Rapid Sample Preparation of Pharmaceutical Drugs in Serum and Blood by Automated µSPE

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Introduction: Sample preparation in medical diagnostics and forensics settings, from extraction and concentration, to dilution and isolation is vital for reliable and accurate analyses. Sample preparation is often the most time consuming physical job a chemist performs [1]. Automation of sample preparation minimises errors, frees analysts from liquid handling tasks, and the ePrep Sample Preparation Workstation (Figure 1) provides a simple and effective way to introduce automation into a laboratory. Micro solid-phase extraction (µSPEed) is an effective sample preparation technique that utilises small bed volumes and particle size (<3µm), enabling smaller sample volume analyses than other forms of SPE along with advantages in speed, efficacy and solvent use [2]. Blood is well known to be a complex and troublesome matrix that requires significant sample preparation, and the use of µSPEed has the potential to simplify these streamlining laboratory procedures.

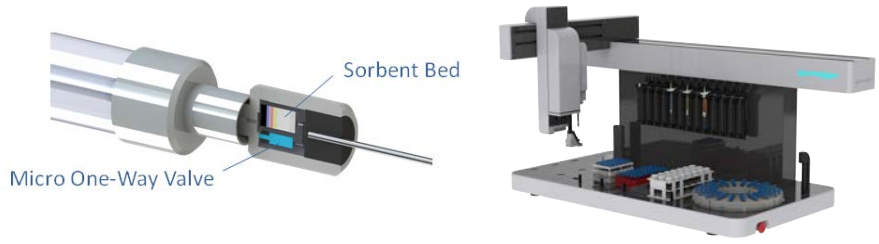


Figure 1: µSPEed cartridges with one-way check valves and the ePrep Sample Preparation Workstation.

Experimental: An automated sample handling workflow depicted in Table 1 was developed with a mixture of 8 compounds listed in Tables 2 and 3, which incorporated processing of serum and whole blood samples spiked with a mixture of drugs at ranges between 0.1 to 10 ppm, and 1 to 200 ppb. The samples were analysed on (1) a Thermo Fisher Scientific Vanquish UHPLC system through a C18+ 100x2.1mm 1.5µm column with UV detector (@ 210 and 254 nm) interfaced to a mass spectrometer (Thermo Fisher Scientific, MSQ), and (2) a Shimadzu 8060 triple quadrupole mass spectrometer equipped with a Shimadzu HPLC Nexera X2 (LC-30AD) utilising a Shimp-pack (XR-ODS III) 50x2.0 mm 1.6µm column.

Table 1 – ePrep Sample Preparation Workstation Workflow

	Volume µL	Reagent
Activation	200	Methanol 0.1% formic acid
Equilibration	200	Water 0.1% formic acid
Load	100 to 250	Serum : Drug Standard
Wash	100	Water 0.1% formic acid
Elute	40 to 50	Methanol 0.1% formic acid
Diluent	50 to 210	Water 0.1% formic acid

Results and Discussion:

1. Serum with 10ppm drug standards by UHPLC-UV Detection - Thermo Fisher Scientific HPLC-UV system (Figure 3) was used to confirm adsorption of compounds to µSPEed C18RPS-3µm/120Å cartridges. Cartridge reproducibility was excellent with an average RSD of 5.8% (Table 2). Aqueous and biological samples were used to test the effectiveness of the trapping and clean up procedures. The analysis was limited by UV detection at 5ppm.

Figure 2: UHPLC chromatogram of 10ppm Drug Standards in Serum.

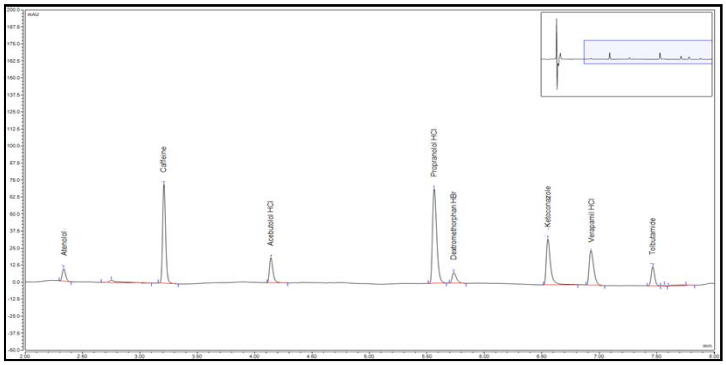
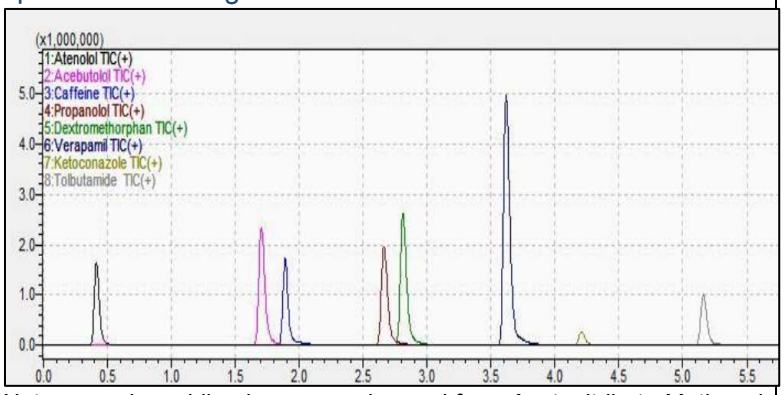


Table 2: Recoveries of 10ppm Serum Drug Standards.

Compound	%Recovery	%RSD
Atenolol	58.2	6.0
Caffeine	96.0	5.3
Acebutolol	104.6	5.3
Propranolol	81.8	5.8
Dextromethorphan	80.2	6.7
Ketoconazole	94.0	5.6
Verapamil	99.4	6.7
Tolbutamide	129.8	5.1

2. Serum with 50ppb drug standards by Multiple Reaction Monitoring (MRM) Mass Spectrometry - Drug standards at 50ppb in serum prepared using µSPEed C18RPS-3µm/120Å cartridges and analysed by MRM Mass Spectrometry. HPLC MRM chromatograms of drugs analysed by electrospray ionization (ESI) on a Shimadzu 8060 triple quadrupole mass spectrometer are presented in Figure 3.

Figure 3: HPLC MRM chromatograms for 50 ppb Drug Standards in Serum prepared with µSPEed C18 RPS-3µm/120Å cartridges.



Note: organic mobile phase was changed from Acetonitrile to Methanol for these experiments resulting in a peak order change in comparison to Figures 2.

Table 3 – Recoveries of 50ppb Drug Standards in Serum

Compound	%Recovery	%RSD
Atenolol	182.3	6.7
Caffeine	88.8	12.2
Acebutolol	53.5	16.0
Propranolol	83.9	12.9
Dextromethorphan	79.6	11.3
Ketoconazole	87.6	9.5
Verapamil	70.6	8.3
Tolbutamide	125.7	6.4

3. Serum with 50ppb drug standards and recoveries with internal standards - Tables 2 and 3 show excellent recoveries of the eight drugs in serum. The increased recovery of some compounds (i.e. acebutolol and tolbutamide of Table 2, and atenolol and tolbutamide of Table 3) are most likely due to enhanced electrospray ionisation following the µSPE purification step. This work was followed with the addition of internal standards (i.e. deuterated analog of drugs) for more accurate recovery measurements. The analysis of serum spiked with propranolol and its deuterated analog both at 10ppb with four µSPEed (C18RPS-3µm/120Å) cartridges revealed a %RSD of 1.12 when comparing the ratios of MRM peak areas for propranolol to its deuterated analog. These results support the use of internal standards for accurate quantitation of drugs.

4. Raw blood sample preparation (proof-of-concept) - Fresh whole human blood was spiked with drug compounds and mixed, then loaded on to µSPEed cartridges for clean-up and trapping/concentrating of drug compounds (data not shown). Recoveries were low due to the analysis of non-protein bound drug components, with the remaining compounds being removed in the filtering and trapping process. The clean up process was highly effective for the eight compounds with little interference from other components of blood and with no further clean-ups. These analyses are being optimised for solvent extraction and recovery by the use of internal standards.

Figure 4: Raw blood in µSPE Cartridge

Conclusion: The application of µSPEed cartridges has the potential to streamline sample preparation in the forensic and medical fields through simplified sample preparation techniques. The small particle (3µm) C18 sorbent and one way flow of µSPEed cartridges results in a very clean sample extract even from a raw serum sample at low analyte concentrations. The ability to automate or remove the most time consuming components allows for decreased error and still provides a comparable method. Further experiments are being performed on other biological fluids including saliva, urine and validation of raw blood. An internal standard will also be added for more accurate recovery calculations.

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References:

1. Kataoka, H., 2003. New trends in sample preparation for clinical and pharmaceutical analysis. TrAC Trends in Analytical Chemistry, 22(4), pp 232-244.

2. Vlčková, H., et al., 2017. Micro-SPE in pipette tips as a tool for analysis of small-molecule drugs in serum. Bioanalysis 9(11), pp 887-901.