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The use of solid phase extraction columns in fatty acid purification

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Abstract

Solid phase extraction (SPE) columns with an aminopropyl bonded stationary phase are commonly used to isolate fatty acids. We tested SPE columns with tubes composed of three different materials, high density polyethylene (HDPE), HDPE coated with a fluorinated polymer similar to Teflon® (FP-HDPE) and glass, to assess the effects of these products on the purity of fatty acid extracts derived from the different SPE columns. Products made of HDPE released significant levels of short chain (mainly C_{16} and C_{18}) fatty acids during elution, while glass and FP-HDPE yielded little contamination. These results urge caution in the use of HDPE products during analysis of short chain fatty acids, especially in applications involving the use of stable isotope ratio values that could be significantly altered by small amounts of contaminants.

1. Introduction

Fatty acids are widely occurring lipids used as energy stores, cellular membrane components and external coatings by plants and animals. They are ubiquitous in nature and are commonly studied by the life sciences/biomedical industry, biogeochemists, paleoecologists and paleoclimatologists (e.g. Russell et al., 1997; Gong and Hollander, 1997; Sessions et al., 2002; Huang et al., 2002; Hu et al., 2003; Hughen et al., 2004;). The analysis of free fatty acids from naturally occurring materials generally involves: (1) extraction of lipids; (2) separation of free fatty acids; (3) conversion of free fatty acids to methyl esters (FAMEs); (4) analysis of FAMEs using gas chromatography (GC), GC/mass spectrometry (GC/MS and/or GC/isotope ratio MS (GC–IRMS); e.g. Huang et al., 2002; Sessions et al., 2002; Hughen et al., 2004).

Fatty acid purification is often accomplished using SPE columns with a bonded aminopropyl stationary phase, which may be used to separate organic samples into three fractions: lipids of low to medium polarity, free fatty acids and a phospholipids fraction (Kaluzny et al., 1985). Currently, pre-fabricated SPE columns are commonly used in this procedure and consist of a fixed volume of aminopropyl-silyl stationary phase packed into high density polyethylene (HDPE) barrels with polyethylene frits to hold the packing in place. Teflon® coated and glass barrels, Teflon® frits and stainless

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Table 1
Yields (ng) of C_{16} and C_{18} FAMEs for samples in Fig. 1A–F

Sample	Chromatogram	C_{16}	C_{18}
HDPE column 1	Fig. 1A	1016	2585
HDPE column 2	Fig. 1B	225	354
HDPE column adaptor	Fig. 1C	1898	4262
FP-HDPE column 1	Fig. 1D	23	39
FP-HDPE column replicate	Fig. 1E	26	32
Aminopropyl-packed glass column	Fig. 1F	8	12

steel frits are also used in place of polyethylene. HDPE may degrade in the presence of some of the solvents used in the elution of lipids from these columns (O'Toole, 1983) but, despite the variety of SPE columns and their common use in the biogeosciences, we know of no study comparing different types of SPE columns for potential analytical artifacts such as polyethylene-derived contaminants.

Here, we compare the contaminants in the fatty acid fractions derived from different brands of high density polyethylene, HDPE with a fluorinated polymer coating (similar to Teflon®) and glass aminopropyl SPE columns. The results show varying but significant levels of contamination released by HDPE solid phase extraction columns, with significant implications for their use in fatty acid analysis.

2. Methods

All chemicals used were Fisher Optima* grade, or were distilled to remove organic contaminants. Each reagent was methylated (see below) and analyzed using GC to ensure purity prior to usage.

Four types of aminopropyl SPE columns (50 mg phase, 3 mL barrels) were tested. Two consisted of HDPE barrels with polyethylene frits manufactured by two different companies. The third column used Teflon® frits inside a FP-HDPE barrel. We packed 500 mg of aminopropyl stationary phase into a glass column using a 'frit' of cotton wool pre-extracted with a 2:1 solution of CH_2Cl_2 and MeOH for the fourth column. The procedures followed published methods for isolating fatty acids from natural samples (Sessions et al., 2002; Huang et al., 2004). The barrels of the SPE columns were pre-rinsed repeatedly with Fisher Optima* grade methanol and conditioned with 8 mL MeOH followed by 8 mL 2:1 CH_2Cl_2:isopropanol. Certain tubes were also conditioned with 8 mL 4% glacial acetic acid in diethyl ether in an attempt to remove any fatty acid contaminants potentially bound to the SPE packing. Following column conditioning, 0.5 mL 2:1 CH_2Cl_2:isopropanol was applied to each column, followed by elution with 8 mL 2:1 CH_2Cl_2:isopropanol, 8 mL 4% glacial acetic acid in diethyl ether and 8 mL MeOH. The stationary phase was dried between application of the different solvents. Elution was accomplished using positive pressure from high purity N_2 connected to the column using copper and Tygon™ tubing with an in-line hydrocarbon scrubber and Teflon® adaptors to couple the tubing to the top of the barrels. Pressure was set to yield flow rates of 2 mL/min. In addition to testing SPE columns, manufactured HDPE SPE column adaptors, often used to couple syringes to the columns, were pre-cleaned and soaked for 5 min in 8 mL 4% glacial acetic acid in diethyl ether.

The 4% glacial acetic acid in diethyl ether fractions, containing the fatty acids, were collected in 15 mL glass centrifuge tubes and dried under a stream of high purity N_2. Each fraction was methylated by heating with MeOH/MeCOCl and the resulting solution was diluted using a saturated aqueous NaCl solution; the methyl esters were extracted from the aqueous solution with hexane. The sample in hexane was dried over Na_2SO_4 and evaporated to dryness under N_2.

Samples were all made up to 0.2 mL by addition of hexane and 1 μL of each was co-injected with 1 μL of a 2 ng/μL androstane standard for compound identification and quantification. Identification was performed with a Hewlett-Packard 6890 gas chromatograph (GC) coupled to an HP 5973 mass spectrometer (MS). The GC used a split/splitless injector in splitless mode and an HP-1 fused silica capillary column (25 m × 320 μm i.d., film thickness 0.5 μm). The oven temperature was programmed from 50 °C to 130 °C at 10°/min, and then at 4°/min to 320 °C, where it was held for 10 min. He was used as carrier gas at a constant flow rate of 2 ml/min. Operating conditions for the mass spectrometer were 70 eV ionizing energy and scan range m/z 50–650. C_{16} and C_{18} FAMEs and *n*-alkanes were identified from their mass spectra, retention times and comparison with published spectra. Quantification was performed using the same GC conditions as for GC–MS, except the He flow rate was 2.6 ml/min, by peak area integration and comparison with the standard.

3. Results

None of the methods yielded perfectly clean chromatograms, reflecting trace levels of impurities derived from solvents or other reagents. However, the same set of solvents and equipments was used throughout, so large quantities of contaminants appearing in only some of the chromatograms (Fig. 1) can be attributed solely to compounds introduced by the different SPE columns. Comparisons of results with and without pre-cleaning showed that cleaning had little effect on the results; results are shown for columns that were pre-cleaned using MeOH and pre-conditioned using CH_2Cl_2: isopropanol.

Columns made with HDPE barrels released significant amounts of C_{16} and C_{18} fatty acids into the ether:glacial acetic acid eluent (Fig. 1A and B see Table 1), with C_{16} and C_{18} FAME yields ranging from 226 to 2585 ng in the final product. Trace levels of C_{14}, C_{15} and C_{17} fatty acids were also released. Tests of the ether:glacial acetic acid solution confirmed that the contaminants enter as fatty acids, not methyl esters (the latter were not present in the ether:glacial acetic acid). Soaking pre-cleaned HDPE SPE column adaptors, which are composed only of HDPE, in the ether:glacial acetic acid solution also released large quantities of C_{16} and C_{18} fatty acids (Fig. 1C). Trace levels of *n*-alkanes (peaks between 35 and 45 min in Fig. 1) were also present in many samples, and were most abundant in HDPE products. These alkanes range from C_{24} to C_{30} and, unlike the fatty acids, exhibit little even over odd preference.

In contrast, neither the FP-HDPE nor the aminopropyl-packed glass columns released large quantities of fatty acids (Fig. 1D–F), with C_{16} and C_{18} FAME yields ranging from 40 to 8 ng. Average C_{16} and C_{18} FAME yields from FP-HDPE columns were about 30 times lower than those released by the HDPE columns.

4. Discussion and conclusions

Neither FP-HDPE nor glass SPE columns released significant quantities of fatty acids during

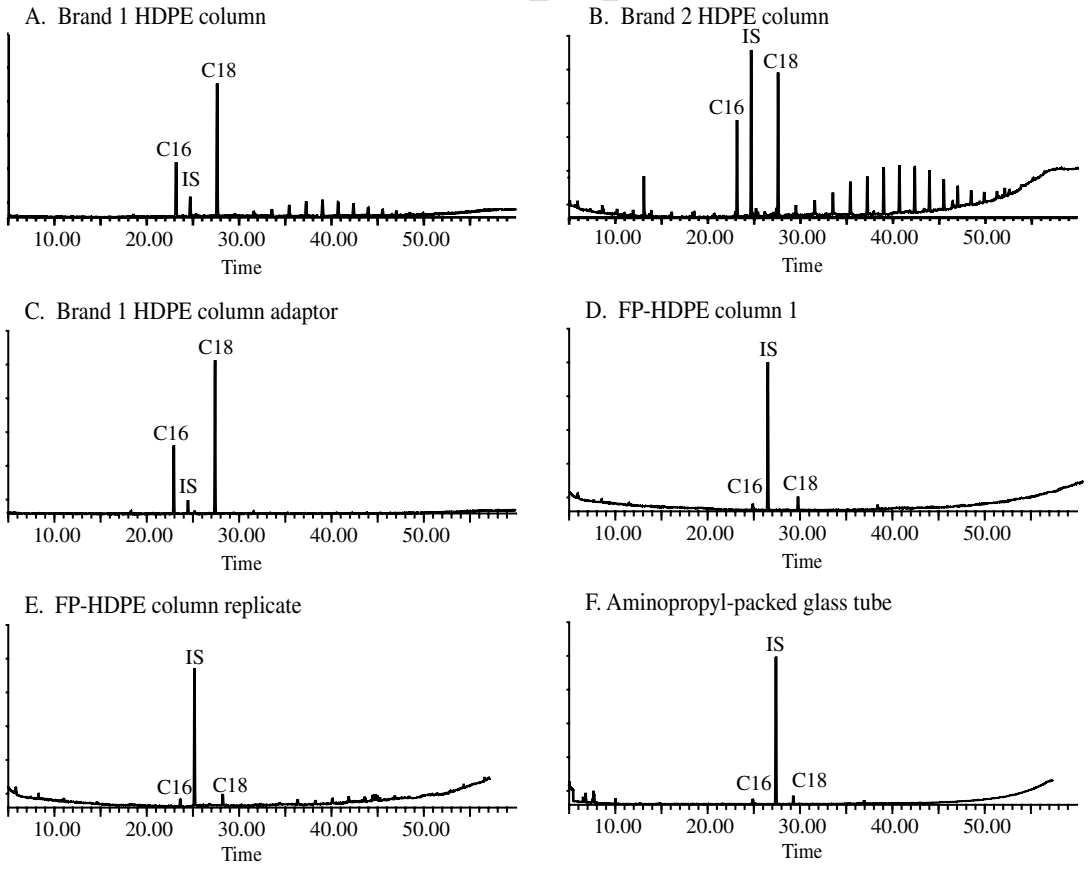

Fig. 1. Chromatograms of acid fractions released by HDPE columns (A and B), HDPE column adaptors (C), fluorinated polymer coated HDPE (D and E) and glass (F). C_{16} and C_{18} indicate C_{16} and C_{18} fatty acid methyl esters, and IS indicates the androstane standard.

the elution procedure, while both HDPE SPE columns and other HDPE products such as the SPE column adaptors did. These results indicate that the source of the contamination is the HDPE itself, as opposed to contamination released by the aminopropyl stationary phase. The results have obvious implications for the analysis of short chain fatty acids using SPE columns. We do not argue that all HDPE products will necessarily yield contaminants: plastic manufacturing procedures could yield variations in the degree of contamination given by different generations of SPE columns. Nevertheless, our results urge caution in the use of HDPE products for purifying fatty acids.

Broadly speaking, we urge caution in developing and applying procedures for isolating commonly occurring compounds from natural samples. Our study was initiated to develop techniques for isolating fatty acids from lake sediments for paleoenvironmental reconstruction. We have applied the procedure outlined above and in Huang et al. (2004) to sediments from Lake Edward, Uganda-Congo (containing between 4% and 16% TOC) using FP-HDPE SPE columns. We found that organic extracts from these sediments contain C_{16} and C_{18} fatty acid concentrations ranging from ~200 to 1150 ng/g TOC (unpublished results). This is very similar to the level of fatty acids released by HDPE SPE columns marketed to purify organic acids.

In conclusion, our results suggest the need for care in isolating short chain fatty acids using aminopropyl SPE columns composed of HDPE. This is especially true for studies involving stable isotopic analysis, in which small amounts of plastic-derived contaminant could significantly bias isotopic measurements. The results do, however, indicate that "off-the-shelf" FP-HDPE or glass SPE columns yield clean short chain fatty acid isolates.

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