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SOLID-PHASE EXTRACTION: SORBENT SELECTION

See **III / SORBENT SELECTION FOR SOLID-PHASE EXTRACTION**

SOLID-PHASE MATRIX DISPERSION: EXTRACTION

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Introduction

Matrix solid-phase dispersion (MSPD) is a patented analytical process for the preparation, extraction and fractionation of solid and/or viscous biological samples prior to instrumental or other forms of analysis. MSPD involves the direct mechanical blending of samples with standard solid-phase extraction (SPE) bonded-phase solid support materials. In this process, the bonded-phase support acts as both an abrasive to produce disruption of sample architecture and as a 'bound' solvent that assists in accomplishing sample disruption. The sample is dispersed over the surface of the bonded-phase support material, producing a unique mixed-character phase for conducting target analyte isolation. This process has been applied to the isolation of a wide range of drugs, pollutants and other compound classes from a variety of sample matrices. The factors affecting the use of MSPD and its applications for sample preparation, extraction and fractionation are addressed.

Development of MSPD

The classical application of all forms of liquid chromatography requires that the sample be applied in a liquid state to the head of the column. Thus, in order to accommodate the use of solid-phase extraction (SPE) columns and discs in the development of an analytical procedure, methodology must also be developed to render the sample and target analytes contained therein into a liquid, relatively non-viscous, particulate-free and homogeneous condition. While some biological fluids are readily obtained in this form, most biological samples do not start out being directly applicable to SPE. This presents the analyst with some rather unique opportunities to apply or develop the best process for rendering a sample into a form compatible with liquid chromatography. Indeed, the most difficult and complex samples to analyse are the solids and semi-solids that are derived from a biological origin. Such samples may be obtained from animal or vegetable material and consist of a non-homogeneous array of fat and/or other tissues, fibre, pulp, etc.

For these and other reasons, the preparation of biological samples for SPE requires an initial disruption of the gross architecture of the sample. This step in the process assures access to all of the components of a sample and initiates the necessary homogeneity required for analysis. Disruption and homogenization also provide a larger overall surface area, generating greater access to solvents and reagents used for analyte isolation, which is the next step in the process. Samples that are by their nature already reasonably homogeneous, such as milk, fruit juices, plasma, etc., are less complicated in this regard but may be too viscous or contain particulates that could hinder rapid SPE extraction and fractionation. For such samples, dilution, filtration and/or centrifugation often solve these problems.

Classical processes for solid or semi-solid sample disruption usually involve one or various combinations

of the following: mincing, shredding, grinding, pulverizing and/or pressurizing of the sample. All of these approaches accomplish the basic requirement of disrupting sample architecture. This initial disruption may be followed or accompanied by the addition of solvents, acids, bases, buffers, abrasives, salts, detergents, chelators, etc., in an effort to more completely disrupt cellular and architectural composition and initiate the extraction and fractionation of various sample components from the analyte(s) of choice. Unfortunately, the creation of often intractable emulsions is often a consequence of these actions and repeated centrifugation, re-extraction and sample manipulation may be required to render the sample suitable for application to an SPE column. Application of this entire process strives to obtain the analyte(s) in solution, free from solids, emulsions or suspensions, reducing the solid sample to a homogenous liquid extract.

In 1989, a technique that remedied many of the complications of dealing with solid samples in their subsequent extraction using solid-phase materials was developed. This was accomplished by literally combining the sample directly with the bonded-phase solid support. This process, designated as matrix solid-phase dispersion (MSPD), was observed to simultaneously accomplish several steps in the more classical approach to sample preparation and SPE extraction/fractionation. A sample (tissue, fruit, etc.) is placed in a glass mortar containing a bonded-phase solid support material, such as octadecylsilyl silica (C_{18}). The solid support and sample are manually blended together using a glass pestle. In this process, the irregularly shaped silica-based solid support serves as an abrasive that promotes disruption of the sample's general architecture, and also acts as a bound solvent which appears to further disrupt the sample by inducing lysis of cell membranes. The blended material is packed into a column suitable for conducting sequential elution with solvents and the blended sample components, and their distribution in the bonded-phase support provides a new phase that exhibits a unique character for performing sample fractionation (**Figure 1**).

Examination of blended tissues by scanning electron microscopy (SEM) showed that sample architecture had been completely disrupted and that sample matrix components had apparently been evenly distributed over the surface of the bonded phase/support, forming an observable layer. The thickness of this 'new phase' of dispersed matrix is approximately 100 μm, similar to that of some micelle or membrane bilayers. Indeed, it appears that this is what occurs in the MSPD process. The sample is distributed over the surface of the bonded phase as a function of interactions with the support and the bonded phase. The tissue matrix components themselves, form a layered phase consisting of support/lipophilic bonded-phase/sample lipids and a further distribution of sample-associated compounds arranged in and on this new phase based on their own relative polarities.

MSPD Versus Other Forms of Chromatography

MSPD is physically and functionally different from classical SPE in the following ways: (1) MSPD is a process that accomplishes sample disruption and dispersal onto particles of very small size, providing an enhanced surface area for subsequent extraction, whereas sample disruption must be conducted as a separate step in preparing samples for SPE; (2) SPE samples must be in a liquid form, relatively free of solids and of moderate viscosity before addition to the column, while MSPD directly handles solid or viscous liquid samples; and (3) The physical and chemical interactions of the components of the system are greater in MSPD and different, in some respects, from those seen in classical SPE or other forms of liquid chromatography.

In applying the MSPD process to a sample, the interactions observed between the individual components and the target analyte(s) involve (1) the sample components with the solid support, (2) the sample components with the bonded phase, (3) the analyte with the solid support, (4) the analyte with the bonded phase, (5) the analyte with the dispersed sample components, (6) all of the above interacting with the elution solvent(s) and their sequence of addition, and (7) the dynamic interactions of all of the above occurring simultaneously. Nonetheless, general chemical principles involved in conducting SPE and other forms of chromatography are also operable in applying MSPD. Thus, the chemical composition and characteristics of the solid support and bonded-phase are expected to affect the retention and elution of the analytes. These same properties will also apply to the dispersed sample components and the unique phase that is created.

Factors Affecting MSPD Extraction and Fractionation of Samples

To date, only the use of silica-based support materials has been reported for MSPD. The use and effect of synthetic polymer-based solid supports is a subject for further study, particularly supports that possess unique surface and pore chemistries, such as

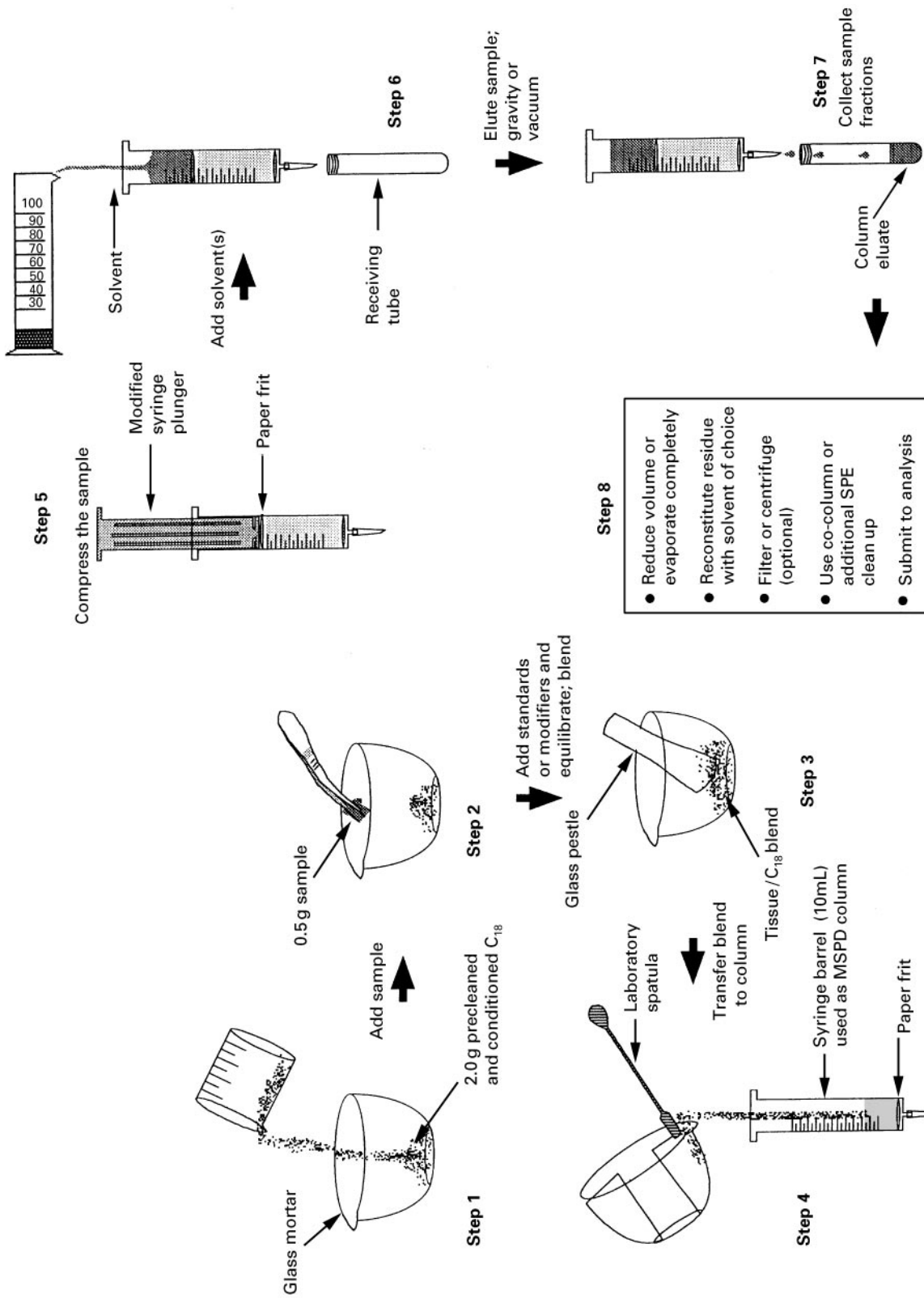

Figure 1 A schematic representation of a MSPD procedure.

hydrophobic interaction supports. For silica-based materials, however, studies have shown that the pore size is of minor importance in MSPD. This effect could vary with the sample and should be considered, however.

The effect of average particle-size diameter has also been examined. As may well be expected, the use of very small particle sizes (3–10 μm) leads to extended solvent elution times for a MSPD column, requiring excessive pressures to obtain adequate flow. However, 40-μm particle size materials (60 Å average pore diameter) have been used most frequently and quite successfully. It has been reported that a blend of silicas possessing a range of particle sizes (40–100 μm) also work quite well, and such materials also tend to be less expensive.

Depending on the application, non-endcapped materials or materials having a range of carbon loading (8–18%) may also be used. It is a simple matter to examine these variables for a given application and should be considered for obtaining the best extraction efficiency and the cleanest sample.

The bonded phase will, of course, play a pivotal role. Depending on the polarity of the phase chosen, rather dramatic effects on the results may be observed and a range of available phases should be examined for each application. It has been reported that in applications requiring a lipophilic bonded phase, C_{18} and C_8 can be used interchangeably and that the best ratio of sample to bonded-phase material is 1 to 4. Most applications have employed lipophilic bonded-phase (C_{18}) materials, blending 2.0 g of solid support with 0.5 g of sample. The best ratio is, of course, dependent on the application and should be examined as a variable during method development. Ratios of bonded-phase to sample less than 4 : 1 have been used successfully and samples have been scaled up to 2 g from the typical 0.5 g used in most MSPD procedures, blended with a proportionately greater amount of solid support.

In general, it has been observed that the isolation of more polar analytes from biological samples is assisted by the use of polar phases (cyanopropyl, for example) and less polar analytes by less polar phases. This would be expected based on retention characteristics of compounds from classical SPE.

Preconditioning of the materials used for MSPD greatly enhances analyte recovery, as has been established with SPE. However, in MSPD it also appears to speed the process of sample blending and dispersal by breaking the surface tension differences that may exist between the sample and bonded-phase solid support. As with SPE, washing or rinsing the solid support materials prior to use also eliminates contaminants from the final eluates obtained for analysis.

MSPD column character may also be altered by modifying the matrix prior to or during the blending step. Several extraction studies designed to isolate a variety of different drugs have shown that addition of chelating agents, acids, bases, etc. at the time of blending affects the distribution and elution of target analytes from the sample. The elution profile of matrix components is likewise affected. This effect can be predicted from basic chemistry and applied in MSPD during sample blending and/or by alteration of the elution solvent composition. This effort to increase or suppress ionization of analytes and sample components greatly affects interactions of specific analytes with the blended phase and the eluting solvent(s). Thus, the use of matrix modification should be considered, as in SPE, as a possible variable to be controlled for attaining reproducible and efficient MSPD extraction.

The correct choice of elution solvents and the sequence of their application to a column is of utmost importance to the success of MSPD or SPE fractionation of samples. Elution solvent sequence and composition can be varied to obtain the best analytical results, when attempting to isolate the analyte or further clean the column of interfering substances with each solvent step. The nature of MSPD columns and the enhanced degree of interaction permit isolation of different polarity analytes or entire chemical classes of compounds in a single solvent or in differing polarity solvents passed through the column. This characteristic makes MSPD amenable to conducting multiresidue isolation and analysis on a single sample. In this regard, true gradient elution of a MSPD column has not been reported to date but should, nonetheless, prove applicable to the complete fractionation of samples.

It has been observed that, in an 8-mL elution of a 2-g C_{18} column blended with 0.5 g of sample, most of the target analytes eluted in the first 4 mL, or in approximately one column volume. This will, of course, vary with each application and with appropriate solvent selection but should be examined to reduce the use of solvent and the elution of other potentially interfering components.

The solid support, bonded phase and solvent elution sequence are all critical in performing MSPD, as they are in SPE. However, they may prove less influential overall than the effect of the sample matrix itself. It should be kept in mind that in MSPD the sample itself is dispersed throughout the column. In contrast, much of the sample is retained only in the first few millimeters of the column bed in SPE. In MSPD, the sample matrix components cover much of the bonded-phase support surface, creating a new phase that is dependent on their interactions with the

solid support and bonded phase, interactions that give the MSPD column its character. This new phase, in association with the analyte's distribution and own interactions with it, are perhaps the most important controlling factors.

This matrix effect has been seen by anyone who conducts chromatography, particularly on large numbers of samples that are less than pristine. Repeated sample injection can create a build-up of non-volatilized or non-eluting sample components at the head of a gas chromatography (GC) or liquid chromatography (LC) column, introducing a new 'phase'. This new phase may subsequently affect the stability, elution and retention character of target analytes as they come into contact with it. The discontinuity of phases within the analytical column may lead to peak tailing, the formation of shoulders or multiple peaks for a single analyte. It can lead, over time, to complete loss of analytes that interact strongly with the new phase. This 'matrix effect', or deposition of sample components as an additional phase, is incorporated throughout the column in MSPD. The dispersion of components establishes a new level of, and consistency in, equilibrium distribution of analytes that is fundamentally different from that seen from limited or discontinuous phases.

Another interesting aspect of this effect is that the analytes tend to co-elute in fractions that are not wholly consistent with predicted solubility behaviour. This observation may underscore a further unique property of MSPD. Elution of a sample is designed to remove the target analyte(s) but, even in SPE, one simultaneously fractionates and co-elutes some of the sample components. In MSPD the total amount of sample components present is much greater. In performing mass-balance experiments, it has been observed that the entire sample, minus a few per cent of what appeared to be denatured macromolecules and connective tissues, can be eluted from an MSPD column. Thus, sequential elution of liver tissue blended with C_{18} recovers 98% of sample triglycerides in hexane and 98% of phospholipids and steroids in dichloromethane. Sugars and polyols are found in the acetonitrile fraction and phosphorylated sugars in water. The presence and concentration of eluted proteins follows the sequence methanol > water > acetonitrile > ethyl acetate. Approximately 7% of the total mass of the sample remains on the column, consisting of connective tissues and denatured macromolecules, including DNA and related nucleotide polymers. Thus, the MSPD process has been used to disrupt otherwise rugged and difficult-to-lyse bacteria, and to simultaneously perform fractionation of the sample for subsequent analysis to identify unique cellular components. These results point to the fact that many of the unique elution properties of MSPD are due, not only to interactions of target analytes with the dispersed matrix but, also, an association of the matrix with the target analyte as specific classes of matrix components are eluted. Therefore, co-elution of target analyte in association with a particular class of matrix component, which is simultaneously interacting with the other materials remaining on the column, seems to be an important factor in the overall chromatographic character of the MSPD process.

The eluates obtained from an MSPD column are often amenable to immediate instrumental analysis, being adequately clean for direct injection. This is, of course, dependent on the sensitivity of the method and whether concentration is required before the analysis. However, in the majority of cases additional steps are required to address the removal of the co-eluting matrix components described above. In a number of cases this additional clean up of the sample has been accomplished by the use of secondary solid-phase materials. For example, bonded-phase material of the same polarity or even of different character than that used in blending can be packed at the bottom of the MSPD column (co-column). Alternatively, the MSPD column may be eluted directly onto a standard SPE column or disc material for the purposes of conducting further sample clean up and/or analyte concentration. Similarly, the eluate may be evaporated and reconstituted for application to another form of chromatography, analysis by immunoassay, etc. Other techniques employed to conduct MSPD eluate clean up have involved the use of classical liquid–liquid extraction, conducted on a small scale, prior to analysis.

While the process of preparing a MSPD column is currently a manual one, the steps of elution, collection and concentration of fractions, etc. are amenable to automation.

Conclusions

A list of MSPD applications for the isolation of a range of compounds from a variety of matrices is shown in **Table 1**. This list illustrates the rather generic character of MSPD for performing the extraction of a variety of matrices for a number of compounds. In most cases, MSPD has been found to provide equivalent results to older official methods conducted by more classical countercurrent and/or SPE techniques. Further, it has been rather consistently observed that MSPD requires 95% less solvent and can be performed in 90% of the time of such classical methods. The use of smaller sample sizes, combined with lower solvent consumption, purchase and disposal, make MSPD competitive

Table 1 Applications of MSPD to the analysis of residues in various matrices

Analyte(s)	*Matrix*
Aminoglycosides	Bovine kidney
Benzimidazoles	Beef liver
Benzimidazoles	Swine muscle
Beta-agonists	Bovine liver
Carbofuran	Corn
Chloramphenicol	Milk
Chlorsulfuron	Milk
Chlorsulon	Milk
Clenbuterol	Bovine liver
Furazolidone	Chicken muscle
Furazolidone	Milk
Furazolidone	Swine muscle
Ivermectin	Fish muscle
Ivermectin	Milk
Ivermectin	Bovine liver
Moxidectin	Bovine tissues
Nicarbazin	Animal tissues
Oxolinic acid	Catfish
Oxytetracycline	Catfish muscle
PCBs	Fish
Pesticides	Beef fat
Pesticides	Catfish muscle
Pesticides	Crayfish
Pesticides	Fish
Pesticides	Fruit, vegetables
Pesticides	Milk
Pesticides	Oysters
Sulfa drugs	Chicken tissues
Sulfadimethoxine	Catfish muscle
Sulfadimethoxine	Catfish, plasma
Sulfonamides	Infant formula
Sulfonamides	Milk
Sulfonamides	Salmon muscle
Sulfonamides	Swine muscle
Sulfonamides	Eggs
Tetracyclines	Milk

with such methods on several levels and should be considered as an alternative when pursuing new analytical methodology. This is especially the case for solid or semi-solid biological materials.

See also: **II / Extraction:** Solid-Phase Extraction; Solvent Based Separation. **III / Solid-Phase Extraction with Cartridges. Sorbent Selection for Solid-Phase Extraction.**

Further Reading

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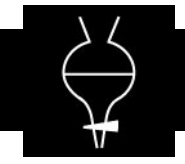
SOLID-PHASE MICROEXTRACTION



Biomedical Applications

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Analysis of drugs in biological samples is growing in importance owing to the need to understand the therapeutic and toxic effects of drugs and to continue the development of more selective and effective drugs. Furthermore, the screening and confirmation of abused drugs in body fluids is important for the detection of potential users of drugs and the control of drug addicts following withdrawal therapy. Simultaneous analysis of these drugs in biological samples