Quantitative Determination of Dipyridamole in Human Plasma Using Liquid Chromatography and Electrospray Ionization Tandem Mass Spectrometry

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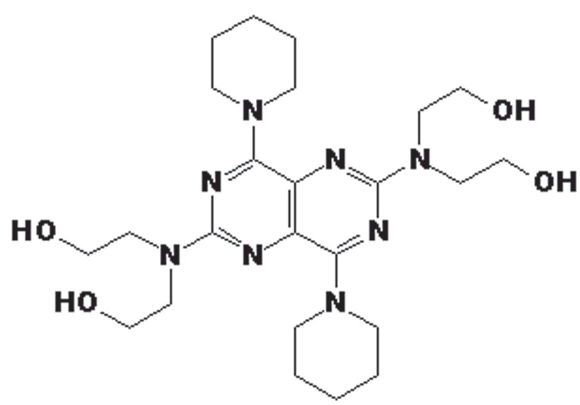
Introduction

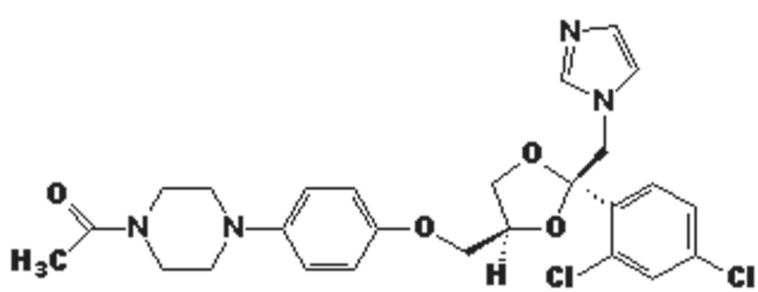
Dipyridamole is a platelet aggregation inhibitor drug. Tandem Labs recently developed an LC/MS/MS assay to quantitate dipyridamole in human plasma to support clinical pharmacokinetic studies. This required the development of a simple, fast, selective, rugged and preferably automated sample preparation procedure.

Dipyridamole was extracted using an automated protein precipitation method. The automated method used a Tomtec Quadra96 to perform the protein precipitation. Dipyridamole was separated on a Phenomenex Luna C_{18}(2) HPLC column (2.1 x 50 mm, 5 µm) using a gradient mobile phase. A Sciex API 3000 was operated in selected reaction monitoring (SRM) mode under optimized conditions for the detection of dipyridamole and ketoconazole (internal standard) positive ions formed by TurboIonSpray™ ionization. The cycle time between injections was approximately 3 minutes for both compounds.

Dipyridamole



Ketoconazole



Quantitation Method

Quantitation was performed by Watson® DMLIMS software. Dipyridamole and ketoconazole chromatographic peaks were integrated using Sciex Analyst software. Concentrations for dipyridamole were then determined from the ratio of the peak areas of the analyte to the peak areas for the internal standard and the comparison of these ratios with weighted quadratic regression curves (weighting factor = 1/concentration). These curves were generated from the analysis of plasma samples fortified with known concentrations of the analyte and internal standard.

The targeted calibration curve range for dipyridamole was from 50.0 ng/mL to 5,000 ng/mL [Figure 3].

Analysis Type:
Positive-ion Selected Reaction Monitoring (SRM)

Transitions Monitored:
Dipyridamole: 505→885
Ketoconazole: 531→82

LC Conditions

HPLC Column:
Phenomenex Luna C_{18}(2),
5 µm, 2.1 x 50 mm

Mobile Phases:
A: 0.1% Formic acid in water
B: 0.1% Formic acid in methanol

LC Conditions:
Gradient

Time (min.)	% B
0.0	40
0.5	40
2.0	90
2.5	90
2.6	40
3.7	Stop

Flow Rate: 500 µL/minute

Column Temperature: 25°C

Injection Volume: 2 to 20 µL

MS Conditions

Source:
TurboIonSpray™
(flow rate ~8 L/min.)

Source Temperature:
350 °C

Ionization Mode:
Positive Ion

Analysis Type:
Selected reaction monitoring (SRM)

Experimental

Both dipyridamole and ketoconazole are available commercially and were purchased from USP. Human plasma with sodium heparin anticoagulant was provided by Biochemed, Winchester, VA. All other chemicals and solvents were provided by VWR Scientific Products, Bridgeport, New Jersey. All chemicals were analytical reagent grade and all solvents were HPLC grade.

Standards/Solutions

Spiking solutions for the calibration curve were prepared from 100,000 ng/mL to 1,000 ng/mL dipyridamole in 90/10 methanol/1M Tris buffer pH~11 (v/v). The calibration curve was prepared fresh daily by spiking of each solution into human plasma.

Tomtec Extraction Procedure

1. Manually aliquot 50.0 µL of standards, quality controls, and subject samples into a 96 well plate. Aliquot 50.0 µL of blank plasma for control blanks and QC0's.
2. Add 100 µL of 50 mM Tris buffer (pH~11) into each well.
3. To all samples except blanks, add 25.0 µL working internal standard (ketoconazole, 20,000 ng/mL in 50/50 MeOH:H2O [v/v])
4. Cover 96 well plate with a capmat. Vortex mix for ~1 minute.
5. Centrifuge 96 square well plate at ~3000 rpm for ~2 minutes.
6. Add 500 µL of acetonitrile to each well.
7. Mix 10 times.
8. Cover 96-well plate with 96-well capmat and centrifuge at ~3000 rpm for ~2 minutes.
9. Transfer top layer (~200 µL) to a clean 96-well plate using Tomtec.
10. Evaporate organic layer to dryness in TurboVap set at ~45°C under nitrogen for ~45 minutes.
11. Reconstitute with 200 µL of 30/70 MeOH:H2O (v/v).
12. Sonicate for ~5 minutes.

Conclusion

The tests met the established acceptance criteria. The automated LC/MS methodology developed is simple and rugged and allows for high-throughput analysis in support of clinical studies.

Table 1. Summary of Calibration Curve Parameters for Dipyridamole
Quadratic weighted 1/x. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
1-Jun-2002	1	0.000000	0.000481	-0.000198	0.9999	50.0	5000
4-Jun-2002	2	0.000000	0.000481	0.003042	0.9986	50.0	5000
6-Jun-2002	3	0.000000	0.000424	0.001821	0.9990	50.0	5000
Mean		0.000000	0.000462	0.001562	0.9992		
S.D.		0.000000	0.000033	0.001638	0.0007		
%CV		-----	7.1	104.9	0.1		
n		3	3	3	3		

Table 2. Intra-Assay Precision for Dipyridamole Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 150 ng/mL	Medium QC 2000 ng/mL	High QC 4000 ng/mL	Dilution QC DF=10 20000 ng/mL
21-Jun-2002	1	147	2010	4060	20000
		146	2080	4070	19600
		151	2050	4030	19700
		139	2050	4150	20500
		139	2080	4060	20500
		141	2050	4150	20500
Mean		144	2050	4090	20100
SD		4.92	25.8	50.9	423
%CV		3.4	1.3	1.2	2.1
%Theoretical		96.0	102.5	102.3	100.5
%Bias		-4.0	2.5	2.3	0.5
n		6	6	6	6
Run Date	**Run Number**	**Low QC 150 ng/mL**	**Medium QC 2000 ng/mL**	**High QC 4000 ng/mL**	**Dilution QC DF=10 20000 ng/mL**
24-Jun-2002	2	153	2080	3960	
		156	2080	4090	
		150	2020	3970	
		151	2010	4140	
		150	2010	3990	
		142	2090	3980	
Mean		150	2050	4020	
SD		4.68	38.7	74.7	
%CV		3.1	1.9	1.9	
%Theoretical		100.0	102.5	100.5	
%Bias		0.0	2.5	0.5	
n		6	6	6	
Run Date	**Run Number**	**Low QC 150 ng/mL**	**Medium QC 2000 ng/mL**	**High QC 4000 ng/mL**	**Dilution QC DF=10 20000 ng/mL**
26-Jun-2002	3	153	2090	4220	
		161	2080	4000	
		148	2070	4000	
		153	2120	4010	
		160	2070	4020	
		159	2130	4110	
Mean		156	2090	4060	
SD		5.13	25.8	88.8	
%CV		3.3	1.2	2.2	
%Theoretical		104.0	104.5	101.5	
%Bias		4.0	4.5	1.5	
n		6	6	6	

Table 3. Back-Calculated Concentrations of Calibration Standards for Dipyridamole

Quadratic weighted 1/x. All concentrations are expressed as ng/mL.

Run Date	Run Number	50.0	100	250	500	1000	2500	4250	5000
21-Jun-2002	1	49.3	93.9	249	507	1000	2490	4290	5020
		50.6	104	†499	509	1010	2460	4230	4990
24-Jun-2002	2	51.1	107	258	540	1080	2480	4410	5110
		43.4	96.3	245	494	981	2330	4200	4880
26-Jun-2002	3	48.2	103	248	494	1040	2470	4270	5190
		49.9	94.5	254	516	1040	2370	4280	4830
Mean		48.8	99.8	251	510	1030	2430	4280	5000
S.D.		2.81	5.57	5.17	17.1	35.4	66.5	72.1	136
%CV		5.8	5.6	2.1	3.4	3.4	2.7	1.7	2.7
%Bias		-2.4	-0.2	0.4	2.0	3.0	-2.8	0.7	0.0
n		6	6	5	6	6	6	6	6

Table 4. Lower Limit of Quantitation for Dipyridamole

Quadratic weighted 1/x.

All concentrations are expressed as ng/mL.

Run Date R	un Number	LLOQ 50.0 ng/mL
24-Jun-2002	2	49.1
		48.0
		48.4
		42.8
		44.4
		44.5
Mean		46.2
S.D.		2.61
%CV	5	.6
%Theoretical		92.4
%Bias		-7.6
n	6	

Figure 1. Plasma Blank

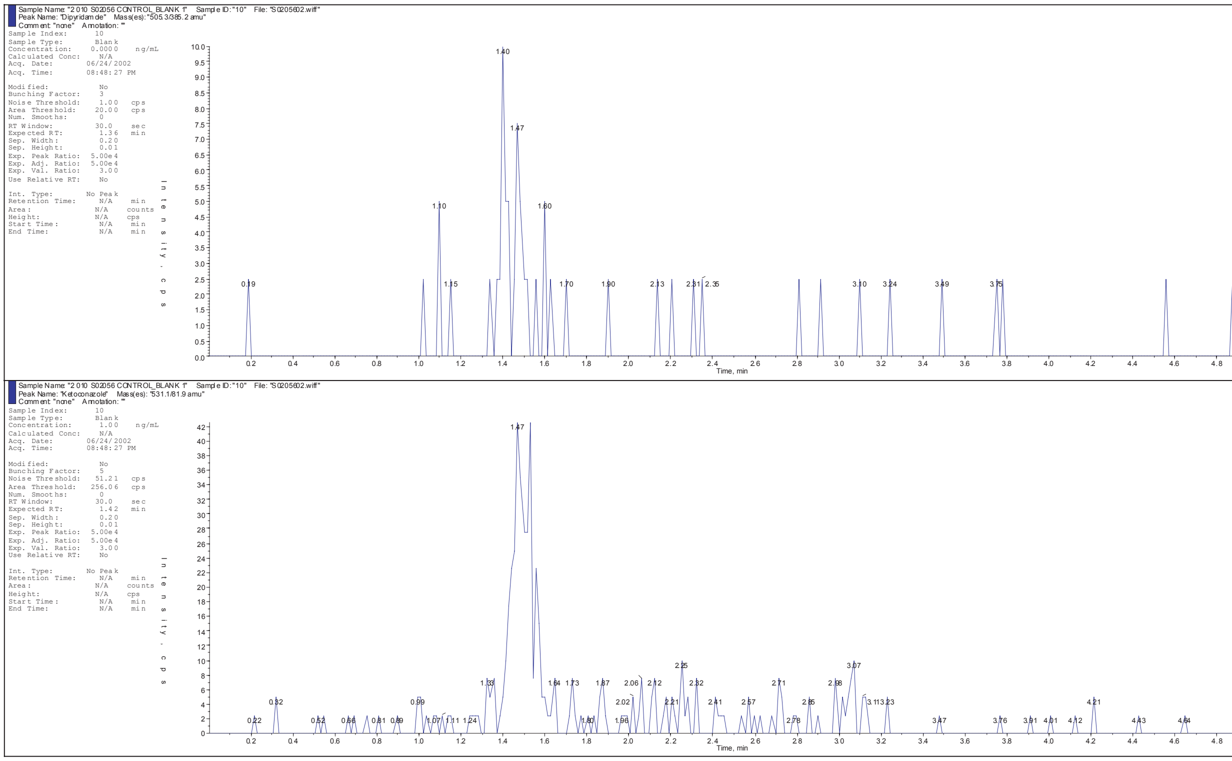


Figure 2. Low Standard (50.0 ng/mL)

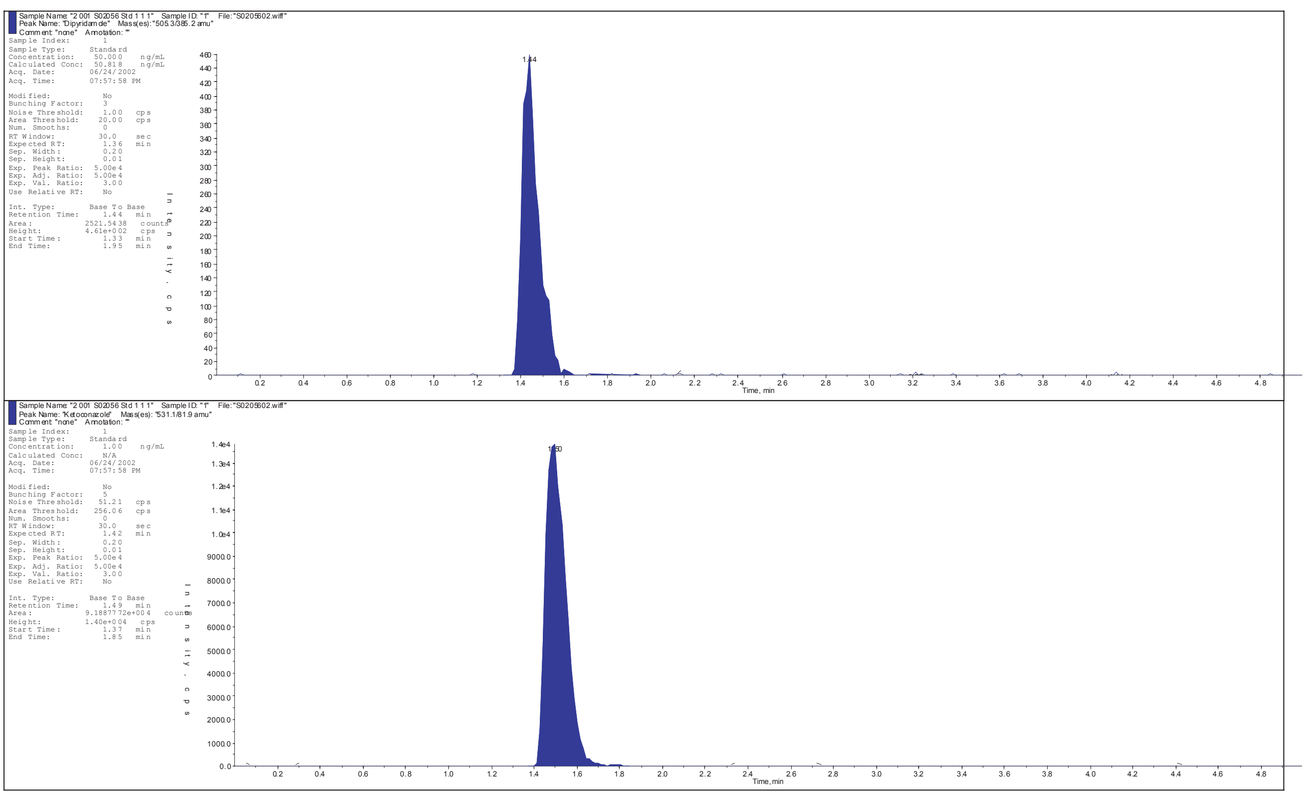


Figure 3. Representative Calibration Curve