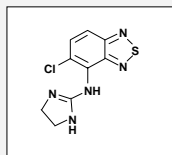


## INTRODUCTION

Tizanidine, with an extensive resonance electron system in structure, can not be fragmented in a collision cell to form significant product ion for selective and sensitive detection using normal MRM mode. In this work, a parent-to-parent transition has been optimized to improve the quantitation limit. The purpose of this work was to develop and validate a reliable method for analyzing tizanidine in human plasma over a concentration range of 0.250 to 10.0 ng/mL to support pediatric studies using only 100 µL of sample.



Tizanidine

## METHOD

In this method, tizanidine and its deuterated internal standard ( $d_4$ ) were extracted from the human plasma by liquid-liquid extraction. The extract was chromatographed on a column (Betasil phenyl, 100x2.1mm, 5µ) with gradient elution using acetonitrile based mobile phase solutions. The tizanidine and its internal standard were detected under positive MRM mode.

### Liquid Chromatography

HPLC : Shimadzu 10ADvp  
 Autosampler : Perkin Elmer 200  
 Column: Betasil Phenyl (100 x 2.1 mm) 5 µ  
 MP A : 10/90 ACN/10 mM Ammonium Acetate pH 4.0  
 MP B : 90/10 ACN/10 mM Ammonium Acetate pH 4.0

### Mass Spectrometry

AB Sciex API 3000 or API 4000  
 Polarity : Positive  
 Scan type : Multiple Reaction Monitoring  
 Tizanidine: m/z 254 → 254  
 Tizanidine- $d_4$ : m/z 258 → 258 (I.S.)

## RESULTS

Figures 1 – 4 show typical chromatograms for selected samples. The S/N ratio for Tizanidine peak at LLOQ level was greater than 15.

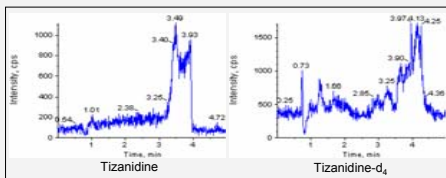


Figure 1. Chromatogram of a Blank (No I.S.)

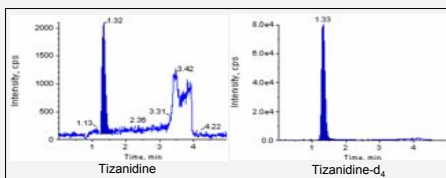


Figure 2. Chromatogram of an Extracted LLOQ sample (0.250 ng/mL)

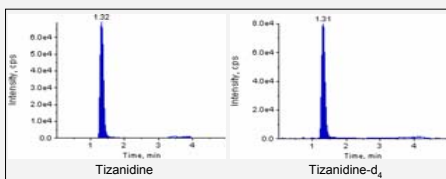


Figure 3. Chromatogram of an Extracted ULOQ Sample (10.0 ng/mL)

The method showed a linear range of 0.250 to 10.0 ng/mL with weighted linear regression (1/x). The correlation coefficients for three precision and accuracy batches were 0.998 or better (Figure 4).

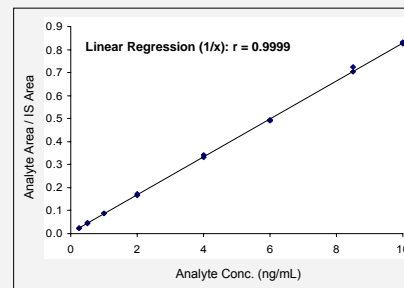


Figure 4. A Typical Calibration Curve for Tizanidine

Validation Summary are presented in Table 1 through Table 3.

Batch		0.25 ng/mL (LLOQ)	0.75 ng/mL (Low)	3.00 ng/mL (Mid)	8.00 ng/mL (High)
1	Mean	0.240	0.754	2.94	8.11
	% CV	2.1	3.4	1.4	1.9
	% Bias	-3.9	0.5	-2.0	1.3
2	Mean	0.240	0.747	2.95	8.00
	% CV	3.9	0.6	2.2	1.7
	% Bias	-4.1	-0.4	-1.6	0.0
3	Mean	0.247	0.744	2.92	8.03
	% CV	2.3	2.4	2.1	2.0
	% Bias	-1.1	3.2	-2.7	0.3
Overall	Mean	0.242	0.758	2.94	8.04
	% CV	3.1	2.8	1.8	1.9
	% Bias	-3.0	1.1	-2.1	0.5

Table 1. Precision and Accuracy Results for Tizanidine Quality Control Samples (n = 6 replicates per batch)

Plasma lot	Avg drug area	Avg I.S. area	Avg area ratio
Lot 1	28801	466898	0.0617
Lot 2	28344	464928	0.0610
Lot 3	29618	478106	0.0619
Lot 4	28444	472915	0.0601
Lot 5	29610	476648	0.0621
Lot 6	29025	475248	0.0611
mean	28974	472457	0.0613
n	6	6	6
SD	553	5387	0.0007
% CV	1.9	1.1	1.2

Table 2. Matrix Effect in Human Plasma at Low QC (0.750 ng/mL)

Matrix (stored in polypropylene)	Stability
Freeze/Thaw (-20 °C/RT)	4 cycles
Freeze/Thaw (-70 °C/RT)	4 cycles
Bench-top (RT)	6.25 hr
Long-term (-20 °C)	119 days
Long-term (-70 °C)	119 days
Extracted matrix	
Autosampler (Ambient)	3 days 16 hr
Refrigeration (4 °C)	3 days 16 hr
Whole Blood	
No instability was observed in ice-water bath or at RT in either glass or polyethylene terephthalate blood collection tubes for up to 1 hour	

Table 3. Summary of Stability

## CONCLUSION

The method was fully validated over a range of 0.250 to 10.0 ng/mL with weighted (1/x) linear regression. The effect of hemolysis and lipemic plasma was also evaluated with no significant effect observed. The overall mean extraction recovery was 91.4%. This method showed acceptable accuracy, precision, selectivity, stability, and reproducibility. The validated method can be demonstrated to be reliable in drug development and pediatric studies. Incurred sample reanalysis (ISR) is under investigation for this on-going pediatric trial.

## ACKNOWLEDGEMENTS

The authors would like to thank Kim Tuyen Nguyen and Kim Jackson for assistance with extraction and data processing.