TRAJAN

TECHNICAL ARTICLE

Automated sample preparation using a digital syringe with embedded SPE capability

Introduction

Popular sample preparation techniques such as solid phase extraction (SPE) can be time consuming and expensive, whether implemented on automated platforms or performed manually. Furthermore, method development to improve the efficiency of the sample preparation process is often difficult and time prohibitive, with long evaporation steps throughout the process.

A key objective for all laboratories is to implement efficient sample preparation where there is a real need to improve productivity and minimize waste. While the introduction of automated and semi-automated platforms into the laboratory for sample preparation enables improved efficiencies, they generally come at a significant cost.

We have combined the advantages of automation and SPE using a digital syringe with an embedded SPE cartridge. This not only improves efficiencies, but virtually eliminates solvent use and waste. The miniaturized format is ideal for small valuable samples such as biological extracellular fluids.

Here we demonstrate the advantages in combining the automation of a hand held, digital syringe with miniaturized SPE sorbent embedded in the needle of the syringe. Method development is rapid and inexpensive, enhancing laboratory workflow, while increasing the accuracy and reproducibility of the SPE process.

Background

MEPS® (Micro Extraction by Packed Sorbent) is a miniaturized version of SPE with several key advantages over traditional SPE (see Table 1). In its original format, MEPS is embedded in an analytical syringe and only suffers from the operator dependent steps of aspiration and dispensing where control of the flowrate typically varies from operator to operator.

Table 1. Comparison table SPE vs MEPS.

	SPE	MEPS
Sample volume	3 mL	50 µL
Time		
Cost		
Solvents	10 mL	500 µL
Evaporation step	Yes	Unnecessary

Coupling MEPS technology with a digitally controlled analytical syringe, eVol (Figure 1), enables semi-automated sample preparation which can be programmed to carry out the whole extraction through to injection into the analysis system.

Figure 1. Programmable digital analytical syringe - eVol XR, with eVol syringe, eVol MEPS syringe and MEPS BIN (Barrel Insert and Needle).

Method, results and discussion

Caffeine in saliva

Sample: Following caffeine consumption, saliva was collected into a clean glass vial. A 1 mL aliquot was diluted with an equal volume of saturated sodium tetraborate solution to buffer the sample to pH 9.5. The sample was then filtered and frozen for subsequent evaluation.

MEPS extraction: The extraction method was first optimized by investigating optimal flow rate, the number of extraction cycles and the number of elution volumes. A C18 MEPS BIN (Barrel Insert and Needle) and eVol MEPS syringe was coupled to the eVol, and used to extract caffeine.

The eVol program was divided into 8 functions (Condition MeOH, Condition H_2O, Load sample, Wash H_2O, pH adjust, Wash H_2O, Air dry, Elute) with a total of 41 steps which took around three minutes (see Table 2). The optimized method was repeated by two additional operators.

Table 2. eVol MEPS method for extraction of caffeine.

Step	Mode	Amount (µL)	Speed
1	**Methanol**		Prime
2	Aspirate	20	4
3	Dispense	20	4
4	Aspirate	20	4
5	Dispense	20	4
6	Aspirate	20	4
7	Dispense	20	4
8	**H_2O**		Prime
9	Aspirate	20	4
10	Dispense	20	4
11	Aspirate	20	4
12	Dispense	20	4
13	Aspirate	20	4
14	Dispense	20	4
15	**Sample**		Bind
16	Aspirate	50	4
17	Dispense	50	4
18	Aspirate	20	4
19	Dispense	20	4
20	Mix (x8)	50	4
21	**H_2O**		Wash
22	Aspirate	20	4
23	Dispense	20	4
24	**Saturated sodium tetraborate**		
25	Aspirate	20	4
26	Dispense	20	4
27	**H_2O**		Wash
28	Aspirate	20	4
29	Dispense	20	4
30	**Air**		Dry
31	Aspirate	50	4
32	Dispense	50	10
33	Aspirate	50	4
34	Dispense	50	10
35	Aspirate	50	4
36	Dispense	50	10
37	**MeOH**		Elute
38	Aspirate	20	4
39	Dispense	20	4
40	Aspirate	50	4
41	Dispense	50	10

Conditions

GCMS of the extracted caffeine from three operators performed on an Agilent 6890/573.

Column part number	054101
Phase	BPX5
Column	30 m x 0.25 mm x 0.25 µm
Oven	
Initial temperature	45°C for 4 min
Rate	10°C/min
Final temperature	300°C for 10 min
Injector	
Carrier gas	He
Carrier gas flow	1.2 mL/min
Constant flow	On
Injection mode	Splitless, 250°C
Purge flow	50 mL/min, nominal inlet pressure 127 kPa
Injection volume	1.0 µL
Liner type	FocusLiner with bottom taper
Detector	**MS**
MS transfer line temperature	280°C
Scan parameters	Low mass: 40.0, High mass: 500.0 Da at 2 scan/sec
MS quad temperature	150°C
MS source temperature	230°C

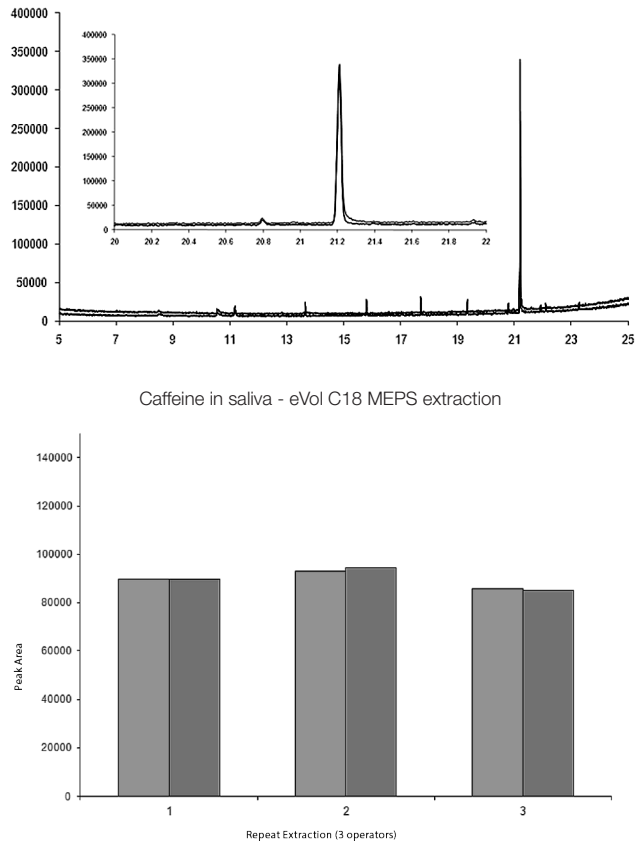


Figure 2. Overlay of caffeine peak extracted on MEPS from three operators. Inter-operator repeatability, three different operators, two independant extractions, same day same MEPS cartridge. Six injections = 4.17% RSD (auto injector repeatability on six standard injections = 2.94% RSD).

Opiate detection in urine

Normal urine contains a high concentration of salts and is not suitable to be injected straight into the MS. Ion suppression limits the detection of codeine and its metabolite morphine in the untreated sample.

A simple dilute and infuse experiment (Figure 3 and 4) demonstrates the inability to detect the ions of interest (476.19 Codeine-6-Glucuronide; 462.18 Morphine-6-Glucuronide; 286.15 Morphine and norcodeine; 272.13 normorphine.

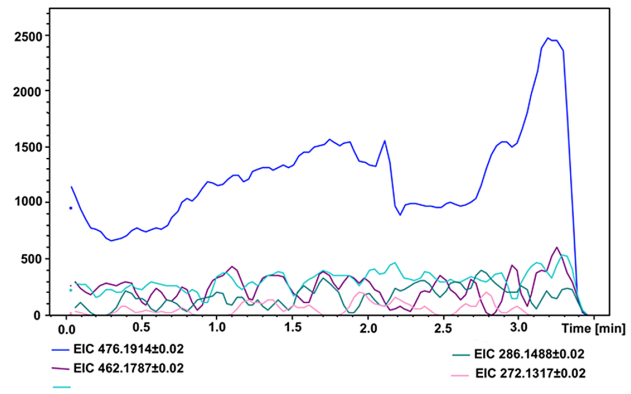


Figure 3. Extracted ion chromatogram of dilute urine infused directly into the ESI source.

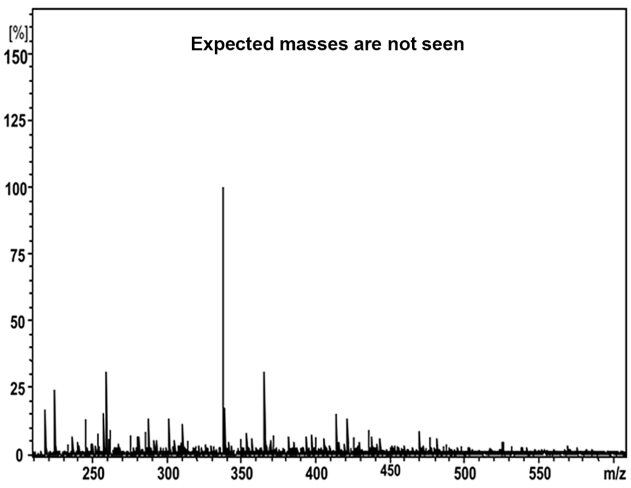


Figure 4. Mass spectra of dilute urineinfused directly into the ESI source.

eVol MEPS direct infusion into ESI

MEPS provides a fast and easy desalting step and the digital syringe format allows convenient sample introduction by direct infusion.

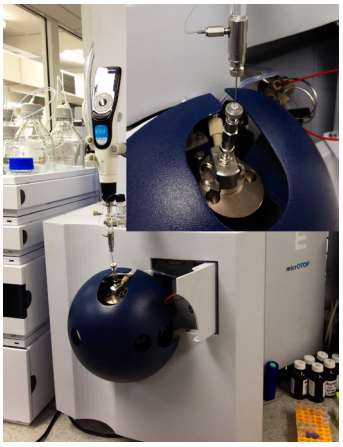


Figure 5. Direct infusion of eVol MEPS into ESI source.

MEPS Method
50 µL MeOH + 0.1% Formic acid
Aspirate 400 µL/min
Dispense 400 µL/min

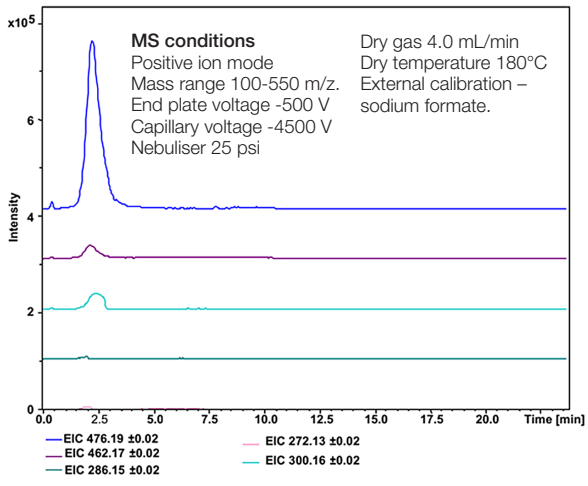
2 x 50 µL Water + 0.1% Formic acid
Aspirate 400 µL/min
Dispense 400 µL/min

40 µL 1:1 Urine:Water 0.1% Formic acid
20 x Aspirate 100 µL/min
20 x Dispense 100 µL/min

2 x 50 µL Water + 0.1% Formic acid
Aspirate 400 µL/min
Dispense 400 µL/min

2 x 50 µL Air
Aspirate 400 µL/min
Dispense 400 µL/min

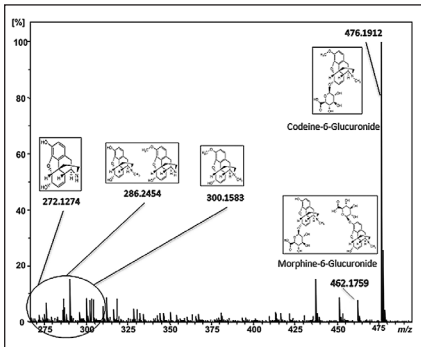
20 µL of 60:40 MeOH:Water + 0.1% Formic acid
Aspirate 100 µL/min
Dispense 5 µL/min



MS conditions
Positive ion mode
Mass range 100-550 m/z.
End plate voltage -500 V
Capillary voltage -4500 V
Nebuliser 25 psi

Dry gas 4.0 mL/min
Dry temperature 180°C
External calibration – sodium formate.

Figure 6. Extracted ion chromatogram of dilute urine processed using MEPS and infused directly into the ESI source.



Errors
Codeine-6-Glucuronide – 0.608 ppm
Morphine-6-Glucuronide – 0.746 ppm
Morphine-3-Glucuronide – 0.746 ppm
Codeine – 3.619 ppm
Morphine – 5.699 ppm
Norcodeine – 5.699 ppm
Normorphine – 2.486 ppm

Figure 7. Mass spectra of dilute urine processed using MEPS and Infused directly into the ESI source.

Conclusion

MEPS is a robust micro SPE technology suitable for high-throughput sample preparation applications.

The digital analytical syringe embodiment delivers controlled flow rates through the programmable drive, minimizing inter-operator error and enabling rapid method development.

The miniature format facilitates direct injection into the LC and/or GC, and as demonstrated here directly into the mass spectrometer source.

References

Esme Candish, Australian Centre for Research on Separation Science (ACROSS). School of Chemistry, University of Tasmania.

Information and support

Visit www.trajanscimed.com or contact techsupport@trajanscimed.com

Specifications are subject to change without notice.