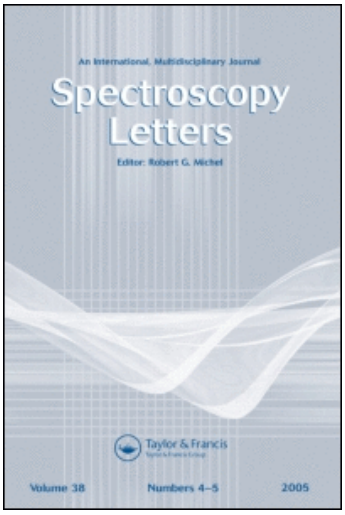

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Simultaneous Synchronous Fluorescence Determination of Carbaryl, Propoxur, and Carbofuran with Multivariate Calibration Methods

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Abstract: A synchronous fluorescence method for the determination of carbaryl (CAB), propoxur (PRO), and carbofuran (CAF) with the aid of multivariate calibration methods, such as classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS), was proposed. Because the synchronous fluorescence spectra (SFS) of these three compounds are seriously overlapped, it is difficult to determine these compounds individually from their mixtures. In this work, the experimental conditions were investigated and optimized to obtain the high sensitivities and well-defined waves for each analyte. Furthermore, chemometrics models were established from a set of known samples and were then applied to the prediction of these three pesticides in a set of validation samples with a relative prediction error (RPE_T) of around 4% and limits of detection of 0.50, 5.5, and 23 ng L^{-1} for carbaryl, propoxur, and carbofuran, respectively. The proposed method was also successfully applied to determine the contents of these three compounds in spiked fruit and vegetable samples.

Keywords: Carbaryl, carbofuran, multivariate calibration methods, propoxur, synchronous fluorescence

INTRODUCTION

Carbamate pesticides are widely used in agriculture due to their activity against numerous insect pests of fruits, vegetables, and crops. Carbaryl

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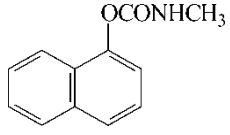
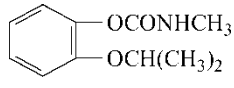
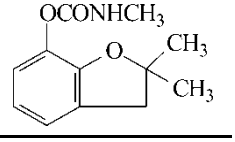
(CAB), propoxur (PRO), and carbofuran (CAF) are powerful carbamate pesticides; their structures (Table 1) are based on N-substituted carbamic acid esters ($R_1OCONHR_2R_3$). Carbaryl is used for cotton, fruit, forests, nuts, and other crops, and is inherently toxic to humans by skin contact, inhalation, and/or ingestion.[1] Propoxur is used on farms as a killing agent for jassides, bugs, and aphids and also in houses against flies and mosquitoes.[2] Carbofuran is commonly used for potato and rotation crop farms to control Colorado potato beetle, flea beetles, and leafhoppers.[3]

Many techniques have been used for analysis of these residues, such as thin-layer chromatography (TLC),[4] gas chromatography (GC),[5] high-performance liquid chromatography (HPLC),[6] and gas chromatography–mass spectrometry (GC-MS).[7,8] Because these carbamate pesticides and their derivatives are thermally labile and can be easily decomposed, it is difficult to analyze them directly by GC at relatively high temperature. HPLC is generally preferred for the determination of these carbamates. However, some preprocessing steps, derivatization of compounds, and post column fluorimetric labeling are generally needed for HPLC and sometimes for GC methods. Recently, GC- and LC-MS methods, the most powerful for pesticide residue analysis, are used, but the instrumentation is quite costly.

Spectrophotometric methods also have been employed for determining carbamate pesticides. Usually, measurements are carried out in the visible region.[9,10] Sastry and Vijaya[11] reported a spectrophotometric method to determine some insecticides (carbaryl, propoxur, fenitrothion and methyl parathion) with the use of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of an oxidant (Ce^{4+} or Fe^{3+}). Jan et al.[12] developed a spectrophotometric method for the determination of carbofuran pesticide based on the hydrolysis of the pesticide. The hydrolyzed product, methylamine, on reaction with sodium nitroprusside solution in acetone

Table 1. Chemical structures of the three carbamate pesticides

Pesticides	Molecular formula	Structure
Carbaryl (CAB)	$C_{12}H_{11}NO_2$	
Propoxur (PRO)	$C_{11}H_{15}NO_3$	
Carbofuran (CAF)	$C_{12}H_{15}NO_3$	

medium gives a purple-colored solution, and the absorbance was measured at 530 nm. Conditions for the complete hydrolysis of pesticide and quantitative determination of methylamine were optimized, and the limit of detection was found to be 0.804 ppm. Manjubhashini et al.[13] described a simple spectrophotometric technique for the analysis of carbaryl in its formulations, water, and food grains. New coupling/complexing agents namely 2-amino, 4-nitrophenol, and 2,4-dimethoxy aniline were employed. The colored complexes formed with these coupling agents are instantaneous and stable for 24 and 10 h, respectively. The λ_{max} observed for these complexes were respectively at 520 and 510 nm.

The spectrophotometric-kinetic approach has also been applied for the multicomponent analysis of the carbamate pesticides.[14,15] It was based on the different rate constants of the reactions between *p*-aminophenol, in the presence of potassium metaperiodate, and the phenolic and naphtholic compounds obtained from the alkaline hydrolysis of the pesticides. Garcia et al.[16] developed a method based on the application of partial least-squares analysis to bilinear data for the simultaneous determination of propoxur, carbaryl, ethiofencarb, and formetanate using a stopped-flow injection procedure. Mixtures containing 2–10 $\mu g\ mL^{-1}$ of propoxur, 2–8 $\mu g\ mL^{-1}$ of carbaryl, 2–10 $\mu g\ mL^{-1}$ of ethiofencarb, and 2–10 $\mu g\ mL^{-1}$ of formetanate were successfully resolved with errors of less than 5%. Ni et al.[17] applied 10 chemometrics methods, including artificial neural networks and multivariate calibration, to the simultaneous determination of carbamate pesticides (carbofuran, isoprocarb, and propoxur) in fruit and vegetable samples. Fluorescence spectroscopy is a versatile analytical technique, which provides high sensitivity of detection. Selectivity is usually improved through spectrofluorimetric strategies such as synchronous fluorescence[18,19] or variable angle scanning fluorescence.[20] Synchronous fluorescence spectroscopy (SFS) was described by Lloyd in 1971 and was further developed with the aid of the Vo-Dinh theory.[21] In SFS, the excitation and emission monochromators are scanned simultaneously in such a manner that a constant wavelength interval is kept between emission and excitation wavelengths ($\Delta\lambda$). Using suitable offset values ($\Delta\lambda$), SFS reduces spectral overlaps by narrowing spectral bands and simplifies spectra by preferentially amplifying strong fluorescence bands.[22,23]

In this paper, SFS was used to determine three pesticides, carbaryl, propoxur, and carbofuran, in mixtures. Carbaryl, propoxur, and carbofuran have strong inner fluorescence, and their signal is overlapped seriously because their structures are similar (see Table 1). It is generally difficult to determine them individually if conventional fluorescence spectroscopy is used. Hence, multivariate calibration methods were used to resolve their overlapping fluorescence spectra and quantitatively determine them. The experimental conditions were investigated and several multivariate calibration methods, such as classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS), were used to build the

calibration models and applied to the prediction of the three pesticides in validation and vegetable samples.

THEORETICAL BACKGROUND

Multivariate calibration, employing relationship between concentration of samples and many variables (e.g., fluorescence intensity at different wavelengths), may be an efficient method to construct predictive model capable of resolving multicomponent mixtures because it allows the rapid and simultaneous determination of each component in the mixture, with minimum sample preparation, reasonable accuracy and precision, and without the need of time-consuming separations. The full-spectrum multivariate calibration methods, such as CLS, PLS, and PCR, offer advantages of speed of determination for the components of interest in a mixture, without the time-consuming preseparation steps.

All these methods comprise two separate stages. In the first step, termed calibration, an empirical model is built, representing the relationship between the response spectra data generated from a set of known samples and the respective concentrations of their component(s) of interest. This is followed by a second step called prediction, in which the calibration model is used to determine the concentration of the components in the unknowns from their spectral data.

CLS is a very common multivariate calibration approach, being a multivariate least-squares procedure based directly on Beer's law, which model accounts for errors in the spectral measurements. This method has generally presumed that there is a linear relationship between the response signals and the component concentrations. The mathematical formulations of CLS, in the matrix compact form can be written as $F = C \times K$.[24] Here, the F, C, and K represent the fluorescence intensity, concentration, and calibration coefficient matrices, respectively. The major disadvantage for CLS is that all interfering chemical components in the spectral region of interest need to be known and be included in the calibration models.

PCR and PLS are factor analysis multivariate statistical tools that have many of the full-spectrum advantages of the CLS method. These methods are efficient to construct the predictive models when there are many variables with high collinearity to be treated, and the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and applicability of quantitative spectral analysis of mixtures. As a matter of fact, PCR and PLS have been successfully applied for analysis of multicomponent mixtures.[25]

The PLS calibration technique using the orthogonallized PLS algorithm developed by Wold[26] and extensively discussed by Martens and Naes[27] involves simultaneously the independent and the dependent variables on the data compression and decomposition operations. PLS is the method normally used for multivariate calibration, where the multivariate signal, in

this case relative fluorescence intensity, measured at different wavelength, and the concentration of the samples are used to establish a linear regression model.

The PCR decomposition is based entirely on spectral variations without regard to the component concentrations. PCR decomposition is significantly influenced by variations, which have no relevance to the analyte concentrations, and in PLS, the spectral decomposition is weighted to the concentration. In PCR, as a first step, covariance in fluorescence intensity matrix is decomposed into factors without using the information about concentration. In the second step, the multivariate multiple linear regression (MLR) modeling between selected factors and concentration is performed.

MATERIALS AND METHODS

Apparatus

SFS measurements were performed with the use of a 10-mm quartz cell on a LS55 Spectrofluorometer (Perkin-Elmer Instruments, MA, USA) equipped with a 150-W continuous xenon lamp. The excitation and emission slits were both maintained at 10 nm and the scanning rate was 1500 nm min^{-1}. The pH of the solutions was measured with a Model SA 720 pH (Orion, LA, USA). The measured fluorescence data were processed on a Pentium IV computer with programs written in MATLAB 6.0 (Mathworks, MA, USA) by the authors.

Reagents

The three pesticides (Shanghai Pesticide Research Institute) were used without further purification. Stock solutions of each pesticide (100 mg L^{-1}) were prepared by dissolving 0.010 g crystals of each crystalline compound in methanol and diluted to 100 mL with doubly distilled water. Britton-Robinson (B-R) buffer solutions with different pH (2.1–8.4) were prepared by adding different amounts of 0.2 mol L^{-1} sodium hydroxide solution into 100 mL of a mixed acid, containing 0.04 mol L^{-1} of each of boric, *ortho*-phosphoric and acetic acids. All reagents were of analytical reagent grade, and doubly distilled water was used for dilution.[28]

General Procedure

A suitable amount of each pesticide or that of their mixtures, together with 2 mL of B-R buffer, were pipetted into a 10-mL volumetric flask, diluted to 10 mL with doubly distilled water. The solution was mixed thoroughly, and then a portion of each solution was transferred into a 10-mm quartz cell.

The SFS for samples were measured and recorded with $\Delta\lambda = 30$ nm, over excitation wavelength range 220–340 nm at 0.5-nm intervals (total 241 wavelengths); from this region the maximum analytical information can be obtained. The recorded data were then subtracted by the one of blank (i.e., buffer solution without the pesticides). The data collected within the selected wavelength range were used for building the calibration and prediction models. The spectrofluorimetric spectra of each carbamate compound and their mixture are shown in Fig. 1. The spectrofluorimetric measurements were carried out at a constant room temperature ($20 \pm 2^{\circ}$C).

Pretreatment of Fruit and Vegetable Samples

The concentration of pesticides in fruit and vegetable samples is too low to detect directly. In order to evaluate the applicability of the proposed method for practical application, the vegetable and fruit samples were spiked with the target compounds and analyzed according to the experimental procedure described above after a pretreatment. In this work, 2.0 mL of 10 mg L^{-1} carbaryl, 100 mg L^{-1} propoxur and carbofuran standard solutions were added into 20 g of each vegetable sample. After an incubation period of 5 h, the samples were chopped, blended, and triturated to form a homogeneous mixture, and the resulting aqueous slurry was transferred to a 250 mL Erlenmeyer flask. To the slurry was added 50 mL of dichloromethane and

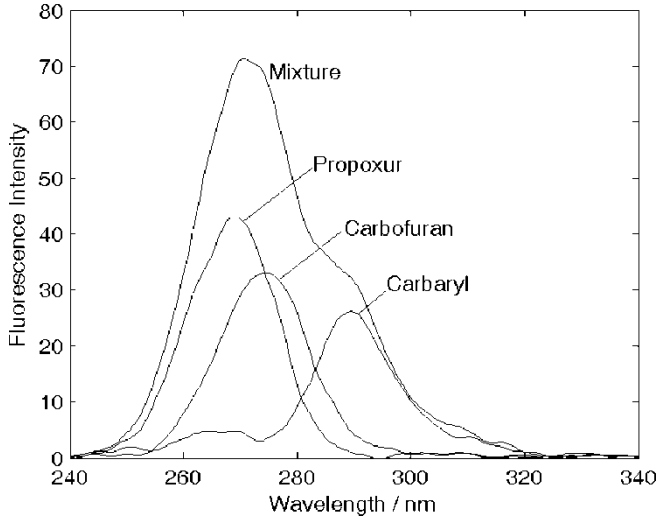


Figure 1. Synchronous fluorescence spectra of carbaryl (0.01 mg L^{-1}), propoxur (0.1 mg L^{-1}), and carbofuran (0.4 mg L^{-1}) and their mixtures in Britton–Robinson buffer of pH 4.10. $\Delta\lambda = 30$ nm.

20 g of anhydrous sodium sulfate, and then 0.2 g activated charcoal was also added to facilitate the removal of interfering coloring matter. The mixture was then shaken for 30 min (Model HY-4 oscillator, Shanghai, China) before the organic phase being filtered on an evaporating dish. The dichloromethane was removed by evaporation, and the residues were diluted to 10 mL with methanol.

RESULT AND DISCUSSION

The Selection of Δλ

In most synchronous spectrofluorimetric studies for chemical systems, the selection of Δλ is very important. The influence of Δλ can be substantial on the shape, location, and signal intensity of a fluorescence peak, as well as on interferences attributed to light scattering.[29] In this work, in order to get the best value of Δλ, three-way synchronous fluorescence data were sampled and studied (Fig. 2). As is evident from Fig. 2, the shape and intensity of SFS of compounds depend on Δλ, the highest intensity of carbaryl is observed at Δλ of 60 nm, and the optimal Δλ for propoxur and carbofuran are almost the same, 30 nm. Moreover, it can be noted that

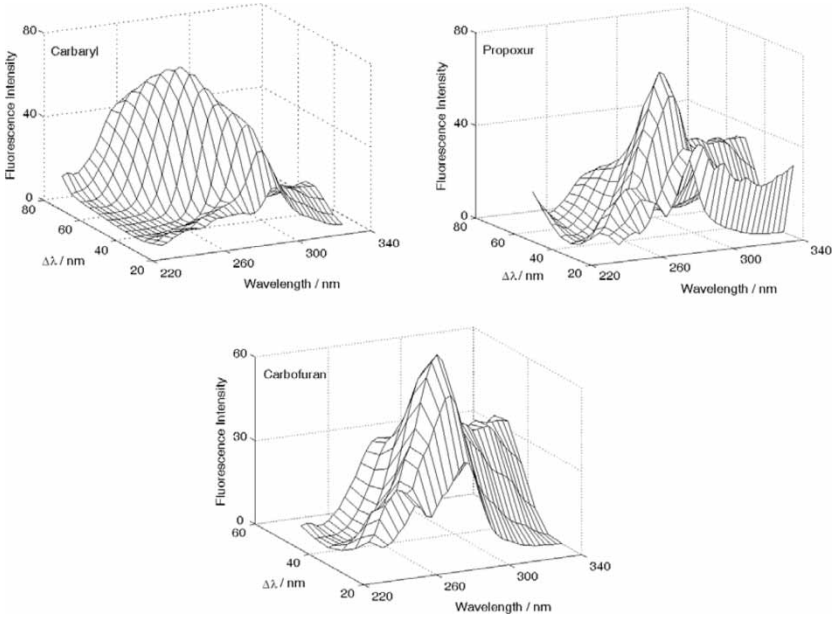


Figure 2. Influence of Δλ on the fluorescence intensity for each compound: $c_{\text{carbaryl}} = 0.02\ \text{mg L}^{-1}$, $c_{\text{propoxur}} = 0.14\ \text{mg L}^{-1}$, and $c_{\text{carbofuran}} = 0.6\ \text{mg L}^{-1}$. Experimental conditions were as in Fig. 1.

relative high sensitivity for carbaryl can be obtained at $\Delta\lambda$ of 30 nm for its flat SFS peak. Thus, the $\Delta\lambda$ of 30 nm was selected in this work based on the similarity of position, shape, and intensity of synchronous fluorescence scans observed for these compounds.

Influence of pH on Fluorescence Intensity

The effects of pH on the relative fluorescence intensity were studied with the use of a set of B-R buffers (pH range 2.1–8.4). It was found that the influence of pH on fluorescence intensity was slight. Because these carbamate pesticides were hydrolyzed in an alkaline medium, thus, the pH 4.1 was selected as a suitable analytical medium for this work.

Interferences

The influence of foreign species was investigated by analyzing a standard mixture solution of 0.01, 0.1, and 0.4 $\mu g\ mL^{-1}$ of carbaryl, propoxur, and carbofuran, respectively, to which increasing amounts of interfering species were added. The tolerable concentration on the determination of this mixture for interference at 10% relative error level were 10 $\mu g\ mL^{-1}$ for isocarbophos and acephate; 5 $\mu g\ mL^{-1}$ for trichlorfon, parathion, and fenitrothion; and 0.4 $\mu g\ mL^{-1}$ for isoprocarb. The results demonstrate that the proposed method has relatively good selectivity; only isoprocarb, whose chemical structure is similar to the analytes analyzed, showed positive interference because isoprocarb also has inner fluorescence around 220–340 nm.

It should also be noted that most of the carbamate compounds are easily decomposed to corresponding metabolites after used on farms; some of those metabolites usually have the similar fluoresphores as the matrix compounds, so it is necessary to take their interference into account during the analysis. These metabolites' interference will be further discussed as another topic.

Calibration Curves and Limits of Detection for Single Component Analysis

The fluorescence spectra (Fig. 3) were obtained at the analytical wavelengths 289, 268, and 274 nm for different concentrations of carbaryl, propoxur, and carbofuran, under conditions discussed in the previous sections above. Thus, the linear calibration curves were also obtained for different concentrations of carbaryl, propoxur, and carbofuran, respectively. Table 2 summarizes the results for the analysis of each pesticide. The limits of detection were 0.50, 5.5, and 23 $ng\ L^{-1}$ for carbaryl, propoxur, and carbofuran, respectively, and the correlation coefficients suggested good linearity over the concentration range of each pesticide.

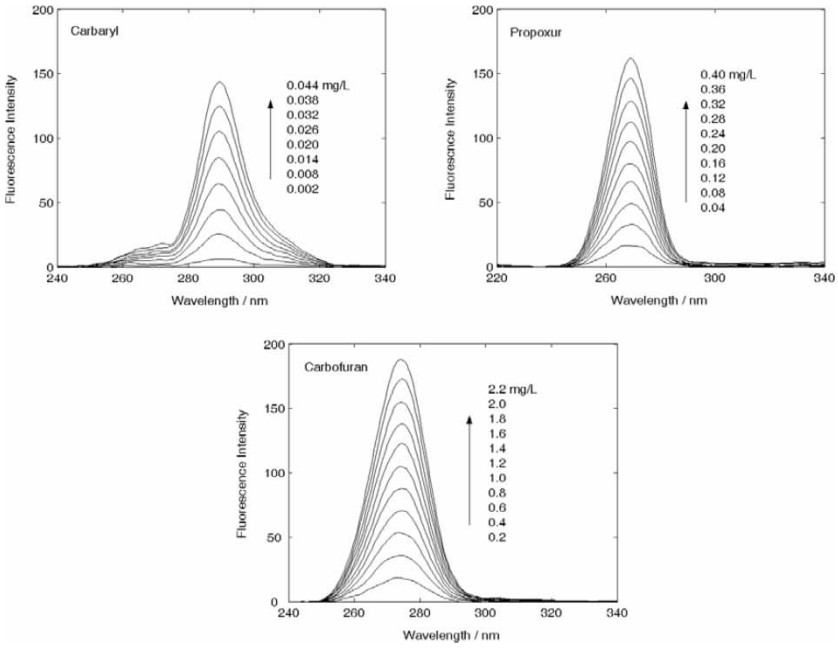


Figure 3. Synchronous fluorescence spectra of the carbamate compounds as a function of concentration (mg L^{-1}). Experimental conditions were as in Fig. 1.

Prediction of Carbamate Pesticides in a Synthetic Mixture

In order to extract maximum quantitative information from the calibration procedure, the composition of the calibration sets (Table 3) was orthogonally designed,[31] according to a four-level orthogonal array, denoted by OA_{16} (4^5). This indicated that a data set of 16 samples was required. The concentration levels for the carbamate pesticides were carbaryl, 0.025, 0.012, 0.018, and

Table 2. Parameters of the linear models for each pesticide compound

Parameters	Carbaryl	Propoxur	Carbofuran
Sample number (n)	8	10	11
Linear range (mg L^{-1})	0.002–0.04	0.04–0.4	0.2–2.2
Intercept	−0.72	0.39	2.56
Slope (L mg^{-1})	3292	403	84.6
Correlation coefficient	0.9999	0.9999	0.9999
SD of intercept	0.38	0.51	0.44
SD of slope	14.1	2.04	0.35
Limit of detection (ng L^{-1})[a]	0.50	5.5	23

[a]Calculated according to the method described by Miller and Miller.[30]

Table 3. Composition of the calibration samples (mg L^{-1})

Number of sample	Carbaryl	Propoxur	Carbofuran
1	0.025	0.05	0.90
2	0.025	0.28	0.60
3	0.025	0.12	0.20
4	0.025	0.18	0.40
5	0.012	0.05	0.60
6	0.012	0.28	0.90
7	0.012	0.12	0.40
8	0.012	0.18	0.20
9	0.018	0.05	0.20
10	0.018	0.28	0.40
11	0.018	0.12	0.90
12	0.018	0.18	0.60
13	0.005	0.05	0.40
14	0.005	0.28	0.20
15	0.005	0.12	0.60
16	0.005	0.18	0.90

0.0050 mg L^{-1}; propoxur, 0.050, 0.28, 0.12, and 0.18 mg L^{-1}; and carbofuran, 0.90, 0.60, 0.20, and 0.40 mg L^{-1}. The synchronous fluorescence for each of the mixture samples were measured and recorded between 220 and 340 nm to produce a data matrix with 16 rows and 241 columns. Another set of samples (Table 4) consisting of 16 synthetic mixtures was then used to evaluate the prediction ability of the calibration models used. In this work, CLS, PCR, and PLS models were established for all the analytes simultaneously to simplify the calibration procedures, and four factors were selected for both PLS and PCR methods in iterative calculation. The relative prediction errors (RPE) of each chemometrics method are listed in Table 5. It was found that all the CLS, PLS, and PCR methods gave good results ($RPE_T < 5\%$) in the predictive model. The RPE for a single component in the mixtures can be calculated by:

$$RPE_S = \left[\frac{\sum_{i=1}^{n} (c_{ij(found)} - c_{ij(added)})^2}{\sum_{i=1}^{n} (c_{ij(added)})^2} \right]^{0.5}$$

and the total prediction error can be expressed by

$$RPE_T = \left[\frac{\sum_{i=1}^{n} \sum_{j=1}^{m} (c_{ij(found)} - c_{ij(added)})^2}{\sum_{i=1}^{n} \sum_{j=1}^{m} (c_{ij(added)})^2} \right]^{0.5}$$

where $c_{ij(added)}$ indicates the concentration of the ith component in the jth mixture and $c_{ij(found)}$ is its estimate.

Table 4. Composition of the prediction samples (mg L^{-1})

Number of sample	Carbaryl	Propoxur	Carbofuran
1	0.008	0.24	0.30
2	0.008	0.20	0.80
3	0.008	0.06	0.50
4	0.008	0.14	0.70
5	0.015	0.24	0.80
6	0.015	0.20	0.30
7	0.015	0.06	0.70
8	0.015	0.14	0.50
9	0.010	0.24	0.50
10	0.010	0.20	0.70
11	0.010	0.06	0.30
12	0.010	0.14	0.80
13	0.024	0.24	0.70
14	0.024	0.20	0.50
15	0.024	0.06	0.80
16	0.024	0.14	0.30

Determination of Carbamate Pesticides in Fruit and Vegetable Samples

In this work, carbaryl, propoxur, and carbofuran in several fruit and vegetable samples (pear, apple, and cabbage) were determined by application of the proposed method. Each sample was pretreated as previously described, and then 0.1 mL of the extract was transferred to the 10-mL volumetric flask for analysis. The PCR method, which was superior to the others on the basis of the %RPE criterion, was chosen and applied for the analysis of the carbamate pesticides in the extracts. The results (Table 6) showed that contents of the pesticides found in the spiked samples were in the range

Table 5. Comparison of %RPEs, $\%RPE_T$, and %Recovery values for pesticides for different chemometrics models

Method	Carbaryl	Propoxur	Carbofuran	RPE_T[a]
CLS	6.34 (94.8)[c]	6.79 (96.0)	3.86 (98.9)	4.15
PLS[b]	6.67 (95.4)	6.91 (104)	3.82 (98.4)	4.13
PCR[b]	6.61 (95.4)	5.95 (103)	3.82 (98.4)	4.02

[a]RPE_S and RPE_T are relative prediction errors and were calculated according to Ref. 32.

[b]Four factors were selected for both PLS and PCR.

[c]The values in the parentheses correspond with the mean %Recovery.

Table 6. Determination of three carbamate pesticides in commercial vegetable samples by PCR method

	Found ($\mu g\ g^{-1}$)[c]			Added ($\mu g\ g^{-1}$)			Found ($\mu g\ g^{-1}$)[d]			%Recovery		
Sample[a]	CAB[b]	PRO	CAF	CAB	PRO	CAF	CAB	PRO	CAF	CAB	PRO	CAF
Pear	0.52	7.43	7.50	0.50	10.0	10.0	1.03	17.6	17.8	102	101	103
Apple	0.52	7.26	7.20	0.50	10.0	10.0	1.02	17.6	17.9	99	104	107
Cabbage	0.52	7.38	7.42	0.50	10.0	10.0	1.02	17.3	17.4	101	100	100

[a]All the samples were obtained from a supermarket in Nanchang.
[b]CAB, carbaryl; PRO, propoxur; and CAF, carbofuran.
[c]The values found in the spiked samples.
[d] The values found after the standard additions.

0.52–7.5 $\mu g\ g^{-1}$, and the procedure was further validated by standard addition of the three pesticides with %Recovery in the range 99–104%.

CONCLUSIONS

In this paper, the chemometrics interpretation of carbamates of carbaryl, propoxur, and carbofuran in synthetic and vegetable samples by the SFS approach in acid medium was investigated. The results showed that the combination of synchronous fluorescence method and multivariate calibration makes it feasible to effectively determine individual carbaryl, propoxur, and carbofuran, the widely used pesticides whose SFS seriously overlap. The developed method is practically useful because of its high sensitivity and selectivity and its cost-effective feature.

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REFERENCES

1. Extension Toxicology Network, Oregon State University. Availabel at http://extoxnet.orst.edu/pips/carbaryl.htm.
2. Hubert, M.; Worthing, R. C. *Pesticide Manual*, 5th ed.; British Crop Protection Council, 1977.
3. Dwyer, J. D.; Johnson, S. B.; Morro, L. S.; Plissey, E. S. *1994 Maine Potato Pest Control Guide*; University of Maine, Cooperative Extension, 1994.
4. Ameno, K.; Lee, S. K.; In, S. W.; Yang, J. Y.; Yoo, Y. C.; Ameno, S.; Kubota, T.; Kinoshita, H.; Ijiri, I. Blood carbofuran concentrations in suicidal ingestion cases. *Forensic Sci. Int.* **2001**, *116* (1), 59–61.
5. Delgado, M. J. S.; Barroso, S. R.; Fernandez-Tostado, G. T.; Polo-Diez, L. M. Stability studies of carbamate pesticides and analysis by gas chromatography with flame ionization and nitrogen-phosphorus detection. *J. Chromatogr. A* **2001**, *921* (2), 287–296.
6. Li, H. P.; Li, J. H.; Li, G. C.; Jen, J. F. Simultaneous determination of airborne carbamates in workplace by high performance liquid chromatography with fluorescence detection. *Talanta* **2004**, *63* (3), 547–553.

7. Gardner, M. S.; Voyksner, R. D.; Haney, C. A. Analysis of pesticides by LC-electrospray-MS with postcolumn removal of nonvolatile buffers. *Anal. Chem.* **2000**, *72* (19), 4659–4666.
8. Richter, P.; Sepulveda, B.; Oliva, R.; Calderon, K.; Seguel, R. Screening and determination of pesticides in soil using continuous subcritical water extraction and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2003**, *994* (1–2), 169–177.
9. Sastry, C. S. P.; Vijaya, D.; Mangala, D. S. Spectrophotometric determination of carbaryl and propoxur using aminophenols and phenylenediamine. *Analyst* **1987**, *112*, 75–78.
10. Naidu, D. V.; Naidu, P. R. A simple spectrophotometric method for determination of carbosulfan and propoxur. *Talanta* **1990**, *37* (6), 629–632.
11. Sastry, C. S. P.; Vijaya, D. Spectrophotometric determination of some insecticides with 3-methyl-2-benzothiazolinone hydrazone hydrochloride. *Talanta* **1987**, *34* (3), 372–374.
12. Jan, M. R.; Shah, J.; Khan, H. Investigation of new indirect spectrophotometric method for the determination of carbofuran in carbamate pesticides. *Chemosphere* **2003**, *52* (9), 1623–1626.
13. Manjubhashini, A. B.; Raman, G. K.; Kumar, A. S.; Chiranjeevi, P. A simple spectrophotometric technique for the determination of carbaryl pesticides in various environments. *Talanta* **2003**, *59* (5), 1015–1019.
14. Khalaf, K. D.; Morales-Rubio, A.; de la Guardia, M.; Garcia, J. M.; Jimenez, F.; Arias, J. J. Simultaneous kinetic determination of carbamate pesticide after derivatization with *p*-aminophenol by using partial least squares. *Microchem. J.* **1996**, *53* (4), 461–471.
15. Bai, L.; Ni, Y. N. Simultaneous determination of binary mixtures of propoxur and isoprocarb by partial least squares-kinetic spectrophotometry. *Chin. J. Anal. Chem.* **2002**, *30* (9), 1088–1091.
16. Garcia, J. M.; Jimenez, A. I.; Arias, J. J.; Khalaf, K. D.; Morales-Rubio, A.; de la Guardia, M. Application of the partial least-squares calibration method to the simultaneous kinetic determination of propoxur, carbaryl, ethiofencarb and formetanate. *Analyst* **1995**, *120*, 313–317.
17. Ni, Y. N.; Huang, C. H.; Kokot, S. Application of multivariate calibration and artificial neural networks to simultaneous kinetic-spectrophotometric determination of carbamate pesticides. *Chemom. Intell. Lab. Syst.* **2004**, *71* (2), 177–193.
18. Lloyd, J. B. F.; Evett, I. W. Prediction of peak wavelengths and intensities in synchronously excited fluorescence emission spectra. *Anal. Chem.* **1977**, *49* (12), 1710–1715.
19. Inman, E. L.; Winefordner, J. D. Constant energy synchronous fluorescence for analysis of polynuclear aromatic hydrocarbon mixtures. *Anal. Chem.* **1982**, *54* (12), 2018–2022.
20. Miller, J. N. Recent developments in fluorescence and chemiluminescence analysis. *Analyst* **1984**, *109*, 191–198.
21. Vo-Dinh, T. Partial least-squares regression: a tutorial. *Anal. Chim. Acta* **1986**, *185*, 1–17.
22. Vo-Dinh, T. Multicomponent analysis by synchronous luminescence spectrometry. *Anal. Chem.* **1978**, *50* (3), 396–401.
23. Patra, D.; Mishra, A. K. Recent developments in multi-component synchronous fluorescence scan analysis. *Trends Anal. Chem.* **2002**, *21* (12), 787–798.
24. Kramer, R. *Chemometric Techniques in Quantitative Analysis*; Marcel Dekker Inc: New York, 1998, pp. 51–130.

25. Feudale, R. N.; Woody, N. A.; Tan, H.; Myles, A. J.; Brown, S. D.; Ferre, J. Transfer of multivariate calibration models: a review. *Chemom. Intell. Lab. Syst.* **2002**, *64* (2), 181–192.
26. Wold, H. Estimation of principal components and related models by iterative least squares. In *Multivariate Analysis*; Krishnaiah, P. R., Ed.; Academic Press: New York, 1966, pp. 391–420.
27. Martens, H.; Naes, T. *Multivariate Calibration*; Wiley: New York, 1989.
28. Chang, W. B.; Li, K. A. *Handbook of Analytical Chemistry*; Beijing University Press: Beijing, 1981.
29. Ni, Y. N.; Lin, D. Q.; Kokot, S. Synchronous fluorescence and UV-vis spectrometric study of the competitive interaction of chlorpromazine hydrochloride and Neutral Red with DNA using chemometrics approaches. *Talanta* **2005**, *65* (5), 1295–1302.
30. Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 4th ed.; Pearson Education Limited: London, 2000.
31. Lan, W. G.; Wong, M. K.; Chen, N.; Sin, Y. M. Orthogonal array design as a chemometric method for the optimization of analytical procedures. Part 2. Four-level design and its application in microwave dissolution of biological samples. *Analyst* **1994**, *119*, 1669–1675.
32. Otte, M.; Wegscheider, W. Spectrophotometric multicomponent analysis applied to trace metal determinations. *Anal. Chem.* **1985**, *57*, 63–69.