APPLICATION NOTE

FALSE POSITIVE DRUG TESTS FROM CONTAMINATED FEED

An On-line and Off-line Application of Micro-SPE (MEPS)

INTRODUCTION

Papaver somniferum (opium poppy) is a feed contaminant that can result in positive drug tests for racing horses.

A Micro-Extraction Packed Sorbent (MEPS) / GCMS method is described below for the regulatory testing of equine and human urine samples. The method allows for the separation of morphine and its metabolites from the metabolites of potential botanical markers that indicate the ingestion of poppy seeds or straw. 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 7 µL, the MEPS elution is compatible with GC and LC inlets making it ideal for integration into an automated sampling system for on-line SPE.

EXPERIMENTAL CONDITIONS

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

RESULTS

The extract was analyzed by GCMS on a BPX5 column (Figure 1a and 1b).

CONCLUSIONS

This application of mixed mode C8/SCX MEPS for a complex biological fluid, 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).

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.

Also like conventional SPE, the number of times the device can be re-used is dependent on the sample matrix. For simple applications, MEPS devices have been used successfully for more than 50 cycles.

REFERENCES

(1) Wynne PM, The accidental contamination of animal feed by naturally occurring opiates with particular reference to morphine. An independent review and analysis of current knowledge. The British Equestrian Trade Association (London), 2005; pp1-143.

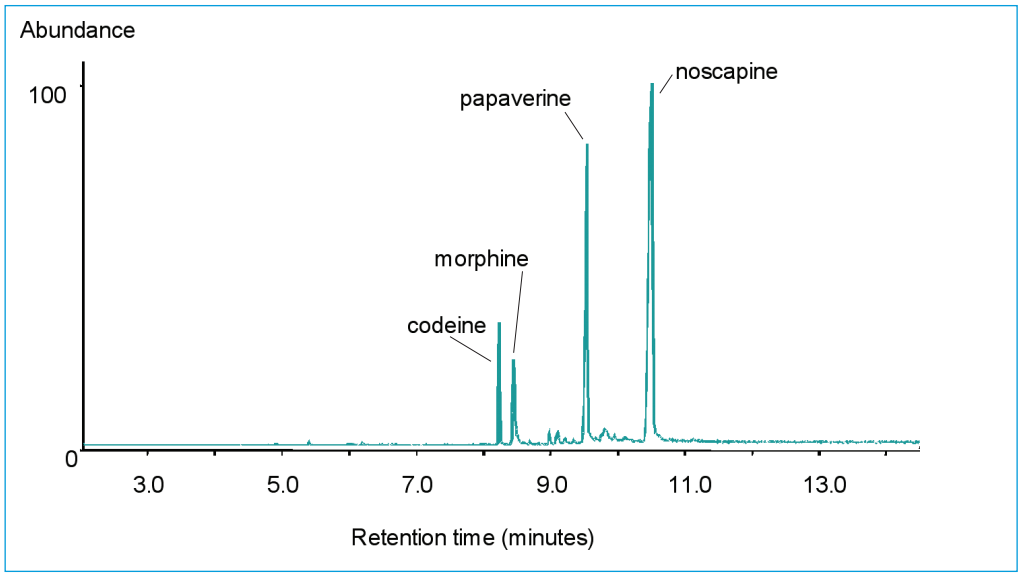

Figure 1a: The analysis of *Papaver somniferum* ssp. *setigerum* by GCMS following micro-SPE extraction of poppy residues and GCMS analysis of the recovered fraction.

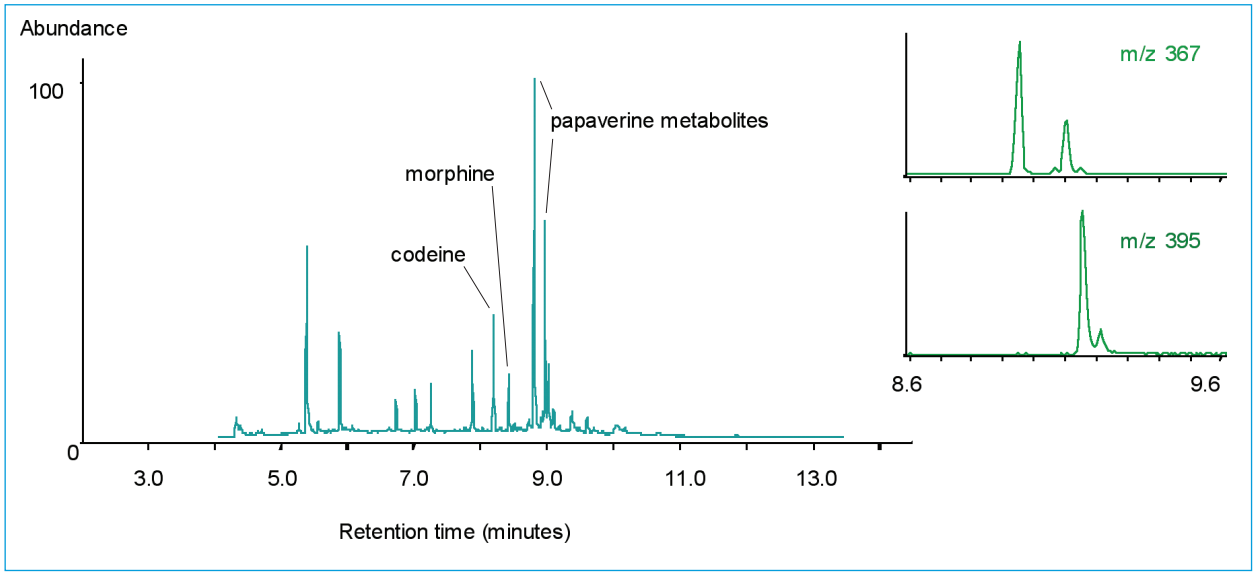

Figure 1b: The analysis of enzyme hydrolyzed horse urine by GCMS following micro-SPE extraction and micro-derivatization (peracetylation) of the recovered basic fraction. (Inset m/z 367 corresponds to O-desmethylpapaverine metabolites and m/z 395 is the O,O'-didesmethylmetabolites)

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