Towards unbiased dried blood spot analysis using temperature-enhanced flow-through desorption coupled online to solid-phase extraction and mass spectrometry

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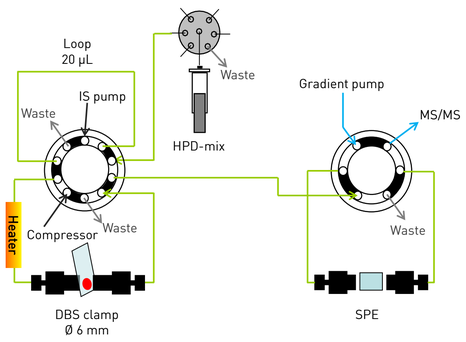
Introduction

Variation of the blood hematocrit (Ht) level still is a serious issue for analyte quantitation in DBS analysis. It influences spot size and consequently causes deviations in the sample aliquot when only a part of the spot is analyzed. The most obvious approach to solve this problem is using the entire spot for analysis. A multi-dispenser prototype is evaluated in this regard with respect to ease of use and volumetric precision. As not only spot size but also analyte recovery varies with blood Ht, temperature-enhanced desorption conditions are applied to re-dissolve the entire dried blood sample.

Methods

Online FTD-SPE-MS/MS



DBS analysis is carried out using a DBS autosampler (DBSA) prototype. The DBS card is transferred from the card rack to the sliding card holder by the x-y-z robot of the DBSA. A picture is taken of the card for determining the exact spot position. Then, the blood spot is clamped for flow-through desorption (FTD). The clamp has an internal rim diameter of 6 mm enabling the desorption of the entire blood spot. Internal standard is added via loop and is flushed over the DBS card together with the desorption solvent. The entire sample is subsequently flushed towards a SPE cartridge for clean-up. Afterwards, the same SPE cartridge is used as "mini LC column" eluting the analytes directly by gradient towards the MS (work has partly been carried out with experimental device instead of DBSA prototype; principle of these measurements is the same as described).

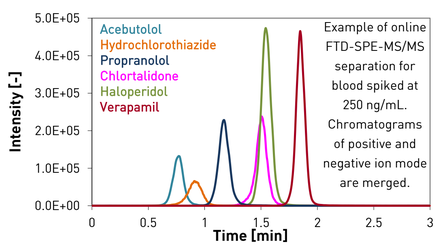
DBS (final temperature-enhanced conditions)

Filter card: Whatman FTA™ DMPK-C Card (Cat No Wb129243)
Sample: 5 µL human blood (K_3-EDTA)
DBS clamp: 6 mm diameter
Desorption: 1 mL water 0.2% FA at 2 mL/min (80°C); 1 mL 5/95 ACN/water 0.2% FA at 5 mL/min (80°C)
DBS clamp flush: 1 mL 80/20 ACN/water 0.2% FA at 5 mL/min; 1 mL water 0.2% FA at 5 mL/min

SPE

Cartridge: HySphere C18HD 10x2 mm (7 µm) (positive MS mode)
HySphere Resin GP 10x2 mm (5-15 µm) (negative MS mode)
Conditioning: 1 mL ACN at 5 mL/min
Equilibration: 1 mL water 0.2% FA at 5 mL/min
Elution:

Time [min]	Flow [ml/min]	Water 0.2% FA [%]	ACN 0.2% FA [%]
0:01	1	95	5
0:05	1	95	5
1:45	1	75	25
2:50	1	75	25
3:10	1	95	5
3:20	1	95	5



ESI-MS (API 4000, ABSciex)

Compound	Q1 [m/z]	Q3 [m/z]	DP [V]	CE [V]	CXP [V]	General Settings	
Positive ion mode						IS [V]	3000 / -4200
Acebutolol	337.2	116.1	26.0	33.0	10.0	TEM [°C]	550
Haloperidol	376.3	165.2	66.0	35.0	30.0	CAD	4
Verapamil	455.4	165.1	76.0	39.0	30.0	CUR [psi]	15
Propranolol	260.1	116.1	61.0	27.0	22.0	GS1 [psi]	55
Negative ion mode						GS2 [psi]	70
Chlortalidone	336.8	190.0	-90.0	-24.0	-11.0	EP [V]	10 / -10
Hydrochlorothiazide	296.0	268.8	-95.0	-26.0	-21.0	Dwell time [msec]	100

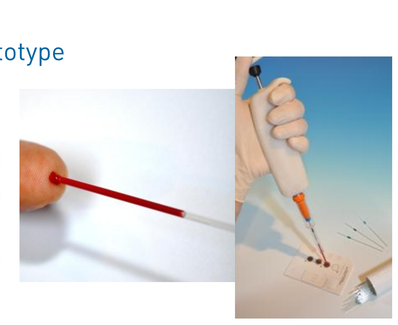
Experimental

Spiked blood of different Ht levels

The Ht level of human K_3-EDTA blood is determined (approximately) after centrifugation (15 min, 3000 rpm) by dividing the volume of red blood cells by the total blood volume. Blood of different Ht levels is then prepared by addition of plasma (low Ht) or red blood cells (high Ht).
Blood is mixed with a standard solution in 40% acetonitrile (ratio 20:1) to obtain blood spiked with Chlortalidone (3.0 µg/mL), Hydrochlorothiazide (0.5 µg/mL), Acebutolol (0.1 µg/mL), Haloperidol (0.1 µg/mL), Verapamil (0.1 µg/mL) and Propranolol (0.2 µg/mL). After the spiked sample is equilibrated for at least 1 hour 5-µL blood is applied to filter paper cards and is allowed to dry at room temperature for ≥ 2 hours.

Sample application using Drummond multi-dispenser prototype

The capillary is held horizontally into a blood drop and is filled by capillary suction. Blood is then dispensed in 5 serial volumes of 5 µL (dispenser strokes are pre-set) onto the DBS card. Touching the card with the capillary is uncritical, in fact, spot size is smaller when touching the card. Spot size of sample volumes up to 10 µL can thus be kept small enough to allow full-spot analysis using the 6-mm clamp.



Standard mixture for loop injection

The volume of the "20 µL" loop is accurately determined to be 21.87 µL. For loop injection of equivalent amounts of compounds as present in 5-µL blood spots the following standard mixture is prepared in 10% acetonitrile with 0.2% FA: Chlortalidone (0.68 µg/mL), Hydrochlorothiazide (0.11 µg/mL), Acebutolol (0.023 µg/mL), Haloperidol (0.023 µg/mL), Verapamil (0.023 µg/mL), Propranolol (0.46 µg/mL).

Measurement of recovery and MS matrix effects

Recovery of the analytes from the blood spot and MS matrix effects are measured using the loop attached to the 10-port valve. The procedure involves three subsequent experiments:

1. A "normal" analysis of a spiked blood spot is performed.
2. The loop is filled with the standard mixture and a blood spot analysis is performed using blank blood as the sample. By switching the loop into the desorption solvent stream at the moment of starting the desorption, blank blood and analyte mix are flushed over the SPE cartridge simultaneously. By comparing 1 and 2, any loss of analyte due to incomplete recovery from the blood spot can be determined independently of SPE recovery and MS ionization suppression.
3. See step 2, but now a totally blank card (no blood spots) is clamped. By comparing 3 and 2, signal loss or enhancement due to matrix effects on MS ionization can be determined.

Results and Discussion

Optimization and exploration

Effects of hematocrit and spot aging on analyte recovery

Dried blood spots are initially analyzed using "standard" conditions for desorption (1 mL water 0.2% FA at 2 mL/min; no heater used). Recoveries are determined for spots made of blood with Ht levels 0.3 and 0.7 after 1 and, respectively, 4 days of storage. For comparison blood spots (Ht 0.7) are analyzed using optimized desorption conditions (see Methods).

Recovery [%]				
Compound	**Ht**	**1 day storage Standard DBS desorption**	**4 days storage Standard DBS desorption**	**4 days storage Optimized DBS desorption**
Haloperidol	0.3	91	88	-
	0.7	65	59	94
Amitriptyline	0.3	92	89	-
	0.7	69	55	96
Verapamil	0.3	92	90	-
	0.7	69	58	95

- Standard conditions: Recovery is significantly lower for DBS of high Ht level.
- Standard conditions: Spot-aging slightly decreases analyte recovery for DBS of high Ht level.
- Optimized temperature-enhanced desorption conditions: ≥94% recovery for DBS of high Ht level even after 4 days aging.

Precision of sample application by multi-dispenser prototype

The 5-µL spotting precision of the Drummond multi-dispenser is investigated taking the FTD-SPE-MS/MS measurement of Haloperidol for calculation. Five capillaries were filled with blood and each one was used to dispense 5x5 µL onto a DBS card.

Precision Drummond Dispenser			
Capillary	**RSD [%] Haloperidol** 5 spots per capillary	**RSD [%] Haloperidol** 4 spots per capillary (first 5 µL discarded)	**Overall RSD [%] Haloperidol** 20 spots, 4 spots per capillary (first 5 µL discarded)
1	28.3	2.7	
2	5.0	2.9	
3	3.7	3.6	3.7
4	20.7	4.6	
5	3.2	3.6	

- RSDs are acceptable for all individual capillaries in case the first spot is not used for calculation. (Drummond is working on an improved version of the dispenser to solve this issue.)
- A good overall precision is obtained for a series of 20 spots from all 5 capillaries.

Measurements temperature-enhanced desorption

Precision of FTD-SPE-MS/MS method

Precision, RSD [%] (n = 4)					
Chlortalidone	Acebutolol	Hydrochloro-thiazide	Haloperidol	Verapamil	Propranolol
5.5	4.6	4.4	3.7	2.2	5.4

- Very good precisions are obtained for all test compounds.

FTD Recovery

Recoveries of the analytes spiked to blood of different Ht levels are determined as described in experiments 1 and 2 (see Methods). Experiment 2 is carried out with blank blood at Ht level 0.45.

Recovery [%]			
	Ht 0.3	**Ht 0.45**	**Ht 0.7**
Chlortalidone	96.8	96.0	97.2
Hydrochlorothiazide	95.6	103.6	92.6
Acebutolol	98.5	95.8	94.5
Haloperidol	101.1	96.9	88.9
Verapamil	106.7	101.3	99.7
Propranolol	91.0	104.3	107.9

- Recoveries for all Ht levels are ranging between 88.9% and 107.9%.
- No dependency of recovery on the Ht is observed. Dried blood is efficiently removed from the card by optimized desorption at high temperature.

Matrix Effects

Matrix effects are determined as described in experiment 3 (see Methods)

MS matrix effects [%] (n = 3)					
Chlortalidone	Acebutolol	Hydrochloro-thiazide	Haloperidol	Verapamil	Propranolol
83.7	109.9	102.7	105.4	88.4	90.4

- Matrix effects are within the bioanalytical acceptance range, except for Chlortalidone. However, the effect is considered to be small enough to enable reliable analysis.

Conclusion

- The Drummond multi-dispenser can conveniently be handled to sample blood from a finger prick and to dispense 5-µL aliquots afterwards onto DBS cards. Touching the DBS card does not result in any analytical issues; in contrast, it helps to keep the spot diameter small.
- The entire bloodspot is analyzed so that no sample is wasted.
- Sample clean-up by online SPE reduces MS matrix effects to an acceptable level.
- By using temperature-enhanced desorption conditions recoveries close to 100% are attained independent of the blood Ht level.
- Flow-through desorption (FTD) at high temperatures combined with online SPE-MS/MS of full bloodspots overcomes the Ht-issue in DBS analysis. This micro-sampling concept therefore enables a robust and reliable quantitative DBS analysis.

Acknowledgement
Drummond Scientific is gratefully acknowledged for loan of the prototype multi-dispenser.

References
[1] Denniff P, Spooner N. Bioanalysis, 2 (8), 2010 , pp 1385-1395.
[2] Ooms JA, Knegt L, Koster EH. Bioanalysis, 3(20), 2011, pp 2311-2320.

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