Solid-Phase Extraction Columns for Lipid Analysis

Abstract: Solid-phase extraction methods are being used for increasing numbers of applications in lipid analysis, but especially as a rapid means of isolating particular components for further analysis. Many different types of packing material are available enabling most different modes of chromatography to be used, including adsorption, reversed-phase, silver-ion and ion-exchange. However, there are occasions when too much is expected of the methodology.

Solid-phase extraction columns makes use of small columns made of an impermeable plastic material and packed with a variety of adsorbents held in place by frits, under trade names such as Bond Elut™, Sep-Pak™, etc. The first reaction of most analysts to their introduction was probably one of pleasant surprise and wonderment that no one had had such a simple idea before. This was certainly my first thought, then my Scottish blood (we have a reputation for thriftiness) asserted itself and I began to wonder whether the costs could be justified in comparison to purchasing adsorbents separately and using disposable pasteur pipettes as mini-columns. In time, I have come to the conclusion that both approaches have their merits. In simple clean-up procedures with silica gel or Florisil™ as the adsorbent, the pasteur pipette technique is often suitable. The commercial pre-packed columns are available in such a wide range of packing materials, with uniform reproducible properties, that their value for specific purposes cannot be denied. I will discuss some of their applications here.

I reviewed the methodology in relation to lipids more comprehensively earlier [1], and this review is available online here. Others have also reviewed the topic [2]. In addition, you will find more than 300 references to the use of the technique in the literature survey section of the website.

The term "solid-phase extraction column" now appears to be widely accepted for the commercial columns, largely because one of the main uses is in the extraction of materials of interest from biological fluids such as plasma or urine. It is a rather clumsy expression, which can perhaps be made more tolerable by abbreviating it to "SPE column", although "sorbent column" might be neater. Apart from silica gel and Florisil™, which have been much used by lipid analysts as adsorbents over the years, SPE columns are available packed with materials to which various functional groups are bonded by chemical means to the silanol groups on the surface of the silica gel particles. Those most widely used are probably those with chemically bonded octadecylsilyl (ODS or C_{18}) moieties, which are of particular value in the extraction of lipids from aqueous media. In addition, there is a variety of packing materials with cationic or anionic functional groups. Only a few of these appear to have been explored in analytical applications with lipids, but I will describe some examples below.
Advantages of Commercial Columns

An important virtue of SPE columns from reputable manufacturers is that they are very reproducible in their properties from batch to batch. They are supplied in sealed packs that rigidly exclude atmospheric moisture. This last feature is of particular importance with the conventional adsorbents, such as silica gel, as the properties are markedly affected by degree of hydration. A bottle of silica gel, used to prepare home-made mini-columns and sitting on the bench in the laboratory for long periods, will slowly pick up water from the air, its retention capacity will be reduced and its elution characteristics will change. Also, commercial columns are packed by machine to give a constant density so that elution conditions are fairly reproducible from batch to batch.

It should not be assumed that all SPE columns of the same nominal type and size from different manufacturers will have the same properties. With bonded-phases especially, the chemistry utilized in bonding may vary and the end result may differ. In my experience, some brands can give better results in some applications than others.

Advantages and Limitations of SPE Methodology

A major problem in surveying the literature on the use of SPE columns is in judging what is really an advance, and what is wishful thinking on the part of the authors. For example, a typical SPE column contains no more than 0.5 g of silica gel of a relatively large particle size. It is simply not possible for them to offer separations comparable to those obtainable by high-resolution thin-layer chromatography (TLC) or high-performance liquid chromatography. Yet, it is easy to find descriptions in the literature of separations involving every conceivable lipid class from complicated mixtures from such columns. By a careful choice of mobile phase, low sample loads and slow solvent flow rates, some remarkable separations may occasionally be possible, but in general it is not advisable to ask too much of SPE methods.

Small sample size is not necessarily a disadvantage. For example, in the analysis of insect pheromones, minute amounts of sample may be all that is available. Clean-up and concentration can often be effected on a SPE column more efficiently than by more cumbersome conventional methods, especially if special micro-extraction procedures are used.

While it is possible to purchase manifolds that utilize suction from below to speed up flow rates and permit large numbers of samples to be fractionated simultaneously, I do not advise use for most lipid applications. These were devised for aqueous extractions mainly, and are less suited to organic solvents, which may evaporate rapidly under such conditions causing a partial drying out of the columns. For most separations with organic solvents, gravity elution with perhaps a little help from pressure applied from above via a flexible pipette bulb for more polar solvents is to be preferred.

On the other hand, SPE columns are often suited to robotic analytical systems. So far, I have only seen a few applications to lipids, but more will no doubt be described in the future.

Applications of ODS columns

SPE columns packed with an ODS phase are not widely utilized in the main-stream of lipid analysis, but they do have some important uses, especially in the extraction of prostaglandins, glycolipids and related lipids from biological fluids. Such compounds are often present at trace levels only, but relatively large volumes of an aqueous medium can be passed through a column, concentrating the lipophilic material on the stationary phase. The lipids are subsequently recovered by elution with an organic solvent. It is essential before using these or other bonded-phase
columns to recognize that they must be subjected to solvation to ensure that the sorbent layer is wetted prior to use. This is accomplished by passing through the column several bed volumes of a solvent such as methanol, which can interact both with the carbon atoms of the bonded functional group and the free silanols on the silica surface. The excess solvating agent is then removed by elution with a solvent that prepares the sorbent layer to receive the sample. The choice of solvents for these purposes will depend on the nature of the lipids to be separated or extracted.

Powell [3] was one of the first to use ODS columns for the extraction and isolation of prostaglandins from urine, plasma and tissue homogenates. The acidified fluids are passed through the column, which retains the required compounds, then phospholipids, proteins and other polar impurities are removed by elution with aqueous ethanol; non-polar lipids are eluted with hexane and the prostaglandins are finally recovered with methyl formate. This basic method with occasional modifications has now been accepted in many laboratories. At about the same time, a similar procedure was introduced for the isolation of water-soluble complex lipids such as gangliosides from aqueous extracts [4].

Subsequently, SPE columns packed with ODS phases were utilized for such applications as the isolation of fatty acids from dilute solution in sea water, for separating isotopically-labelled phospholipids from the water-soluble precursors, to eliminate silica gel from lipids separated by preparative thin-layer chromatography, to separate cyclic monomers from higher polymers in oxidised oils, and for the isolation of polar lipids, such as free acids, sphingosine-1-phosphate, platelet-activating factor and lysophospholipids from cellular homogenates or even directly from plasma. This list is far from exhaustive, but it indicates the main types of application at least.

ODS columns of this type have also been used for reversed-phase separations of fatty acid methyl esters. Approximately 1 mg of samples rich in eicosapentaenoate has given useful enrichment of components of interest by elution with acetonitrile-water mixtures [5]. Pure components were not isolated, but the method may be of value in preparing concentrates of specific components for further analysis. Comparable methods have been used for isolation of a fraction enriched in very-long-chain fatty acids (> C22). One practical advantage of columns of this type is that they can often be used several times before they need to be discarded.

Silica Gel Columns

SPE cartridges packed with silica gel have been much used by lipid analysts for the fractionation of total lipid extracts into simpler classes. For example, the phospholipids in milk fat amount to less than 1% of the total lipid; after eluting the simple lipids first with hexane-diethyl ether, the phospholipids can be recovered by washing the column with a chloroform-methanol-water mixture [6]. This is an important type of separation for lipid analysts, and one not readily achieved by HPLC. It can be done easily enough by TLC, but not in such a clean and efficient manner, as spray reagents and other impurities from the TLC adsorbents often contaminate the fractions. Similar methods have been developed by many others, adapted if need be to recover the individual simple lipids and glycolipids. Again, further examples could have been selected, but as cautioned above it is easy to expect too much from the technique.

Similar SPE methods have been developed for the isolation of oxidized lipid classes, such as sterol oxides, where the simplicity and rapidity of the methodology reduces the opportunities for artefact formation.

The main application in our laboratory is to clean up methyl ester preparations from clinical samples to remove cholesterol and non-lipid impurities that might otherwise interfere with gas chromatographic analysis. To my mind this is the ideal type of application for SPE columns, as the large difference in polarity between the required analyte and the impurities means that the separation is accomplished quickly and efficiently. The labour time thus saved more than
compensates for the cost of the columns. Similar methods can be used to purify other types of derivative prior to more refined analyses by instrumental methods.

**Anion-exchange columns**

Better separations of lipid classes have been accomplished on SPE columns with bonded aminopropyl groups (anion-exchange columns). Columns of this type offer greater potential for specific separations, as there are then opportunities for ionic forces and hydrogen bonding effects to come into play. For example, Kaluzny *et al.* [7] were able to isolate up to ten different lipid classes in a reasonable degree of purity by sequential elution with solvents of increasing polarity. It is important to note that their claim that acidic phospholipids, such as phosphatidylinositol and phosphatidylserine, can be recovered quantitatively from columns of this type by simple elution schemes has been refuted by a number of analysts.

However, it is possible to get good recoveries by adding ionic species to the mobile phase. By a careful choice of mobile phases, it is possible to use aminopropyl columns to separate complicated mixtures of lipids into five clean fractions, i.e. containing only simple lipids, free fatty acids, glycolipids, zwitterionic phospholipids (phosphatidylcholine and phosphatidylethanolamine) and acidic phospholipids [8]. A similar method has been used to isolate phosphatidylglycerol from lung surfactant [9].

Columns of this type have been much used for separations of oxidized lipids and their derivatives, where hydrogen-bonding effects with the bonded NH$_2$ groups improve the specificity of separations. They have also been used for sphingolipid classes.

**Silver Ion Columns**

My last general example is one developed in my own laboratory, *i.e.* to convert SPE columns containing a bonded phenylsulphonic acid phase to the silver ion form in order to separate lipids by degree of unsaturation [10]. By eluting in a stepwise fashion with dichloromethane-acetone-acetonitrile mixtures of increasing polarity, it is possible to separate methyl esters of fatty acids with zero to three double bonds cleanly from each other, and with care acceptable separations of fatty acids with four to six double bonds. With the standard small column available at that time (0.5 g adsorbent), it was only possible to work on the 0.25 mg scale), but this was sufficient for gas chromatography-mass spectrometry purposes. Supelco (Sigma-Aldrich) are now selling solid-phase extraction columns packed with an ion-exchange medium pre-impregnated with silver ions under the trade name 'Discovery Ag-ION SPE'. They recommend this for the separation of cis- and trans-monoenoic fatty acids, especially.

It is not hard to envisage the use of this methodology for prostaglandins, steroids, insect pheromones and a host of other applications. It is reviewed from a practical standpoint elsewhere on this website.

**Other Potential Applications**

Weak cation-exchange columns containing bonded propionic acid groups have been employed to obtain distinctive separations of sphingoid bases [11], and there must be scope for further novel applications.

It is easy to predict that many more applications of SPE columns of silica gel and ODS will be described. More use could surely be made of the latter for separating lipids according to molecular size, and there are other hydrophobic bonded-moieties available (octyl, cyclohexyl and phenyl) to
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be tried. Indeed, I am certain that much more could be made of the many other SPE columns available commercially with bonded functional groups. For example, diethylaminoethyl-cellulose and carboxymethyl-cellulose have proved of great value for the separation of complex lipids in conventional column chromatography. Without doubt, some of the structurally related amine and acid-containing bonded phases in SPE columns would be of value in such applications. Phenylboronic acid SPE columns might permit the isolation of di- and monoacylglycerols while preventing acyl migration; they could also prove of value in the separation of glycolipids or perhaps of phosphoinositides. Perhaps diol or cyanopropyl columns could be utilized for separations of simple lipids, as in HPLC. We shall see what transpires in time.

References


This article has been updated appreciably from two earlier papers (now amalgamated) by the author that first appeared in Lipid Technology (Christie, W.W. Lipid Technology, 3, 31-33 (1991) and 15, 16-19 (2003)).

WW Christie

Scottish Crop Research Institute (and Mylnefield Lipid Analysis), Invergowrie, Dundee (DD2 5DA), Scotland

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