The Extraction of Saliva for The Analysis of Basic Drugs Residues Using MEPS™-GCMS

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Introduction

Oral fluid is considered a desirable sample for regulatory screening of drugs of abuse and for clinical monitoring because it may be collected in a non-invasive fashion when compared with the procedures used for collection of urine and blood. Unlike urine, the appearance of the drug residues in saliva may be directly correlated with plasma drug concentrations. The relatively low concentration of most drugs in saliva and the small sample volume that is typically available for analysis makes micro-extractive techniques both attractive and necessary for this matrix.

MEPS™ is a micro-scaled SPE device that is incorporated directly into a liquid handling syringe and may be used with robotic autosamplers 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.

Experimental

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. Noticeable anaesthetic effects (numbness and inverted sensitivity) were no longer observed at 2 hours following administration. A three hour washout period was allowed following administration of the drug to remove any drug residues from the site of injection.

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.

A C18 MEPS™ BIN on a 100 µL syringe was conditioned with methanol (20 µL) and water (20 µL) at 10 µL/sec. The diluted sample (100 µL) was loaded to waste in 1 cycle at 10 µL/sec. 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 (20 µL) and the fraction analyzed without further modification.

Gas Chromatography Mass Spectrometry was performed on a 6890GC-5973N MSD (Agilent Technologies, CA, USA) equipped with an ETP electron multiplier (SGE Analytical Science, VIC, Australia) and a BPX5 column (30 m x 0.25 mm ID, 0.25 µm film thickness, SGE). Injections of 2 µL (equivalent to 10 µL of saliva) 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 flow rate of 1.2 mL/min in constant flow mode. EI mass specta were collected over the range 40-500 Da at 2 scan/sec. The transfer line temperature was 280 °C, the quadrapole was 150 °C and the source was 230 °C.

Results and Discussion

Mepivacaine hydrochloride is an effective local anaesthetic of the amide class that is used for local anaesthesia, including intraoral administration for dental procedures (Figure 1). The free base form of the drug is hydrophobic and the amine centre sufficiently unshielded with a pKa of 7.65 for both reversed-phase and mixed-mode SPE strategies to be employed in its extraction. The complexity of the saliva matrix coupled with the small sample volumes that are available makes micro-extraction an attractive alternative to 'dilute and shoot' methodology for most testing applications.

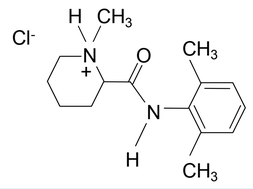


Figure 1: Mepivacaine hydrochloride is a local anaesthetic used for nerve block in dental anaesthesia.

The extraction of mepivacaine from saliva 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).

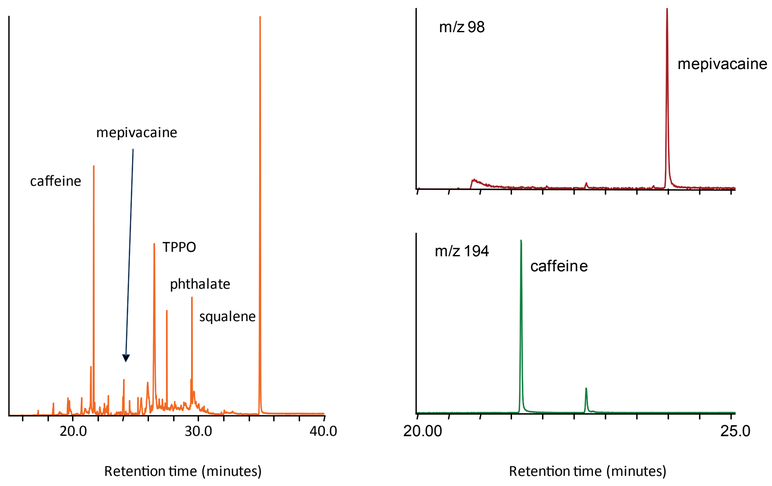
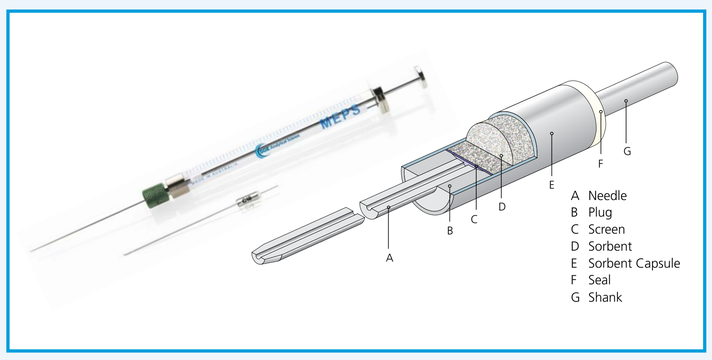


Figure 1: The Total Ion Chromatogram (left) and Extracted Ion Chromatograms (right) for mepivacaine and caffeine obtained from the analysis of a saliva sample extracted using C18 MEPS™.

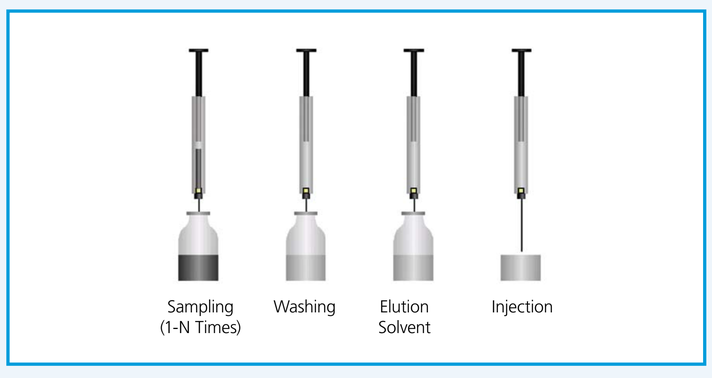
Conclusion

MEPS™ is a fast and sample sparing method that is suitable for the extraction of basic drugs such as mepivacaine from volume limited 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 MEPS™ Principle



The MEPS™ consists of a small (~7 µl) compartment, the 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.



MEPS™ works like other sample preparation tools with the common steps being 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.

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 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.

Parameter	MEPS™	SPE
Time taken for extraction	3 min	20 min
Sample consumption	0.4 mL	3 mL
Organic solvent consumption	0.3 mL	7 mL
Elution volume	20-50 µL	2 mL

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