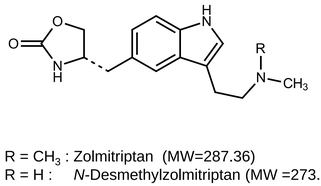
Simultaneous Determination of Zolmitriptan and Its Metabolite in Human Plasma Using LC-MS/MS Techniques

Moo-Young Kim, Marsha Luna, Rochelle Burke, Julie Showalter and Yansheng Liu – KCAS LLC, Shawnee, Kansas

INTRODUCTION

Zolmitriptan is a synthetic tryptamine derivative developed for the acute treatment of migraine. It is metabolized to *N*-desmethylzolmitriptan which is an active and major metabolite. This metabolite's contribution to the drug's therapeutic effect is very high because its plasma concentration is two-third of the concentration of the main drug and its potency is 2-6 times more than main drug. In this presentation, a sensitive and robust LC-MS/MS method is described to quantify both zolmitriptan and its metabolite, *N*-desmethylzolmitriptan in human plasma with an LLOQ of 0.1 ng/mL.



R = CH_3 : Zolmitriptan (MW=287.36)
R = H : *N*-Desmethylzolmitriptan (MW =273.34)

Figure 1. Zolmitriptan and *N*-Desmethylzolmitriptan

METHOD

In this method, zolmitriptan, *N*-desmethylzolmitriptan and their deuterated internal standards (d6 and d3, respectively) were extracted from human plasma by liquid-liquid extraction (using MTBE). The extract was chromatographed on a C8 column with gradient elution using acetonitrile based mobile phase solutions.

Liquid Chromatography

HPLC : Shimadzu 10ADvp
Autosampler : Perkin Elmer 200
Column : Alltima C8 (150 x 2.1 mm) 5µ
MP A : 0.1% Formic acid in DI H_2O
MP B : 0.1% Formic acid in ACN

Mass Spectrometry

AB Sciex API 4000
Polarity : Positive
Scan type : Multiple Reaction Monitoring
Zolmitriptan: m/z 288 → 58
Zolmitriptan-d_6: m/z 294 → 64
N-Desmethylzolmitriptan: m/z 274 → 243
N-Desmethylzolmitriptan-d_3: m/z 277 → 243

RESULTS

The method was fully validated over a range of **0.1 to 15 ng/mL** with weighted ($1/x^2$) linear regression. The correlation coefficients for precision/accuracy validation batches were 0.995 or better (**Figure 2** and **3**).

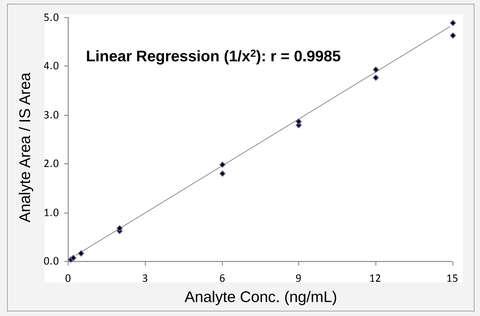


Figure 2. A Typical Calibration Curve for Zolmitriptan

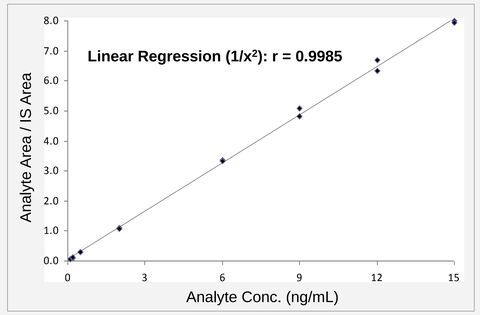


Figure 3. A Typical Calibration Curve for *N*-Desmethyl-zolmitriptan

Figures 4~6 show typical chromatograms for selected samples. The HPLC condition was optimized to separate two analytes in the chromatogram to minimize the interference. The S/N ratio for zolmitriptan and *N*-desmethylzolmitriptan peak at LLOQ level was greater than 100. Both compounds had a small carryover, which was less than 5% of LLOQ.

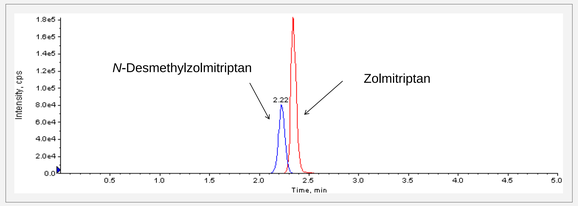


Figure 4. Chromatogram of an Extracted ULOQ Sample (15 ng/mL)

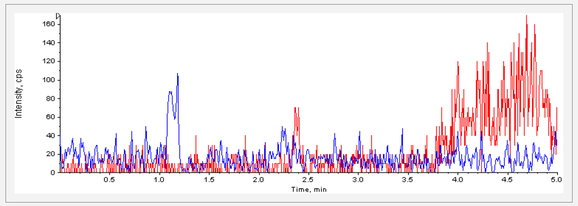


Figure 5. Chromatogram of a Recon Blank (No I.S.) Injected after ULOQ

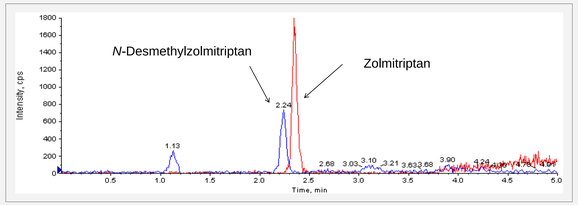


Figure 6. Chromatogram of an Extracted LLOQ sample (0.1 ng/mL).

	Avg. $MF_{analyte}$	Avg. MF_{IS}	Avg. IS *Normalized* MF
ME 1	0.9375 (0.9100)	1.0118 (0.9457)	0.9265 (0.9622)
ME 2	0.9176 (0.8383)	0.9821 (0.8641)	0.9343 (0.9701)
ME 3	0.9333 (0.9465)	1.0317 (0.9490)	0.9046 (0.9973)
ME 4	0.9642 (0.9119)	0.9963 (0.9386)	0.9678 (0.9715)
ME 5	0.9786 (0.9319)	1.0090 (0.9333)	0.9699 (0.9985)
ME 6	0.9519 (0.9318)	1.0507 (0.9345)	0.9060 (0.9972)
		mean	**0.9349 (0.9828)**
		n	**6**
		SD	**0.0288 (0.0166)**
		%CV	**3.1 (1.7)**

(1) Matrix Factor (MF) = (Peak response in presence of matrix substances)/(Peak response in absence of matrix), (2) Avg. IS Normalized MF = (Avg. $MF_{analyte}$)/(Avg. MF_{IS}), (3) The values in parentheses are for *N*-Desmethylzolmitriptan.

Table 1. Matrix Effect for Zolmitriptan and *N*-Desmethyl-zolmitriptan in Human Plasma at concentrations of 0.30 ng/mL.

Batch		0.1 ng/mL (LLOQ)	0.3 ng/mL (Low)	4.5 ng/mL (Mid)	10 ng/mL (High)
1	**Mean**	0.088 / 0.102	0.292 / 0.299	4.58 / 4.54	9.81 / 9.65
	% CV	6.4 / 5.4	1.2 / 6.2	5.5 / 2.0	2.7 / 4.9
	% Bias	-12.1 / 2.4	-2.6 / -0.4	1.8 / 0.8	-1.9 / -3.5
2	**Mean**	0.099 / 0.100	0.299 / 0.294	4.43 / 4.67	9.53 / 9.74
	% CV	7.8 / 9.8	8.3 / 7.0	3.7 / 5.6	4.2 / 4.8
	% Bias	-0.5 / -0.5	-0.5 / -2.1	-1.6 / 3.7	-4.7 / -2.6
3	**Mean**	0.092 / 0.101	0.295 / 0.323	4.25 / 4.60	9.52 / 10.0
	% CV	3.1 / 5.3	4.7 / 4.1	3.6 / 5.0	2.8 / 4.1
	% Bias	-8.4 / 0.9	-1.8 /7.7	-5.5 / 2.2	-4.8 / 0.0
Overall	**Mean**	0.093 / 0.101	0.295 / 0.305	4.42 / 4.60	9.62 / 9.80
	% CV	7.8 / 6.8	5.4 / 6.9	5.2 / 4.4	3.4 / 4.6
	% Bias	-7.1 / 0.9	-1.6 /1.7	-1.8 / 2.2	-3.8 / -2.0

Table 2. Precision and Accuracy Results for Zolmitriptan/*N*-Desmethylzolmitriptan Quality Control Samples (n = 6 replicates per batch).

	Zolmitriptan		*N*-Desmethylzolmitriptan	
	0.3 ng/mL	10 ng/mL	0.3 ng/mL	10 ng/mL
Mean	0.287	9.59	0.306	9.63
% CV	2.2	3.8	4.5	2.2
% Bias	-4.3	-4.1	1.9	-3.7

Table 3. Hemolytic Effect Evaluation (n=6 replicates per batch).

	Zolmitriptan		*N*-Desmethylzolmitriptan	
	0.3 ng/mL	10 ng/mL	0.3 ng/mL	10 ng/mL
Mean	0.282	9.71	0.304	9.81
% CV	2.6	4.8	3.8	5.0
% Bias	-5.9	-2.9	1.4	-1.9

Table 4. Lipemic Effect Evaluation (n=6 replicates per batch).

The matrix effect was evaluated using matrix factor described in **Table 1**, for which low-level QCs (0.30 ng/mL) were prepared from six different lots of human plasma. The intra- and inter-assay precision and accuracy data presented in **Table 2** met the required criteria. The results in **Table 3** and **4** indicate that hemolytic and lipemic plasma do not affect the analysis of the analyte in human plasma.

CONCLUSION

A robust and sensitive method has been established for the measurement of zolmitriptan and *N*-desmethylzolmitriptan in human plasma, with K_2EDTA as anticoagulant, by LC-MS/MS in the concentration range of 0.100-15 ng/mL for both analytes.