg^{-1} for CBZ and OPP, respectively. The detection limits were 0.60 and 0.33 mg kg^{-1} for CBZ and OPP, respectively.

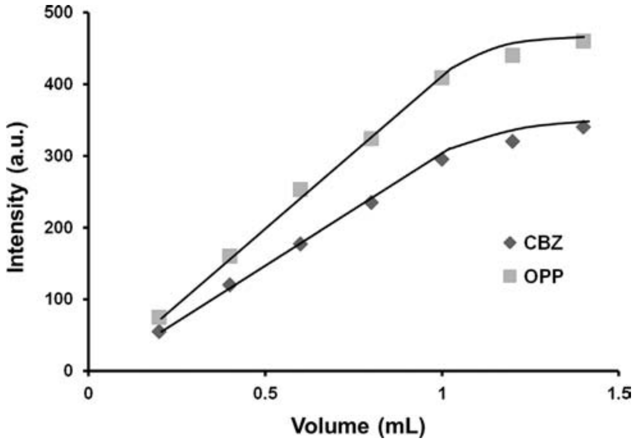

Fig. 4. Effect of the sample volume on the analytical signal; 10 and 8 mg kg^{-1} of CBZ and OPP, respectively.

Table 1
Analytical parameters.

Parameter	CBZ	OPP
Linear dynamic range (mg kg^{-1})	2–30	1,1–20
Calibration graph		
Intercept	5.7125	2.5286
Slope (kg mg^{-1})	24.0435	41.3788
Correlation coefficient	0.9992	0.9994
Detection limit (mg kg^{-1})	0.6	0.33
Quantification limit (mg kg^{-1})	2	1,1
Sample throughput (h^{-1})	12	

Inter- and intra-day repeatabilities were established for 10 independent analyses of CBZ/OPP solutions at three different levels: 5 and 4; 10 and 8; and 22 and 16 mg kg^{-1} for CBZ and OPP, respectively On one hand, the intra-day repeatabilities were in the 3.0–4.2% and 2.3–3.3% ranges for CBZ and OPP, respectively. On the other hand, the inter-day values (%) were in the 6.1–6.8 and 5.7–6.2 ranges for CBZ and OPP, respectively. A sample throughput of 12 samples per hour was obtained.

3.5. *Ruggedness and robustness*

The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results undertaken by two analysts. The RSD values (%) for intra- and inter-day assays did not exceed 5% and 8%, respectively, indicating the ruggedness of the method.

The robustness of the method, which can be described as its capacity to remain unchanged when there are small variations in experimental conditions, was also studied. In this case, small changes were carried out in the carrier solution (22–28% MeOH: H_2O, v-v), eluting solution (47–53%), flow-rate (20–24 μL s^{-1}) and fluorescence wavelengths (± 2 nm in both excitation and emission). Fluctuations lower than $\pm 5\%$ were observed when compared to the optimum parameters, so demonstrating the robustness of the proposed method.

3.6. *Selectivity study*

In order to check the usefulness of the proposed method for the analysis of real samples, the potential interference from other pesticides was studied in this section. For this study, extracts from real samples (checked by the HPLC–MS method in order to confirm the absence of the analytes) were used, spiking the extracts after the extraction procedure. Then, different pesticides (fluorescent and non-fluorescent) that are widely used were added to extract solutions containing CBZ and OPP, and their influence on the analytical signal was investigated. Tolerance level was defined as the amount of foreign species that produced an error not exceeding $\pm 2\sigma$ in the determination of the analyte, being σ the standard deviation. The obtained results are summarized in Table 2. It can be observed that both analytes could be analyzed, without significant errors, in the presence of high levels of potentially interfering compounds that could be present in real samples.

The potential interference from CBZ on OPP and viceversa when an excess of one of them is present was also evaluated. Under the optimized conditions, CBZ tolerated a 5-fold level of OPP, whereas OPP tolerated a 9-fold concentration of CBZ.

3.7. *Applications*

The proposed system was applied to the determination of CBZ and OPP in cherries, pineapples and mangos. All samples (6 cherries, 5 pineapples and 5 mangos) were obtained from local markets.

In all cases, CBZ and OPP levels were below the detection limit of the proposed method. In addition to the proposed method, we also used a reference HPLC–MS method for the analysis of the samples. No presence of CBZ or OPP was detected with the HPLC method either. As a result, we decided to perform recovery studies to evaluate the accuracy of the method. Two samples from each fruit were selected for the recovery study. These experiments were accomplished by spiking the samples at different concentration levels (3–22 mg kg^{-1}), which are close to the legislated values [33,34]. In all cases, the sample (previously homogenized as described in Section 2.3.) was spiked with the required volumes of the 100 μg mL^{-1} CBZ and OPP stock solutions, the mixture was gently blended to assess the homogeneity of the sample and it was allowed to stand at room temperature for 12 h in order to evaporate the solvent (MeOH). After that, it was kept in the fridge until the extraction method and analysis were performed.

The experimental results obtained by the proposed method were compared with those ones obtained by the HPLC reference method by means of a *t*-test and an *F*-criterion at a 5% significance level. In all cases, the average results obtained by both methods were statistically identical, as the difference between the averages had no significance at the probability level indicated. All results are shown in Table 3.

Thanks to the high dilution (80-fold) and the use of the solid support, no matrix effect was found and matrix-matched calibrations were not required. The absence of matrix effect can be observed in Fig. 3, in which similar signals are obtained for sample and standard solutions with the same levels of CBZ and OPP.

Table 2
Interference study.

Foreign species	Tolerated interferent/analyte (w/w) ratio	
	CBZ[a]	OPP[b]
Imazalil, simazine, morestan[c], chlorsulfuron	> 1000	> 100
Bendiocarb[c], aminocarb[c]	> 1000	80
Propoxur, quinmerac[c], imazaquin	200	> 100
Carbaryl[c]	> 100	9
Carbofuran [c]	100	50
Carbendazim[c]	—	9
O-phenylphenol[c]	5	—

[a] For a 8 mg kg^{-1} CBZ concentration.
[b] For a 5 mg kg^{-1} OPP concentration.
[c] Fluorophore.

Table 3
Applications.

	CBZ					OPP				
	Added (mg kg^{-1})	**Found (mg kg^{-1})[a] Proposed method**	**Found (mg kg^{-1})[a] Reference method**	t_{calc}[b]	F_{calc}[c]	**Added (mg kg^{-1})**	**Found (mg kg^{-1})[a] Proposed method**	**Found (mg kg^{-1})[a] Reference method**	t_{calc}[b]	F_{calc}[c]
Cherry-1	4	4.2 ± 0.2	4.0 ± 0.2	1.4	1.0	5	5.0 ± 0.1	4.8 ± 0.2	2.0	0.3
	15	14 ± 0.6	14.7 ± 0.4	1.7	1.8	8	8.2 ± 0.4	8.3 ± 0.3	0.2	1.4
Cherry-2	5	4.6 ± 0.2	4.8 ± 0.1	1.2	1.0	4	3.8 ± 0.2	4.1 ± 0.2	2.3	1.0
	10	10.2 ± 0.4	10.4 ± 0.4	0.6	0.9	10	9.3 ± 0.4	9.5 ± 0.5	0.6	0.7
Mango-1	5	5.0 ± 0.2	5.1 ± 0.2	0.6	1.0	5 12	5.4 ± 0.2	5.1 ± 0.2	1.6	1.5
	15	14.4 ± 0.6	14.8 ± 0.4	0.9	1.8		11.0 ± 0.4	11.3 ± 0.4	0.8	0.6
Mango-2	4	4.1 ± 0.1	4.3 ± 0.2	1.0	0.3	3	2.6 ± 0.1	2.7 ± 0.1	0.8	2.2
	22	23.3 ± 0.5	22.8 ± 0.9	0.9	0.3	10	11.5 ± 0.2	11.0 ± 0.6	1.3	0.5
Pineapple-1	3	2.8 ± 0.2	2.7 ± 0.1	0.8	1.2	4	4.2 ± 0.2	4.2 ± 0.1	0.0	2.5
	12	11.2 ± 0.5	11.9 ± 0.5	1.9	1.0	10	9.5 ± 0.5	9.1 ± 0.4	1.1	1.9
Pineapple-2	15	14.9 ± 0.6	15.3 ± 0.8	0.8	0.6	7	6.9 ± 0.4	7.2 ± 0.3	1.0	1.5
	20	19.9 ± 0.9	20.2 ± 0.7	0.5	1.6	16	17.3 ± 0.6	11.1 ± 0.7	0.3	1.4

[a] Means of three determinations ± SD.
[b] Theoretical value $t=2.776$, $p=0.05$.
[c] Theoretical value $F=19$; $p=0.05$.

The obtained results showed the suitability of the proposed method for the determination of CBZ and OPP in fruits using the QuEChERS extraction procedure.

4. Conclusions

In this work, a flow-through optosensor was described for the determination of a binary mixture of pesticides in fruits. The on-line separation of CBZ and OPP was performed using an additional amount of solid support in the flow-through cell, which is a strategy scarcely described in scientific literature. The use of the solid support provided additional selectivity and sensitivity when compared to homogenous solution. As a result, no matrix effect was found, allowing the use of solvent calibration instead of matrix-matched standards. The obtained detection limits also allowed fulfilling the MRLs established by USA and Europe in cherries, pineapples and mangos. As a result, flow optosensing revealed itself as useful for the analysis of complex samples for specific applications, such as the analysis of a small number of target compounds in food.

Acknowledgments

The authors are grateful to the University of Jaén, Project UJA 2011/12/31, for financial support, and to Centro de Instrumentación Científico-Técnica (CICIT) for the availability of the HPLC system.

References

[1] G.T. Miller, Sustaining the Earth, Thompson Learning, Inc. Pacific Grove, California, 2004, pp. 211-216.
[2] R. Romero-González, A.G. Frenich, J.L.M. Vidal, Talanta 76 (2008) 211–225.
[3] C. Ferrer, M.J. Martínez-Bueno, A. Lozano, A.R. Fernández-Alba, Talanta 83 (2011) 1552–1561.
[4] K. Banerjee, D.P. Oulkar, S.B. Patil, M.R. Jadhav, S. Dasgupta, S.H. Patil, S. Bal, P. G. Adsule, J. Agric. Food Chem. 57 (2009) 4068–4078.
[5] A. Lazartigues, C. Fratta, R. Baudot, L. Wiest, C. Feidt, M. Thomas, C. Cren-Olivé, Talanta 85 (2011) 1500–1507.
[6] G.C. Bedendo, I.C.S.F. Jardim, E. Carasek, Talanta 88 (2012) 573–580.
[7] N. Yoshioka, Y. Akiyama, T. Matsuoka, T. Mitsuhashi, Food Control 21 (2010) 212–216.
[8] X. Ye, L.J. Tao, L.L. Needham, A.M. Calafat, Talanta 76 (2008) 865–871.
[9] J. Yang, D. Li, C. Sun, Anal. Methods 4 (2012) 3436–3442.
[10] K. Takahashi, M. Horie, Y. Hirose, J. Food Hyg. Soc. Jpn. 49 (2008) 403–406.
[11] P.Q. Tranchida, F.A. Franchina, M. Zoccali, S. Pantò, D. Sciarrone, P. Dugo, L. Mondello, J. Chromatogr. A 1278 (2013) 153–159.
[12] M. del Pozo, L. Hernández, C. Quintana, Talanta 81 (2010) 1542–1546.
[13] A. Waseem, M. Yaqoob, A. Nabi, Anal. Lett. 43 (2010) 603–617.
[14] A.M. Ashrafi, J. Dordević, V. Guzsvány, I. Švancara, T. Trtić-Petrović, M. Purenović, K. Vytřas, Int. J. Electrochem. Sci. 7 (2012) 9717–9731.
[15] P.L.A. Sundari, S.P. Palaniappan, P. Manisankar, Anal. Lett. 43 (2010) 1457–1470.
[16] E.J. Llorent-Martínez, P. Ortega-Barrales, M.L. Fernández-de Córdova, A. Ruiz-Medina, Anal. Chim. Acta 684 (2011) 21–30.
[17] V.L.R.G. Abreu, J.L.M. Santos, J.L.F.C. Lima, Food Anal. Methods 5 (2012) 867–873.
[18] S.M. Oliveira, H.M. Oliveira, M.A. Segundo, A.O.S.S. Rangel, J.L.F.C. Lima, V. Cerdá, Anal. Methods 4 (2012) 1229–1236.
[19] S. Matsuoka, K. Yoshimura, Anal. Chim. Acta 664 (2010) 1–18.
[20] L.M. Shen, M.L. Chen, X.W. Chen, Talanta 85 (2011) 1285–1290.
[21] A. Valero-Navarro, A. Salinas-Castillo, J.F. Fernández-Sánchez, A. Segura-Carretero, R. Mallavia, A. Fernández-Gutiérrez, Biosens. Bioectron. 24 (2009) 2305–2311.
[22] G.N. Piccirilli, G.M. Escandar, F.C. Cañada, I.D. Merás, A. Muñoz de la Peña, Talanta 77 (2008) 852–857.
[23] E.J. Llorent-Martínez, M.L. Fernández-de Córdova, A. Ruiz-Medina, P. Ortega-Barrales, Talanta 96 (2012) 190–194.
[24] L. Molina-García, A. Ruiz-Medina, M.L. Fernández-de Córdova, Anal. Chim. Acta 689 (2011) 226–233.
[25] L. Molina-García, M.L. Fernández-de Córdova, A. Ruiz-Medina, Talanta 96 (2012) 195–201.
[26] E.J. Llorent-Martínez, P. Ortega-Barrales, A. Molina-Díaz, Anal. Bioanal. Chem. 383 (2005) 797–803.
[27] E.J. Llorent-Martínez, P. Ortega-Barrales, A. Molina-Díaz, Spectrosc. Lett. 39 (2006) 619–629.
[28] P. Ortega Barrales, A. Domínguez Vidal, M.L. Fernándezde Córdova, A. Molina Díaz, J. Pharm. Biomed. Anal. 25 (2001) 619–630.
[29] G.N. Piccirilli, G.M. Escandar, Analyst 135 (2010) 1299–1308.
[30] J.F. García Reyes, E.J. Llorent-Martínez, P. Ortega Barrales, A. Molina Díaz, Talanta 64 (2004) 742–749.
[31] E.J. Llorent-Martínez, J.F. García Reyes, P. Ortega-Barrales, A. Molina-Díaz, Anal. Chim. Acta 555 (2006) 128–133.
[32] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412–431.
[33] ⟨www.mrldatabase.com/⟩, January 2013.
[34] FAO, WHO, ⟨www.codexalimentarius.org/standards/pesticide-mrls/en⟩, January 2013.