ON-LINE AND OFF-LINE APPLICATION OF MICRO-SPE (MEPS)

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Introduction

Solid-phase extraction (SPE) has revolutionized sample preparation. Variations on the technique offer enhanced recovery, greater speciation and reduced solvent and sample consumption over other techniques. Micro-Extraction Packed Sorbent (MEPS) is the miniaturization of conventional SPE from milliliter to microliter bed volumes that allows SPE to be used with very small samples. The manipulation of the small volumes is achieved with a precision gas tight syringe. With a typical void volume of 7µL, the MEPS elution is compatible with GC and LC inlets making it ideal for integration into an automated sampling system for on-line SPE.

In most cases, MEPS allows the same level of sample concentration as is possible with off-line conventional SPE while providing opportunities for truly hybrid multi-dimensional methods. MEPS methods may be readily adapted from established SPE methods including those based on mixed mode or complex chemistries.

Like SPE, MEPS is for use with liquid samples (either normal or reversed phase) and yields four fractions: the unretained, weakly bound, strongly bound and irreversibly bound. However, because MEPS is a double pass system (sample and solvent enter and exit from the bottom of the bed, the weakly bound fraction (commonly the interferences eliminated by washing) is less strongly bound. The irreversibly bound fraction affects MEPS and conventional SPE and is usually associated with sorbent wetting rather than sample purification and so the irreversible binding of matrix material from one sample does not preclude reuse of the device for a sample of the same type.

Like conventional SPE, the number of times the device can be reused is dependent on the sample matrix. For simple applications, MEPS devices have been used successfully for >50 cycles.

Benefits of MEPS

- MEPS allows SPE methodology to be applied to small sample volumes.
- MEPS can be integrated into autosampler robotics and allows on-line use of SPE.
- MEPS can reduce sample and reagent consumption and waste disposal.
- Double pass flows can reduce the weakly bound fraction.
- MEPS is field portable for remote sampling with or without the use of automated equipment.
- MEPS is adaptable for other analytical techniques including immunoassay and off-line analysis by NMR, IR and other methods.

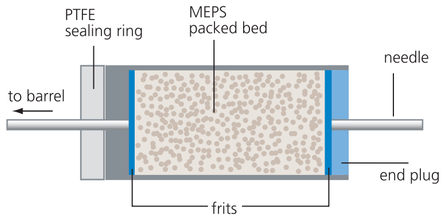

Figure 1: MEPS needle with integral 30µm C18 packed bed SPE device (void volume approximately 7µL)

Plant extracts

The plant Phytolacca octandra (Figure 2) is one of the inkweeds or Pokes. In this application, the aerial portions of the plant were homogenized in acidified methanol, allowed to percolate for 12 hours, filtered and then extracted using a C2 MEPS cartridge. The MEPS was conditioned with methanol (30µL), water (30µL) and then 100µL of the plant extract was passed through the sorbent at 5mL/sec. The exhausted fraction was ejected at the same rate and the sorbent washed with 100mL water. The sorbent was dried with air (3x80µL at 50mL/sec) and eluted sequentially with hexane (10µL), dichloromethane (10µL) and methanol (10µL). The eluates were analyzed directly by GCMS on a BPX5 column (Figure 3).

The C2 MEPS method allowed the one step isolation of the FAME fraction (hexane) and the elimination of the highly polar sugar fraction (see Figure 3). Speciation of polar and non-polar analytes from a single sample digest was readily achieved without the need for off-line sample preparation.

Figure 2: Phytolacca octandra

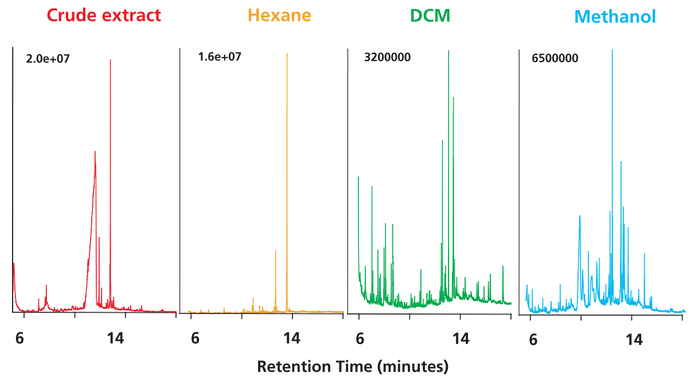

Figure 3: Effectiveness of the water wash in a GCMS analysis of Phytolacca octandra extract before and after fractionation on MEPS C2.

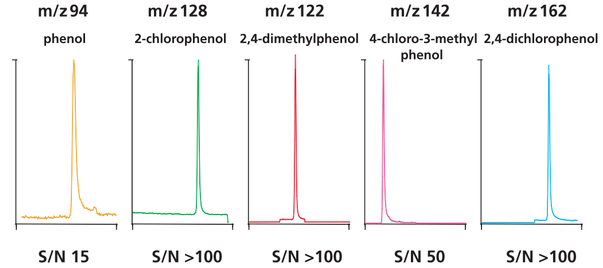

Figure 4: Phenols in waste water at 250 ppt.

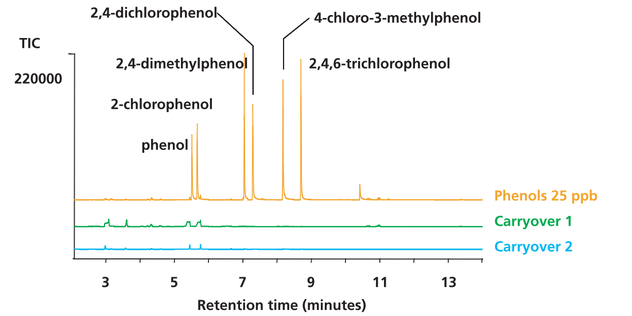

Figure 5: Phenols in waste water at 25 ppb.

Waste Water

To demonstrate the usefulness of MEPS for dilute samples with a relatively simple matrix, a surrogate wastewater sample was prepared from clear phenol free waste water spiked with either 25ppb or 250ppt phenols. The water was extracted with a C18 MEPS cartridge conditioned with methanol (30µL), water (30µL) and then 10 x 100µL of the water at 5µL/sec. The exhausted water was ejected at the same rate after each cycle and the sorbent dried with air (3x80µL at 50µL/sec). The analytes were eluted with methanol (10µL) and the fraction analyzed directly by GCMS on a BPX5 column. Chromatograms for the water samples are shown in Figures 4 and 5. Carryover was examined following the extraction of the 25ppb sample by elution of a second and third portion of methanol without any intervening wash steps (Figure 5).

The C18 MEPS method allowed the one step isolation of phenols from water with good recovery, linearity and little carryover. The sorbent was reusable for the application for a large number of samples with no loss of performance after 10 analyses.

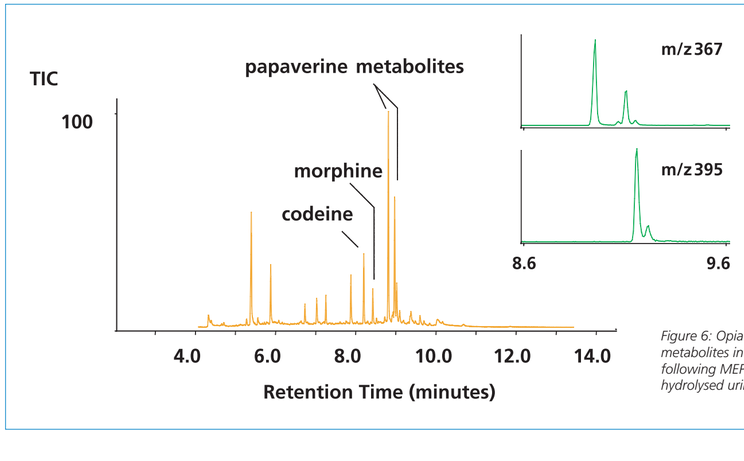

Figure 6: Opiate metabolites in urine following MEPS of hydrolysed urine.

Toxicology samples

Papaver somniferum (opium poppy) is a feed contaminant that can result in positive drug tests for racing horses. We describe the extraction of a urine sample from an animal receiving contaminated feed as a demonstration of the off-line application of mixed mode C8/SCX MEPS for complex biological fluids.

Here, 300µL of a diluted equine urine was hydrolyzed with b-glucuronidase or acid, filtered and extracted on a C8/SCX MEPS cartridge conditioned with methanol (30µL), potassium phosphate buffer (0.2M, pH6, 30µL) at a flow rate of 5µL/sec. The exhausted fraction was ejected at the same rate and the sorbent washed with 100µL phosphate buffer, 50µL acetic acid (1%v/v) and 100µL methanol. The sorbent was dried with air (3x80µL at 50µL/sec) and the sorbent eluted with 20µL dichloromethane-isopropanol-ammonia (49:49:2). The organic phase was evaporated under nitrogen and derivatized with 10µL of acetic anhydride-pyridine (1:2) at 80°C for 30 minutes before evaporation and reconstitution in 5µL of ethyl acetate. The extract was analyzed by GCMS on a BPX5 column (Figure 6).

The C8/SCX MEPS method allowed the microscale preparation of a small volume sample with comparable performance to conventional SPE techniques. Used off-line with derivatization and GCMS here, the sample was also suitable for on-line ESI-LCMSMS analysis by changing the elution solvent to methanol-ammonia (98:2) or methanol-trimethylamine (98:2).

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