Plasma Drug Testing for D&L Isomers of Amphetamine and Methamphetamine

Robbins, B[1], Long, M[1], Carpenter, R[1,2]

[1] Advanta Genetics, 10935 CR 159, Tyler, TX 75703, [2] University of Texas at Tyler, Soules College of Business, 3900 University Blvd, Tyler, TX 75799

THE HUMAN ADVANTAGE IN LABORATORY TESTING

Introduction

- Existing in two enantiomeric forms, D- or L-methamphetamine, produce radically different effects on the human nervous system.
- The D- form can be used therapeutically as a treatment for overeating disorders, narcolepsy, and Attention Deficit Disorder. But it also produces habit-forming central nervous system effects engendering potential illegal abuse.
- There is a need to develop a plasma matrix assay for determining the enantiomeric form of methamphetamine D- and L- isomers.

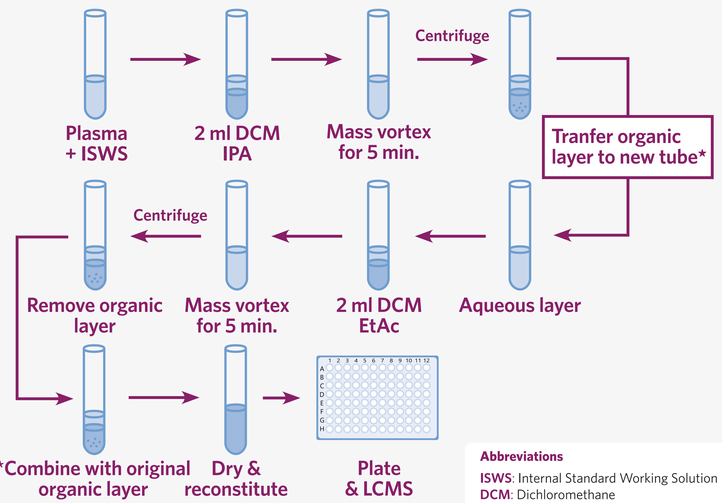
Objective

- Develop and validate an assay to measure the enantiomeric forms of methamphetamine in human K2EDTA plasma to evaluate the prescription vs illicit potential.

Materials & Methods

- The D- and L- isomers of amphetamine and methamphetamine for quality controls and standard preparation were from Cerilliant Corporation (Round Rock, TX). Drug free plasma was obtained from Bio IVT (Westbury, NY). Other high-performance liquid chromatography and American Chemical Society grade chemicals were obtained from Fisher Scientific (Pittsburgh, PA). Chiral separation was performed using isocratic separation with a Supelco Astec CHIROBIOTIC® V2 (25 cm x 2.1 mm, 5 ×m). Separation and detection was done by LC-MS/MS using an Shimadzu Prominence LC system and an AB Sciex API 4000 mass spectrometer.
- This assay was developed with an ES positive assay for 63 other drugs of interest.

Extraction Process



Abbreviations

ISWS: Internal Standard Working Solution
DCM: Dichloromethane
IPA: Isopropyl alcohol
EtAc: Ethyl acetate

Study Design

Interassay Mean and SD

Drug / Metabolite	LLOQ	%CV	%E	LQC	%CV	%E	MQC	%CV	%E	HQC	%CV	%E
D-Amphetamine	2.7 ± 0.5	17.09	9.6	7.8 ± 0.4	6.95	3.8	296.3 ± 7.9	2.67	-1.2	898.6 ± 21.0	2.34	-0.2
L-Amphetamine	2.8 ± 0.3	9.9	13.7	7.6 ± 0.6	8.34	0.8	293.4 ± 10.9	3.71	-2.2	866.6 ± 25.7	2.76	-3.7
D-Methamphetamine	2.5 ± 0.5	19.48	-0.5	7.5 ± 0.7	9.88	-0.3	286.1 ± 15.2	5.3	-4.6	834.2 ± 59.3	7.11	-7.3
L-Methamphetamine	2.6 ± 0.4	14.24	2.4	7.9 ± 0.6	7.57	5.6	323.4 ± 19.7	6.09	7.8	904.2 ± 59.3	6.56	0.5

Interassay Precision and Accuracy

	LLOQ				LQC				MQC				HQC			
	%CV		%E		%CV		%E		%CV		%E		%CV		%E	
Drug / Metabolite	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
D-Amphetamine	11.76	19.25	0.35	18.33	3.88	8.16	-1.04	7	2.25	3.21	-1.69	-0.47	0.64	3.17	-1.83	1.47
L-Amphetamine	8.02	11.63	6.82	17.88	5.76	11.22	-2.39	5.95	2.7	3.85	-4.75	-0.4	1.26	2.39	-6.06	-1.28
D-Methamphetamine	12.53	18.3	-15.24	17.72	2.38	7.66	-10.26	9.72	2.57	6.99	-5.3	-3.42	4.75	8.87	-10.33	-4.35
L-Methamphetamine	7.14	15.1	-7.05	18.82	5.36	9.09	1.32	9.75	1.95	5.96	2.29	14.42	4.07	5.21	-3.3	7.35

Stability

	F/T 3 Cycles		Overnight Stability			Post Preparation Stability		
Drug / Metabolite	QC	%Diff	RT	4 °C	-20 °C	Init % Diff Nom	%Diff Init Day 3	%Diff Init Day 7
D-Amphetamine	QC 37.5	-4.44	0.82%	0.77%	1.05%	-0.56	-10.46	0.56
	QC 400	-1.66	1.17%	0.54%	0.50%	-2.51	-3.97	2.54
L-Amphetamine	QC 37.5	-2.53	0.35%	0.25%	0.45%	-4.41	-4.03	4.51
	QC 400	-0.09	0.94%	0.44%	0.43%	-4.10	-2.36	4.19
D-Methamphetamine	QC 37.5	1.12	1.86%	1.19%	1.59%	-1.24	9.71	1.25
	QC 400	-1.15	1.00%	1.06%	1.76%	-6.30	5.44	6.50
L-Methamphetamine	QC 37.5	4.13	1.77%	1.08%	2.24%	8.76	5.16	-8.39
	QC 400	-3.99	1.17%	0.59%	2.07%	5.20	6.78	-5.06

Linearity of Dilution

	% Diff from Expected			
Drug / Metabolite	1:5 Dilution	1:10 Dilution	1:20 Dilution	1: 100 Dilution
D-Amphetamine	-2.90	1.44	-8.24	-11.20
L-Amphetamine	-4.40	1.28	-7.28	-9.96
D-Methamphetamine	-2.84	0.32	-9.04	-10.08
L-Methamphetamine	1.44	1.32	-8.16	-8.76

Results

The assay had a dynamic range from 2.5 to 1000 ng/ml for all four analytes. It had %error (%E) and % coefficient variability (%CV) <20 at the lower limit of quantitation (LLOQ) (2.5 ng/ml) and <15 over the range of the assay for the standard curve. Validation samples at 7.5, 300, and 900 ng/ml exhibited inter and intra-assay %CV and %E of <15. The analytes exhibited excellent room temperature, refrigerated and frozen stability characteristics with less than 15% degradation over 7 days. There were negligible effects of matrix, carryover, freeze-thaw, concomitant medications and dilution.

Conclusion

The novelty of this study is the development and validation of a reliable method for the determination of D- and L- isomers of amphetamine and methamphetamine in human plasma samples with a straightforward general extraction schema applied to a much larger analyte assay. The D- and L- assay proved to be sensitive, stable and produce %CV and %E statistics within 20% at the LLOQ and within 15% for the standard curve and validation samples.