A new sample introduction technique for mass spectrometry that allows for dried blood spot analysis and online SPE

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Introduction

Over the last few years, the collection and storage of blood samples onto specially designed filter paper cards has gained a lot of interest. The sampling technique is considered to be very convenient, easy to use and cheap. The workload within the lab has increased by this approach, however, due to punching out and manual off-line extraction procedures. Consequently, other application areas also suitable for this way of sampling are hardly explored. In this paper we present a new flow through concept that allows for the direct analysis of sample spots from paper into the mass spectrometer via online solid-phase extraction.

Experimental conditions

Online DBS-SPE (Spark Holland)

DBS

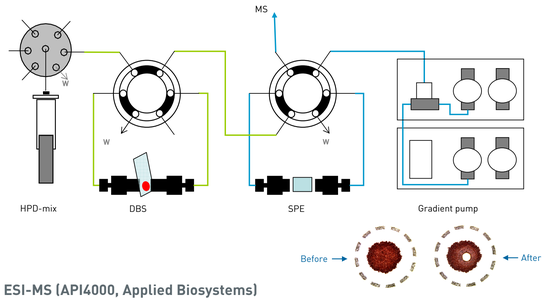
Filter card:	Whatman Protein SaverTM 903® Card
Sample volume:	15 µL
Sample matrix:	Blood (Na2-EDTA)
Desorption solvent:	1 mL water 0.2% FA at 2 mL/min (= sample transfer SPE)
Clamp flush:	1 mL 80/20 acetonitrile/water 0.2% FA at 5 mL/min
	1 mL water 0.2% FA at 5 mL/min

SPE

Cartridge:	HySphere C18HD 10x2 mm
Conditioning:	1 mL acetonitrile at 5 mL/min
Equilibration:	1 mL water 0.2% FA at 5 mL/min
Sample transfer:	1 mL water 0.2% FA at 2 mL/min
Cartridge wash:	1 mL 5/95 acetonitrile/water 0.2% FA at 5 mL/min
Elution:	3 min gradient A) water 0.2% FA; B) acetonitrile 0.2% FA
Clamp flush:	1 mL 80/20 acetonitrile/water 0.2% FA at 5 mL/min

Gradient:

time (m:s)	flow (mL/min)	A %	B %
00:01	1.0	95	5
00:05	1.0	95	5
01:35	1.0	60	40
01:45	1.0	60	40
02:00	1.0	95	5
03:00	1.0	95	5



ESI-MS (API4000, Applied Biosystems)

ESI-MS/MS conditions (positive mode)

MS-settings

Compound	Q1 mass	Q3 mass	DP	CE	CXP
Propranolol	260.1	116.1	31	25	8
Haloperidol	376.1	165.2	6	35	12
Amitriptyline	278.1	233.1	16	25	16
Verapamil	455.3	165.2	66	37	8

General settings: IS 5500 TEM 450 CAD 4 CUR 15 GS1 80 GS2 40 EP 10 Dwell 100

Results and Discussion

Test data proved that the developed system can be used to clamp and seal filter paper cards up to pressures of about 300 bar. Samples were directly flushed from the paper onto a C18 SPE cartridge by means of acidified water delivered by a high pressure solvent dispenser which is also used to perform an automated cleanup step. Subsequently, the disposable SPE cartridge is directly eluted towards the MS by means of a gradient. A throughput of at least 20 samples per hour could be obtained already (non optimized). Preliminary experiments show that no LC column is required as online SPE provides sufficient clean-up and separation.

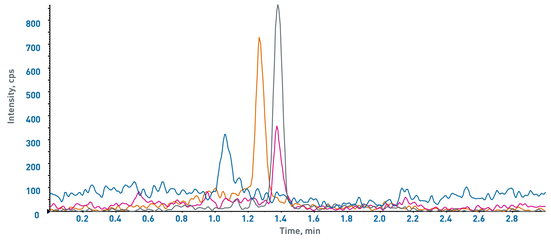


Figure 1: DBS-SPE-MS/MS chromatogram of **Propranolol**, **Haloperidol**, **Amitriptyline** and Verapamil in blood at lowest limit of quantitation (1 ng/mL)

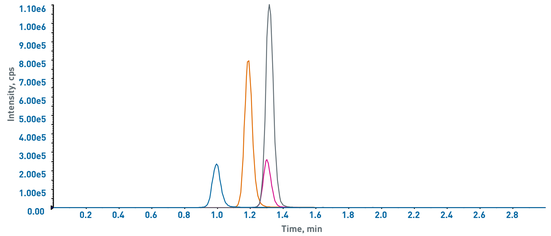


Figure 2: DBS-SPE-MS/MS chromatogram of **Propranolol**, **Haloperidol**, **Amitriptyline** and Verapamil in blood at highest limit of quantitation (1000 ng/mL)

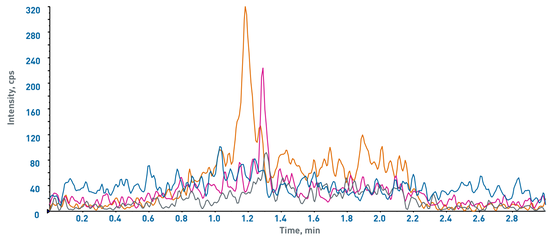


Figure 3: DBS-SPE-MS/MS chromatogram of blank blood analyzed directly after a 1000 ng/mL sample

The data below show that sensitivity of the technique is sufficient to determine the compounds at therapeutic level, despite the fact that only a small aliquot of the original sample (~0.25 µL) is introduced into the MS. In addition, sensitivity can be easily ~10 times higher than with a punch-out and extract methodology where only a part of the re-dissolved sample is injected.

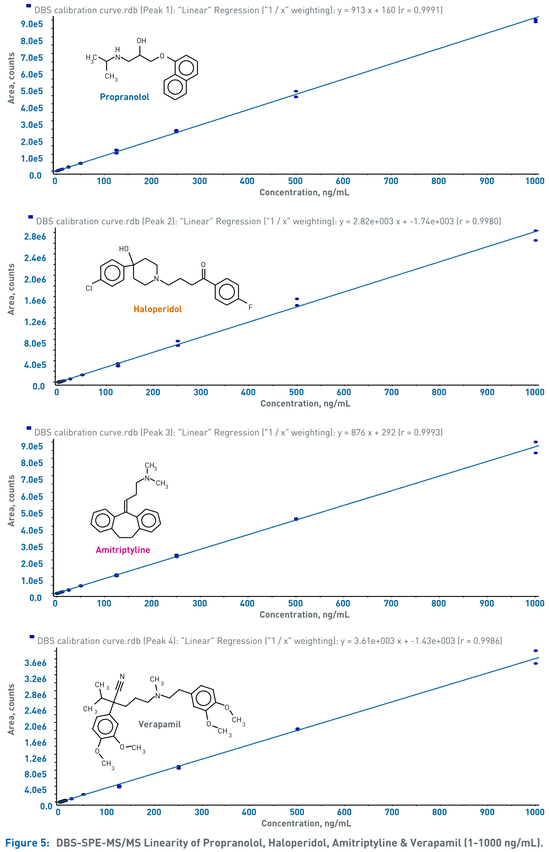


Figure 5: DBS-SPE-MS/MS Linearity of Propranolol, Haloperidol, Amitriptyline & Verapamil (1-1000 ng/mL).

Another part of the pre-validation test included the measurement of the relative standard deviation (RSD) and carry-over from a previously analyzed high concentration sample (1000 ng/mL). For the latter, a generic high and low organic solvent wash solution was used instead of optimizing the wash conditions to the dedicated analytes.

Relative standard deviation (%) of DBS-SPE-MS/MS at low, medium and high concentration (n=9)

Concentration level		Propranolol	Haloperidol	Amitriptyline	Verapamil
Low	(10 ng/mL)	4.11	5.48	6.84	7.20
Medium	(400 ng/mL)	5.55	5.75	4.32	3.24
High	(800 ng/mL)	5.38	4.44	5.09	6.12

Carry-over of DBS-SPE-MS/MS after analysis of high concentration sample (n=2)

Sample	Propranolol	Haloperidol	Amitriptyline	Verapamil
Spiked blood (1000 ng/mL)	9.11E+05	2.95E+06	9.78E+05	3.80E+06
Blank blood	< LOD	1.08E+03	5.17E+02	< LOD
Carry-over (%)	n.d	0.04	0.05	n.d

LOD = limit of detection defined as 2 times peak to peak noise; n.d. = not detected

Conclusion

- The concept of automated dried blood spot desorption by means of a new flow through concept proved to be easy to use and capable of analyzing at least 20 samples per hour.
- Blood samples were directly flushed from the paper towards a SPE cartridge and sufficient clean-up was obtained to measure Propranolol, Haloperidol, Amitriptyline and Verapamil by means of MS/MS without the need of an LC separation.
- The new DBS-SPE-MS/MS methodology can also be considered as DBS-LC-MS/MS with a disposable mini LC column and additional clean-up tools.
- As the completely desorbed blood sample is taken into analysis an LLOQ of 1 ng/mL was obtained even though only about 0.25 µL of the original sample spot was in use.
- Good linearity was obtained for all 4 compounds in blood over the 1-1000 ng/ml range (r > 0.998)
- Pre-validation data also showed that the relative standard deviation for low, medium and high concentration levels is well within the required guidelines for bioanalysis (3.2 – 7.2%) even though no internal standard was used.
- As filter paper cards can be spotted with any liquid, future work will be done to test the current approach for other application areas such as environmental, forensic and food.

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