Cleanup of pharmaceutical drugs in biological fluids by automated microSPE prior to LC/MS

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Introduction

Micro Solid-Phase Extraction (µSPE) is an effective sample preparation technique utilising small bed volumes and particle size (<3 µm) that enable low sample volume analyses than other forms of SPE along with advantages in speed, efficacy and solvent use. Biological fluids (e.g. blood, serum) are complex and troublesome matrices that require significant sample preparation. The ePrep Sample Preparation Workstation provides fully automated liquid handling and greatly reduces the time and labour required for accurate preparation of standards at a wide concentration range. The use of µSPEed has the potential to simplify and streamline laboratory procedures.

In this work, we demonstrate the effectiveness of a novel µSPE technique combined with the Eprep workstation for the simultaneous analysis of seven pharmaceutical drugs in biological samples.

Figure 1 – µSPEed cartridges with one-way check valves and ePrep sample preparation Workstation

Methods

An automated sample handling workflow was developed for the detection and quantification of 7 pharmaceutical drugs in a complex biological matrix. The samples were loaded onto the µSPEed cartridge, (C18, 3µm/120Å), washed with ultrapure water and then eluted with MeOH. All standards and samples were prepared using the ePrep automated Workstation prior to analysis by on a Shimadzu 8060 triple quadrupole mass spectrometer equipped with a Shimadzu UHPLC Nexera X2 utilising a Zorbax SB-C18 (2.1 x 50 mm 1.8 µm). The biological samples were spiked with a 25 ppb Standard containing the 7 drugs.

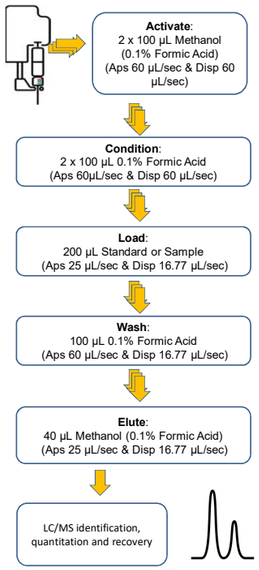

Figure 2 – µSPE Workflow

Experimental

1) **LC-MS separation of the 7 drugs in biological samples:** Gradient separation of the 7 drugs was performed on a Shimadzu (Rydalmere, Australia) Nexera MP UHPLC using a a Zorbax SB-C18 (2.1 x 50 mm 1.8 µm). Mobile phases consisted of ultra-pure water (A) and acetonitrile (B), each with 0.1% formic acid. Linear gradient from 10-80% of ACN in 8 min, then 2 min at 10% for equilibration step prior next injection.

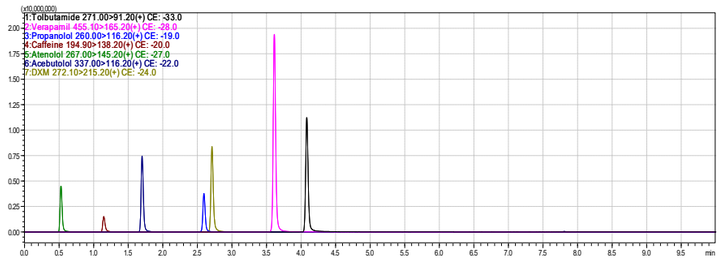

Figure 3 shows a typical HPLC chromatogram for a standard mix containing 7 target compounds at 25 ppb after µSPE extraction by LC-MS

2) **Method validation:** Calibration linearity for MS detection of the seven drugs was investigated by making 3 consecutives injections of a mix standard prepare at 5 different concentrations (each standard was prepare in triplicate), showing excellent linearity in the concentration range of 5-45 ppb.

Sample	R^2	LOD (ng/mL)	LOQ (ng/mL)
Atenolol	0.9938	0.88	3.29
Acebutolol	0.9750	0.05	0.14
Caffeine	0.9929	1.54	4.68
Propranolol	0.9903	0.08	0.23
DXM	0.9880	0.29	0.88
Verapamil	0.9837	0.05	0.14
Tolbutamide	0.9871	0.02	0.06

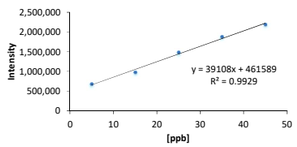

Table 1 Linearity, LOD and LOQ of the 7 drugs after µSPE extraction by LC-MS. Figure 3 Representative calibration curve for caffeine by HPLC MRM analysis of drug calibration standards after µSPE

Method precision was estimated using LC-MS detection by making 10 consecutives 2 µL injections of a calibration standard with a concentration of 25 ppb of each analyte. The obtained results show good precision for the µSPE extraction method (see Table 2).

	Atenolol	Acebutolol	Caffeine	Propranolol	DXM	Verapamil	Tolbutamide
Avg	27.32	25.25	25.58	27.33	25.21	24.95	25.41
SD	0.72	1.92	1.13	1.73	1.20	1.11	1.26
%RSD	2.6%	7.6%	4.4%	6.3%	4.7%	4.4%	4.9%

Table 2 Reproducibility of a 25 ppb standard containing the 7 drugs after extraction procedure quantified by area.

3) **Quantification of drugs in biological samples by µSPEed-LC/MS analysis:**

	Water		Serum		Urine		Saliva		Blood	
	Recovery%	%RSD	Recovery%	%RSD	Recovery%	%RSD	Recovery%	%RSD	Recovery%	%RSD
Atenolol	126.6	6.4	54.7	6.5	100.7	7.2	n.d.	n.d	n.d.	n.d
Caffeine	28.7	7.3	71.5	10.1	*	*	32.5	1.5	122.3	5.6
Acebutolol	100.9	4.7	110.5	8.1	113.5	3.1	95.2	2.7	n.d.	n.d
Propranolol	131.3	9.6	95.6	8.7	108.7	7.5	86.1	1.5	21.4	2.9
DXM	131.6	7.6	90.4	9.4	94.6	5.6	81.7	1.4	13	8.9
Verapamil	85.2	8.5	100.1	7.4	100.8	3.1	92.2	1.7	60.8	5.7
Tolbutamide	77.3	7.8	76.6	8.9	76.7	3.8	72.2	1.9	66.1	7.8

n.d. non detectable signal
* Caffeine concentration in the blank higher than in the spike sample (possible matrix effects)

Table 3 Average recoveries from spiked biological samples after µSPE extraction.

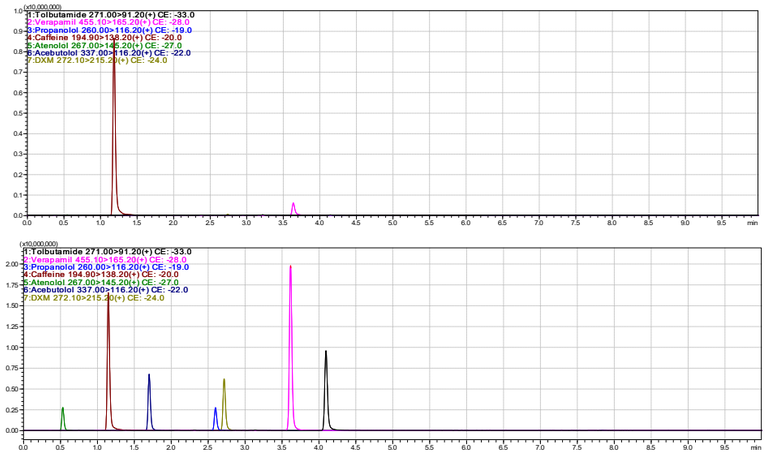

Figure 4 Typical HPLC chromatogram for a blank urine (a) and a spike urine sample containing 7 target compounds at 25 ppb (b) after µSPE extraction by LC-MS

Conclusions

The accurate and fast automated microSPE allows the quantification of 7 target pharmaceutical drugs, showing even acceptable recoveries in matrices as complexes as saliva or blood. Demonstrating that µSPEed on an automated platform can be used to prepare and quantify target drugs in "difficult" matrices.