

Separating organochlorine pesticides by high performance liquid chromatography. A technical note

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Abstract

Nitrile and silica phases were tested as column packing for normal phase liquid chromatographic separations of organochlorine pesticides in mobile phase consisting of n-heptane. Excellent separation for the pp'-DDD, op'-DDD (isomers), op'-DDT and pp'-DDT (isomers) was achieved using a nitrile column, but no isomer separation was attained using a silica column. Evaluation of the chromatographic parameters (α =selectivity, R=resolution, N=theoretical plate number, and k' =capacity factor) was performed for each column.

Key words: Organochlorine pesticides, high performance liquid chromatography.

Separación de pesticidas organoclorados por cromatografía líquida de alta precisión. Nota técnica

Resumen

Columnas empacadas con ciano y sílica se usaron en cromatografía líquida de alta precisión para la separación de pesticidas organoclorados, como fase móvil se usó el n-heptano. Los organoclorados pp'-DDD, op'-DDD (isómeros), op'-DDT y pp'-DDT (isómeros) fueron eficientemente separados usando columnas de ciano, sin embargo, ninguna separación se logró usando columnas de sílica. Para cada columna se evaluaron los parámetros cromatográficos (α = selectividad, R = resolución, N = número de platos teóricos, y k' = factor de capacidad).

Palabras clave: Pesticidas organoclorados, cromatografía líquida de alta precisión.

Introduction

The determination of the levels of chlorinated hydrocarbon residues is important because of their persistence in the environment. Levels of organochlorine pesticides residues in a range of food stuffs entering the UK have been monitored [1,2]. Ogata et al. [3] and Estep et al. [4] used a classical method for determining pesticides in non-fatty foods involving extraction of organochlorine pesticides with an organic solvent, elution with two solvents and analysis by gas chromatography. Several analysts [5-7] injected the clean-up into one column of the gas chromatography, then into another with a different packing material which alters

the ratio of the retention time. Recently, Fernandez et al. [8] have reported a method for the detection and quantitative determination of organochlorine pesticides. After extraction with hexane, the pesticides were cleaned-up by adsorption chromatography. The detection of organochlorine pesticides was performed by capillary gas chromatography with electron-capture detector. More recently, Fingler et al. [9] reported the analysis of organochlorine pesticides in samples of rain and snow. All samples were analysed by capillary gas chromatography using an electron-capture detector. Finally, Trevisani et al. [10] proposed a gas chromatographic multi-residue method for the study of residues

from several groups of pesticides. This method involves complicated clean-up steps that make it extremely slow.

Since all the above methods are either insufficiently effective or too complicated, many researchers prefer a preliminary separation of the chlorinated hydrocarbons by liquid chromatography. Some [11,12] chose Florisil as the stationary phase. Most [13-18] select silica gel, which has remarkable adsorbing power.

The main objective of this work was to investigate the selective separation of organochlorine pesticides using a nitrile and silica column by normal phase HPLC.

Experimental

Apparatus and chemicals

The HPLC system consisted of a Waters 510 pump equipped with a U6K universal injector. Detection was performed using a Waters 484 turnable absorbance detector operating at 230 nm. The columns used were: (1) stainless steel, 25 cm x 4.6 mm I.D., Lichrosorb Si 60, 10 mm, manufactured by Hibar-Merck, (2) stainless steel, 25 cm x 4.6 mm I.D., Zorbax CN, 10 mm, made by Dupont. n-hep-

tane and hexane used as mobile phase was HPLC grade from Fisher Scientific. All solvents from HPLC analysis were redistilled from glass before use.

High purity standard of: pp'-DDD (p,p'-dihydroxydichlorodiphenylmethane), op'-DDD (o,p'-dihydroxydichlorodiphenylmethane), op'-DDE (o,p'-dichlorodipenyldichloro-ethylene), pp'-DDT (p,p'-dichlorodiphenyltrichloroethane), op'-DDT (o,p'-dichloro-diphenyltrichloroethane) aldrin, and heptachlor, were purchased from Alltech Associates. Stock solutions were prepared by dissolving the organochlorine pesticides in hexane. Peaks were identified by comparison of retention time with those of standards.

Results and Discussion

Separation of organochlorines using silica (Fig. 1A) and nitrile columns (Fig. 1B) are illustrated in Figure 1. This figure shows a good separation of organochlorines using both silica and nitrile column. As expected, the retention time of each organochlorine was longer using a silica column because of the highest polarity of this stationary phase. It is worth noting in this figure that the first four peaks corresponding to aldrin (peak 1), hep-

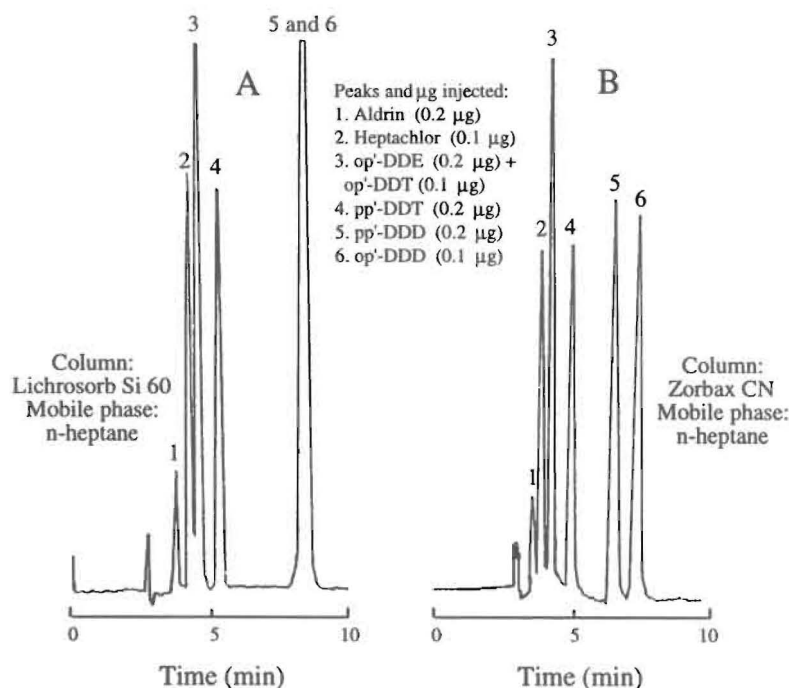


Figure 1. Chromatograms of organochlorine pesticides using a silica column (A) and a nitrile column (B).

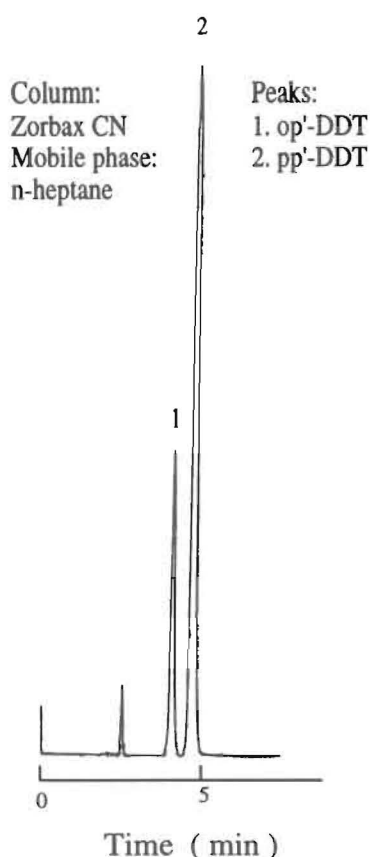


Figure 2. Chromatogram of *op'*-DDT (peak 1) and *pp'*-DDT (peak 2).

tachlorine (peak 2), *op'*-DDE (peak 3), and *pp'*-DDT (peak 4) show almost the same separation, but for the last two peaks corresponding to the isomers *pp'*-DDD (peak 5) and *op'*-DDD (peak 6), no separation was attained using a silica column. Nevertheless, a complete separation was achieved using a nitrile column for the six organochlorine compounds. This is a good finding because of most researchers used silica gel column for these type of analysis.

Figure 2 shows the HPLC separation of the DDT isomers using the same nitrile column and n-heptane as mobile phase. In this separation, the DDT isomers, are separated into two main peaks corresponding to *op'*-DDT (first peak) and *pp'*-DDT (second peak). A complete separation of these isomers was attained using these chromatographic conditions.

Evaluation of the chromatographic parameters (α = selectivity, R = resolution, N = theoretical plate number, and k' = capacity factor) was performed for each column used in this analysis. Results for each pair of organochlorine pesticides are shown in Table 1 and Table 2. The separation attained shows a high selectivity for both statio-

Table 1
Chromatographic parameters α and R for each pair of organochlorine pesticides using Lichrosorb-Si and Zorbax-CN columns

| Organochlorine Pesticides | Lichrosorb-Si α | Zorbax-CN α | Lichrosorb-Si R | Zorbax-CN R |
|-----------------------------------|---------------------------|-----------------------|----------------------|------------------|
| Aldrin-Heptachlor | 1.45 | 1.13 | 1.67 | 0.29 |
| <i>op'</i> -DDE - <i>pp'</i> -DDT | 1.30 | 1.82 | 1.71 | 2.00 |
| <i>pp'</i> -DDD - <i>op'</i> -DDD | 1.00 | 1.29 | 0 | 1.54 |

Table 2
Chromatographic parameters N and k' using Lichrosorb-Si and Zorbax columns

| Organochlorine Pesticides | Lichrosorb- Si N | Zorbax- CN N | Lichrosorb- Si k' | Zorbax- CN k' |
|---------------------------|-----------------------|-------------------|------------------------|--------------------|
| Aldrin | 2139 | 1806 | 0.42 | 0.307 |
| Heptachlor | 1726 | 3402 | 0.62 | 0.346 |
| <i>op'</i> -DDE | 5625 | 3802 | 0.73 | 0.423 |
| <i>pp'</i> -DDT | 4225 | 1469 | 1 | 0.769 |
| <i>pp'</i> -DDD | - | 2584 | 2.23 | 1.34 |
| <i>op'</i> -DDD | - | 2572 | 2.23 | 1.73 |

nary phases with a run time of 10 min. Because of their polarity properties, the pp'-DDD and op'-DDD molecules showed strong retention for both stationary phases. Nevertheless, the nitrile column was more selective for this pair of compounds. Also, these table shows the influence of the stationary phase such as silica, the separation of polar organochlorines is incomplete, therefore the selectivity factor and resolution decrease. But on the other hand, as the stationary phase polarity decrease such as nitrile, separation of more polar organochlorines is easily achieved, increasing resolution and selectivity.

Conclusion

Nitrile and silica column can be used successfully for preliminary separation of organochlorines pesticides. As the polarity of organochlorine increase, better separation can be achieved using nitrile column than silica column because of the highest polarity of the silica column. Thus, selectivity and resolution decrease for this stationary phase.

Excellent separation for the pp'-DDD, op'-DDD (isomers) op'-DDT and pp'-DDT (isomers) was achieved using a nitrile column, but none separation was attained using a silica column.

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