The Extraction of Saliva for The Analysis of Basic Drugs Residues using MEPS™-GC/MS

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Introduction

Oral fluid is considered a desirable sample for regulatory screening of drugs of abuse and for therapeutic drug monitoring because it can be collected in a non-invasive fashion when compared with the procedures used for collection of urine and blood. Unlike urine, the dominant species in oral fluid is the parent drug and as a general rule there is a correlation between oral fluid concentration and blood/plasma concentrations. The relatively low concentration of most drugs in saliva and the small sample volume that is typically available for analysis makes micro-extraction techniques both attractive and necessary for this matrix.

MEPS™ is an SPE device that is incorporated directly into a liquid handling syringe and may be used with robotic auto-samplers for on-line chromatographic analysis. The small scale of the MEPS™ device is effective for the extraction of small volume samples and is therefore potentially valuable for the extraction of oral fluids for GC-MS confirmatory analysis.

We present here a simple reversed-phase C18-MEPS™ extraction for saliva collected from a patient that had been administered the local anaesthetic mepivacaine for a dental procedure several hours previously.

The MEPS™ Principle

The MEPS™ device consists of a small (~ 7 µl) compartment: "Barrel Insert and Needle Assembly (BIN)" that contains the stationary phase, and is built into the syringe needle. The packing material is 40-50 µm silica with 60 Å pore size and a range of common surface modifications.

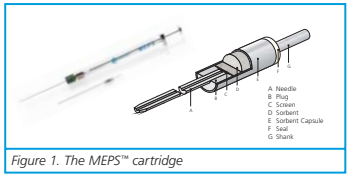

Figure 1. The MEPS™ cartridge

MEPS™ works like other sample preparation tools with the common steps being conditioning, sampling, washing and elution with the difference that the glass syringe design allows these steps to be performed by a robotic system (such as an autosampler) with the needle being robust enough to penetrate standard septa.

Figure 2: Standard operation of a MEPS™ extraction

Advantages of MEPS™

- Sample Size and Sensitivity:
  Sample volumes may be as little as 10 µL, or by taking multiple aliquots of 100 µL or 250 µL, samples of 1 mL or larger may be concentrated.
- Robustness:
  Samples can be drawn and dispensed through septa.
- Automation:
  The capability to extract samples and make injections on-line using a single device reduces both sample processing times and the need for operator intervention.
- Sorbent Life:
  Typical BIN life for extraction of whole plasma sample is conservatively about 40 to 100 samples. This significantly increases for cleaner samples.
- Carry Over:
  The small quantity of phase in the MEPS™ BIN can be easily and effectively washed between samples to reduce the possibility of carryover. This washing process is simply not practical with off-line SPE devices. With automation of MEPS™ washing can occur while the previous sample is running.
- Flexible and easy to use:
  The dimensions of the sorbent bed ensure that the performance remains identical to conventional SPE devices when used for extraction of similar samples.

Experimental

The principle of the detection of basic drug substances in saliva was investigated with saliva samples spiked with lignocaine, articaine and mepivacaine hydrochloride. Samples were 2.2 ml vials for injection containing (a) 44 mg lignocaine HCl plus 27.5µg adrenaline, (b) 66 mg mepivacaine HCl and (c) 88 mg atricaine HCl plus 20 µg adrenaline (Septodont, Lancaster PA). Saliva samples were diluted with a concentrated sodium tetraborate solution (1:1) and spiked with a mixture of the three anesthetics to a final concentration of 100 pg/ml.

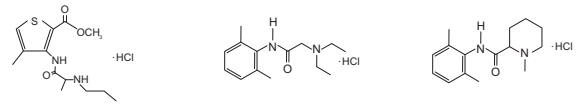

Figure 3. Structures of the anaesthetic drugs used in this study

Patient Sample: Mepivacaine hydrochloride (10 mg, 0.5 % w/v, AstraZeneca Pty Ltd, NSW, Australia) was administered into the gum for local anaesthesia of a 80 kg male undergoing minor remedial dentistry. Saliva was collected without a wash solution or use of a stimulating agent into a clean glass vial. A 1 mL portion of the sample was diluted with an equal volume of saturated sodium tetraborate solution to buffer the sample to pH 9.5.

MEPS™ Extraction: A C18 MEPS™ BIN on a 100 µL syringe was conditioned with methanol (20 µL) and water (20 µL) at 10 µL/sec. The spiked sample (1000 µL) was loaded in 10 cycles at 10 µL/sec. The expelled fraction was passed through the MEPS™ cartridge a second time before being discarded. The sorbent was washed with water (20 µL) and the pH adjusted with saturated sodium tetraborate solution (20 µL) and the sorbent again washed with water (20 µL) and dried with air (3 x 80 µL) at 80 µL/sec. The cartridge was eluted with methanol (10 µL) and the fraction analyzed without further modification.

Gas Chromatography Mass Spectrometry: GCMS was performed on a GP2010/QP2010 (Shimadzu Corporation, Kyoto, Japan) and a BPX5 column (30 m x 0.25 mm ID, 0.25 µm film thickness, SGE). Injections of 1 µL were splitless at a temperature of 250 °C. Purge flow was 50 mL/min with a nominal inlet pressure of 127 kPa. The oven temperature was programmed from 40 °C (hold for 4 min) to 300 °C (hold for 10 min) at 10 °C/min. The carrier gas was helium at a flowrate of 1.2 mL/min in constant flow mode. Mass spectra were collected over the range 40-500Da at 2 scan/sec. The transfer line temperature was 280 °C, the quadropole was 150 °C and the source was 230 °C.

Results

The extraction of mepivacaine from saliva of the dental patient using MEPS™ was fast (extraction time was 2 – 3 minutes for a concentration factor of 5 x) and gave sufficient sensitivity to detect mepivacaine in full scan mode. Sensitivity was enhanced for either less concentrated samples or for smaller samples by using SIM. Carryover into a second elution of methanol or isopropanol was less than 10 % on the basis of the height of the base peak (m/z 98).

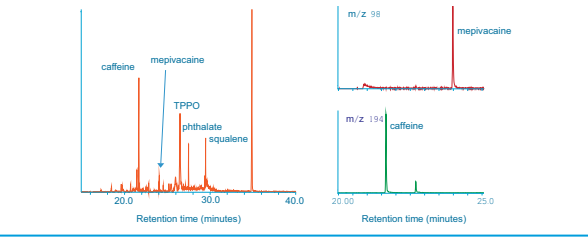

Figure 4: The Total Ion Chromatogram (left) and Extracted Ion Chromatograms (right) for mepivacaine and caffeine obtained from the analysis of a saliva sample extracted using C18 MEPS™

In order to determine the limits of the method, saliva samples were spiked with decreasing amounts of a mixture of articaine, lignocaine and mepivacaine. The total ion chromatogram of this sample is shown in Figure 5 with the main signal belonging to caffeine. The limit of detection was reached at about 100 pg/ml (0.1 ppb) the extracted ion chromatograms of the extracted saliva samples for m/z 86 (articaine and lignocaine) and m/z 98 for mepivacaine are shown in the bottom two traces of figure 6.

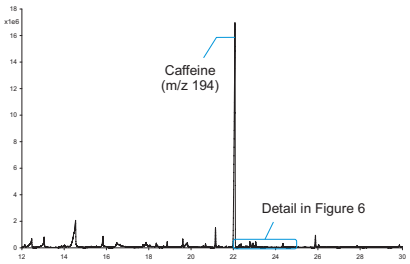

Figure 5. The total Ion Chromatogram obtained from the analysis of a spiked saliva sample extracted using C18 MEPS™

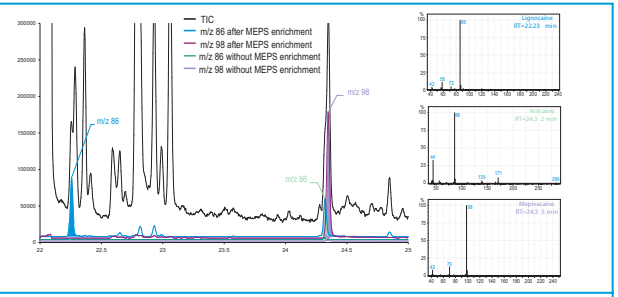

Figure 6. Detail of the Chromatogram in Figure 5 showing TIC, EICs and mass spectra for the three anesthetic drugs.

After enrichment of the same sample (0.1 ppb) with the MEPS™ device, the peak areas for the EICs have increased significantly. Enrichment factors were 27 x for mepivacaine, 40 x for articaine and 83 x for lignocaine. The recovery between the sample components differed significantly (from 27 to 83 %). However, it should be noted that the method was initially optimised for lignocaine and the other two drugs were added later. These investigations indicated that the sample loss occurred during the loading step with no loss observed in the wash solutions and the mass balance data supported a complete elution. Further investigations are needed to optimise the sample binding rate.

Conclusion

MEPS™ is a fast and low volume method that is suitable for the extraction of basic drugs such as mepivacaine from limited samples in aqueous matrices such as saliva. Unlike other on-line techniques including those based on the molecular weight of matrix components, MEPS™ retains the advantages of reversed-phase solid-phase extraction in a format that is suitable for small volume samples. Elution volumes of 20 µL or less also provide for the use of MEPS™ on-line with suitably equipped instruments.

The following table shows the comparison of experimental parameters of SPE compared to MEPS™:

Parameter	MEPS™	SPE
Time taken for extraction	3 min	20 min
Sample consumption	10 to 1000 µL	1 - 3 mL
Organic solvent consumption	0.3 mL	7 mL
Elution volume	10-50 µL	2 mL

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