Automated Sample Preparation Using a Digital Syringe with Embedded SPE Capability

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Abstract

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 which 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

	SPE	MEPS™
Sample Volume	3 mL	50 µL
Time		
Price		
Solvents	10 mL	500 µL
Evaporation Step	Yes	Unnecessary

Table 1. MEPS™ compared to traditional cartridge SPE.

MEPS™ 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 dependant steps of aspiration and dispense where control of the flowrate typically varies from operator to operator.

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. The programmable digital analytical syringe - eVol® XR.

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 of Caffeine: 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™ syringe was coupled to 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 ~ 3minutes (see Table 2). The optimized method was repeated by two additional operators.

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

Step	Mode	Amount (µL)	Speed
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

Table 2. eVol Method for Caffeine extraction on MEPS .

- Gas Chromatography Mass Spectrometry:
  GCMS of the extracted caffeine from three operators was performed on an Agilent 6890/5973 using a BPX5 column (30 m x 0.25 mm ID, 0.25 µm df, see Figure 2). Injection - 1.0 µL, splitless @ 250 °C. FocusLiner with bottom taper
  Purge flow - 50 mL/min with a nominal inlet pressure of 127 kPa.
  Oven - 45 °C (hold for 4 min) to 300 °C (hold for 10 min) @ 10 °C/min. Carrier gas – He @ 1.2 mL/min in constant flow mode.
  MS - Mass spectra were collected over the range 40-500 Da at 2 scan/sec. Transfer line was 280 °C, quadropole was 150 °C and the source was 230 °C.

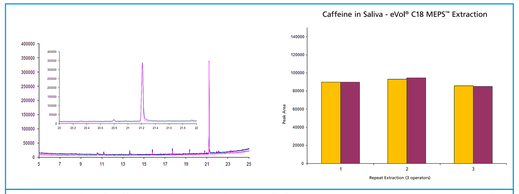


Figure 2. Overlay of caffeine peak extracted on MEPS™ from 3 operators. Inter-operator repeatability, three different operators, two independant extractions, same day same MEPS™ cartridge. 6 injections = 4.17 % RSD (auto injector repeatability on 6 standard injections = 2.94 % RSD).

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 it's metabolite morphine in the untreated sample. A simple dilute and infuse experiment (Figure 4 and 5) 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.

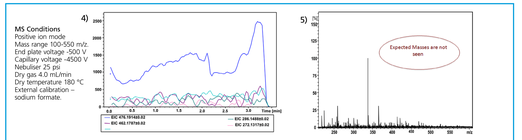
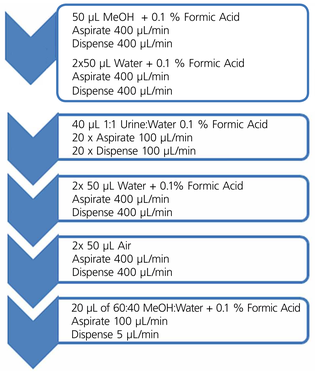


Figure 4. Extracted Ion Chromatogram of Dilute Urine Infused Directly into the ESI Source.
Figure 5. Mass Spectra of Dilute Urine Infused Directly into the ESI Source.

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.



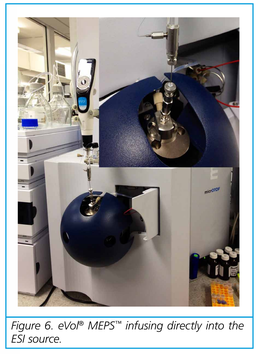


Figure 6. eVol® MEPS™ infusing directly into the ESI source.

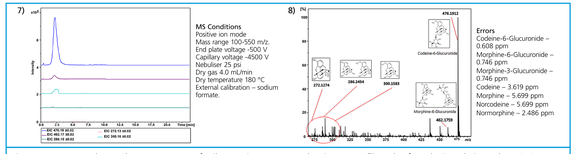


Figure 7. Extracted Ion Chromatogram of Dilute Urine Processed Using MEPS™ and Infused Directly into the ESI Source.
Figure 8. Mass Spectra of Dilute Urine processed using MEPS™ and Infused Directly into the ESI Source.

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 direct into the mass spectrometer source.

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