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Investigation of preconcentration strategies for the trace analysis of multi-residue pesticides in real samples by capillary electrophoresis

Clóvis L. da Silva, Elizabete C. de Lima, Marina F.M. Tavares*
Institute of Chemistry, University of Sao Paulo, C.P. 26077, 05513-970 Sao Paulo, SP, Brazil

Abstract

In this work, on-line preconcentration strategies were investigated for the multi-residue analysis of pesticides in drinking water and vegetables using micellar electrokinetic chromatography. Among the on-line strategies, sweeping and stacking with reverse migration of micelles (SRMM), with and without the insertion of a plug of water before sample injection, were contrasted. A new version of SRMM was also introduced. The modification consisted of momentarily applying a positive voltage at the inlet vial right after sample has been injected, increasing the efficiency by which the analytes are captured. Nine pesticides from different classes, carbendazim (benzimidazole), simazine, atrazine, propazine and ametryn (triazine), diuron and linuron (urea), carbaryl and propoxur (carbamate), were baseline separated in less than 6 min with a electrolyte composed of 20 mmol l^{-1} phosphate buffer at pH 2.5, containing 25 mmol l^{-1} sodium dodecyl sulfate and 10% methanol. Limits of detection (LODs) in the order of 2–46 μg l^{-1} for the pesticides under investigation were obtained solely using the on-line strategies. Enrichment factors of 3–18-fold were obtained. These factors were computed as the improvement of the concentration LODs with respect to the reference condition (injection of 10 s at 2.5 kPa pressure). The proposed methodologies were applied to the analysis of pesticides in complex matrices such as carrot extracts where the detection of 2.5 μg l^{-1} was illustrated. By combining off-line solid-phase extraction and the proposed on-line strategies, the detection of pesticides in drinking water at the 0.1 μg l^{-1} level was conceived.

Keywords: Sample stacking; Sweeping; Food analysis; Pesticides

1. Introduction

Pesticide is a generic term used to describe a large number of widely differing biological, inorganic and organic compounds, including positional, geometric and optical isomers, employed in the control, prevention and elimination of plagues, that attack plantations and herds, as well as vectors of diseases in human beings [1]. Organic pesticides, the most commonly employed group, present pronounced physiological activity, and are constituted mostly by organochlorine, organophosphorous, carbamate and triazine, among others [2]. Due to their widespread use in agriculture associated with their persistence and toxicity, pesticides are a source of environmental contamination, presenting serious hazards to human health, through incorporation of residues in waters, soils and crops.

The fate of pesticides in the environment is determined by factors such as adsorption, absorption, lixiviation and volatilization, as well as microbiological, photochemical and chemical transformations. The importance of each factor depends

*Corresponding author. Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes 748, 05508-900 São Paulo, SP, Brazil. Tel.: +55-11-3091-2056x216; fax: +55-11-3815-5579.
E-mail address: mfmtavar@iq.usp.br (M.F.M. Tavares).

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upon the pesticide physical–chemistry properties, such as vapor pressure, hydrophilicity, partition coefficient, thermal stability and photochemical stability. Additionally, the type of interaction between the pesticide and the environment (soil, sediment, water), which depends on the biomass, organic matter content, pH, ionic strength capability, texture and hydrogeology, also determines the degree of environment contamination.

The continuous monitoring of pesticides residues in environmental samples is of great importance and demands high efficiency, unique selectivity and high sensitivity techniques. Gas chromatography [3] and high-performance liquid chromatography [4] have been established for years as the techniques of choice for the analysis of pesticides. Capillary electrophoresis (CE) in its various modes of operation has proven to be a resourceful alternative to the analysis of great variety of solutes due to its advantageous characteristics of high efficiency, fast analysis and low consumption of reagents and solvents [5]. However the major challenge for the implementation of CE in the pesticide analysis scenario [6] is its poor concentration limit of detection. The low concentration sensitivity is associated with the use of capillary columns, due to the micrometer-range optical pathlength represented by the inner diameter, in addition to the small injection plug, necessary to preserve the technique high efficiency.

Preconcentration procedures have always been implemented when trace analysis of compounds in real matrices is contemplated. In CE, a variety of on-line preconcentration strategies have been reported aiming at decreasing limits of detection by the insertion of a large volume of sample in the capillary without compromising peak efficiency and resolution [7]. In general, preconcentration strategies can be classified into two categories depending upon the physical phenomenon associated to the analyte concentration. One category involves the criterious manipulation of the analyte electrophoretic velocity. A collection of strategies grouped by the name of sample stacking and transient isotachophoresis are a few examples of this category thoroughly discussed in recent review articles [7–9]. The other group of preconcentration strategies explores the analyte ability of partitioning into a pseudo-stationary phase. Sweeping [10] and analyte concentrator devices [11] are representative of this group.

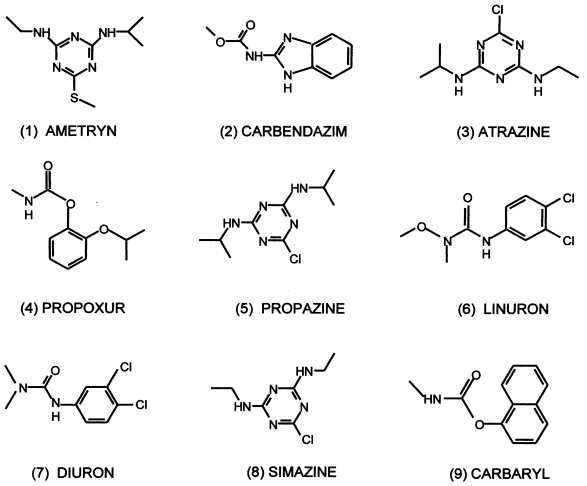

Fig. 1. Structures of the pesticides under investigation in this work.

Depending on the nature of the sample analyte, enhancement factors of the order of 100- up to 1000-fold, even approaching a million-fold sensitivity increase, have been reported [7–11], making viable the trace analysis of pesticides [6] and other compounds of environmental importance by CE methodologies. Recently, the separation of triazines [12], urea-derived pesticides [13,14], carbamates [15,16], among others [6,17] in a variety of environmental matrices have all been investigated by capillary electrophoresis, using several preconcentration schemes to achieve ppb-level concentrations.

In this work, three on-line preconcentration strategies as well as their combination with off-line solid-phase extraction were investigated for the multi-residue analysis of pesticides in drinking water and vegetables. Among the on-line strategies, sweeping (SW) and stacking with reverse migration of micelles (SRMM), with and without the insertion of a plug of water prior to the sample, were investigated and contrasted. A new version of SRMM was also introduced. Nine pesticides, whose structures are depicted in Fig. 1, were selected for the studies.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade, solvents

were of chromatographic purity and water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA). Nine pesticides (carbendazin, simazine, atrazine, propazine, ametryn, diuron, linuron, carbaryl and propoxur) and sodium dodecyl sulfate (SDS) were acquired from Riedel-de Haën (Seelze, Germany). Individual pesticide stock solutions were prepared at concentration of 500 mg l^{-1} in methanol. Working solutions were prepared by mixing appropriate volumes of the stock solutions to give final mixtures containing 0.5 mg l^{-1}, 2.5 μg l^{-1} and 0.1 μg l^{-1} of each pesticide. The electrolyte system consisted of a pH 2.5 phosphate buffer solution at 0.020 mol l^{-1} concentration containing 0.025 mol l^{-1} SDS and 10% methanol.

2.2. Sample preparation

2.2.1. Drinking water

Octadecylsilica cartridges of 1 g capacity were obtained from Supelco (Bellefonte, PA, USA). The cartridge was connected to a vacuum manifold system (Visipred, Supelco). As preconditioning, the cartridges were rinsed with 12 ml of acetonitrile (ACN) and 10 ml of deionized water, consecutively. A 250-ml volume of drinking water was fortified with a mixture of nine pesticides to give a final concentration of 0.1 μg l^{-1} each. The whole volume sample was loaded to the cartridge at a flow-rate of 3 ml min^{-1}. The cartridge was then disconnected from the vacuum line and dried under nitrogen. Exactly 5 ml of methanol was used to elute the pesticides from the cartridge. The full procedure was repeated three times in a similar manner and distinct eluates were obtained. Each eluate was dried under nitrogen, resuspended in 1 ml of appropriate solutions and saved for the sweeping and stacking studies.

2.2.2. Carrots

Carrots were acquired in local grocery stores. The extraction procedure followed that described by Minelli et al. [18], with modifications. To a 15-g portion of a carrot sample homogenized in blender, 30 ml of a mixture of solvents: acetone–petroleum ether–dichloromethane (1:1:1, v/v) and 4 g of NaCl were added. The mixture was sonicated for 15 min. The extraction procedure was repeated twice. The organic layers were separated and transferred to 15-ml centrifuge flasks containing 2 g of anhydrous Na_2SO_4, mixed gently, and centrifuged for 3 min. Aliquots of the dry organic extracts were transferred to 25-ml cylindrical tubes immersed in a heating bath at 35 °C to evaporate to approximately 1 ml final volume, under nitrogen stream. As a clean-up procedure, the concentrated extract (1 ml) was applied to a cartridge containing 1 g of amino modified octadecylsilica stationary phase (Strata, Phenomenex, Torrance, CA, USA), previously conditioned by 25 ml of dichloromethane. The cartridge was dried under nitrogen and the compounds were eluted with 7 ml CH_2Cl_2–MeOH (99:1). The eluate was evaporated to dryness and ressuspended in 1 ml of appropriate solutions (independent procedures) for latter injection.

2.3. Apparatus

All experiments were conducted in a capillary electrophoresis system (Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector set at 220 nm, a temperature control device, maintained at 25 °C and a acquisition and treatment data software supplied by the manufacturer (HP ChemStation, rev A.06.01). Samples were injected hydrodynamically at 2.5 kPa pressure. Injection times were manipulated for signal enhancement as described in the figure legends. The power supply was operated under reverse polarity and constant voltage conditions of −25 kV. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 58.3 (50.0 cm effective length)×75 μm I.D.×375 μm O.D. was used. At the beginning of each day, the capillary was conditioned by flushing 1 mol l^{-1} NaOH solution (30 min), followed by a 20 min flush with deionized water and electrolyte solution (30 min). In between runs, the capillary was just replenished with the electrolyte (4 min).

The electrolyte pH was measured by a pH meter (Model DM-21, Digimed, São Paulo, Brazil), while solutions conductivities were measured by a conductivity meter (Model DM-31, Digimed).

3. Results and discussion

3.1. Preconcentration strategies

The aim of this work was to evaluate comparative-

ly the performance of on-line preconcentration strategies for the separation of nine pesticides of different classes and to assess the viability of CE when the methodologies were applied to real sample matrices. Before hand, an electrolyte composition was optimized. Fig. 2 presents the separation of a mixture of pesticide standards in the optimized electrolyte, where an appropriate injection condition was arbitrarily defined, which for now on will be referred as the reference condition. Three preconcentration strategies were studied: sweeping [10] and two variations of stacking with reverse migrating micelles [9].

SW was described by Quirino and Terabe in 1998 [19] and presents the most impressive signal enhancement factor among all strategies implemented so far for CE separations [10,11]. Quirino and Terabe's approach is based upon the capture and accumulation of neutral analyte molecules by a charged pseudostationary phase that penetrates the solute zone during application of voltage. Sample matrix may present lower, similar or higher conductivity as compared to the background electrolyte. However, by keeping constant the resistance across the capillary through preparation of the sample in a medium of similar conductivity as the background electrolyte, sweeping efficiency is improved. The condition for sweeping to occur is that the sample zone must be free of additives, and that includes micelles, cyclodextrins, or any other organic modifier. In that condition, a long plug of sample can be introduced. In this work, sweeping was tested in a low pH medium in which electroosmotic flow was practically null. By applying a negative voltage at the capillary inlet, micelles enter the capillary from the cathodic end towards the anode (detection side), capturing and accumulating the analyte molecules, until they reach the interface between the sample zone and the anodic side of the electrolyte. Concentrated zones proceed throughout the capillary column being separated by micellar electrophoresis principles.

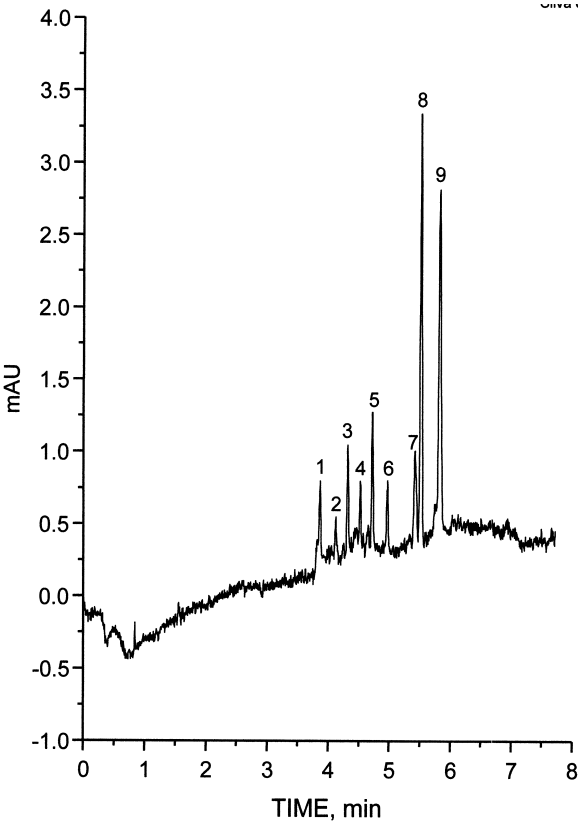

Fig. 2. Reference separation of nine pesticide standards at 0.5 mg l^{-1} concentration each by reverse micellar electrokinetic chromatography in 20 mmol l^{-1} phosphate buffer at pH 2.5 containing 25 mmol l^{-1} SDS and 10% methanol. Other conditions: hydrodynamic injection of 10 s at 2.5 kPa pressure, applied voltage of −25 kV, 25 °C, and direct detection at 220 nm. Peak legends as in Fig. 1.

Sample stacking, a preconcentration mechanism first conceived for ionic solutes, was extended to the separation of neutral analytes in micellar electrokinetic chromatography as described by Liu et al. in 1994 [20]. After this pioneering work, several clever manipulation of the micelle mobility was envisioned and a great number of stacking procedures was described. In a recent review, Quirino and Terabe categorized these procedures according to injection mode [9]. In this work, two variations of sample stacking for neutral analytes were tested, namely, stacking using reverse migrating micelles and a water plug (SRW) and a modified version of the stacking with reverse migration micelles (m-SRMM).

The SRW strategy consists basically in the introduction of a long plug of water followed by a long plug of sample (usually twice as much) in a capillary preconditioned with a low pH background electrolyte. Sample is prepared in a low-conductivity micellar matrix. When the electric field is established, micelles at the cathodic end migrate towards

de anode (detection end), capturing analyte molecules of the sample zone and conducting them towards the water region. By penetrating the water plug, micelles are accelerated until they reach the interface between the water plug and the electrolyte, where they stack.

The stacking with reverse migration micelles proposed in this work is a variation of the original SRMM described by Quirino and Terabe [9]. In the original SRMM, samples were preferably prepared in water and injected in a capillary preconditioned with an acidic micellar electrolyte (pH<5). Under this condition, the micelle electrophoretic mobility surpasses the flow mobility. On application of a negative voltage at the capillary inlet, stacking of the micelles at the anodic interface sample matrix/electrolyte, removal of the sample matrix from the capillary, by the effect of the counterelectroosmotic flow and separation of zones occur. The modification introduced in this work consisted in applying momentarily a positive voltage at the inlet vial right after sample has been introduced. Current increases and it is monitored until it reaches 80% of the value initially measured for the background electrolyte. The voltage is switched to negative and the partially stacked micelles somewhere near to the capillary inlet are then accelerated towards the anodic interface sample matrix/electrolyte. In this m-SRMM, the efficiency by which the analytes are captured is enhanced, due to the double trajectory micelles are forced to experience.

The performance of SW, SRW and the m-SRMM was evaluated for a mixture of nine pesticides standards prepared at 0.5 mg l^{-1} concentration, as depicted in Fig. 3. During sweeping (Fig. 3A), the pesticide mixture was diluted in a 25 mmol l^{-1} phosphate buffer at pH 2.5 to match the conductivity of the electrolyte (2.35 mS cm^{-1}). For the SRW, the standard mixture was diluted in a 4 mmol l^{-1} phosphate buffer at pH 2.5 containing 10 mmol l^{-1} SDS, whereas for the m-SRMM, sample was diluted in plain water. By inspection of Fig. 3 it can be observed that m-SRMM was the procedure that could be performed in the smallest amount of time, with highest efficiency. However, the fact that all peaks are close together might not be adequate for all sort of samples, where matrix constituents are likely to interfere with the separation of the pesticide major peaks. Another observation from Fig. 3 is that the relative predominance of peaks differs for each strategy, indicating that analyte–micelle interactions are diverse, as expected from the large range of hydrophobicity of the studied analytes.

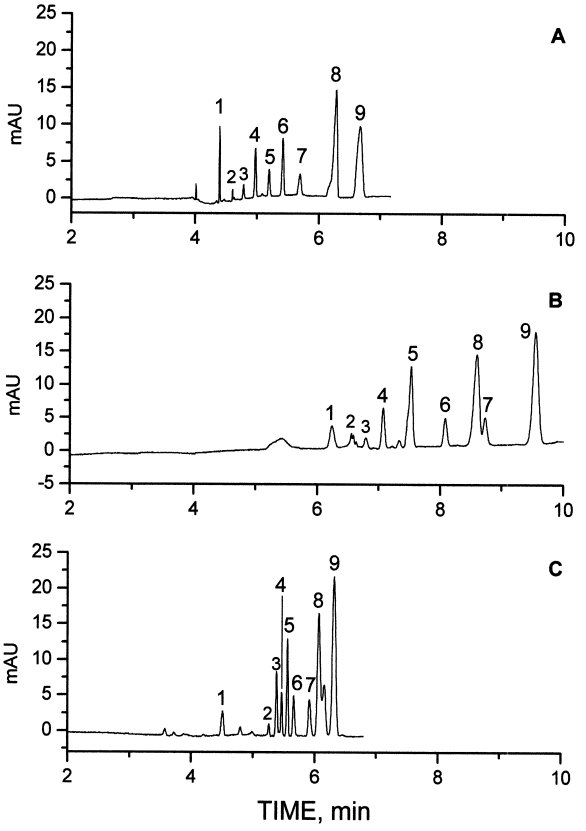

Fig. 3. Evaluation of the preconcentration strategies: (A) sweeping (SW), (B) stacking with reverse migrating micelles and a water plug (SRW) and (C) modified stacking with reverse migrating micelles (m-SRMM) for the analysis of nine pesticide standards at 0.5 mg l^{-1} concentration. Sample preparation: (A) standard mixture was prepared in 25 mmol l^{-1} phosphate buffer at pH 2.5 (sample conductivity = 2.06 mS cm^{-1}, buffer conductivity = 2.12 mS cm^{-1}), (B) standard mixture was prepared in 4 mmol l^{-1} phosphate buffer at pH 2.5 containing 10 mmol l^{-1} SDS and (C) standard mixture was prepared in water. Injection conditions: (A) hydrodynamic injection of 1.6 min at 2.5 kPa pressure; (B) injection of 50 s water followed by 1.6 min sample at 2.5 kPa pressure; (C) hydrodynamic injection of 1.6 min at 2.5 kPa pressure followed by applied voltage of +20 kV by 2 min. Other conditions as in Fig. 2. Peak legends as in Fig. 1.

In order to compare the effectiveness of the preconcentration strategies, limits of detection (LODs) and enrichment factors were computed. The

Table 1
Comparative evaluation of the preconcentration strategies under investigation in this work based on enrichment factors calculated by limits of detection (LODs) and peak heights (PKHs)

Pesticide	Reference LOD ($\mu g\ l^{-1}$)	SW			SRW			m-SRMM		
		LOD ($\mu g\ l^{-1}$)	γ_{LOD}	γ_{PKH}	LOD ($\mu g\ l^{-1}$)	γ_{LOD}	γ_{PKH}	LOD ($\mu g\ l^{-1}$)	γ_{LOD}	γ_{PKH}
Ametryn	93	5.2	18	19	27	3.4	6.3	17	5.5	6.3
Carbendazin	170	43	4.0	4.1	36	4.7	8.7	35	4.8	5.5
Atrazine	71	28	2.5	2.6	46	1.6	2.9	6.5	11	12
Propoxur	110	8.1	13	15	15	7.5	15	9.6	12	14
Propazine	56	17	3.2	3.4	7.6	7.4	14	4.4	13	15
Diuron	100	6.6	15	16	20	5.3	9.6	10	10	11
Linuron	80	15	5.1	5.2	20	5.0	7.2	11	7.1	8.0
Simazine	18	3.6	4.9	5.0	6.6	2.6	4.9	3.5	5.0	5.6
Carbaryl	22	5.6	4.0	4.1	5.3	4.2	7.7	2.7	8.4	9.6

SW, Sweeping; SRW, stacking with reverse migrating micelles and a water plug; m-SRMM, modified stacking with reverse migrating micelles.
$\gamma_{LOD} = LOD_{reference}/LOD_{strategy}$.
$\gamma_{PH} = PKH_{reference}/PKH_{strategy}$.
Reference condition: injection of 10 s at 2.5 kPa pressure.

results are compiled in Table 1. The LOD for each pesticide was calculated as the concentration that gives a signal-to-noise ratio of 3. Enrichment factors were calculated as a ratio of LOD and peak height, separately, considering as reference, the condition presented in Fig. 2, i.e., hydrodynamic injection of the pesticide mixture by 10 s at 2.5 kPa pressure. It is worth mentioning that the calculation of enhancement factors based on LOD ratios is not thoroughly adopted in the literature and a comparative evaluation of preconcentration strategies may result in discrepancies. We feel that LOD ratios as enhancement factors are good comparative parameters to be computed because concentration LOD is ultimately what a method can offer in terms of detection capability for a given analyte. As can be observed in Table 1, the strategies described in this work provided roughly a 3–18-fold sensitivity increase, depending on the pesticide. Even though these factors were not as impressive as those found in the literature, it must be considered that the selected pesticides comprise a wide range of hydrophobicity and polarity and, therefore, their partition coefficients differ considerably.

3.2. Application of the preconcentration strategies to real samples

The use of solid-phase extraction as a means of off-line preconcentration of pesticides in natural waters and other matrices is widespread [21]. Three separate samples of drinking water, fortified with a mixture of nine pesticides, were submitted to solid-phase extraction procedures, as described in the Experimental section. The resulting eluates were further concentrated on-line by the SW and SRW and m-SRMM strategies described previously. The electropherograms of the combined off-line and on-line preconcentration strategies are presented in Fig. 4. In all cases, it is possible to detect analytes 3, 4, 6, 7, 8 and 9 in the water matrix. Ametryn and carbendazim were detected by both stacking procedures while propazine was detected only when sweeping was employed. Also, migration time varied considerably for the stacking procedures, recommending the use of spiking procedures for peak identification in case these methods were to be validated and applied for quantitative purposes with this kind of sample matrix. By combining solid-phase extraction with either sweeping or stacking strategies, the detection of 0.1 $\mu g\ l^{-1}$ concentration for several pesticides under investigation was achieved.

The proposed preconcentration strategies were also applied to the determination of pesticides in carrots, a much more complex matrix. Extraction and clean-up procedures were described in the experimental section and followed the procedure proposed by Minelli et al. [18] with modifications. In

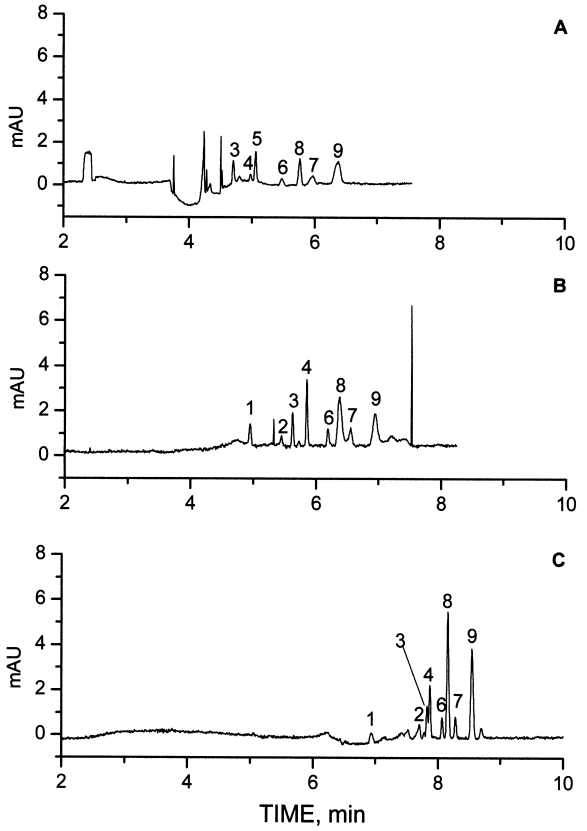

Fig. 4. Application of the preconcentration strategies: (A) sweeping (SW), (B) stacking with reverse migrating micelles and a water plug (SRW) and (C) modified stacking with reverse migrating micelles (m-SRMM) to the analysis of drinking water enriched with nine pesticide standards at 0.1 μg l^{-1} concentration. Sample was pre-concentrated off-line by solid-phase extraction prior to the electrophoretic analysis as described in the Experimental section. Sample dilution and electrophoretic conditions as in Fig. 3. Peak legends as in Fig. 1.

the original extraction procedure, sample is blended with a mixture of solvents. However, according to Babic et al. [22], sonication of the sample in the presence of solvents is much more effective, therefore, sonication was selected for the extraction step described in this work. Other modifications in Minelli's extraction procedure comprise the amount of sample and solvents that were increased from 5 to 15 g and 15 to 30 ml, respectively, and the stationary phase used during clean-up, where aminopropyl substituted cyanopropyl. Additionally, the use of Na_2SO_4 for desiccation of the extraction solvents was not described in the original procedure.

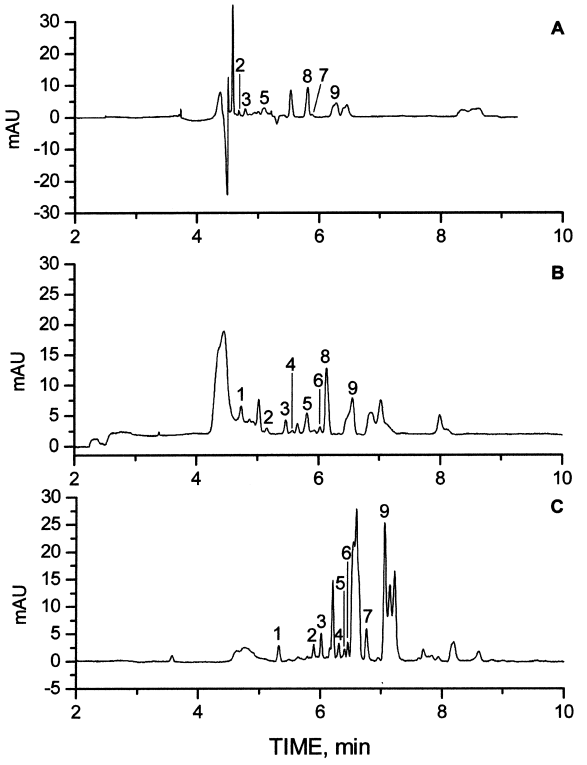

Fig. 5. Application of the preconcentration strategies: (A) sweeping (SW), (B) stacking with reverse migrating micelles and a water plug (SRW) and (C) modified stacking with reverse migrating micelles (m-SRMM) to the analysis of carrots enriched in natura with nine pesticide standards at 2.5 μg l^{-1} concentration. Sample preparation is described in the Experimental section. Sample dilution and electrophoretic conditions as in Fig. 3. Peak legends as in Fig. 1.

Fig. 5 presents the electropherograms of extracts of carrot samples, fortified in natura with nine pesticides at 2.5 μg l^{-1} concentration, following each of the proposed on-line preconcentration strategies. For this kind of sample, matrix effects are relevant. Nevertheless, several pesticides under investigation in this work could be clearly identified in the sample with no interference from matrix constituents.

4. Conclusion

On-line preconcentration of pesticides from different classes (triazine, benzimidazole, urea and carbamate) during micellar electrokinetic chromatographic

separation was established, providing detection limits of the order of 2–46 μg l^{-1}. These results indicate that CE is a perfectly eligible technique for pesticide residue analysis in complex matrices such as fruits and vegetables. Moreover, the use of solid-phase extraction, a common practice in pesticide analysis, in combination with any of the proposed on-line preconcentration strategies allowed the determination of pesticides at the 0.1 μg l^{-1} level in drinking water.

Acknowledgements

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