

Overview

- ▶ **Purpose** –To demonstrate the utility of conventional bioanalytical liquid handling tools to facilitate off-line automation of internal standard addition and dried blood spot (DBS) analysis
- ▶ **Methods** –DBS methanol extraction and HPLC/MS/MS (API5500)
- ▶ **Results** – Range from 1-500 ng/mL with accuracies and precision better than $\pm 15\%$ using HPLC/MS/MS

Introduction

The benefits of using DBS for the determination of drug concentrations in whole blood have been widely reported.^{1,2} The technique continues to evolve and gain acceptance for DMPK support in the pharmaceutical industry. However, one of the disadvantages of the technique is the additional time and labor involved for the analyst during the sample preparation process when compared to traditional preparation techniques.

Here we report on an accurate and precise method for the semi-automated DBS extraction and HPLC/MS/MS analysis of Alprazolam, α -Hydroxyalprazolam, and Midazolam from human whole blood. The method shows that conventional liquid handling tools found in a bioanalytical laboratory can be used to semi-automate the DBS extraction. This technique includes adding internal standard/color indicating dye to the cards with a liquid handling instrument³, followed by extraction in the pipette tips of automated liquid handling instruments.

Methods

Extraction

- ▶ Alprazolam, α -Hydroxyalprazolam, and Midazolam extracted from human whole blood using a DBS extraction procedure
- ▶ Card type: Ahlstrom 226 (ID Biological Systems)
- ▶ Internal standard: contains color indicating dye and added to DBS card using liquid handling robot
- ▶ Sample volume: 30 μ L
- ▶ A TOMTEC device was used to directly punch spots into a 96 well plate or tips of liquid handling robot prior to extraction
- ▶ Punch diameter: 3 mm
- ▶ Solvent: Methanol

HPLC

- ▶ Gradient HPLC using acetonitrile and water
- ▶ Flow rate: 0.7 mL/minute
- ▶ HPLC column: HSC18 2.1x50 mm (Supelco)
- ▶ Column temperature: 50°C

Mass Spectrometry

- ▶ Sciex API5500 operating in MRM mode
- ▶ ESI
- ▶ Positive ion mode
- ▶ MRM transitions:
Alprazolam: 308.9 \rightarrow 280.0
 α -Hydroxyalprazolam: 325.1 \rightarrow 297.7
Midazolam: 326.0 \rightarrow 290.3

Table 1. Quality Control results for DBS extractions performed manually and with liquid handling instruments.

	Avg Accuracy (%)		CV (%)
	MANUAL	APRICOT	
Alprazolam			
LQC	96.4	6.0	
MQC	100	6.8	
HQC	92.4	4.6	
α-OH Alprazolam			
LQC	98.6	5.9	
MQC	97.8	6.6	
HQC	92.4	4.4	
Midazolam			
LQC	91.7	2.7	
MQC	95.3	5.5	
HQC	92.4	5.1	

	Avg Accuracy (%)		CV (%)
	APRICOT	TOMTEC	
Alprazolam			
LQC	99.2	5.8	
MQC	98.4	3.0	
HQC	93.9	4.0	
α-OH Alprazolam			
LQC	88.0	7.1	
MQC	96.2	3.0	
HQC	98.4	5.3	
Midazolam			
LQC	98.4	5.5	
MQC	99.9	7.4	
HQC	92.6	5.1	

	Avg Accuracy (%)		CV (%)
	TOMTEC	MANUAL	
Alprazolam			
LQC	109	10.3	
MQC	103	8.6	
HQC	95.4	5.5	
α-OH Alprazolam			
LQC	104	8.1	
MQC	98.8	5.9	
HQC	99.2	4.8	
Midazolam			
LQC	106	6.4	
MQC	102	5.1	
HQC	90.5	4.7	

Table 2. Comparison of internal standard added to DBS card manually and with a liquid handling instrument.

α -OH-Alprazolam D ₅	CV (%)	% Difference
TOMTEC IS Addition	6.5	0.0
Manual IS Addition	8.7	-
Alprazolam D₅		
TOMTEC IS Addition	6.9	1.5
Manual IS Addition	10.2	-
Midazolam D₄		
TOMTEC IS Addition	6.8	-6.5
Manual IS Addition	9.0	-

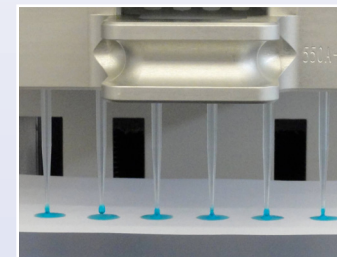


Figure 1. Internal standard being added to the DBS card by Apricot Designs liquid handling instrument.

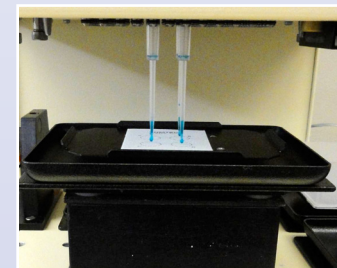


Figure 2. Internal standard being added to the DBS card by TOMTEC liquid handling instrument.

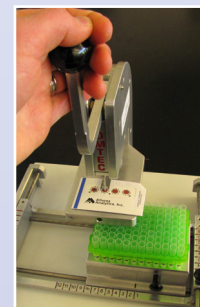


Figure 3. TOMTEC punching device used to punch DBS spots directly into tips of liquid handling instrument.

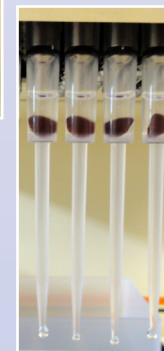


Figure 4. Dried blood spot punch extraction in tips of liquid handling instrument.

Conclusions

- ▶ Semi-automated DBS extraction of Alprazolam, α -Hydroxyalprazolam, and Midazolam from human whole blood demonstrated equivalent accuracy and precision to manual extraction. Results are acceptable per bioanalytical industry guidance
- ▶ Internal standard can be added to DBS cards using a liquid handling instrument.
- ▶ The use of the punching device and semi-automated method reduced the extraction time by more than 50% when compared to manual methods.

References

1. Barfield, et al. *J. Chromatogr B* 2008, 870:32-37.
2. Spooner, et al. *Anal. Chem.* 2009, 81:1557-1563.
3. Christianson, et al. *Bioanalysis* 2010, 2(11):1829-1837.2007, 377: 1-13.