Investigation of a new concept for dried blood spot analysis without punching by means of online DBS-SPE-MS/MS

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Objective

Investigate feasibility of on-line extraction of Dried Blood Spots (DBS) for pharma bioanalysis using:
- **On-line DBS-LC-MS/MS**
- **On-line DBS-SPE-MS**

Experimental On-line DBS extraction instrument

An experimental device has been built to test the concept of on-line DBS extraction. A common DBS filter card is inserted into a specially designed clamping system (patent pending). A 2 mm circular area of the spot center is clamped witch sufficient force to seal the spot against at least 4000 Psi (~280 bar). For On-line DBS-LC-MS/MS, the extract is directly loaded onto the LC column by the LC-pump for gradient LC-MS/MS analysis (see fig 1). For On-line DBS-SPE-MS/MS, a High Pressure Dispenser (HPD) is used to direct the extraction solvent through the clamped spot area. The extract is trapped on an SPE cartridge for clean-up and then eluted directly into the MS by the gradient LC-pump (see fig 2)

Conclusion

- **A new DBS clamping device allows on-line (flow-through) dried blood spot desorption directly coupled to LC-MS/MS or SPE-MS/MS**
- **On-line DBS-LC-MS showed serious matrix interference and does not seem suitable for reliable quantitative bio-analysis at adequate sensitivity.**
- **On-line DBS-SPE-MS/MS showed good linearity and precision for 4 model compounds with sensitivity down to sub-ng/mL level.**
- **Matrix components are effectively flushed to waste before eluting the SPE trap to the MS/MS system.**
- **A fresh SPE cartridge for every analysis eliminates built-up of matrix constituents from consecutive analyses**
- **On-line DBS-SPE-MS/MS is a simple straightforward system capable of analyzing at least 20 samples per hour.**

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Experimental setup

ESI-MS/MS

MS: API 4000 (ABSciex) in positive mode
General settings: IS 5500; TEM 450; CAD 4; CUR 15; GS1 80; GS2 40; EP 10; Dwell 100

Compound specific 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

On-line DBS-LC-MS/MS

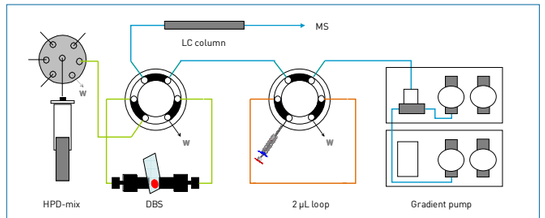

Figure 1 System configuration for On-line DBS-LC-MS (the HPD is used for clamp wash only)

Experimental conditions

DBS
Filter card: Whatman FTA® DMPK-C Card
Sample volume: 15 µL
Sample matrix: Human Blood (K3-EDTA)
Desorption solvent: first 1 min of LC gradient
Clamp flush: 1 mL 80/20 ACN/water 0.2% FA at 5 mL/min
1 mL 5/95 ACN/water 0.2% FA at 5 mL/min

LC
Column: Intersil ODS-3 column, 3 µm 4.6 x 50 mm (GL Sciences Inc.)
Mobile phase A: Acetonitrile 0.2% FA
Mobile phase B: Water 0.2% FA

LC Gradient:

time (m:s)	flow (mL/min)	A %	B %
00:01	1.0	95	5
01:00	1.0	95	5
02:00	1.0	40	60
03:00	1.0	40	60
04:00	1.0	95	5
04:30	1.0	95	5

On-line DBS-LC-MS/MS

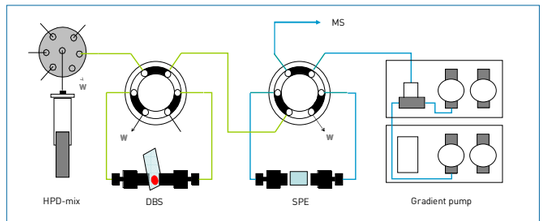

Figure 2 System configuration for On-line DBS-SPE-MS

Experimental conditions

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

SPE 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

Results

On-line DBS-LC-MS/MS

Figure 3 shows a typical chromatogram of an On-line DBS-LC-MS run. A throughput of about 12 samples per hour was obtained.

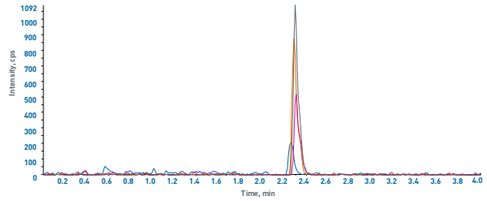

Figure 3 On-line DBS-LC-MS of Propranolol, Haloperidol, Amitriptyline and Verapamil in blood at 750 ng/ml

Linearity was investigated over the 50-1000 ng/mL range. After completion of the first calibration series, the sequence was repeated 4 more times because the first series showed a bad regression coefficient. Subsequent series show an improving coefficient of regression but much lower sensitivity as is shown for Haloperidol and Verapamil in figure 4

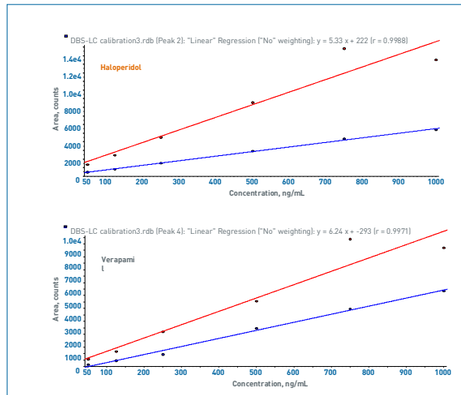

Figure 4. linearity of the first [red trace] and fifth [bleu trace] series for haloperidol and verapamil

It was suspected that blood components are building up on the LC column or MS source, causing increasing levels of ionization-interfering compounds eluting from the column. In order to investigate this, 2 µL of neat analyte solution was manually injected 5 times immediately after the calibration series (using the 2 µL loop; see fig 1). The results (see figure 5) indicate that indeed matrix components had built-up and are gradually washed off the column under clean LC conditions.

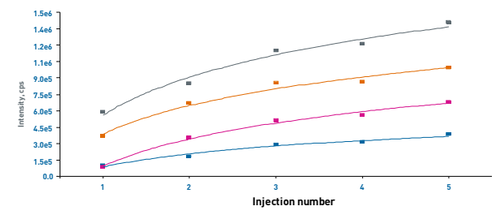

Figure 5. Signal from 5 subsequent injections of neat solutions of Propranolol, Haloperidol, Amitriptyline and Verapamil immediately after calibration series of blood samples

It is clear from the above, that direct elution of blood extracts onto the LC column, without any clean-up, results in significant interference from matrix components, seriously hampering assay reliability and sensitivity.

On-line DBS-SPE-MS/MS

Figure 6 shows a typical chromatogram of an On-line DBS-SPE-MS run. A throughput of at least 20 samples per hour was obtained.

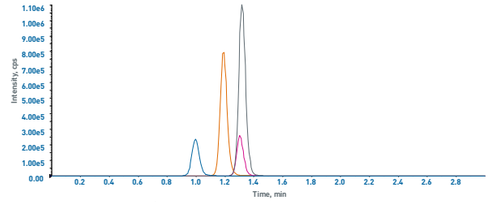

Figure 6. DBS-SPE-MS/MS chromatogram of Propranolol, Haloperidol, Amitriptyline and Verapamil in blood at upper limit of quantitation (1000 ng/mL)

Linearity was measured over the 1-1000 ng/mL range. Good regression coefficients were obtained as shown in Table I show.

Table I. Linearity of DBS-SPE-MS/MS for 1 – 1000 ng/mL

Compound	regression coefficient (r)
Propranolol	0.9991
Haloperidol	0.9980
Amitriptyline	0.9993
Verapamil	0.9986

Precision of the DBS-SPE-MS/MS method was measured at 3 different concentration levels. As shown in Table II, the RSD values obtained are well within common acceptance criteria. The LOQ was calculated te be below 1 ng/mL for all compounds.

Table II. 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 from a previously analyzed high concentration sample (1000 ng/mL) was determined after a generic high and low organic solvent wash step. As shown in figure 7 and by the data in Table III, only Haloperidol and Amitriptyline gave some measurable carry-over. Optimization of wash solvents will likely further reduce carry over.

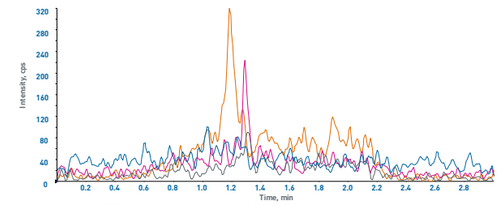

Figure 7: DBS-SPE-MS/MS chromatogram of blank blood analyzed directly after a 1000 ng/mL sample

Table III. Carry-over of DBS-SPE-MS/MS (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

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