POSTER # T2312

LC-MS/MS Analysis of Valproic Acid in Human Plasma Using Derivatization Techniques

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INTRODUCTION

Valproic acid, chemically known as 2-propylpentanoic acid, is an anticonvulsant drug widely used in the treatment of epilepsy and bipolar disorder. The quantitation of valproic acid in plasma utilizing LC/MS techniques traditionally has been challenging due to weak retention in chromatography and lack of sensitivity and specificity in detection by directly monitoring unfavorable ion transition pair under negative ion mode. Here, we explore the derivatization method that changed the detection polarity of the analyte from negative to positive mode and also chromatographic properties to improve robustness of the method. The signal-to-noise ratio and thus the specificity of detection were significantly improved by monitoring the different ion transition pair under positive ion mode.

METHOD

Following a protein precipitation procedure, the extract was subjected to a derivatization reaction using N-(3-Dimethyl aminopropyl)-N'-ethylcarbodiimide (EDC) and 2,2,2-trifluoro ethylamine (TFEA) in phosphate buffer. The resulting solution was diluted and chromatographed on a Betasil Phenyl column using a valve switching system preventing the introduction of reaction salts into the LC/MS system. Derivatized valproic acid and its internal standard (valproic acid-d_6) were detected by an API 3000 LC/MS system under positive MRM mode.

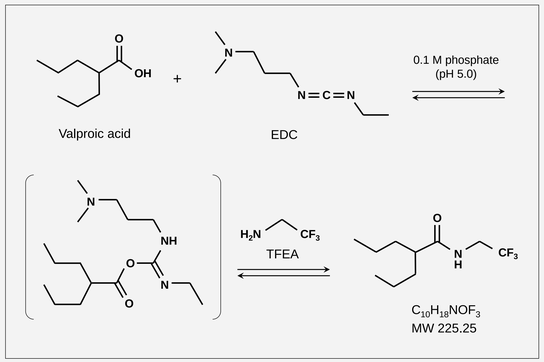

Figure 1. Derivatization Reaction of Valproic acid (Reference for derivatization of carboxylic acid: Q. L. Ford et al. *J. Chromatogr. A* 1145 (2007) 241-245)

Liquid Chromatography

HPLC : Shimadzu 10ADvp
Autosampler : Perkin Elmer 200
Column : Betasil Phenyl (100 x 2.1 mm) 5µ
MP A : 0.1% Formic acid in DI H_2O
MP B : 0.1% Formic acid in MeOH
MP C : 30/70/0.1 MeOH/DI H_2O/Formic acid

Mass Spectrometry

AB Sciex API 3000
Polarity : Positive
Scan type : Multiple Reaction Monitoring
Der-Valproic acid: m/z 226 → 206
Der-Valproic acid-d_6: m/z 232 → 212 (I.S.)

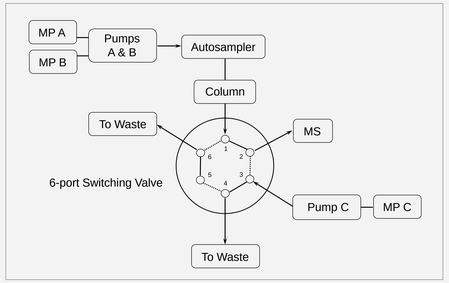

Figure 2. Diagram of LC/MS Instruments.

RESULTS

The method showed a linear range of **0.500** to **150 µg/mL** with weighted linear regression ($1/x^2$). The correlation coefficients for three validation batches were 0.997 or better (Figure 3).

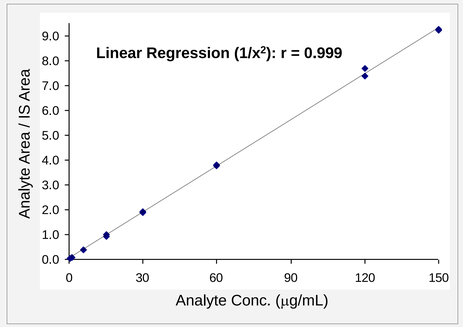

Figure 3. A Typical Calibration Curve for Valproic acid

Figures 4 through 6 show typical chromatograms for selected samples. The S/N ratio for Der-Valproic acid peak at LLOQ level was greater than 200.

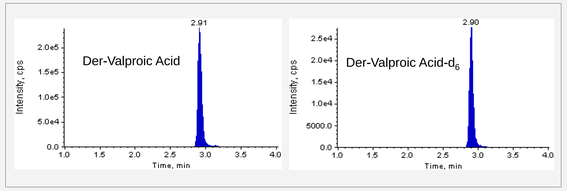

Figure 4. Chromatogram of an Extracted ULOQ Sample (150 µg/mL)

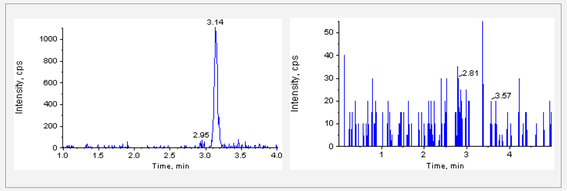

Figure 5. Chromatogram of a Blank (No I.S.)

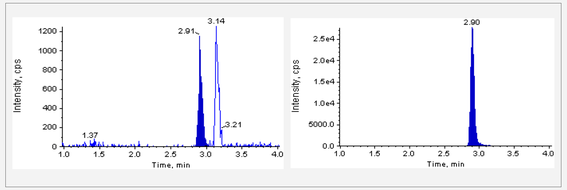

Figure 6. Chromatogram of an Extracted LLOQ sample (0.500 µg/mL)

Validation Summary are presented in **Table 1** through **Table 3**.

Batch		0.500 µg/mL (LLOQ)	1.50 µg/mL (Low)	45.0 µg/mL (Mid)	90.0 µg/mL (High)
1	**Mean**	0.465	1.43	45.9	90.0
	% CV	4.7	4.7	2.6	0.9
	% Bias	-7.0	-4.7	2.1	0.0
2	**Mean**	0.480	1.41	45.0	87.0
	% CV	5.2	5.1	2.9	1.5
	% Bias	-4.0	-5.7	0.0	-3.3
3	**Mean**	0.481	1.49	46.0	88.9
	% CV	4.4	3.2	2.1	2.1
	% Bias	-3.9	-0.6	2.2	-1.2
Overall	**Mean**	0.475	1.45	45.6	88.7
	% CV	4.8	4.7	2.6	2.1
	% Bias	-5.0	-3.6	1.4	-1.5

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

Plasma lot	Avg drug/IS ratio (1.50 µg/mL)	Avg drug/IS ratio (90.0 µg/mL)
Lot 1	0.1018	5.9493
Lot 2	0.0980	5.9965
Lot 3	0.1009	5.7984
Lot 4	0.1000	5.7630
Lot 5	0.1002	5.8852
Lot 6	0.0992	5.9618
mean	**0.1000**	**5.8924**
n	**6**	**6**
SD	**0.0013**	**0.0944**
% CV	**1.3**	**1.6**

Table 2. Matrix Effect in Human Plasma

Matrix (stored in polypropylene)	Stability
Freeze/Thaw (-20 ºC/RT)	4 cycles
Freeze/Thaw (-70 ºC/RT)	4 cycles
Bench-top (RT)	24 hr
Long-term (-20 ºC)	97 days
Long-term (-70 ºC)	97 days
Extracted matrix	
Autosampler (Ambient)	6 days
Refrigeration (4 ºC)	6 days
Whole Blood	
No instability was observed in ice-water bath or at RT in polyethylene terephthalate blood collection tubes for up to 90 minutes	

Table 3. Summary of Stability

CONCLUSION

The method was fully validated over a range of 0.500 to 150 µg/mL with weighted ($1/x^2$) linear regression. The effect of hemolysis and lipemic plasma was also evaluated with no significant effect observed. This method showed acceptable accuracy, precision, selectivity, stability, and reproducibility.

ACKNOWLEDGEMENTS

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