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SOLID PHASE EXTRACTION IN FOOD ANALYSIS

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In this study, a Solid Phase Extraction (SPE) as a method of sample preparation has been described. A typical procedure, the mechanisms involved in SPE and factors which affect effectivity have also been discussed. Possible uses of SPE in fragrance and food chemistry have been presented.

1. Introduction

Solid phase extraction (SPE) is an increasingly useful technique for the isolation, separation and concentration of analytes from liquid samples, which allows avoiding many problems associated with liquid/liquid extraction, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, emulsion formation and disposal of large quantities of organic solvents. It also offers several advantages: reduced lab time, easy manipulation, higher concentration factor, no problem with the miscibility of solvent, easy adaptable for very selective extraction, easy automatisation.

Although SPE in the column mode is very effective, it has also some drawbacks, such as channeling, limited flow rates, insufficient equilibration time for quantitative uptake, incomplete elution, and memory effects from previous extractions. Though these disadvantages are more pronounced only in chelating resins.

2. SPE theory

An SPE device consists of a resin bed packed into a small extraction tube, usually made of plastic. The resin is packed between two frits to hold the resin bed securely in place. A liquid sample is passed through the resin bed by applying either positive pressure or vacuum to the column.

A typical procedure of solid phase extraction involves four steps. First, the column is conditioned with an appropriate solvent to solvate functional groups of the sorbent. After the sorbent is further conditioned with the sample matrix solvent, the sample solution is forced through the sorbent by aspiration or positive pressure. The column containing retained analyte is subsequently washed with an appropriate solvent that selectively elutes impurities but leaves the analyte on the column. The purified analyte is finally eluted with a solvent strong enough to displace the analyte from the sorbent.

The mechanisms involved in solid phase extraction are:

1) normal phase chromatography whose procedures typically involve a polar analyte, a mid- to nonpolar matrix (e.g. acetone, chlorinated solvents, and hexane), and a polar stationary phase. Polar-functionalized bonded silicas (e.g. LC-CN, LC-NH2, and LC-Diol), and polar adsorption media (LC-Si, LC-Florisil, ENVI-Florisil, and LC-Alumina) are typically used under normal phase conditions. Retention of an analyte under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding, pi-pi interactions, dipole-dipole interactions, and dipole-induced dipole interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism — usually a solvent that is more polar than the sample's original matrix.
2) reversed phase chromatography whose procedures involve a polar (usually aqueous) or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid- to nonpolar. Several SPE materials, such as alkyl- or aryl-bonded silicas (LC-18, ENVI-18, LC-8, ENVI-8, LC-4, and LC-Ph) are in the reversed phase category.
3) Ion exchange chromatography that can be used for compounds which are charged when in a solution (usually aqueous, but sometimes organic). Anionic (negatively charged) compounds can be isolated on LC-SAX or LC-NH2 bonded silica cartridges. Cationic (positively charged) compounds are isolated by using LC-SCX or LC-WCX bonded silica cartridges. The primary retention mechanism of the compound is based mainly on the electrostatic attraction of the charged functional group on the compound to the charged group that is bonded to the silica surface. In order to retain a compound by ion exchange from an aqueous solution, the pH of the sample matrix must be such at which both the compound of interest and the functional group on the bonded silica are charged. Also, there should be few, if any, other species of the same charge as the compound in the matrix that may interfere with the adsorption of the compound

of interest. A solution having a pH that neutralizes either the compound's functional group or the functional group on the sorbent surface is used to elute the compound of interest. When one of these functional groups is neutralized, the electrostatic force that binds the two together is disrupted and the compound is eluted. Alternatively, a solution that has a high ionic strength, or contains an ionic species which displaces the adsorbed compound, is used to elute the compound.

Factors affecting ion-exchange selectivity are:

- pH – the retention of ionic compounds is achived by promoting ionization. The optimum pH for 100% ionization of an ionic analyte depends on its pKa, defined as the pH at which 50% of the ionizable groups are charged and 50% are neutral. Adjustments of the pH of an acid analyte solution to two pH units higher than the pKa of the analyte results in approximately 99% ionization.
- ionic strength – a measure of the total concentrations of ionic species in the matrix, influences retention of an ionic analyte. High concentrations of extraneous cations in the sample matrix, for example, will compete with the cationic analyte for available acidic sites.
- organic solvent – in some instances, the solubility of the neutral form of an acid or base is much lower in water compared to the ionic form. Consequently, the analyte may become insoluble when an elution solvent is used which converts it to the neutral form. A water-miscible organic solvent must be added to the elution solvent to effectively elute such compounds.
- flow rate – since ion-exchange interactions occur at a slower rate than polar or nonpolar interactions, a flow rate less than 5 mL/min for sample solution is suggested.

4) size exclusion which is used for removing unbound radioisotope, desalting or buffer exchange of protein solutions. Smaller molecules enter the pores of the hydrated carbohydrate polymer and are significantly retarded as the sample solution percolates through the gel [1, 2].

3. SPE applications

Solid phase extraction is widely used in analytical chemistry, for example in the fields referring to environment, pharmacy, food and cosmetics.

3.1. Analysis of volatile components

As far as essential oils are concerned, SPE is used for their pre-separation and fractionation prior to gas chromatographic analysis as well as after superheated water extraction (SWE). Both of these applications – before GC analysis and after SWE, can be illustrated by examples given below.

According to Antonelli and Fabbri [3], essential oils can be divided in two categories – the former one includes essential oils which are very complex mixtures whose components belong to different classes of compounds and the latter one consists of essential oils with one major component comprising up to 90% with a few other minor compounds which can be important for the quality and difficult to identify. That is why silica SPE is needed for pre-separation of essential oils which consequently leads to formation of three fractions: the first one containing all hydrocarbons, both saturated and unsaturated, the second one which contains carbonyl compounds, ethers, esters and tertiary alcohols and the third one containing primary alcohols, acids and diols. Such a fractionation helps to avoid problems with peak overlapping or coeluting substances as well as to facilitate the concentration of remaining fractions after removal of the major compound in the latter group of essential oils.

As a frequent aim of SWE is to avoid the use of organic solvents, it is suggested to apply extraction methods that are solvent free or use minimal amounts of solvents. SPE is exactly such a technique which enables extraction of retained analytes with a small volume of elution solvent [4].

An endcapped C_{18} SPE cartridge was used after superheated water extraction of fragrance compounds from *Rosa canina*. In this case, a solvent used for elution was hexane and identified compounds were mainly benzyl alcohol, benzaldehyde, phenylethyl alcohol, 2,6,11-trimethyl dodecane, eicosane, tetrahydroional and limonene [5].

The most frequently used sorbent in the analysis of volatile components is octadecyl (C_{18}) that enables reversed phase extraction of mid- to nonpolar analytes.

A column with such a stationary phase was utilized in the analysis of volatile compounds of *Rosa damascena* after extraction with superheated water, which is obligatory when extracting essential oils on a laboratory scale. For elution, a mixture of hexane and ethyl acetate was used and there were 37 eluted and identified components with percentage higher than 0.05%. The major ones were linalool, phenylethyl alcohol, citronellol, nerol and geraniol [6].

An identical procedure – with the same sorbent and eluent – was applied for reextracting analytes from the aqueous extract of *Origanum onites*, which are mainly carvacrol, borneol, terpinen-4-ol, α-terpineol, thymol and linalool [7]; as well as in the work concerning essential oils of *Achillea monocephala* leaves and flowers. The major compounds were camphor and borneol for the leaf oil and camphor, borneol, 1,8-cineole and α-campholenal for the flower oil [8]. Dichloromethane was used for elution of compounds retained on C_{18} cartridge while isolating the volatile and semi-volatile compounds of *Salvia officinalis* leaves infusion. These compounds were α-thujone, camphor, 1,8-cineole, 6-oxobornyl acetate, β-thujone, 1-borneol, exo-2-hydroxycineole acetate [9].

Octadecyl sorbent was also proved to be the best sorbent for retaining hexanal and hexanol − the compounds responsible for beany flavour of soymilk. Many sorbents were tested − considering polarity of hexanol and hexanal reverse-phase

sorbents were preferable, but only one adsorbed both mentioned compounds, which were then eluted with methanol [10].

A solid phase extraction on a trifunctional silane SPE C_{18} cartridge was utilized to evaluate residues of essential oil components in honey. Some of essential oils have been tested successfully against the mite and are applied to control varroosis but the drawback of such a natural treatment is a high level of residues found in honey that may change its taste. Retained compounds such as thymol, menthol, eucalyptol, and camphor were eluted with acetone [11].

An endcapped C_{18} SPE cartridge and hexane as eluent were used for reextracting the analytes after subcritical water extraction of essential oils from *Thymbra spicata*, which were carvacrol and thymol − more than 90%, (E)-car-3-en-2-ol and enantiomers of α-pinene. In this work, also the efficiency of the C_{18} material was tested with steam-distilled sample of essential oil of *T. spicata* with a known composition, which showed that there were almost no changes in the sample composition after the application of SPE of C_{18} material [12].

Another cartridge used in the analysis of volatile compounds is a florisil cartridge with MgO/SiO_2 phase, that enables reversed phase extraction of polycyclic aromatic hydrocarbons. An example of such an use can be a separation of *Smyrnium olusatrum* stem essential oil from aqueous phase after hydrodistillation. Three fractions were collected using dichloromethane, dicholoromethane–methanol and methanol in turn. As NMR and GC-MS analysis showed, the first fraction was constituted by furanodiene, while the second fraction was constituted by another compound which remained unknown [13].

Styrene-divinylbenzene as a solid phase and dichloromethane as an eluting solvent turned out to be the best combination for isolation of terpenoids, which was proved in the work concerning determination of terpenoids in wines, where the authors studied the influence of several extraction variables, including the solid phase employed (C_{18} versus divinylbenzene-based) and eluting solvent (n-pentane, dichloromethane, ethanol and methanol) [14].

In the analysis of volatile compounds in eucalyptus honey the cartridge containing polypropylene-divinylbenzene as stationary phase was used. Five groups of these compounds: terpenes and derivatives, ketones, furan and pyran compounds, norisoprenoids, and other compounds were then eluted with dichloromethane [15].

Among many different branches of science that can take advantage of solid phase extraction there is even pharmacy which uses SPE for determination of biologically active components in plant materials − an excellent example of such use can be a work on the determination of triptolide in root extracts of *Tripterygium wilfordii*. Extracts were applied to a weak anion-exchange Strata-NH_2 column and then triptolide-containing fraction was eluted with dichloromethane–methanol [16].

3.2. Determination of residues of noxious substances

SPE can also be utilized to determine residues of noxious substances like pesticides, insecticides, toxins or antibiotics in food and environment. For such a purpose, octadecyl SPE cartridge can be successfully used as well. As an example, separation from water sample and concentration of picloram herbicide can be given. Picloram was retained on such a column and then eluted with acetic acid [17]. The same sorbent was used to evaluate the amount of pesticides in natural water. Although the authors observed that many sorbents like, for example macroreticular amberlite XAD resins, C_8- or C_{18}-modified silica and graphitized carbon black can be used for this aim, they decided to use an octadecyl cartridge. Eight different nitrogen- and phosphorus-containing pesticides such as: alachlor, azinphos-ethyl, chlorfenvinphos, chlorpyriphos, deltamethrin, ethoprophos, fenamiphos and malathion pesticides were retained on the sorbent and then eluted with ethyl acetate which was proved to be the most effective solvent [18].

As far as antibiotics are concerned, solid phase extraction on RP-18 cartridge – applied for compounds with nonpolar groups – was used as a pre-separation technique for high-performance liquid chromatography of tetracyclines in meat, milk and cheese, which allowed us to replace microbial methods of detection. In this case methanol served as an elution solvent [19].

In determination of pyrethroid insecticide residues in vegetable oils a combined column packed with deactivated basic alumina and C_{18} was proved to be the most effective. The use of acetonitrile extract as an elution solvent provided the best results [20].

Another cartridge utilized in such analysis is the above-mentioned florisil. This sorbent was used to extract pesticide residues from essential oils of citrus fruit. Pesticides used in the citrus industry were eluted with dichloromethane as using this solvent enables the best compromise between elution volume and the separation of pesticides from polar compounds in the matrix [21].

Florisil was also used to analyse polychlorinated dibenzo-p-dioxins/ dibenzofurans (PCDDs/PCDFs) and polychlorinated biphenyls (PCBs) in spieces, as one of the clean-up steps [22].

For compounds that are charged when in solution, an ion exchange sorbents are applied like for example aminopropyl (NH_2) weak anion exchange sorbent, which was used for separation from water and concentration of dicamba pesticide, which was then eluted with K_2HPO_4. What is more, the authors of this work found that pesticide recovery was enhanced by about 10% when pesticides were eluted from the cartridges using several small aliquots as compared to one large aliquot [17].

Tandem graphitized carbon black (GCB) and anion exchange SPE columns were utilized for the cleanup of plant extracts so as to determine organochlorine and organophosphorus pesticides in fresh fruits and vegetables. To elute pesticides, an acetonitrile-toluene mixture was used, however there were few pesticides (e.g. acephate, bromophos ethyl, leptophos) that required a larger volume of solvent

than others. These pesticides can be more rapidly eluted with an acetone-toluene mixture [23].

For separation of aflatoxin B_1 in the research for optimal cooking treatment for reduction of aflatoxin AFB_1 contamination in wheat, an Oasis HLB cartridge with nonpolar sorbent was used. As washing was considered to be one of such methods, it was necessary to determine the amount of AFB_1 in water after washing. AFB_1 was then extracted from water, retained on the column and eluted with methanol consecutively [24].

3.3. Food analysis

Solid phase extraction is also used in food analysis. For instance, this method was used to clean-up cholesterol after classical Soxhlet ectraction and a supercritical carbon dioxide extraction from egg-containing food. In the former case the cholesterol fraction was purified by SPE using a silica gel cartridge that enables adsorbtion of polar compounds, and then four fractions were eluted – the first two fractions of hexane and hexane-diethyl ether mixture were discarded and the third and fourth fractions were eluted with a hexane-diethyl ether mixture and methanol – these fractions were collected. In the latter case the solid trap was rinsed with methanol and then with hexane. The methanol fraction was collected [25]. Cholesterol was also determined in animal fats. It had to be purified from glycerides on Sep-Pak cartridge (Strata-NH_2) – a weak anion exchange sorbent. Triglycerides were washed off the cartridge with a mixture of n-hexane-ethyl acetate and finally cholesterol was eluted with a chloroform-methanol mixture [26].

In the work concerning detection of irradiated meats, the solid phase florisil cartridge was utilized so as to retain the hydrocarbons, formed due to irradiation, which were consecutively eluted with n-hexane [27].

One of the latest applications of SPE in food chemistry is fractionation of *cis/trans* fatty acid methyl esters (FAMEs). According to appropriate regulations, fat content, considering type of fats, must be listed on the Nutrition Facts panel of food, so it is necessary to find an effective and reliable technique of analysis. As *trans* fats are difficult to resolve on capillary GC columns, it becomes necessary to reduce the sample complexity prior to GC analysis. For this purpose, silver-ion solid phase extraction (Ag-ion SPE) is used. Silver ions are anchored onto SCX SPE functional group as a counter-ion and act as an electron acceptor that forms polar complexes with double bonds of unsaturated FAMEs. As *cis* substituted double bonds are more steric accessible than *trans*, they can form stronger complexes with Ag-ions and be longer retained on stationary phase, which makes fractionation possible [28].

4. Conclusion

As it was shown, solid phase extraction is a useful technique applied in many fields of chemistry. Its future is closely related to improvement of sorbents that can

be even more effective and selective, however even now this method has so many advantages that it should become increasingly popular.

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ESTRAKCJA DO FAZY STAŁEJ W ANALIZIE ŻYWNOŚCI

Streszczenie

Opisano technikę ekstrakcji do fazy stałej (Solid Phase Extraction, SPE), stosowaną jako metoda przygotowania próbek do analizy za pomocą chromatografii gazowej. Omówiono zasadę działania, scharakteryzowano mechanizmy odpowiedzialne za proces rozdziału i czynniki wpływające na efektywność. Przedstawiono możliwe zastosowania SPE w chemii żywności i substancji zapachowych, ze wskazaniem rodzajów wypełnienia i zatrzymywanych związków chemicznych.