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Letter

Fatty acid contaminations originating from commercially available solid phase-extraction columns

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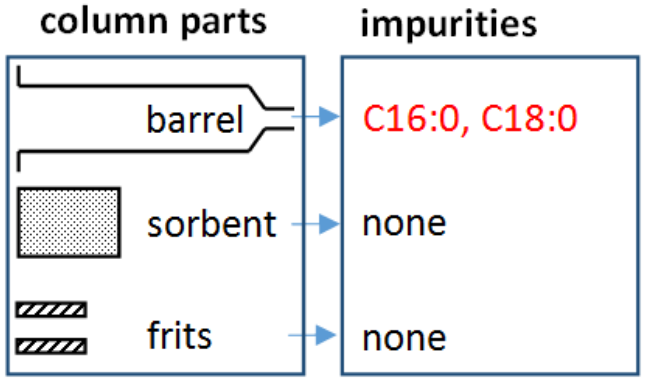
Abstract

It has been shown previously that commercially available solid phase extraction (SPE) columns release contaminants such as palmitic acid and stearic acid during separation of lipid classes. The presence of contaminating fatty acids in the fatty acid fraction is particularly troublesome. We here confirm that the overwhelming majority of the contaminants originate from the barrels, and have identified two contaminants as palmitic acid and stearic acid or their equivalents. We urge readers to take fatty acid contaminants into careful consideration when planning their experiments, and if necessary use sorbent packed in glass barrels instead of plastic such as polypropylene.



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Introduction

Solid phase extraction (SPE) is widely used to separate lipid phases: the original method published by Kaluzny *et al.* [1] was cited over 700 times as of October 2015. Although the method of Kaluzny *et al.* fractionates the lipid classes into many group, for many studies it is sufficient to fractionate the lipids into three groups: i) triacylglycerols by eluting with chloroform-2-propanol (2:1), ii) free fatty acids by eluting with 2% acetic acid in diethyl ether and iii) polar lipids by eluting with methanol *e.g.* [2]. Already three years after publication of Kaluzny *et al.*[1], Prasad *et al.* reported the presence of contaminants in fractions eluted by 2% acetic acid in diethyl ether from a commercially available SPE column [3]. By using the aminopropyl sorbent packed in glass columns, Prasad *et al.* demonstrated that the majority of contaminations originated from the other parts of the commercially available column, most likely the polypropylene barrels, since stainless steel frits were used [3]. In a similar vein, Notter *et al.* reported that collection of eluent in polypropylene tubes resulted in contaminations by palmitic and stearic acid [4]. Contamination was significantly reduced by collecting eluent in glass tubes, though some contamination remained in all fractions and was attributed to the commercial SPE columns.

The discovery by Notter *et al.* of fatty acid contaminations (or possibly, but less likely, their methyl-ester equivalents) in all fractions is troublesome, though it is particularly unfavorable in the free fatty acid-fraction: fatty acids originating from the SPE column are indistinguishable from fatty acids originating from the analyzed material. Depending on the amount of lipid initially added to the SPE column, the contaminants may have a major impact on the results: The data of our previous study [5] was marred by the presence of palmitic and stearic acid. For the sum of all fatty acids, the presence of the contaminants was negligible, *i.e.* less than 3%. However, for stearic acid, a minor constituent of the material under investigation in [5], the contamination by stearic acid in the SPE blank was in the same order of magnitude as the endogenous stearic acid, as assessed by direct transmethylation (*i.e.* the sample was not in contact with an SPE column). Although a blank was included in [5], the variance between triplicates was large, possibly a result of different lengths of time the solvent was in contact with the polypropylene barrels, since solvents eluted at different rates under the influence of gravity.

Although the presence of contaminants has been reported before, we are of the opinion that the subject has not received sufficient attention. We herein set out to illustrate the presence of contaminants in three commercially available SPE columns which may be used with the method of Kaluzny *et al*. [1] or variations thereof.

Experimental

The following three SPE columns were used: i) BondElut, 500 mg NH2 sorbent, 6 ml reservoir from Agilent Technologies, ii) Isolute, 500 mg NH2 sorbent, 3 ml reservoir from Biotage, iii) Telos, 500 mg NH2 sorbent, 6 ml reservoir from Kinesis. Working in duplicates, the columns were dismantled into barrels, frits and sorbents, see **Figure 1**. The barrels were placed in 60 ml glass jars with Teflon-lined screw-caps; the upper rims of BondElut and Telos columns were trimmed to fit into the jars with trimmings added into the jar. The frits were placed into separate 60 ml glass jars. The sorbents were placed inside glass Pasteur pipettes, with a small wad of glass wool acting as frit at the bottom. To each of the glass jars, 10 ml of 2% acetic acid (Scharlau, ≥ 99.8% purity) in diethyl ether (Sigma ≥ 99.8% purity) was added, 0.1 mg nonadecanoic acid (Larodan, ≥ 99% purity) as internal standard in 0.1 ml chloroform (Sigma, ≥ 99.8% purity) was added, jars were capped and tumble-agitated (Heidolph Reax 2, speed 3) for 19 h at room temperature. Thereafter, the solvents were transferred to 12 ml glass test tubes with Teflon-lined screw caps. The sorbent in Pasteur pipettes was conditioned with 6 × 1.0 ml hexane (Sigma, ≥97.0% purity). The neutral lipid-fractions were eluted with 4 × 1.0 ml 2:1 (v/v) chloroform and 2-propanol (Fisher, HPLC-grade) and collected in glass test tubes containing 0.1 mg nonadecanoic acid in 0.1 ml chloroform. The fatty acid-fractions were eluted with 4 × 1.0 ml 2% acetic acid in diethyl ether and collected in glass test tubes containing 0.1 mg nonadecanoic acid in 0.1 ml chloroform. Solvents in all glass test tubes were evaporated at 40 °C under $N_{2(g)}$. Residuals were re-suspended in toluene (Sigma, 99.9% purity) and methylated and separated by gas chromatography-mass spectroscopy according to the HCl-method given in [6]; nonadecanoic acid was used to quantify peaks, and peaks were identified by primarily comparing retention times to those of a standard mix (GLC 463, NuChek Prep.) and secondarily by confirming that the ion fragmentation pattern was typical of a fatty acid.

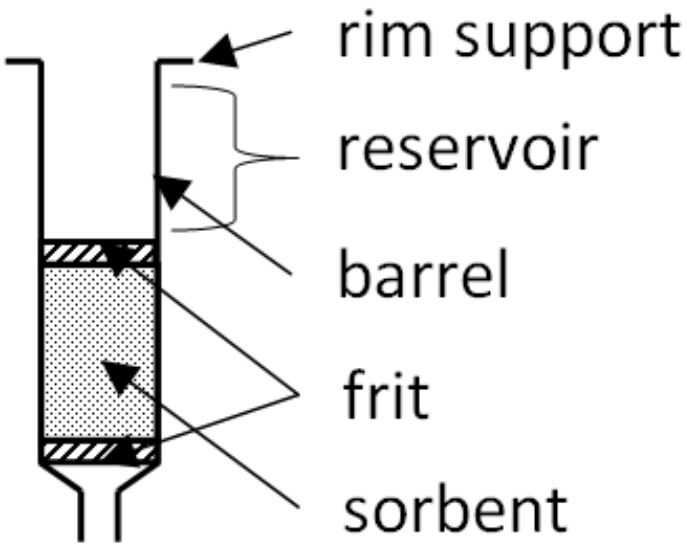

Figure 1 parts of a commercially available SPE column

Results and Discussion

From the frits in contact with 2% acetic acid in diethyl ether, sorbent in contact with chloroform-2-propanol and sorbent in contact with 2% acetic acid in diethyl ether, methyl esters of palmitic acid and stearic acid were present below the limit of quantification (10 × baseline) or even below the limit of detection (3 × baseline), see **Table 1** and **Table 2**. Equally small amounts of other compounds were detected, though these were not identified as fatty acids. This presence of only minute amounts of palmitic acid and stearic acid suggests that the frits and the sorbent are not the main source of the contamination; possibly, the contaminants were carry-over from the barrels.

Table 1 Presence of palmitic acid equivalents in the barrels, frits and sorbents of three different commercially available SPE-columns.

C16:0	barrel	frits	sorbent, NL-fraction	sorbent, FA-fraction
BondElut	0.7	n.q.	n.q.	n.q.
Isolute	0.7	n.q.	n.q.	n.d.
Telos	1.4	n.q.	n.q.	n.q.

n = 2, with means reported; n.q. = not quantified, n.d. = not detected;
amounts are given in mg for 10 ml solvent in contact with the barrels for 19 h;
note that barrel dimensions differ

Table 2 Presence of stearic acid equivalents in the barrels, frits and sorbents of three different commercially available SPE-columns.

C18:0	barrel	frits	sorbent, NL-fraction	sorbent, FA-fraction
BondElut	0.7	n.q.	n.q.	n.d.
Isolute	1.5	n.q.	n.q.	n.d.
Telos	1.9	n.q.	n.q.	n.q.

n = 2, with means reported; n.q. = not quantified, n.d. = not detected;
amounts are given in mg for 10 ml solvent in contact with the barrels for 19 h;
note that barrel dimensions differ

The 2% acetic acid in diethyl ether in contact with the barrels contained at least 0.7 mg palmitic and stearic acid equivalents each, and a myriad of other compounds, though these were not identified as fatty acids and would therefore not interfere with fatty acids analysis. Under regular circumstances, the barrel would not be exposed to solvent for 19 h, and furthermore, the exposed surface area would be smaller under regular conditions, with only the inner part of the barrel in contact with the solvent. None-the-less, the results serve to illustrate the leaching of undesirable compounds from the barrel to the solvent. The difference in dimension precludes comparing the Isolute barrel to the BondElut and Telos barrels: the latter provided a greater surface area because of both a larger reservoir and that they were cut to fit into the extraction jars; with a greater contact area between barrel and solvent, diffusion rates are likely to differ. In spite of the difference in barrel dimension, the BondElut column releases overall the least amount of fatty acids and would therefore appear to be the preferable choice. However, readers are urged to confirm that the amounts of fatty acids leached from the columns is acceptable for their purposes: in our case [5], unpublished data, 200-230 µg fatty acid equivalents (sum of palmitic and stearic acid equivalents) was found during a regular blank run on Telos columns, which was unacceptable for our purposes. We suggest that readers intent on using pre-made SPE columns with polypropylene barrels for lipid class separation, investigate if the amount of contaminants can be reduced to an acceptable level by reducing the time that the solvents are in contact with the barrel, *e.g.* by using a vacuum chamber. For applications in which no contaminating fatty acid equivalents can be tolerated, we recommend using glass barrels, as demonstrated by Prasad *et al.*[3].

Conclusions

Palmitic and stearic acid or their equivalents were extracted from the barrels of three different types of commercially available solid phase extraction columns. To avoid these contaminations, we suggest using sorbent packed in glass barrels.

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