GC-MS IN FORENSIC DRUG ANALYSIS:

Application to Opium Contaminated Animal Feeds

Paul Wynne, Dan di Feo and Peter Dawes. SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria, Australia 3134 SGE Inc. 2007 Kramer Lane, Austin, TX 78758

Introduction

Forensic drug analysis faces increasing pressure to provide sound evidence in the prosecution of illicit drug use. Reliable differentiation of opiate abuse from the ingestion of poppy products is a continuing goal of regulatory drug testing laboratories to avoid contentious debate. The requirement is particularly important in horse and camel racing where zero-tolerance thresholds are exceeded by very low levels of contaminant but is also of significant importance to the growing field of human workplace drug testing.

A recent study [1] in the area of animal feeds has found that it is unlikely that accidental contamination of feed can be detected by low frequency testing and that contamination of feed is more likely in many cases to be detected during drug screening of biological fluids. Consequently novel strategies to deal with positive cases are required. Frequent testing requires methods that are robust for preparation of diverse sample types (allowing detection of both parent alkaloids and their metabolites) as well as being suitable for on-line or automated use. Cost, transport and storage limitations also demand methods that are sparing in their consumption of reagents and sample.

Figure 1. *Papaver somniferum* ssp.*somniferum in flower and bud.*

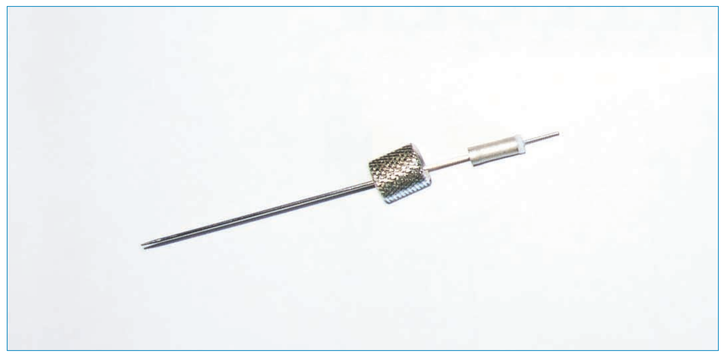

Figure 2. *A micro SPE column ready to be fitted to an SGE syringe.*

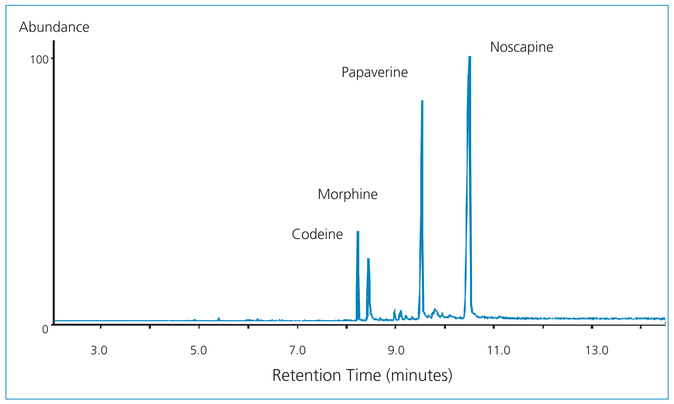

Figure 2. *The analysis of enzyme hydrolysed horse urine by GCMS following micro-SPE extraction and micro-derivatisation (peracetylation) of the recovered basic fraction. (Inset m/z 367 corresponds to O-desmethylpapaverine metabolites and m/z 395 is the O,O'-didesmethylmetabolites)*

Method

Micro extraction of urine and plant specimens was scaled from methods reported for conventional SPE devices. Typically, urine (0.3ml) was diluted with 0.2M sodium phosphate buffer (0.3ml, pH 6.0) and the sample enzyme hydrolysed by incubation with 50IU of glucuronidase enzyme (E.coli) at 50 °C for 1 hour. The specimen was centrifuged at 2000g to remove suspended materials and the supernatant drawn into a micro-SPE syringe with a mixed mode sorbent (equivalent to C8/SCX) that was previously conditioned with methanol (30µl) and phosphate buffer (30µl). The exhausted sample was expelled from the syringe and the sorbent rinsed with 2% v/v aqueous acetic acid (30µl). Excess reagent was displaced with air (100µl), the sorbent washed with methanol (2x30µl) before elution of the retained analytes with isopropanol containing 2% w/v ammonia. The eluted bases were suitable for evaporation and derivatisation in a sealed tube or direct analysis by LCMS.

In this study, analytes were derivatised with 30µl of acetic anhydride – pyridine (1:2) at 80 °C for 1 hour prior to evaporation to dryness and reconstitution in ethyl acetate (100µl) for GCMS analysis. The method was also effective for crude aqueous extracts prepared by soaking feed samples.

Limitations and thresholds

Effective management of the problem may be achieved with a combination of drug-testing methods that include the analysis of bio-markers (the 'post-metabolic chemotaxonomy' technique) and multiple levels of field testing of feed prior to use.

A micro-SPE / GCMS method is described for the regulatory testing of equine and human urine samples. The method describes columns that are ideal for the analysis and allow the separation of morphine and its metabolites from the metabolites of potential botanical markers that indicate the ingestion of poppy seeds or straw.

As feed contamination of animal feed with opiates is inevitable even with the implementation of feed testing, successful and ethical management of morphine positive drug investigations should be biased in favour of detecting other evidence that might demonstrate the contamination hypothesis and attempt to destigmatise the investigation and prosecution of such cases, particularly where there is compelling evidence to suggest that opiate contamination of animal feeds has occurred.

This application details a method for sample preparation that is suitable for chemotaxonomic classification of poppy residues and the analysis of horse urine for either GC or LC inlet.

Because the method is suitable for both plant and biofluid samples and allows minimisation of sample and reagent consumption, it is ideal for use in quality control or regulatory environments and also allows a significant reduction in sample transport storage and reagent consumption. This application is a specific demonstration of a technique that is generally useful in areas of chemotaxonomy, toxicology and pharmaceutical analysis, particularly where there is limited sample for analysis.

Conclusion

The micro-SPE technique is suitable for on-line or off-line sample preparation for the chemotaxonomic identification of opiates in urine and animal feed extracts. While offering all the advantages of conventional SPE techniques, the micro-SPE method minimises consumption of reagents and waste disposal, is suitable for samples of limited volume and may be adapted for automation using laboratory robotics designed for autosampler syringes. The technique may be adapted for GC or LC inlet and for enhancing immunoassay performance.

As micro-SPE is based on fully scaled conventional SPE devices and uses precision syringes for fluid handling, the technique is suitable for use with most convention SPE techniques. The mixed mode application described exemplifies the most demanding extraction type.

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.

www.sge.com