Analysis of entire dried blood spots by flow through desorption coupled to online solid-phase extraction and mass spectrometry

Lena Knegt, Bert Ooms and Christel Hempen ▶ Spark Holland, Pieter de Keyserstraat 8, P.O. Box 388, 7800AJ, Emmen, The Netherlands, Phone (+31) 591 631 700, E-mail: christel.hempen@sparkholland.com, Website: www.sparkholland.com

Overview

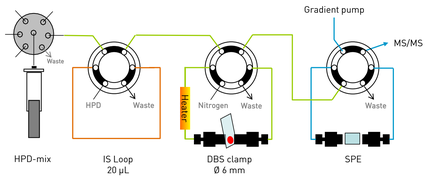
- A sample introduction technique for mass spectrometry based upon flow through desorption of filter paper cards and online SPE is presented.
- A method for the analysis of entire five-µL blood spots is developed. The conditions for desorption of the dried sample from filter paper cards are optimized.
- Validation is carried out with respect to precision, accuracy, linearity, carry-over, recovery and matrix effects.
- The effect of different blood hematocrit levels on the analyte recovery is investigated.
- The results of the validation experiments are within the bioanalytical acceptance criteria.

Introduction

Increasing interest in dried blood spot (DBS) analysis is based on less-invasive sample collection, small sample volumes and easy sample shipping and storage. Automated sample preparation of DBS is preferable to traditional manual procedures due to throughput and error prevention. Therefore, a novel technique for DBS analysis based on flow through desorption coupled online to solid-phase extraction and MS detection is applied. Because sufficient sensitivity as well as avoidance of sample loss are desirable, analysis of whole DBS is carried out. In this work the development and validation of a new method for analysis of entire DBS is presented. The effect of different hematocrit levels is investigated.

Methods

Online DBS-SPE (Spark Holland)



DBS

Filter card:	Whatman FTA™ DMPK-C Card (Cat No Wb129243)
Sample:	5 µL Human blood (K_3-EDTA)
Desorption solvents:	1 mL water 0.2% FA at 2 mL/min, Heater 80°C
	1 mL 5/95 acetonitrile/water 0.2% FA at 5 mL/min, Heater 80°C
DBS 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
Clamp drying:	Nitrogen at 1.5 bar

SPE

Cartridge:	HySphere C18HD 10x2 mm, 7 µm
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, Heater 80°C
	1 mL 5/95 acetonitrile/water 0.2% FA at 5 mL/min, Heater 80°C
	(= desorption solvents)
Elution:	A) water 0.2% FA; B) acetonitrile 0.2% FA

Gradient:

Time [mm:ss]	Flow [mL/min]	A [%]	B [%]
00:01	1.0	95	5
00:05	1.0	95	5
01:45	1.0	75	25
02:50	1.0	75	25
03:20	1.0	95	5
03:30	1.0	95	5

LC flow split to 0.2 mL/min

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

ESI-MS (Acquity TQD, Waters)

ESI-MS/MS conditions (positive mode)

MS-settings				
Compound	**Parent (m/z)**	**Daughter (m/z)**	**General settings**	
Analytes of final method				
Acebutolol	337.2	116.1	Capillary voltage	2.00 kV
Imipramine	281.3	85.9	Cone voltage	28 V
Verapamil	455.3	165.2	Cone voltage	28 V
			Cone gas	50 L/hr
Internal standard (IS) added by loop			Source temp	150 °C
Propranolol	260.3	116.1	Desolvation temp	250 °C
			Desolvation gas	500 L/hr
Analytes used in optimization experiments			Collision gas	0.30 mL/min
Amitriptyline	376.1	165.1	Dwell time	0.05 sec
Haloperidol	278.1	90.8		

Desorption of whole DBS from card

Five-µL blood spiked with Acebutolol, Imipramine and Verapamil is pipetted on a filter paper card and is allowed to dry at room temperature for at least two hours.

For analysis the blood spot is clamped in an experimental device for flow through desorption (FTD) without punching.

The clamp has an internal rim diameter of 6 mm enabling the desorption of the entire blood spot.

The complete sample is thus subsequently flushed towards a SPE cartridge for clean-up.

Afterwards, the same SPE cartridge is used as "mini LC column" so that the analytes are directly eluted by gradient to MS.

Internal standard is added via loop and is flushed over the DBS card together with the desorption solvent.

Results and Discussion

Optimization of desorption conditions

DBS desorption conditions have been investigated with respect to the number of desorption steps, desorption solvent composition, flow rate and solvent temperature.

Data shown in the Table were generated using 1 mL solvent for each desorption step at a flow rate of 2 mL/min.

Desorption optimization for DBS spiked with 4 analytes (Ht 0.7); Peak area (n = 2)					
1. Desorption step	**2. Desorption step**	**Haloperidol**	**Amitriptyline**	**Verapamil**	**Propranolol**
0.2% FA	-	1052.8	3088.7	3961.5	6158.6
5% ACN 0.2% FA	-	1025.1	3233.6	4002.2	6119.5
0.2% FA	5% ACN 0.2% FA	1326.5	4116.2	5180.9	7772.6
0.2% FA, 80°C	5% ACN 0.2% FA	1328.5	3979.1	5086.6	7478.6
0.2% FA, 80°C	5% ACN 0.2% FA, 80°C	1393.7	4407.9	5588.2	8444.7

- Performing two desorption steps using heated solvents yield highest peak areas for all compounds.

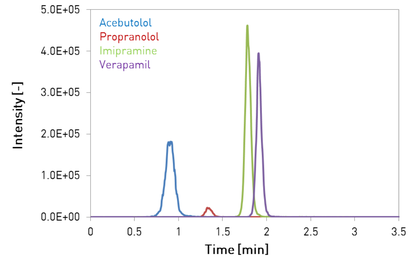
Is analyte recovery dependent on Hematocrit?

Desorption dependence on Ht (Peak area, n = 2)				
Ht	**Acebutolol**	**Imipramine**	**Verapamil**	**Propranolol (IS)**
0.3	14219.9	23046.6	19508.6	1725.7
0.45	14391.6	21350.0	18735.9	1706.2
0.7	15585.1	22100.9	18635.2	1701.7
Ratio Ht 0.45 / Ht 0.3 [%]	101.2	92.6	96.0	98.9
Ratio Ht 0.45 / Ht 0.7 [%]	92.3	96.6	100.5	100.3

- Small differences in analyte recovery for blood spots of Ht 0.3 and Ht 0.7, respectively, in comparison to DBS of Ht 0.45.

Chromatogram online DBS-SPE-MS/MS

Measurement of blood standard spiked at 250 ng/mL.



Validation

Intra-day Precision

The repeatability of the analyte measurement is determined for DBS spiked to concentration levels low, medium and high (1 ng/mL, 25 ng/mL and 500 ng/mL).

The repeatability of the IS loop addition (12.5 ng/mL Propranolol) is determined at each concentration level.

Precision [% CV, n = 6]				
Concentration [ng/mL]	**Acebutolol**	**Imipramine**	**Verapamil**	**Propranolol (IS)**
1	8.0	10.4	16.3	4.7
25	4.7	3.6	3.5	1.7
500	5.9	4.7	4.5	4.0

- Acceptable precisions are obtained for all compounds at all concentration levels and also for the IS addition.

Inter-day Precision and Accuracy

Inter-day precision and accuracy are determined for DBS spiked to levels 1 ng/mL, 25 ng/mL and 500 ng/mL.

Measurements are carried out in threefold on four consecutive days.

Inter-day Precision and Accuracy				
	Concentration [ng/mL]	**Acebutolol**	**Imipramine**	**Verapamil**
Inter-day precision [% CV, n = 4]	1	10.1	8.1	10.9
	25	3.3	2.3	4.9
	500	4.4	5.3	6.4
Accuracy [%]	1	112.6	103.9	101.7
	25	97.3	96.2	94.6
	500	92.3	95.4	94.8

- Good inter-day precisions and accuracies are obtained for all concentration levels of the three analytes.

Carry-over

Investigation of carry-over is carried out by measuring a blank DBS after a sample of high analyte concentration.

For the high standard, blood spiked with analytes to a concentration of 800 ng/mL (ULOQ) is used.

Carry-over			
Peak area (n = 2)	**Acebutolol**	**Imipramine**	**Verapamil**
spiked DBS (800 ng/mL)	41706.8	64439.5	50321.6
blank DBS	< LOD	< LOD	< LOD
Carry-over [%]	n.d.	n.d.	n.d.

- No carry-over was detected as no analyte was measured at all in the blank DBS measurements.

Desorption Recovery

Approach: A number of consecutive analyses of the same DBS are carried out.

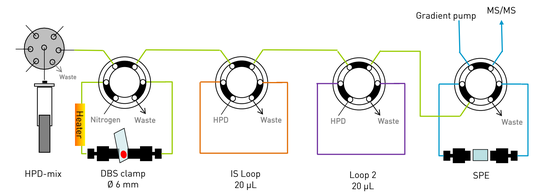
The sum of the analyte signals of all analyses is close to 100% recovery.

Desorption Recovery [%]			
Desorption No.	**Acebutolol**	**Imipramine**	**Verapamil**
1	100.0	99.8	99.6
2	0.0	0.2	0.4
3	0.0	0.0	0.0

- The desorption recoveries obtained are almost 100% for all compounds.

Matrix Effects

For the determination of matrix effects positions of IS loop and DBS clamp are exchanged and a second loop (Loop 2) is added to the system set-up:



Loop 2 is filled with analyte standard solution (12.5 ng/mL Acebutolol, Imipramine and Verapamil in 10% ACN 0.2% FA) by means of a High Pressure Dispenser (HPD).

The desorption solvents are flushed over a blank DBS, via the IS loop and loop 2 and subsequently towards a SPE cartridge.

In a second measurement the heated desorption solvents are directly flushed to the IS loop. The DBS clamp is by-passed.

Matrix effects				
Peak area (n = 2)	**Acebutolol**	**Imipramine**	**Verapamil**	**Propranolol (IS)**
DBS clamp by-passed	7551.6	13403.6	9372.6	1869.2
Blank DBS	7615.5	11292.6	9630.0	1703.9
Matrix effects [%]	100.8	84.3	102.7	91.2

- For Imipramine ion suppression slightly outside the acceptance range is observed.

Conclusion

- The concept of dried blood spot desorption by means of the flow through technique proved to be easy to use.
- The blood sample is directly flushed from the paper towards the SPE cartridge and sufficient clean-up is obtained to measure Acebutolol, Imipramine and Verapamil by means of MS/MS.
- The whole DBS is enclosed by the clamp and entirely desorbed and analyzed. No sample is wasted.
- The use of heated desorption solvents results in highest analyte peak areas. Under these conditions analyte recoveries are comparable for DBS with Ht levels between 0.3 and 0.7.
- Internal standard addition via a loop during desorption of a blood spot provides a good overall precision. It is flexible and comparable to the traditional way of IS addition but might not compensate for some dedicated assay aspects such as desorption recovery, however.
- Intra-/Inter-day precision and accuracy are well within the bioanalytical acceptance criteria for low, medium and high analyte concentrations.
- Good linearity (R^2 > 0.99) is observed for all analytes over the concentration range from 1 ng/mL to 800 ng/mL. (data not shown)
- No carry-over is detected at all for DBS analysis executing a clamp flush step between the measurements.
- Matrix effects determined for Acebutolol, Verapamil and Propranolol are within the bioanalytical acceptance range. For Imipramine ion suppression was observed (84.3%) that probably could be compensated by the use of an isotope-labeled IS.
- The data suggest that an effect of the hematocrit level on the analyte recovery is circumvented by analyzing entire blood spots of known volume under optimized harsh conditions. The concept therefore could have the potential to allow for reliable quantitation in DBS analysis.

BETTER **SAMPLE** CARE

0053.881-1