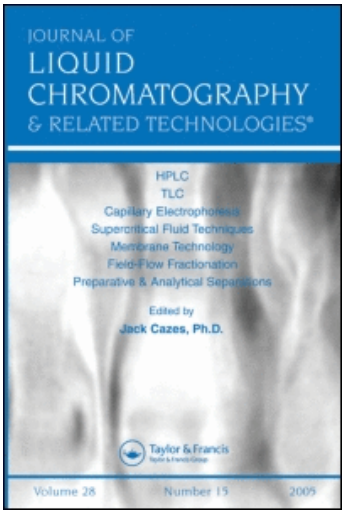

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Analysis of Organochlorine Pesticides in Plain Milk Using Direct Injection on an ISRP Column, with Column Switching

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ANALYSIS OF ORGANOCHLORINE PESTICIDES IN PLAIN MILK USING DIRECT INJECTION ON AN ISRP COLUMN, WITH COLUMN SWITCHING

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ABSTRACT

A simple and rapid procedure for extraction and separation from aldrin, DDT, endrin, heptachloro- and methoxychloro-organochlorine pesticides in raw milk has been developed by direct injection into an HPLC system without pretreatment of the samples, using an ISRP column.

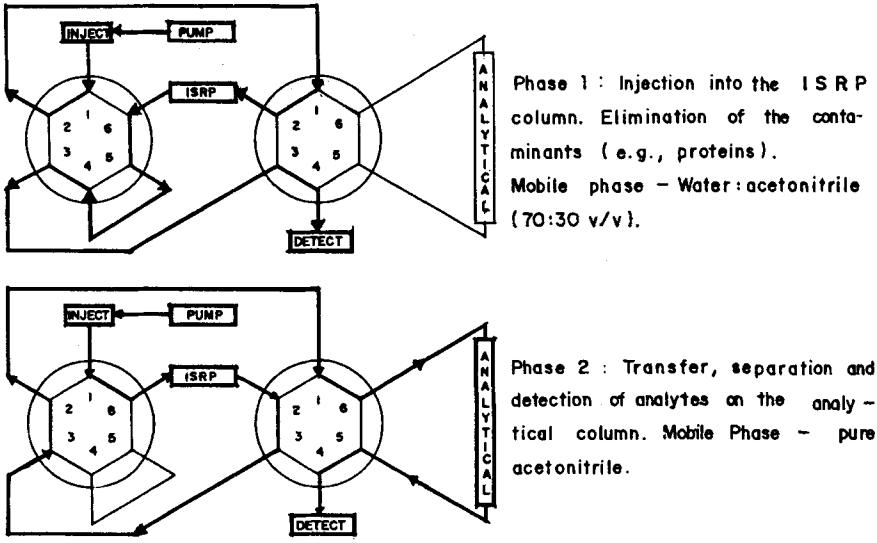

Figure 1. Schematic Representation of the HPLC column switching system.

INTRODUCTION

The determination of organochlorine pesticide residues in milk has always presented problems because the most common approach has required total extraction of fat, together with lipophilic compounds, including organochlorine pesticide residues.[1] The analytical methods generally involve initial solvent extraction of lipids, proteins and contaminants. The subsequent separation of pollutants from lipids often presents considerable problems in the analyses of organochlorine pesticides, partitioning between solvents of different polarities,[1] precipitation of proteins[2] and chromatography using aluminium oxide,[3] silica gel and florisil[4] have been employed.

These traditional methods are time consuming and expensive because of the high cost of the solvents and adsorbents. In contrast to such conventional extraction procedures, on-line extraction and clean-up procedures have been described using normal solid-phase extraction[5,6] which allows a significant decrease in the number of manual operations involved, but has the disadvantage of requiring large amounts of solvents.

The employment of steam distillation, solvent extraction at normal pressure has been described for recoveries of organochlorine compounds in water samples,[7] but require various operations to obtain pure samples.

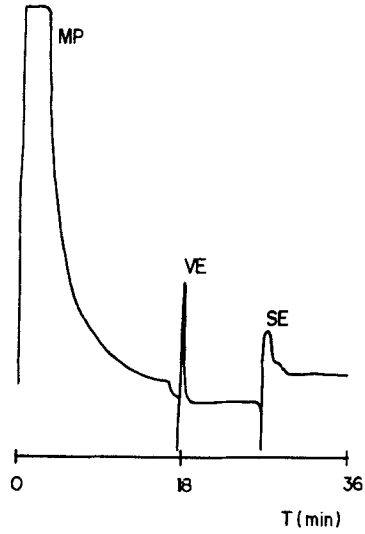

Figure 2. Chromatogram of the plain milk blank. MP = milk proteins; VE = valve effect: SE = solvent effect.
Chromatographic conditions: Extraction column - HSA-Si-C_{18} (30 x 4.6 mm), mobile phase - water:acetonitrile (70:30 v/v); analytical column - ODS-Hypersil (30 x 4.6 mm), mobile phase - acetonitrile; flow rate 1.5 mL/min, room temperature, detection = UV at 220 nm; injection volume - 20 μL.

Various extraction methods have been mentioned and, depending on their concentrations, the final analyses of organochlorine contaminants are performed by electron-capture GC[1,3-10] or GC-MS.[6,8,10]

The development of internal surface reversed phase (ISRP) silica materials by Pinkerton[11,12] for serum, milk and urine injection assays of drugs by HPLC have been successful for analysis of biological samples.

The use of human serum albumin (HSA), immobilized on the surface of silica, has also been employed for the resolution of drug enantiomers.[13,14]

This work describes a procedure for the extraction and separation of organochlorine pesticides by direct injection into an HPLC system, without treatment of samples using a new ISRP column.

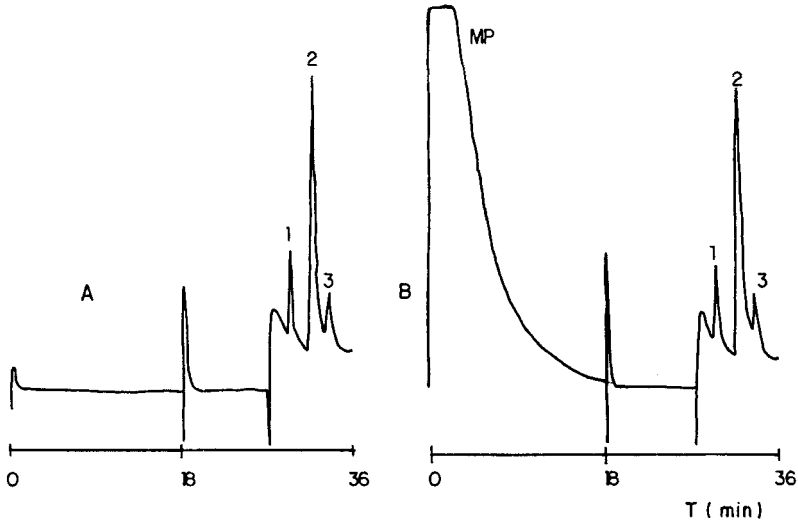

Figure 3. Chromatograms of the first group of pesticides.
(A) A standard sol'n of 50 μg/μL each of Endrin (1), DDT (2), and Aldrin (3). (B) Plain milk fortified with 50 μg/μL for Endrin (1), DDT (2), and Aldrin (3); MP = milk proteins. Chromatographic conditions as in Fig. 2.

EXPERIMENTAL

Chemicals and Solvents

Acetonitrile (HPLC grade) was obtained from Sharlau (ICS, Lapeyrousse Fossat, France). The standards (aldrin, DDT, endrin, heptachloro- and methoxychloro-) organochlorine pesticides, were obtained from Polyscience Corporation (Niles, Illinois, USA). The human serum albumin was obtained from Sigma Chemical Company (St. Louis, MO, USA), the trichlorooctadecylsilane, sodium cyanoborohydride and 25% (v/v) glutaraldehyde solution were obtained from Aldrich Chemical (Strasbourg, France). The pure water was prepared with an Elgastat UQH II (Cofralab, Bordeaux, France). The silica gel Merckosorb (pore diameter 60 Å and particle size 10μm) was obtained from Merck (Germany).

The natural raw milk was obtained from a grocery store in Bordeaux, France and was diluted with mobile phase [water:acetonitrile (70:30 v/v)] to 50% (v/v) and 25% (v/v) milk solutions. Stock standard solutions were prepared by dissolving known amounts of aldrin, DDT, endrin, heptachloro in acetonitrile to obtain 50 and 100 μg/μL.

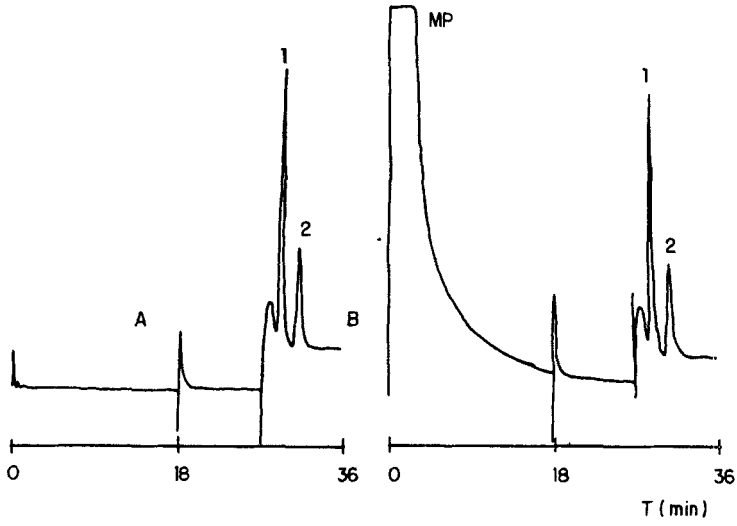

Figure 4. Chromatogram of the second group of pesticides.
(A) A standard sol'n of 50μg/μL each of Methoxychlor (1) and Heptachlor (2). (B) Plain milk fortified with 50μg/μL of Methoxychlor (1) and Heptachlor (2); MP = milk proteins. Chromatographic conditions as in Fig. 2.

Fortification of Milk Sample

The milk sample was fortified by adding 100 μL aliquots of standards aldrin, DDT, endrin, heptachloro and methoxychloro (100 μg/μL) solution into 100 μL aliquots of 50 % (v/v) milk solution, resulting in a milk sample at 50 μg/μL for aldrin, DDT, endrin, heptachloro and methoxychloro.

Chromatouraphic System and Conditions

The HPLC system consisted of a Philips Model 4015 pump, Philips Model 4025 Multi-Wavelenth UV-Vis detector (Atl, Bobigny, France) and a Kipp & Zonen BD 40 recorder (Enraf-Nonius, Gagny, France). The extraction of the proteins was carried with a ISRP-C_{18} column (30 x 4.6 mm), synthesized according to the reported protocol[15] and chromatographic separations were carried out on an ODS-Hypersil (30 x 4.6 mm, 3 μm) column, (Hewlett Packard) according to the schematic representation in Figure 1, maintained at room temperature, at a flow-rate of 1.5 mL/minute.

The initial mobile phase composition was water:acetonitrile (70:30 v/v). After 18 minutes, the switching valve (Model 7000-Rheodyne-AII Tech), was opened and the mobile phase was changed to HPLC grade acetonitrile.

The system was equilibrated at the initial mobile phase composition for 15 minutes before injecting the next sample. A manual injector (Model 7125-075 fitted with a 20 μL loop, Rheodyne, Cotati, CA,USA) was used for direct injection into the ISRP column. The detection of the eluted organochlorine pesticides was carried out at 254 nm with a UV-Vis detector.

RESULTS AND DISCUSSION

The employment of an HSA-C_{18} ISRP column with 60 Å pore and 10 μm particle size showed good results in the extraction of milk proteins, due to two factors. First, the milk proteins are not adsorbed by human serum albumin immobilized on the surface of the silica gel,[13,14] and, second, the milk proteins are large molecules that are not able to get into the small pore silica.

Various sample solutions were tried for direct injection: plain milk, and milk spiked with 50 % and 25 % (v/v). The 25 % (v/v) sample solution showed the best results, considering the time for the in-line column extraction and the minimized clogging of the HPLC system or column.

Figure 2 shows the extraction of milk proteins in 18 minutes, which is followed by the baseline disturbance at 18 ± 0.3 minutes, caused by the valve change, and at 28 ± 0.5 minutes, caused by the solvent change. The same effects were observed in Fig. 3 (A and B chromatograms).

The mobile phase, water:acetonitrile (70:30 v/v) gave good results, a separation without interference, with the use of these two columns (one for pre-concentration and the other for separation) with column switching (Fig. 3, A chromatogram).

The use of HPLC grade acetonitrile as the carrier for the organochlorine pesticides from the ISRP to the analytical column gives the best results, permitting the aldrin, endrn, and DDT separation, (see Fig. 3, A and B chromatograms) and the methoxychloro and heptachloro separation, (see Fig. 4, A and B chromatograms) when employing the analytical column mentioned.

On the ISRP column the extraction of these organochlorine pesticides was excellent, with recoveries of 50 ~g/ml additions to the milk samples beig 99.3 % (n=5). Elimination of any sample treatment, as described by others (1-10) make the procedure much simplier, and quicker to do, and, above all, minimizes sample loss. Such a facile method shoul appeal to many laboratories doing such analyses of pesticides.

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