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White Paper

Qualification of an LC-MS/MS Method for the Simultaneous Determination of Desipramine and 2-Hydroxydesipramine in Human Plasma

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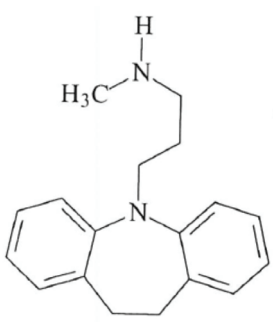
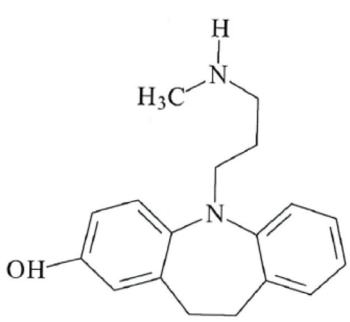
Introduction:

Desipramine is a tricyclic antidepressant actively involved in blocking the reuptake of norepinephrine and serotonin within the brain. Desipramine can also be used to treat neuropathic pain associated with damage to the somatosensory nervous system. A method with very high throughput utilizing low sample volume has been qualified for the simultaneous determination of both desipramine and a major metabolite, 2-hydroxydesipramine, in human plasma.

Desipramine:



2-Hydroxydesipramine:



Qualification Components:

For the method qualification and to ensure ruggedness, this assay was subject to a wide range of analytical tests. Initial testing focused on determining any potential conversion of the metabolite to the parent compound both in neat solution and during matrix processing, as well as adsorption of both compounds to polypropylene. Qualification of the method was demonstrated by the successful testing of matrix effect, recovery, accuracy, precision, hemolyzed and lipemic plasma, dilution integrity, and freeze-thaw stability. All samples were processed and analyzed using the procedure outlined in the "Sample Preparation" section.

Methods:

Sample Preparation:

1. Manually aliquot 0.0500 mL of human plasma (EDTA) sample and 0.0250 mL of internal standard (ISTD)
2. Add a basic buffer to all samples and mix.
3. Perform Liq-Liq extraction with MTBE using automated liquid handler software (Sciclone) and mix, centrifuge, and transfer supernatant to a clean 96-well polypropylene plate.
4. Evaporate samples and reconstitute in 90% ACN.

HPLC Chromatography

Column: Thermo Scientific, BioBasic SCX, 50 x 3.0 mm, 5 µm

Mobile Phase: 80:20 ACN:10 mM $HCOONH_4$, pH 2.5 w/HCOOH

Run Time: 2.5 minutes

Retention Time: 0.9 minutes (Desipramine)

1.0 minutes (2-Hydroxydesipramine)

LC-MS/MS Detection

Mass Spectrometer: API 4000

Source: ESI

Ion Mode: Positive

Ions monitored: Desipramine (267.3 → 72.2 m/z)

2-Hydroxydesipramine (283.3 → 72.2 m/z)

d_4-Desipramine (271.3 → 72.2)

Results:

Table 1. Inter-Batch Statistics for Accuracy and Precision:

Desipramine Quality Control Samples		Precision (% CV)	Accuracy (% Bias)
Inter-batch	LLOQ	8.1	-7.0
	Low	3.7	-4.7
	Medium	2.1	1.3
	High	2.6	-2.7
2-Hydroxydesipramine Quality Control Samples		**Precision (% CV)**	**Accuracy (% Bias)**
Inter-batch	LLOQ	16.1	-6.4
	Low	7.6	-9.7
	Medium	3.8	-2.7
	High	4.8	-2.9

Table 2. Matrix Effect for Desipramine in Human Plasma (Heparin)

		LLOQ		High	
Batch	Lot#	0.500 ng.mL	% Dev.	150 ng.mL	% Dev.
6	1	0.462	-7.6	147	-2.0
	2	0.495	-1.0	144	-4.0
	3	0.475	-5.0	146	-2.7
	4	0.487	-2.6	144	-4.0
	5	0.492	-1.6	151	+0.7
	6	0.447	-10.6	143	-4.7
	7	0.496	-0.8	148	-1.3
	8	0.486	-2.8	148	-1.3
	9	0.502	+0.4	150	+0.0
	10	0.523	+4.6	150	+0.0
Mean		0.487		147	
% CV		4.4		1.9	
% Theoretical		97.4		98.0	
n		10		10	

Table 3. Matrix Effect for 2-Hydroxydesipramine in Human Plasma (Heparin)

		LLOQ		High	
Batch	Lot#	0.250 ng.mL	% Dev.	75.0 ng.mL	% Dev.
6	1	0.297	+18.8	75.2	+0.3
	2	0.282	+12.8	69.7	-7.1
	3	0.260	+4.0	73.0	-2.7
	4	0.263	+5.2	71.8	-4.3
	5	0.275	+10.0	75.0	+0.0
	6	0.268	+7.2	70.6	-5.9
	7	0.281	+12.4	76.3	+1.7
	8	0.286	+14.4	76.3	+1.7
	9	0.257	+2.8	77.0	+2.7
	10	0.281	+12.4	75.5	+0.7
Mean		0.275		74.0	
% CV		4.6		3.5	
% Theoretical		110.0		98.7	
n		10		10	
n		10		10	

Figure 1. Representative chromatograms of control blank matrix (EDTA) for desipramine and ISTD (A) and 2-hydroxydesipramine and ISTD (B)

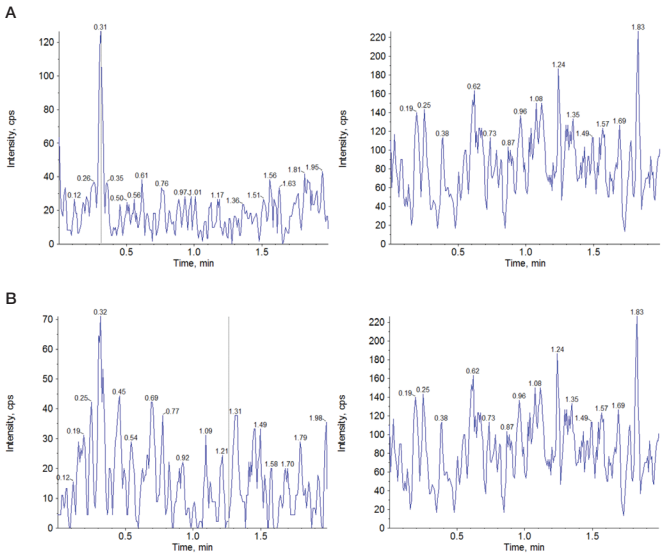


Figure 2. Representative chromatograms of blank with ISTD samples for desipramine and ISTD (A) and 2-hydroxydesipramine and ISTD (B)

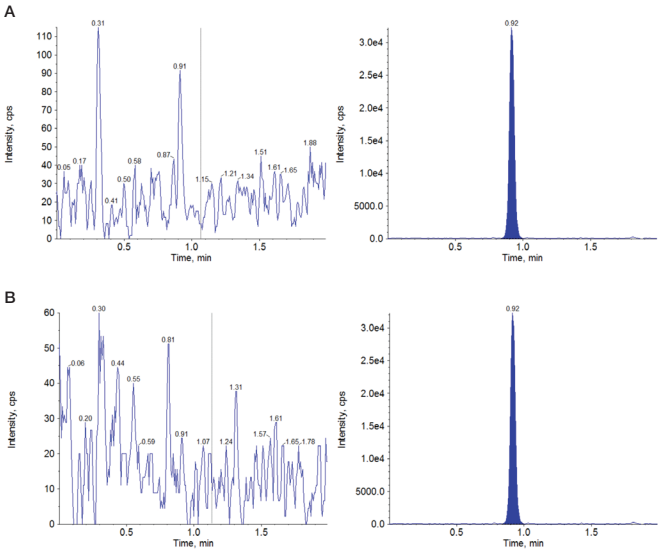
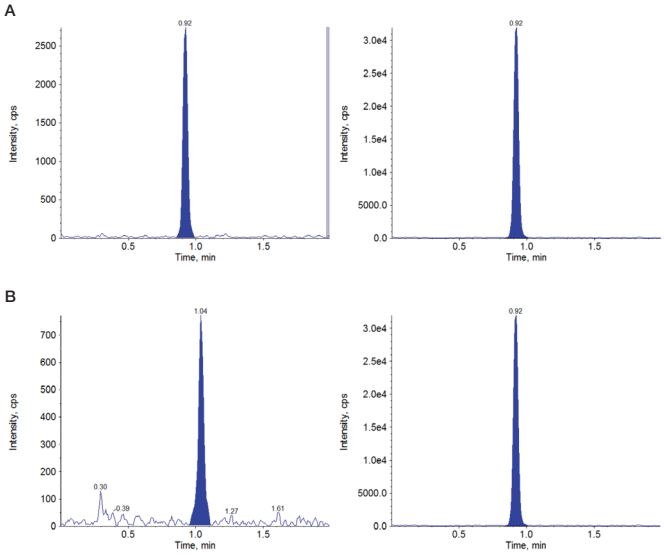


Figure 3. Representative chromatograms of LLOQ for desipramine and ISTD (A) and 2-hydroxydesipramine and ISTD (B)



Development Summary:

Assay Volume Required		0.0500 mL	
Standard Curve Range		0.5 - 200 ng/mL DES 0.25-100 ng/mL 2HD	
Regression Type		Weighted linear (1/concentration2)	
Dilution Integrity		up to 900 ng/mL (DES), up to 450 ng/mL (2OH)	
Batch Size		192 injections	
Recovery		DES Recovery (%)	2HD Recovery (%)
Analyte	Low	93	102
	High	97	100
Internal Standard		100	
Short-term Stability		50 hours in polypropylene at ambient temperature under white light	
Freeze and Thaw Stability		5 cycles in polypropylene at ambient temperature under UV-shielded light at -20°C	
Post-preparative Stability		123 hours in a 96 well polypropylene plate at 5°C	
Sample Collection and Handling Stability		2.5 hours in polypropylene at ambient temperature under white light	

Conclusion:

A high throughput semi-automated method has been qualified for the simultaneous analysis of desipramine and 2-hydroxydesipramine in human plasma. Both analytes meet acceptance criteria for all stability, selectivity, precision, and accuracy evaluations performed. Rapid analysis was achieved by the use of a low sample aliquot volume and automation instruments producing an efficient and cost-effective assay.