

Title: Simultaneous Determination of Tolbutamide, Omeprazole, Midazolam, and Dextromethorphan in Human Plasma by LC-MS/MS – A High Throughput Approach to Evaluate Drug-drug Interactions

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Introduction

Metabolic drug-drug interactions are due to alterations in drug metabolism. Cytochrome P450 system (CYP) is one of the main enzyme system involved in metabolic drug interactions. This system may be affected by enzyme induction or inhibition.

Tolbutamide, omeprazole, midazolam and dextromethorphan are substrates of CYP2C9, CYP2C19, CYP3A4 and CYP2D6 (Figure 1), respectively; therefore these compounds are commonly used to monitor CYP enzyme activities. This poster presents a high throughput LC-MS/MS method for the simultaneous determination of tolbutamide, omeprazole, midazolam and dextromethorphan in human plasma for support of a drug-drug interaction study.

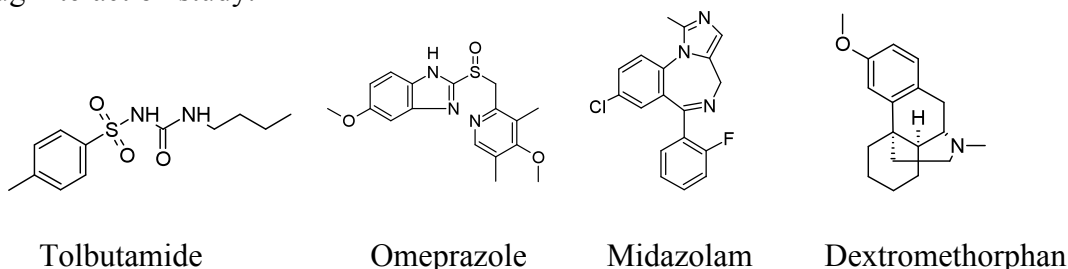


Figure 1. Chemical Structures of Tolbutamide, Omeprazole, Midazolam, and Dextromethorphan

Method

The analytes were extracted using 50 μ L of human plasma with methanol protein precipitation. Reversed phase separation was achieved on a Hypersil GOLD AQ column (50 x 4.6 mm, 5 micron) using a Shimadzu HPLC system coupled to an API 4000 mass spectrometer with turbo ionspray[®] in positive ion MRM mode. The run time was 4.0 minutes. The transitions (precursor to daughter, m/z) were 271.1/171.9, 346.2/198.0, 326.0/291.0, 271.7/171.0 for tolbutamide, omeprazole, midazolam and dextromethorphan, respectively. The transitions were 280.2/171.9, 349.2/198.0, 330.0/295.0, 274.7/171.0 for the stable-isotope labeled internal standards tolbutamide-d₉, omeprazole-d₃, midazolam-d₄ and dextromethorphan-d₃, respectively.

Results

The high throughput LC-MS/MS method was validated for accuracy, precision, sensitivity, stability, recovery, matrix effect, and calibration range. Acceptable intra-run and inter-run assay precision (<10%) and accuracy (<10%) were achieved over a linear range of 50 to 50000 ng/mL for tolbutamide, 1 to 1000 ng/mL for omeprazole, 0.1 to 100

ng/mL for midazolam, and 0.05 to 50 ng/mL for dextromethorphan in human plasma (Table 1). This method has been successfully used in support of a drug-drug interaction study (Table 2). Method robustness was demonstrated by incurred sample reproducibility (ISR) test of 50 clinical study samples with 100% of the results meeting the criterion (%Difference within 20%). The overall ISR results for all compounds showed that over 95% of the samples had a %Difference of less than 10%.

Table 1. Accuracy and Precision of the QC samples for Method Validation

Day ID	N=6	Tolbutamide Concentration, ng/mL			Omeprazole Concentration, ng/mL			Midazolam Concentration, ng/mL			Dextromethorphan Concentration, ng/mL		
		150	4000	39000	3	80	780	0.3	8	78	0.15	4	39
Intraday 1	Mean	163.205	3997.460	35242.810	3.220	81.558	712.077	0.330	7.974	73.205	0.162	3.969	36.701
	SD	6.729	56.129	541.951	0.090	1.760	16.527	0.017	0.161	1.859	0.009	0.089	0.763
	%CV	4.1	1.4	1.5	2.8	2.2	2.3	5.2	2.0	2.5	5.6	2.2	2.1
	%Nominal	108.8	99.9	90.4	107.3	101.9	91.3	110.0	99.7	93.9	108.0	99.2	94.1
Intraday 2	Mean	158.816	4023.344	36297.406	3.137	82.569	727.585	0.307	7.922	450	0.161	3.933	36.625
	SD	8.024	64.038	627.417	0.117	1.248	18.847	0.012	0.094	1.191	0.005	0.077	0.607
	%CV	5.1	1.6	1.7	3.7	1.5	2.6	3.9	1.2	1.6	3.1	2.0	1.7
	%Nominal	105.9	100.6	93.1	104.6	103.2	93.3	102.3	99.0	94.2	107.3	98.3	93.9
Intraday 3	Mean	151.043	4093.942	35407.266	3.138	83.626	715.969	0.299	8.252	74.186	0.155	4.155	37.375
	SD	8.960	74.607	599.665	0.084	1.874	11.356	0.010	0.133	1.515	0.006	0.158	0.908
	%CV	5.9	1.8	1.7	2.7	2.2	1.6	3.3	1.6	2.0	3.9	3.8	2.4
	%Nominal	100.7	102.3	90.8	104.6	104.5	91.8	99.7	103.2	95.1	103.3	103.9	95.8
Interday Results	Mean	157.688	4038.249	35649.161	3.165	82.584	718.544	0.312	8.050	73.614	0.159	4.019	36.900
	SD	9.091	74.365	731.555	0.100	1.777	16.392	0.019	0.194	1.514	0.007	0.146	0.801
	%CV	5.8	1.8	2.1	3.2	2.2	2.3	6.1	2.4	2.1	4.4	3.6	2.2
	%Nominal	105.1	101.0	91.4	105.5	103.2	92.1	104.0	100.6	94.4	106.0	100.5	94.6

Table 2. Accuracy and Precision of the QC samples for Sample Analysis

N=24	Tolbutamide Concentration, ng/mL			Midazolam Concentration, ng/mL		
	150	4000	39000	0.3	8	78
Mean	154.763	4122.339	36207.324	0.306	8.072	73.375
SD	6.298	86.250	1600.366	0.014	0.124	2.497
%CV	4.1	2.1	4.4	4.6	1.5	3.4
%Nominal	103.2	103.1	92.8	102.0	100.9	94.1
N=24	Omeprazole Concentration, ng/mL			Dextromethorphan Concentration, ng/mL		
	3	80	780	0.15	4	39
Mean	3.063	81.463	722.172	0.147	4.001	36.515
SD	0.231	2.130	28.040	0.009	0.102	1.232
%CV	7.5	2.6	3.9	6.1	2.5	3.4
%Nominal	102.1	101.8	92.6	98.0	100.0	93.6

Conclusions

A high throughput LC-MS/MS method for the simultaneous determination of tolbutamide, omeprazole, midazolam and dextromethorphan in human plasma has been developed and validated. The method was fast, robust, sensitive and reliable. This method has been successfully used in support of a drug-drug interaction study during pharmaceutical development. The method robustness was demonstrated by reanalysing 50 incurred samples from the study with 100% of the samples meeting the acceptance criteria.