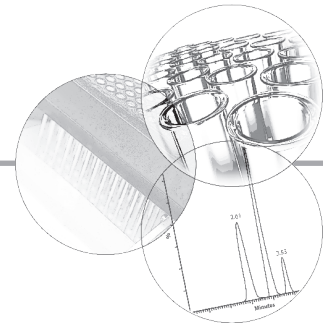

Microextraction by packed sorbent in bioanalysis

"A good sample-preparation method will effectively isolate the analyte of interest from a complex matrix for quantification."

Sample preparation is an important step in the bioanalysis process because of the difficulties that follow when using complex sample matrices, such as plasma and urine. A good sample-preparation method will effectively isolate the analyte of interest from a complex matrix for quantification. It will enhance selectivity and sensitivity, and eliminate the ion suppression, caused by the matrix, in ESI–MS. For the extraction of drugs from urine and plasma matrices, liquid–liquid extraction (LLE) methods require solvent or solvent mixtures of quite high polarity, and these often yield emulsions and a high matrix background in the extracted sample. For even more polar analytes, such as drug conjugates and metabolites, recovery and sample purity is further compromised. SPE is mostly attractive in recovering drug residues because of their ability to efficiently retain highly functionalized compounds from aqueous samples and then to release them into organic solvents on elution. SPE methods are useful for complex biological samples because the main requirements of the extraction (matrix exchange, desalting, removal of macromolecules and highly polar compounds) are well matched to the properties of the sorbent.

"The key aspect of microextraction by packed sorbent is that the solvent volume used for the elution of the analytes is of a suitable order of magnitude to be injected directly into GC or LC systems…"

Microextraction by packed sorbent (MEPS) is a new type of SPE that has been miniaturized to work with sample volumes as small as 10 µl and up to 250 µl. The commercially available presentation of MEPS uses the same sorbents as conventional SPE columns and so is suitable for use with most existing methods by scaling the reagent and sample volumes. The superior performance of MEPS was recently illustrated by online LC–MS and GC–MS assays of drugs and metabolites in urine, plasma and whole blood samples [1–21].

What is MEPS & how does it work?

Microextraction by packed sorbent is a new miniaturized SPE technique that can be connected online to GC or LC without any modifications. MEPS works with much smaller samples (as small as 10 µl) than full-scale SPE. It can be fully automated – the sample processing, extraction and injection steps are performed online using the same syringe. It significantly reduces the volume of solvent and sample needed. In MEPS, the sorbent (1–2 mg) is either inserted into the syringe (100–250 µl) barrel as a plug or between the needle and the barrel **(Figure 1)** as a cartridge. The cartridge bed can be packed or coated to provide selective and suitable sampling conditions. Any sorbent material such as silica-based material (C2, C8, C18 and SCX), restricted-access material, hilic, carbon, polystyrene–divinylbenzene copolymer or molecular imprinted polymers can be used. MEPS is commercially available from SGE Analytical Science, Melbourne, Australia [101].

■ How it works

The plasma or blood sample is drawn through the syringe by an autosampler (which pumps the sample up and down) through the sorbent and then washed once with water (50–250 µl) to remove salts, proteins and other interfering material. The analytes are then eluted with an organic solvent such as methanol or the LC mobile phase (20–50 µl) directly into the instrument's injector GC or LC. The MEPS sorbent can be used for more than 100 extractions before being discarded [1–3].

■ Washing & elution solutions

Water is a typical washing solution and washing volume will be between 50 and 250 µl. The solvent percentage and the pH are important

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Figure 1. Microextraction by packed sorbent product syringe (250 µl), from SGE Analytical Science, Melbourne, Australia, with packing bed (the dead volume is ~7 µl).

factors during the washing. It has been shown that the analyte leakage increases as the solvent percentage in washing solution increases [2,21]. For basic drugs, the optimal washing solution contains 5% methanol and 0.1% formic acid in water, using silica-based benzenesulphonic acid cation exchanger as sorbent [3]. A pure or high solvent percentage (~50%) is a typical elution solution. Furthermore, the pH is an important factor (control charged/uncharged analyte). For the extraction of a basic drug (ropivacaine) from plasma samples, it was found that the optimal elution solution was 0.25% ammonium hydroxide (pH > 10) added to an elution solution containing 95% methanol and 5% water [3].

■ Study of the carry-over with MEPS

Carry-over is one of the common problems with automated systems. It is a limiting step for trace analysis, giving poor accuracy and precision during method validation. The small quantity of phase in the MEPS can easily and effectively be washed out between samples to reduce the possibility of carry-over. With the automation of MEPS, washing can occur while the previous sample is running. Carry-over decreased to less than 0.1% when the sorbent was washed at least five times with elution solution and washing solution between extractions [21].

■ Can MEPS eliminate matrix effects?

Complex matrices such as blood, plasma and urine have potential for ion suppression, particularly with electrospray ionization (ESI) MS [22]. MEPS provides flexibility in different parameters, such as washing solution, elution solution and type of sorbent materials. MEPS was used to investigate matrix effects [18]. **Figure 2** shows a full-scan ESI–MS of blank human plasma that was pretreated using protein precipitation (PPT) and MEPS. The PPT produced the greatest amounts of salts and phospholipids ions, while the MEPS technique eliminated salts and reduced the phospholipid concentration significantly.

MEPS application

■ Drugs of abuse in human urine by DART/TOF

Microextraction by packed sorbent has been evaluated for fast screening of cocaine and its metabolites in human urine samples using MS detection. Several sorbents, such as C8, ENV+, Oasis MCX, and Clean Screen DAU, were investigated using MEPS. In this study, the focus was on fast extraction and preconcentration of the drug and its metabolites with rapid analysis using a direct analysis in real time (DART) source with a time-of-flight (TOF) mass spectrometer. The analysis time was less than 1 min. A concentration factor of 30–50 times was obtained using MEPS and Clean Screen DAU as sorbent. The LOD for ecgonine methyl ester, benzoylecgonine, cocaine and cocaethylene were 22.9, 23.7, 4.0 and 9.8 ng/ml, respectively, using Clean Screen DAU as sorbent. The study has demonstrated that the combination of MEPS with direct analysis in real time DART/TOF can be a very useful tool for screening of drugs of abuse in a biological matrix [20].

■ Anticancer drugs in human plasma by LC–MS/MS

Busulphan and cyclophosphamide (alkylating agents and the most widely used anticancer drugs) were extracted and determined by MEPS–LC–MS/MS. The validation showed that selectivity, accuracy and precision were satisfactory [15,16]. The busulphan bioanalytical method using MEPS–LC–MS showed good accuracy and precision within the range of therapeutically relevant levels (5–2500 ng/ml) [15]. It also reduced the sample preparation time for busulphan (<1 min per sample compared with 40 min using LLE), which is of great importance in adjusting the busulfan dose in clinical settings. Concerning cyclophosphamide, the accuracy of the quality-control samples ranged from 95 to 106%. The interday variation was in the range of 5–9%, while the intraday variation was between 1 and 5%. The method was employed for the quantitation of cyclophosphamide in human plasma samples for patient samples [16].

Local anesthetics in whole blood by LC–MS/MS.

Microextraction by packed sorbent was utilized for the extraction of local anesthetics (lidocaine, ropivacaine and bupivacaine) from human blood samples online with LC–MS/MS. The blood

samples were diluted (1:20 v/v) with 0.1% formic acid before MEPS handling. The lower LOQ was set to 10.0 nM for all the studied analytes. The validation of the method showed that the accuracy of the quality-control samples ranged from 85 to 97%. The interday precision of the studied analytes was in the range of 1–5%. The calibration curve in plasma was constructed in the concentration range of 10–10,000 nM. The regression correlation coefficient (r) was at least 0.995 for all runs [18].

Functional differences

In MEPS, the sample and reagents are drawn and expelled from the bottom of the sorbent bed. In contrast, conventional SPE columns have flow from the top-down for all steps. For biological samples in particular, the MEPS approach offers a number of advantages over the top-down approach. The first is that MEPS allows the ability to load and elute only the bottom of the sorbent with sample volumes of less than one bed volume. Such advanced applications can improve the method's capacity to concentrate analytes and allow the processing of volumes approaching the limit of precision of the syringe. The second advantage of loading the sample through the bottom frit and then expelling depleted matrix along the same path is a reduction in frit fouling. As the expelled sample passes back out through the frit on the compressive out-stroke, fine particulates that would reduce frit performance can be expelled in a manner not possible with conventional top-down approaches. The third advantage offered by the MEPS approach derives from a reduction in the coagulation mechanism that has been proposed as the major cause of 'blockage' for viscous samples, such as plasma, relative to conventional SPE devices. The disruption of the process is physical rather than chemical and comes from decompression of the sorbent on the in-stroke compared with compression of the sorbent during top-down loading.

Conclusion

Microextraction by packed sorbent approach to sample preparation is very promising for many reasons:

- It is easy to use;
- It is online and fully automated;
- It is rapid;
- The cost of analysis is minimal compared with conventional SPE.

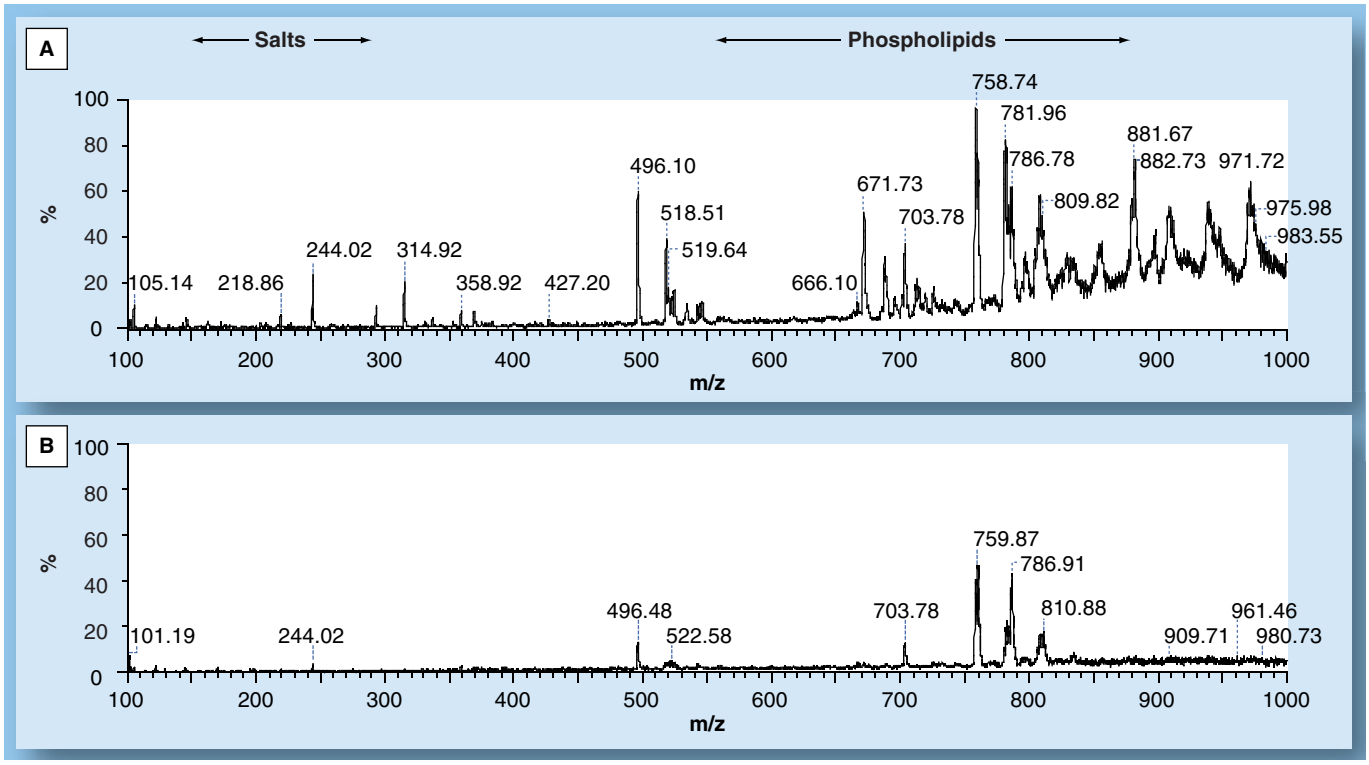

Figure 2. Full-scan ESI–MS resulting from direct infusion of blank human plasma pretreated with (A) protein precipitation and (B) MEPS-C18.

Not only is the automation process with MEPS advantageous, but the much smaller volumes of the samples, solvents and dead space in the system provide other significant advantages, such as the speed and the simplicity of the sample-preparation process. The key aspect of MEPS is that the solvent volume used for the elution of the analytes is of a suitable order of magnitude to be injected directly into GC or LC systems. MEPS significantly reduces the volume of solvent and sample needed.

Future work should be focused on the extraction of more drugs and metabolites from whole blood and plasma. A broad range of applications in different areas, such as environmental and food analysis, will be needed. MEPS could be used for onsite environmental analysis. More selective sorbents and the use of antibodies for more selective extraction will be investigated. MEPS is adaptable to other analytical techniques, including immunoassay and offline analysis by NMR, infra-red and other methods.

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