THE EXTRACTION AND ANALYSIS OF URINARY ANTITUSSIVE METABOLITES USING MEPS™ AND ESI-LCMS^n

Wirth, H.J. Melbourne, Australia, Lahoutifard, N., Courtaboeuf, France, Gooley, A., Melbourne, Australia and Wynne, P.M., Melbourne, Australia.
Dr Paul Wynne, SGE Analytical Science, 7 Argent Place, Ringwood, Victoria, 3134, Australia

ABSTRACT

Microextraction Packed Sorbent (MEPS™) is an adaptation of SPE into a miniaturized device with a typical void volume of less than 10 µL. With operating volumes of this scale and its compatibility with autosampler syringes, MEPS™ allows the specificity of the solid-phase process to be harnessed for digital chromatography using discontinuous changes in solvent polarity. The eluant volumes are sufficiently small to be injected directly into a HPLC system and therefore permit the on-line use of solid-phase extraction methodology in real time with the HPLC.

In this application, we describe the analysis of naturally voided human urine samples that were collected following the administration of single doses of antitussive medicines containing codeine, dextromethorphan or pentoxyverine. Urine specimens were first hydrolysed with beta-glucuronidase and then the analytes of interest were extracted using MEPS™ prior to analysis on a Protecol C18 HQ105 column using a 1 % v/v aqueous acetic acid – methanol mobile phase. Detection of the target analytes was by ESI-MSMS with collision parameters selected for specific analytes. The MEPS™ sorbents were nominally 50 µm silicas modified with C18, C8 or SCX chemistries. Speculative structural elucidation of metabolites was possible by mass fragmentography and MS^3 or MS^4 as required.

The effectiveness of MEPS™ was compared with the same sample prepared off-line using conventional cartridge SPE. The method was used to demonstrate the effectiveness of MEPS™ for on-line extraction of biological fluids for LCMS analysis.

THE MEPS™ PRINCIPLE

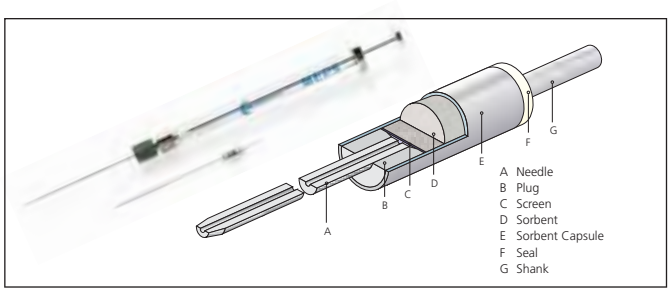


Figure 1: The MEPS™ catridge

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.

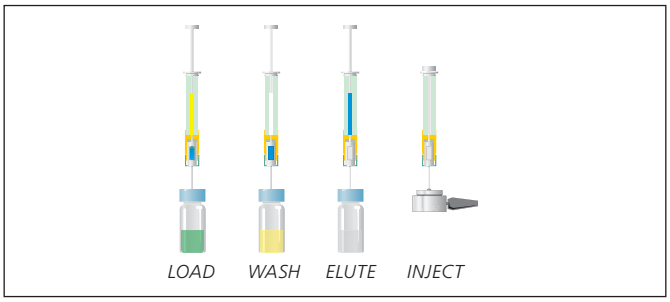


Figure 2: Standard operation of a MEPS™ extraction

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 1mL 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.

SGE Analytical Science

EXPERIMENTAL

Administration and Sample Collection

Oral administration of a single dose of: 10 mL Vicks Cough Syrup (equivalent to pentoxyverine (carbetapentane) citrate 15 mg), 10 mL Robitussin DX Dry Cough Forte syrup (equivalent to 30 mg dextromethorphan hydrobromide) 2 Mersyndol day strength tablets (equivalent to paracetamol 1000 mg and codeine phosphate 19.2 mg) Naturally voided urine samples were collected at 0, 2, 3 and 4 hours following administration. Urine samples were stored frozen at -20 °C until required for analysis.

Sample preparations

3 mL aliquots of urine from the samples collected at 0 and 2 hours after oral administration were diluted with 0.1 M phosphate buffer (pH 6.0, 4.5 mL) and the pH adjusted to 6.2-6.3. The samples were then enzyme hydrolysed with beta-glucuronidase for 2 hours at 50 °C. Samples were extracted by either a conventional mixed mode SPE method or by a reversed-phase MEPS™ method.

SPE extraction was performed on Bond-Elut Certify™ columns using methods described previously. (Wynne PM, Batty DC, Vine JH and Simpson NKJ., Chromatographia, 59 (4/5), S50-S61, (2004)).

MEPS™ extraction was performed on C18 MEPS™ BINS fitted to a 100 µL MEPS™ syringe. MEPS™ BINS were conditioned sequentially with 50 µl methanol and 100 µl water.

- 50 µL methanol conditioning
- 100 µL water conditioning
- 1 mL sample was drawn and expelled in 80 µl steps
- 80 µL water wash
- 50 µL sodium tetraborate pH adjustment
- 80 µl water wash
- 2 x 80 µL air drying
- 2 x 20 µL methanol elution
- 10 µl iso-propanol elution

Mixed Mode MEPS™ for codeine and metabolites

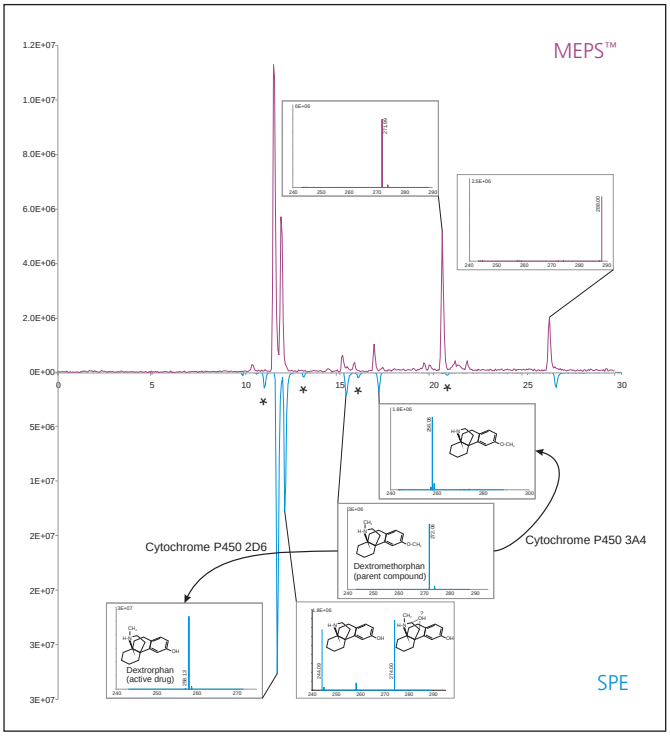
MEPS™ extraction was performed on M1 (C8/SCX mixed mode) MEPS™ BINS. The extractions were the same as for the C18 extractions except for the following: pH adjustment with 50 µl 1 % v/v acetic acid, elution with 100 µl methanol followed by 20 µl 1 % v/v ammonia in methanol.

Chromatographic Conditions and Detection

Column:	SGE ProteCol™-P C18 HQ105 150 mm x 4.6 mm ID
Mobile Phase A:	1 % aq. acetic acid in 10 % methanol
Mobile Phase B:	1 % aq. acetic acid in 90 % methanol
Flow rate:	0.7 ml/min
Gradient:	20 min 0 to 100 % B 10 min at 100 % B
Temperature:	40 °C
Injection volume:	10 µL
Detection:	Thermo LCQ Classic positive ion mode

SAMPLE 1: DEXTROMETHORPHAN AND ITS METABOLITES

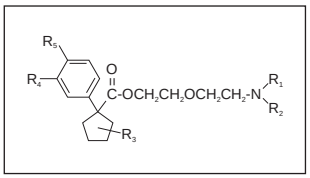
Pharmacokinetics (http://www.tga.gov.au/npmeds/pi-dextromethorphan.rtf): Dextromethorphan is well absorbed from the gastrointestinal tract after oral administration. It is metabolised in the liver, exhibiting polymorphic metabolism involving the cytochrome P450 isoenzyme (CYP 2D6). It is excreted in the urine as unchanged dextromethorphan and demethylated metabolites, including dextrorphan, which has some cough suppressant activity. The plasma elimination half-life of dextromethorphan is 1.2 to 3.9 hours.

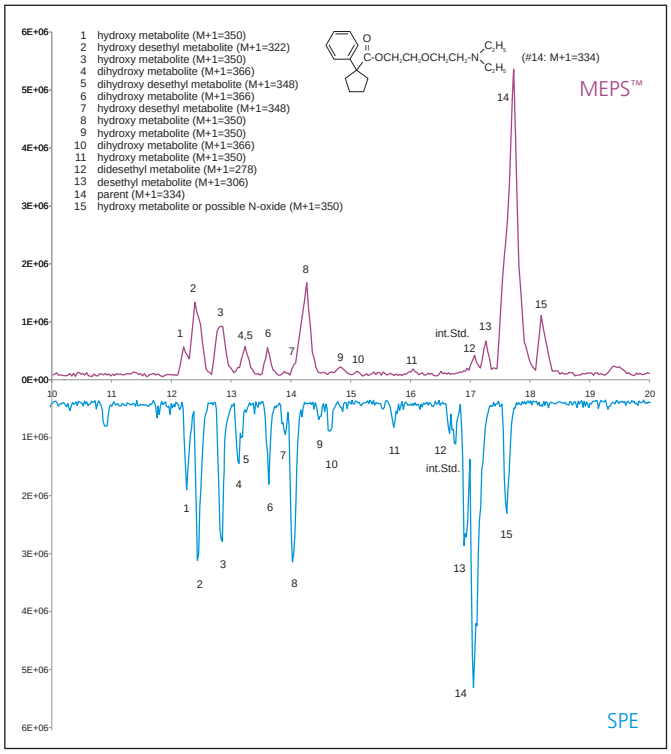


Metabolites of Dextromethorphan

SAMPLE 2: CARBETAPENTANE AND ITS METABOLITES

Carbetapentane exhibits a complex metabolite pattern where R1 and R2 are either ethyl groups or hydrogens and R3 to R5 are either hydrogens or hydroxy groups.

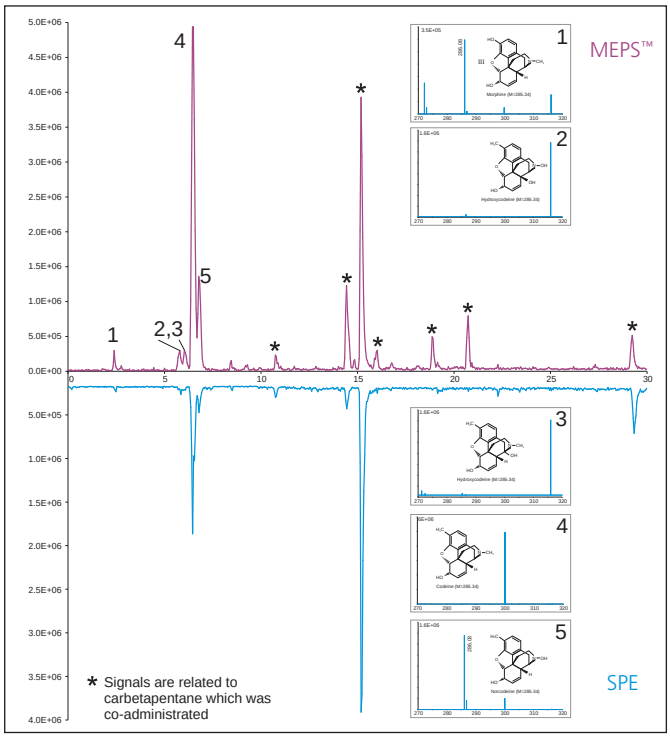




Metabolites of Carbetapentane

SAMPLE 3: CODEINE AND ITS METABOLITES

Codeine is metabolized by Cytochrome P450 2D6 to morphine and by Cytochrome P450 3A4 to norcodeine. Two forms of hydroxy-codeine were also detected.



Metabolites of Codeine

SUMMARY

MEPS™ is a new alternative for sample preparation. While the sensitivity and precision of MEPS™ is comparable or superior to existing methods MEPS™ has the advantage in much shorter extraction times and the easy adoptability to automated systems.

Experimental parameters of the SPE and the MEPS™ extractions in this work highlight the differences between these methods:

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

AUSTRALIA & PACIFIC REGION
SGE Analytical Science Pty Ltd
Toll Free: 1800 800 167
Tel: +61 (0) 3 9837 4200
Fax: +61 (0) 3 9874 5672
Email: support@sge.com

FRANCE
SGE Europe Ltd
Tel: +33 1 69 29 80 90
Fax: +33 1 69 29 09 25
Email: europe@sge.com

INDIA
SGE Laboratory Accessories Pvt Ltd
Tel: +91 22 24715896
Fax: +91 22 24716592
Email: sgeindia@vsnl.com

MIDDLE EAST
SGE Gulf
Tel: +971 6 557 3341
Fax: +971 6 557 3541
Email: gulfsupport@sge.com

UNITED STATES OF AMERICA
SGE Incorporated
Toll Free: (800) 945 6154
Tel: +1 512 837 7190
Fax: +1 512 836 9159
Email: usa@sge.com

CHINA
SGE Shanghai Representative Office
Tel: +86 21 6407 9382
Fax: +86 21 6407 9386
Email: china@sge.com

GERMANY
SGE GmbH
Tel: +49 (0) 6155 / 60746 0
Fax: +49 (0) 6155 / 60746 50
Email: europe@sge.com

JAPAN
SGE Japan Inc
Tel: +81 45 222 2885
Fax: +81 45 222 2887
Email: japan@sge.com

UNITED KINGDOM
SGE Europe Ltd
Tel: +44 1908 568 844
Fax: +44 1908 566 790
Email: europe@sge.com

SGE Analytical Science
www.sge.com

TA-0135-M_Rev8 © SGE Analytical Science Pty Ltd 07/2009