Application of an Automated Microsampling Technique to Trazodone and Paracetamol

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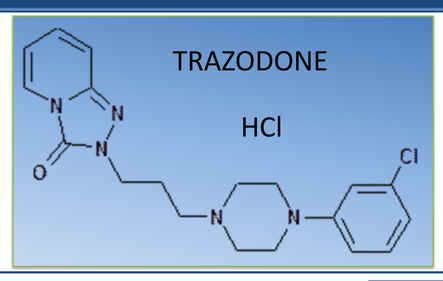
INTRODUCTION and AIM

The use of the microsampling tecniques for non-clinical and pediatric PK studies are well documented. The interest on microsampling continues to increase since it leads to progress in the 3Rs strategy.

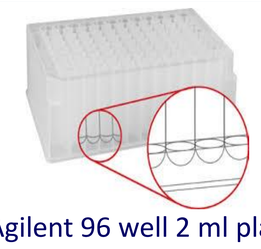
The aim of the present work was to implement a new approach for blood collection by Volumetric Absorptive Microsampling device (VAMS, MITRA™) that allows an accurate collection of fixed and low blood volume (10 µl) and an easy manipulation of the samples. Trazodone (TRZ), an antidepressant discovered by Angelini, and paracetamol (PAR), an antipyretic and analgesic drug, were used as test molecules. In particular, the study was focused on:

1. Sample collection procedure from mouse
2. Analytical method's performance by the evaluation of Matrix Effect, Recovery, Reproducibility, Specificity and Stability of the final extract using fresh and frozen blood
3. Aging of the blood samples absorbed on the device
4. Automation of the extraction procedure

MATERIALS and METHODS



VAMS, Neoteryx, Mitra™



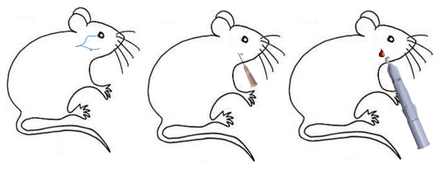
Agilent 96 well 2 ml plates

26-G needles

Liquid Handling Workstation Perkin Elmer, Janus

Agilent series 1200 ABSciex, API4000

In vivo sample collection from mouse



a) Mouse was restrained with the non-dominant hand
b) Blood was collected from submandibular vein: puncture was done behind the point at which the upper and lower jawbones meet
c) The microsampling device tip was quickly positioned to absorb the blood drop that passively flows
d) A gentle pressure to the site was applied with gauze sponge to stop the bleeding

Extraction procedure

10 µl of blood (blank or spiked) absorbed on the VAMS device
↓
drying of VAMS samples at RT (on the benchtop)
↓
transfer of the tip to well plate containing 100 µl of H2O with 1% f.a. and IS (*)
↓
sonication (15') and vortex (30')
↓
addition of 300 µl of acetonitrile and vortex (30')
↓
centrifugation and separation of the clear extract

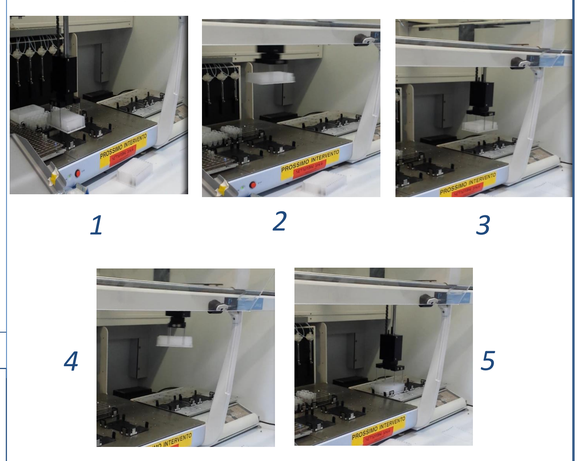
PAR ↓ Dilution (5 fold) of the extract with mobile phase

TRZ ↓ Evaporation of an aliquot of the extract (200µl) ↓ Dissolution of the dried residue with mobile phase (100µl)

↓ **HPLC-MS/MS analysis**

(*) Internal Standards: paracetamol d4 and trazodone d6

Representative automated steps



1 – 2: The gripper pulls up and rotates the extraction plate
3 – 4: The gripper moves the extraction plate and pulls down it over the mixer
5: The gripper transfers the extraction plate to the original position

Chromatography

	PARACETAMOL	TRAZODONE
Analytical column	Waters, X Select CSH XP, C18; 2.5µm, 4.6 x 75 mm	Waters, X Bridge, C18; 3.5µm, 4.6 x 150 mm
Column T.	40 °C	
Flow rate	1000 µl/min	1200 µl/min
Mobile phase	A: water containing 0.1 % formic acid B: acetonitrile containing 0.1 % formic acid	
Gradient	Step / Total Time (min) / A% 0 (equil.) / 2.0 / 95 1 / 1.0 / 95 2 / 3.0 / 10 3 / 4.0 / 10 4 / 4.1 / 95	Step / Total Time (min) / A% 0 (equil.) / 3.0 / 95 1 / 1.0 / 95 2 / 6.0 / 10 3 / 6.1 / 95
Injection V.	5 µl	10 µl
MRM (Q1/Q3)	152.0/110.0 156.0/114.0 (IS)	372.0/176.2 378.4/182.0 (IS)

RESULTS

Sample collection from mouse

- ✓ Reduction of the overall amount of blood volume collected from each mouse
- ✓ Minimal pain and distress with no permanent damage to the animals
- ✓ Reduction of the experimental animals by alternating face sides
- ✓ Better quality of collected samples: no sample processing (centrifugation, transfer) or handling required

Automation

The extraction procedure was easily automated.

The results obtained by automated extraction were comparable with those obtained by manual extraction (data not shown)

Bioanalytical method performance

Performance of the analytical method and representative chromatograms at low concentration are shown below

Analytical Parameter	PARACETAMOL 3 µg/ml (Low Concentration) 200 µg/ml (High Concentration)		TRAZODONE 5 ng/ml (Low Concentration) 1000 ng/ml (High Concentration)	
	FRESH BLOOD (up to 48h at +4°C)	FROZEN BLOOD (-80°C)	FRESH BLOOD (up to 48h at +4°C)	FROZEN BLOOD (-80°C)
Aging of samples on the tip	3 h, 20 h, 6 days	3 h, 20 h, 8 days	3 h, 20 h, 6 days	3 h, 20 h, 8 days
Matrix Effect	MF = 0.84 – 0.90 (CV% ≤ 4.3)	MF 0.78 – 0.90 (CV% ≤ 8.6)	MF = 1.0 (CV% ≤ 2.4)	MF = 0.17 – 0.31 (CV% ≤ 12.6)
	ISn_MF = 1.0 (CV% ≤ 4.2)	ISn_MF = 1.0 (CV% ≤ 4.2)	ISn_MF = 1.0 (CV% ≤ 2.4)	Isn_MF = 0.80 – 0.90 (CV% ≤ 12.9)
Recovery (%)	90.2 – 111.5 (CV% ≤ 6.7)	70.6 – 116.6 (CV% ≤ 6.6	94.8 – 113.0 (CV% ≤ 10.5)	73.8 – 100.9 (CV% ≤ 12.4)
Specificity	No peak was evidenced from blank samples with and without IS			
Stability of the final extract	Clear and stable up to 72 hours in the autosampler (RT)			

Matrix Effect: evaluated by ***Matrix Factor (MF)***

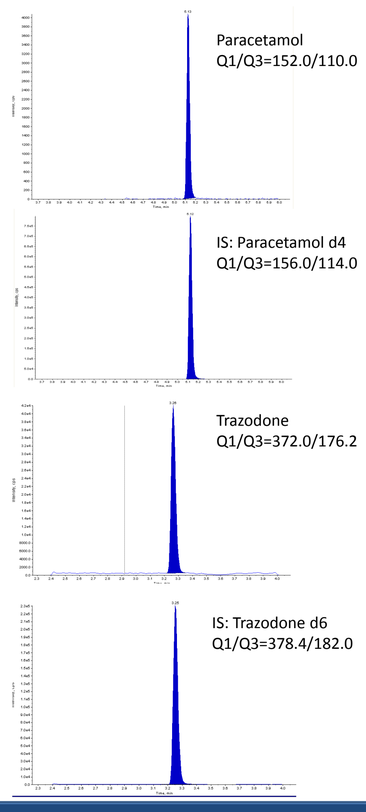
$$MF = \frac{analyte\ /IS\ area\ of\ post-extracted\ samples}{mean\ analyte/IS\ \ area\ of\ clean\ samples}$$

Compensation by IS was also evaluated by Isn-MF

Recovery determined by the following formula:

$$100\ X\ \frac{analyte\ peak\ area\ of\ extracted\ spiked\ sample}{mean\ analyte\ peak\ area\ \ of\ post-extracted\ samples}$$

A correction factor = 2 was applied for trazodone



CONCLUSIONS

The procedure applied for the blood collection from mouse resulted to be efficient with minimal pain and distress for the animals and an improvement of samples and data quality. The extraction procedure was efficient with good and reproducible recovery for both compounds in fresh blood. A negligible matrix effect was obtained for paracetamol with fresh and frozen blood. Matrix effect was not observed for trazodone by using fresh blood; instead it resulted relevant (and not fully compensated by the deuterated IS) with frozen blood. The aging of the fresh blood samples on the device (up to 6 days) did not have an impact on the matrix effect and recovery for both compounds. Moreover, specificity of the analytical method and stability of the final extract were also proven and the extraction procedure was easily automated.

The obtained results confirm the suitability of VAMS for non-clinical PK studies and its advantages towards the implementation of 3Rs strategy.

Acknowledgement

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References

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