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INVESTIGACIÓN

Obtention of highly purified fractions of eicosapentaenoic acid and docosahexaenoic acid from sardine oil by silver-resin chromatography: A semi-preparative procedure

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RESUMEN

Obtención de fracciones altamente purificadas de ácido eicosapentaenoico y docosahexaenoico de aceite de sardina mediante cromatografía en columna utilizando resina impregnada con ión plata: Un procedimiento semi-preparativo.

Mediante cromatografía en columna utilizando resinas impregnadas con ión plata se separaron fracciones puras de ácido eicosapentaenoico (EPA) y ácido docosahexaenoico (DHA) a partir de un concentrado de EPA+DHA obtenido de aceite de sardina. Se utilizaron dos tipos de resinas impregnadas con plata: Amberlite IR-118H y Dowex 50 W-HCR-W2. La columna de Amberlite-plata permite la separación de fracciones casi puras de EPA (98,5%), siendo incapaz de separar el DHA de otros ácidos grasos poliinsaturados. La columna de Dowex-plata no separa el EPA a partir del concentrado, pero permite la obtención de una fracción de DHA de alta pureza. El procedimiento cromatográfico que se describe permite obtener fracciones puras de dos importantes ácidos grasos poliinsaturados de la serie n-3 que pueden ser utilizados para la investigación nutricional o farmacológica, o como sustratos para la obtención de lípidos estructurados.

PALABRAS-CLAVE: Aceite de sardina – Ácido docosahexaenoico – Acido eicosapentaenoico – Cromatografía en columna utilizando resina impregnada con ión plata – Procedimiento semi-preparativo.

SUMMARY

Obtention of highly purified fractions of eicosapentaenoic acid and docosahexaenoic acid from sardine oil by silver-resin chromatography. A semi-preparative procedure.

Pure fractions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were obtained from silver-resin column chromatography of EPA+DHA concentrates obtained from sardine oil. Two types of silver-impregnated resins were assayed; Amberlite IR-118H and Dowex 50 W-HCR-W2. The Amberlite-silver column allows the separation of almost pure fractions (98.5%) of EPA, being unable to separate DHA from other polyunsaturated fatty acids. The Dowex-silver column does not separate the EPA from the concentrate but allows the isolation of a highly purified fraction of DHA. The chromatographic procedure described may provide pure forms of two important n-3 polyunsaturated fatty acids for nutritional or pharmacological research or as substrates for the obtention of structured lipids.

KEY-WORDS: Docosahexaenoic acid – Eicosapentaenoic acid – Sardine oil – Semi-preparative procedure – Silver-resin column chromatography.

1. INTRODUCTION

During the last years, interest in the nutritional and pharmacological effects of dietary polyunsaturated fatty acids and specifically of n-3 polyunsaturated fatty acids has increased (Simopoulus, 1991; Uauy *et al.*, 1992). Two of these n-3 fatty acid are of particular interest; eicosapentaenoic acid (20:5, EPA) and docosahexaenoic acid (22:6, DHA). Both fatty acid have been involved in important physiological actions (Uauy & Valenzuela, 1992). EPA ingestion can has effects on cardiovascular diseases through a variety of mechanisms (Glosmet, 1985) and DHA in essential for the development of the neural and vision function, mainly in neonates (Uauy *et al.*, 1989). Samples enriched in these fatty acids are needed to futher investigate their nutritional, health and biochemical effects and to serve as secondary analytical standards. Different procedures for the obtention of EPA and DHA concentrates at laboratory scale, including urea complexation and interesterification with specific lipases allowing the obtention of concentration up to 90% for both fatty acids, have been developed (Haagsma *et al.*, 1982; Ackman *et al.*, 1988; Haraldsson *et al.*, 1989). However, the nutritional and pharmacological research on these fatty acids needs pure forms of either EPA and DHA.

It has been known for many years that silver ion undergoes a reversible reaction with unsaturated fatty acids to form rather weak and unstable complexes (Scholfield, 1979). This characteristic allows the separation of polyunsaturated fatty acids by silver resin column chromatography obtaining highly purified fractions of different mono, di- and polyunsaturated fatty acids (Hoque *et al.*, 1972; Adlof & Emkem, 1981). In this work we describe two comparative procedure for the obtention of almost pure fractions of EPA and

DHA by silver-resin chromatography starting from a concentrate containing up 90% of EPA+DHA, as free fatty acids, obtained from sardine oil by selective winterization und urea complexation (Nieto *et al.,* 1996).

2. MATERIALS AND METHODS

Polyunsaturated fatty acid concentrates containing up to 90% of EPA + DHA as free fatty acids were obtained from freshely processed sardine oil as previously described (Nieto *et al.,* 1996). Methyl ester derivatives of the fatty acids from the concentrate were obtained by treatment with boron trifluoride-methanol (Morrison & Smith, 1964). Amberlite acid resin (IR 118H), obtained from Rohm and Haas (USA), and Dowex acid resin (50 W-HCR-W2), obtained from Sigma Chemicals (St. Louis, MO, USA), previously washed in a beaker with toluene, acetone and water, were separatedly transferred to 45 cm x 3.5 cm i.d. water-jacketed glass columns. Both resins were transformed to the sodium form by passing a 1M solution of sodium nitrate in water through the column until the eluent was no longer acidic. Then the resins were treated with 0.4M silver nitrate solution added drop by drop by a period of 30-45 min., allowing the addition of 500-600 mL of silver nitrate solution. In this process effluent from the column is first neutral and free of silver ion, then silver ion breaks through (assessed as silver chroride precipitation by hydrochloric acid). Finally when the resins were saturated the effluent becomes acidic, silver ion being still present. The treated resins were washed for excess of silver ion with water, followed by water: methanol (1:1 v/v), methanol and finally petroleum ether (40-60°C). Samples of 2 g of concentrate were added to each column (held at 20°C) and eluted with acetone: acetonitrile (5.5: 4.5 v/v). Fractions of 0.5 mL of eluants were collected and assessed spectrophotometrically at 210 nm for the cualitative identification of the elution profile. Fractions of the eluant corresponding to each peack and showing up to 0.25 optical density units were mixed, rotatory evaporated under nitrogen stream, disolved in hexane, and assessed by gas-liquid chromatography for fatty acid identification. Chromatograms were obtained from a Hewlett Packard HP 5890 Plus gas-chromatograph equiped with a flame-ionization detector and a split injector. The column used was a fused capillary SP 2330 (30 m x 0.25 mm i.d). Helium was the gas carrier (1.5 mL/min). Peack area integration was assessed by a Hewlett Packard integrator (model HP 3396A). The temperature program was as described previously (Nieto *et al.,* 1996).

3. RESULTS AND DISCUSSION

Table I shows the EPA and DHA composition of the concentrate, expressed as percentage of the total fatty acids present in the sample, compared to the original fatty acid composition of sardine oil before the concentration procedure. The concentrate shows a total absence of saturated fatty acids, the presence of less than 2% of monoinsaturated fatty acids, and a minor proportion (4%) of docosatetraenoic acid (22: 4, n-6, DTA) and docosapentaenoic acid (22: 5, n3, DPA), (not shown, Nieto *et al.,* 1996). Individual concentrations of EPA and DHA in the concentrate are 41.1% and 50.2% respectively.

Table I
Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) composition (% methyl esters) of sardine oil before and after the concentration procedure*
(From Nieto *et al.*, 1996)

	Before Concentration Procedure	After Concentration Procedure
EPA	11,8	41,1
DHA	17,7	50,2
EPA + DHA	29,5	91,3

* Results represent a typical concentration procedure.

The chromatographic profile of the EPA + DHA concentrate (as methyl ester derivatives) obtained from the Amberlite-and Dowex-silver impregnated columns is shown in Figure 1A and 1B respectively. It can be observed that the Amberlite-silver column (Fig. 1A) allows the obtention of a narrow and defined peack corresponding almost exclusively to EPA, which after gas chromatograhic analysis shows a purity up to 98.5%. However, the DHA profile is wide and poorly defined, being also contamined with the presence of DTA and DPA. The elution profile obtained from the Dowex-silver column (Fig. 1B), is less defined than the profile obtained from the Amberlite column, but allows the obtention of a peack containing a high concentration of DHA (97%) as assessed by gas chromatography. This column also allows the separation of EPA from the rest of the fatty acids, but with less resolution than obtained from the Amberlite-silver column chromatography. As can be observed from Fig. 1 (A and B), both column allows the separation of highly purified fractions of EPA and DHA as methyl esters when starting from a concentrate containing the two polyunsaturated fatty acids. However, chromatography from Amberlite-silver column appears as more suitable for the separation of EPA, the Dowex-silver column being better for the separation of DHA.

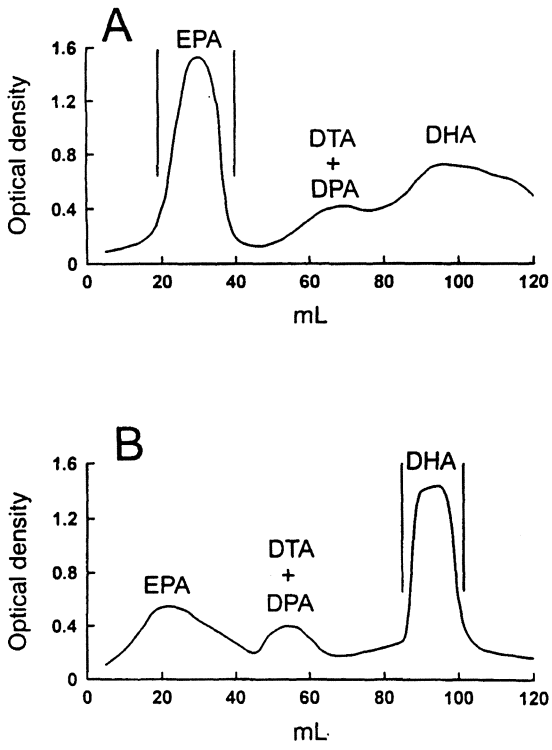

Figure 1
Separation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by silver-resin column chromatography from a concentrate containing both fatty acids (90% EPA + DHA) obtained from sardine oil. (A) Amberlite IR 118H-silver column and (B) Dowex 50 W-HCR-W2-silver column. DTA: docosatetraenoic acid. DPA: docosapentaenoic acid. Fractions between paralel lines were mixed for the recovery of each fatty acid.

Ours results show that it can be possible the separation of almost pure forms of EPA and DHA as methyl ester derivatives by silver-resin column chromatography. These columns normally can be used almost indefinitely without loss of resolution or sample capacity, which is a distinct advantage over other separation system, such as silica gel/silver nitrate system (Adlof & Emkem, 1985). Silver-resin chromatography, which is relatively inexpensive at laboratory scale (the column can be reused and the solvents recovered), may be a useful tool and good help when highly purified fractions of EPA and DHA are needed. As an example these fractions may be used for the obtention of EPA or DHA containing triacylglycerols, or for the specific incorporation of these fatty acids to mono or diacylglycerols. The structural modification of lipids (e. g. «designer lipids» or «taylor-made lipids») by biotechnological procedures (such as lipase transesterification) for specific purposes is now an important challenge for the oleochemical industry (Valenzuela & Nieto, 1994). The chromatographic procedure described here may provide n-3 polyunsaturated fatty acids as substrates for this enzyme technology for the obtention of structured lipids.

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