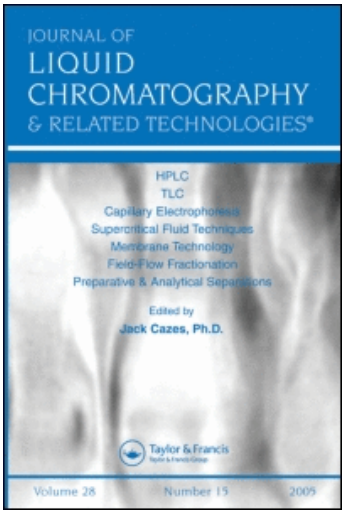

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On Line Extraction and Separation of Bendiocarb, Methomyl, Methylparathion, and Pentachlorophenol Pesticides from Raw Milk

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ON LINE EXTRACTION AND SEPARATION OF BENDIOCARB, METHOMYL, METHYL-PARATHION, AND PENTACHLOROPHENOL PESTICIDES FROM RAW MILK

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ABSTRACT

A high performance liquid chromatography (HPLC) method for extraction and determination of pesticides from raw milk was developed. The method involves direct injection of raw milk samples on a bovine serum albumin-dimethyl-octyl-silica gel (BSA-Si-C_8) column. The mobile phase 0.05 mol.L^{-1} phosphate buffer pH6.0 in acetonitrile (70:30 v/v) was employed for extraction and separation of bendiocarb, methylparathion, pentachlorophenol, and methomyl pesticides. The method shows good results of recovery in the pesticides studied, higher than 99.6%.

INTRODUCTION

Isolation and quantification of small molecules from complex macromolecules matrices has presented significant challenges to the field of liquid chromatography. In particular, the analysis of drugs, metabolites, and pesticides in biological fluids has historically posed problems, because of the need to remove proteins prior to high performance chromatography injection. This sample preparation step is required in order to avoid damaging chromatographic columns.

Conventional sample preparation procedures involve precipitation of proteins, followed by extraction and preconcentration of analytes. In many cases, sample preparation is disadvantageous because of labil of few components, low extraction yields, difficulties with reproductibility, and consumption of analysis time.[1]

The development of Internal Surface Reversed Phase (ISRP) silicas materials by Pinkerton[2,3] for direct serum, milk, and urine injection assays of drugs by HPLC have been used in the field of biological analysis samples. These HPLC packing materials for direct injection isolate small molecules from biological macromolecules on direct sample injection by exerting two separation mechanisms.[4]

Many other direct injection ISRP (columns) assays have been developed and validated for similar applications, such as phenylalanine in human plasma for diagnosis and treatment of phenylketonuria,[5] drug enantiomers in serum,[6,7] extraction and identification of carbamazepine from human serum,[8] and analysis of organochlorine pesticides from raw milk.[9] Many protein-bonded stationary phases based on albumins such as bovine serum albumin,[10] human serum albumin,[11-13] glycoproteins such as alpha-acid glycoproteins,[1] ovomucoid, avidin and conalbumin,[15] and celulas,[16] have been developed in order to separate enantiomers. The immobilization of the BSA on stationary phase has been performed "in situ" by frontal chromatography on a column packed with the appropriate stationary phase.

The final synthesis step involves coupling of the bovine serum albumin by reactive glutaraldehyde followed by small amount of sodium cyanoborohydride solution.[17,18]

This paper describes the application of the bovine serum albumin-dimethyl-octyl-silica gel (BSA- Si-C_8) column for extraction and separation of bendiocarb, methomyl, methylparathion, and pentachlorophenol pesticides from crude milk by direct injection in a high performance liquid chromatography system, (HPLC).

EXPERIMENTAL

Chemicals and Solvents

Acetonitrile, acetone, dioxane, methanol, toluene, cyclohexanol, and xylene were obtained from Sharlau (ICS, Lapeyrousse-Fossat, France), all were HPLC grade. Pesticide standards (bendiocarb, methomyl, methylparathion, and pentachlorophenol) were obtained from Polyscience Corporation (Niles, IL, USA). Bovine serum albumin was obtained from Sigma Chemical Company (St Louis, MO, USA). Chlorodimethylsilane, sodium cyanoborohydride, octene-1, sodium hydroxide, potassium dihydrogen, and 25% (v/v) glutaraldehyde solution were obtained from Aldrich Chemical (Strasbourg, France). The pure water was obtained with an Elgastat UQH II (Cofralab, Bordeaux, France). The silica gel Nucleosil (pore diameter 100 Å and particle size 10μm) was obtained from Macherey-Nagel (Duren-Germany). The natural raw milk was obtained from a local store in Bordeaux (France) and was diluted with mobile phase (phosphate buffer pH 6.0 in acetonitrile (70:30 v/v) to obtain 50% (v/v) and 25% (v/v) milk solutions.

Stock standards solutions were prepared by dissolving known amounts of bendiocarb, methylparathion, and pentachlorophenol in acetonitrile to obtain 50 and 100 μg/mL solutions. The same procedure was used to obtain 50 and 100 $\mu g.mL^{-1}$ for methomyl stock standard solution.

Fortification of Milk Sample

The milk sample was fortified by adding 100 μL aliquots of standard (100 $\mu g.mL^{-1}$) solution to 100 μL aliquots of 50 % (v/v) milk solution, resulting in a milk sample at 50 $\mu g.mL^{-1}$ for bendiocarb, pentachlorophenol and methylparation. The same procedure was used to obtain a milk sample at 50 $\mu g.mL^{-1}$ for methomyl.

Synthesis of Chlorodimethyl-octylsilane

Chlorodimethylsilane (0.06 $mol.L^{-1}$) freshly distilled over magnesium powder was added to a crystal of H_2PtCl_6 followed by drop wise addition of 0.05 $mol.L^{-1}$ of octene-1. The mixture was stirred under reflux for about 15 hours, yielding the desired silane as a white viscous liquid. The oil obtained was then filtered after distillation at 443 K in 15 mm HG pressure. The structure of the silane was characterized by NMR spectrometry.[19]

Synthesis of Bonded Stationary Phase (Si-C_8)

Silica gel 10 g (previously activated by heating for 15 hours at 180°C under 1.0 mmHg a pressure), was added to 0.1 mol.L^{-1} of chlorodimethyl-octylsilane in a dry 60 mL xylene aliquot and the mixture was stirred under reflux for 15 hours. After filtration, the product was washed twice with 40 mL of acetone and then with 40 mL of xylene. Bonded silica obtained was dried at 120°C under vacuum for 15 hours.

Column Packing

The column was packed by the ascending slurry packing method.[20] The colloid was prepared by adding 1.7 g of silica in 60 mL of dioxane:toluene:cyclohexanol (17:17:66 v/v/v) and sonicated for 5 minutes, and quickly transferred to a stainless-steel column (100 mm x 4.6 mm I.D) that was used as slurry reservoir. The packing was performed using a pressure of 300 Bar. The immobilization of the BSA was performed "in situ" by frontal chromatography on a column packed with the appropriate octyl-silica (Si-C_8).

This was done by pumping 0.05 mol.L^{-1} pH 6.0 of phosphate buffer followed by addition of 20 mL of a 1.0 % (v/v) buffered solution of bovine serum albumin (pH 6.0) and 5.0 mL of 25 % (v/v) solution of glutaraldehyde. After five hours, 10.0 mL of sodium cyanoborohydride was pumped into the column. The final stationary phase was washed thoroughly with deionized water and pure acetonitrile. [17,21]

Chromatographic System and Conditions

The HPLC system consisted of a Philips Model 4015 reciprocating pump, Philips Model 4025 Multi-Wavelength UV-Vis detector (ATI, Bobigny, France), and a Kipp & Zonen BD 40 recorder (Enraf-Nonius, Gagny, France). On line extraction and separation of proteins and separation of pesticides was carried out by a BSA-Si-C_8 column (100mm x 4.6 mm), synthesized following the reported protocol.[17,21] Separation was conducted at room temperature at a flow-rate of 2.0 mL.min^{-1}.

The initial mobile phase composition was 0.05 mol. L^{-1} phosphate buffer pH 6.0 in acetonitrile (70:30 v/v). A manual injector (Model 7125-075) fitted with a 5.0 μL loop (Rheodyne, Cotati, CA, USA) was used for direct injection into the ISRP column. Detection of the eluted pesticides was monitored at 220nm with a UV-Vis detector.

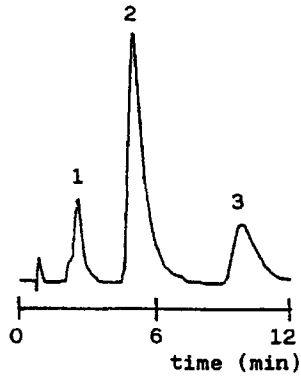

Figure 1. Chromatogram of a standard solution of bendiocarb (50 $\mu g.mL^{-1}$ - peak 1), pentachlorophenol (50 $\mu g.mL^{-1}$- peak 2) and methylparathion (50 $\mu g.mL^{-1}$ - peak 3). Chromatographic conditions: BSA-Si-C_8 (100 mm x 4.6 mm) column; mobile phase: 0.05 $mol.L^{-1}$ phosphate buffer pH 6.0 : acetonitrile (70:30 v/v) flow-rate 2.0$ml.min^{-1}$ at room temperature, detection: UV at 220 nm, injection: 5.0 µL.

RESULTS AND DISCUSSION

The use of a BSA-Si-C_8 (ISRP) column packed with 100 Å pore and 10 µm particle size showed good results in on line extraction of milk proteins for two reasons. First, the milk proteins are not adsorbed by bovine serum albumin immobilized on the surface of the silica gel and, second, the milk proteins are large molecules that are not able to get into the small silica pore.

Various sample solutions were tested for direct injection of raw milk, but the 25% (v/v) milk spiked with sample solution show the best results, considering the time for the in-line column extraction and the minimized clogging of the HPLC system. Figures 2 and 3 (A and B chromatograms) show that the milk proteins were immediately eluted from the BSA-Si-C_8 column with the same retention time (2.2 ± 0.2 min) as from the stationary phase with the mobile phase of 0.05 $mol.L^{-1}$ phosphate buffer pH 6.0 in acetonitrile (70:30 v/v). In Fig.3 (B and C chromatograms) we observed that mobile phase 0.05 $mol.L^{-1}$ phosphate buffer pH 6.0 in acetonitrile (97:03 v/v) was the best for extraction of milk proteins from 25 % (v/v) sample milk solution.

The BSA-Si-C_8 column show efficiency to separate the bendiocarb, methylparathion and pentachlorophenol, employing the mobile phase composed by 0.05 $mol.L^{-1}$ phosphate buffer pH 6.0 in acetonitrile (70:30 v/v). The retention times were: 2.3±0.2, k'-1.23 (bendiocarb), 4.5±0.2, k'-3.50 (pentachlorophenol), and 9.0±0.2, k'-7.70 (methylparathion) minutes and a

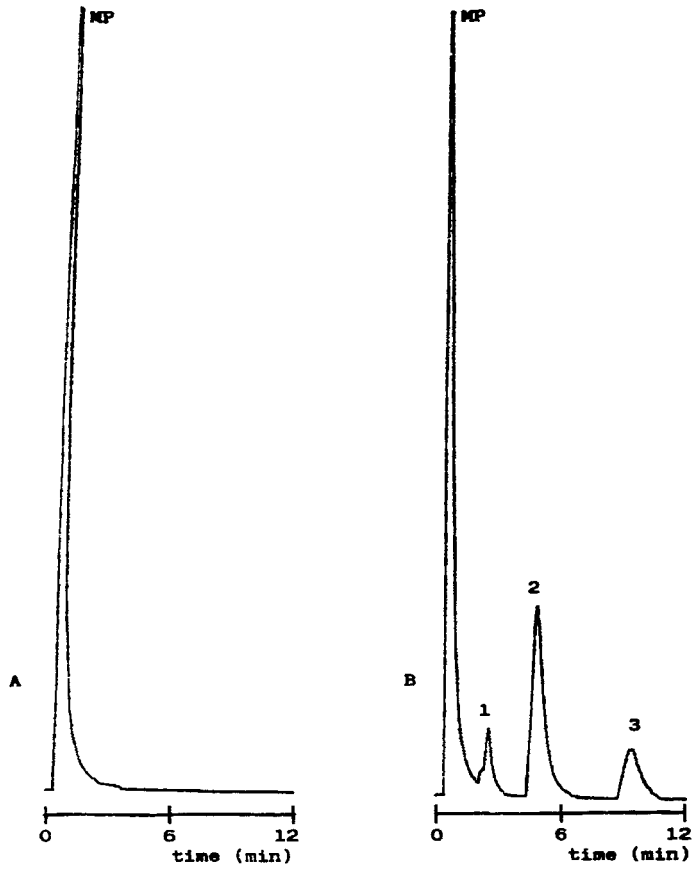

Figure 2. Chromatograms : (A) Raw milk blank, (B) raw milk fortified with 50μg.mL for bendiocarb (peak-1),pentachlorophenol (peak-2) and methylparathion (peak-3). Peak MP- milk proteins. Chromatographic conditions: see Fig1.

separation coefficient (k'), as shown in Figures 1 and 2 (B chromatogram). In the Figure 2, (B chromatogram) and Figure.3 (C chromatogram) Where was no observed any interference of milk proteins to obtain the peaks. During this study ii was observed that the carbamate pesticide (methomyl) showed weak adsorption with BSA-Si-C_8 column. However, when a mobile phase composed by 0.05 mol.L^{-1} phosphate buffer pH 6.0 in acetonitrile (97:03 v/v) was used, a retention time of 3.8±0.2 minutes for the standard methomyl and good results for its extraction from milk samples were obtained, as shown in Figure 3 (A and C chromatograms).

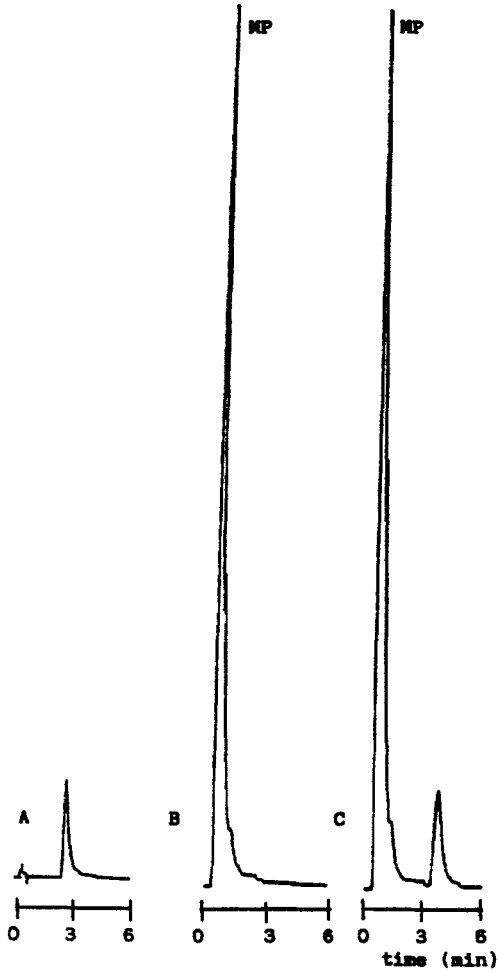

Figure 3. Chromatograms: (A) 50 $\mu g \cdot mL^{-1}$ standard methomyl solution, (B) raw milk blank and (C) raw milk fortified with 50 $\mu g \cdot mL^{-1}$ methomyl pesticide. Chromatographic conditions: see Fig. 1.

The recovery obtained for the extraction method was checked by doing five injections of milk samples containing 50 $\mu g.mL^{-1}$ of bendiocarb, methomyl, pentachlorophenol, and methylparation. The results obtained by checking showed good recoveries; higher than 99.6% in all assayed extractions. The reproducibility of the method was performed by a statistic method [standard deviation, (Sd) and coefficient of variance (CV)], in which obtained results were

satisfactory. The values founded for the standard deviation (Sd-0.62) and coefficient variance (CV-0.31), (n=5), indicated a good precision method. The detection limit was determined by measuring a minimum amount which was injected to provide a peak signal approximately twice the noise. Thus, was found a minimum value of 0.05 $\mu g.mL^{-1}$ of pentachlorophenol and methomyl, 1.0 $\mu g.mL^{-1}$ for malation and methylparathion. The easy-to-perform analysis method described does not require great amounts and prior treatment of milk samples.

CONCLUSION

In this paper our main objective was to study an application of the ISRP column in the extraction from milk raw pesticides and second, the separation. We observed that the column employed is suitable to extraction (99.6 %) and good separation for the study of pesticides. On line extraction is easy, because it doesn't require a conventional sample preparation. The use of on line extraction offers the best results, and does not require samples manipulation procedures.

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