Chiral analysis of Methamphetamine in Hair Samples

JOHN JAY COLLEGE OF CRIMINAL JUSTICE

Cordant Health Solutions®

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Introduction

Methamphetamine (MAMP) is a highly addictive illicit drug typically abused for its nervous system stimulating effects. Conversely, methamphetamine has therapeutic use treating attention deficit hyperactivity disorder, controlling appetite and assisting with weight loss, and is available as the pure *l*-isomer in over the counter (OTC) nasal inhalers due to its decongestant activity. Because *l*-Methamphetamine (*l*-MAMP) is available in OTC form, forensic guidelines require a sample to contain greater than 20% *d*-Methamphetamine (*d*-MAMP) when classifying results as consistent with illicit MAMP use. Chiral chromatographic analysis is capable of distinguishing between *l*-MAMP and *d*-MAMP. Previous literature has shown that *d/l*-MAMP can be distinguished in urine and oral fluids. Hair has become a standard matrix for drug testing as the window of detection is larger in comparison to other matrices such as urine, saliva, blood. This study sought out to develop and validate a method to detect *d/l*-MAMP in hair using a chiral derivatizing agent and traditional reverse phase liquid chromatography mass spectrometry (LC/MSMS).

Materials and Method

Standards and Reagents

Analytical standards (*d*-methamphetamine, *l*-methamphetamine, methamphetamine-d5) were obtained from Cerilliant Corp. (Round Rock, TX). Negative hair was obtained from a donor and was tested negative for methamphetamine and drugs of abuse prior to this study. Chemical reagents including dichloromethane, isopropyl alcohol, methanol, water, acetic acid, ammonium hydroxide, hydrochloric acid, sodium bicarbonate, and sodium phosphate were purchased from VWR International (Bridgeport, NJ). 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide was purchased from Sigma-Aldrich (St. Louis, MO). All solvents were HPLC grade or better. Three working calibration standards were prepared in methanol from stock material at concentrations of 100,000 ng/mL, 10,000 ng/mL, and 1,000 ng/mL. Three working quality control standards were prepared in methanol from separate stock material at concentrations of 100,000 ng/mL,10,000 ng/mL, and 1,000 ng/mL. A deuterated internal standard was prepared in methanol from stock material at a concentration of 10,000 ng/mL. All working standards were stored at -20°C when not in use. Calibration and quality control specimens were prepared by preparing hair extraction buffer with the appropriate volume of working calibration or quality control material. Calibration curves were generated over the range of 25 – 10,000 ng/mL. Quality control samples were included in each batch of analyzed specimens at concentrations of 40, 125, and 750 ng/mL. Authentic hair samples for correlation testing were obtained from Cordant Health Solutions.

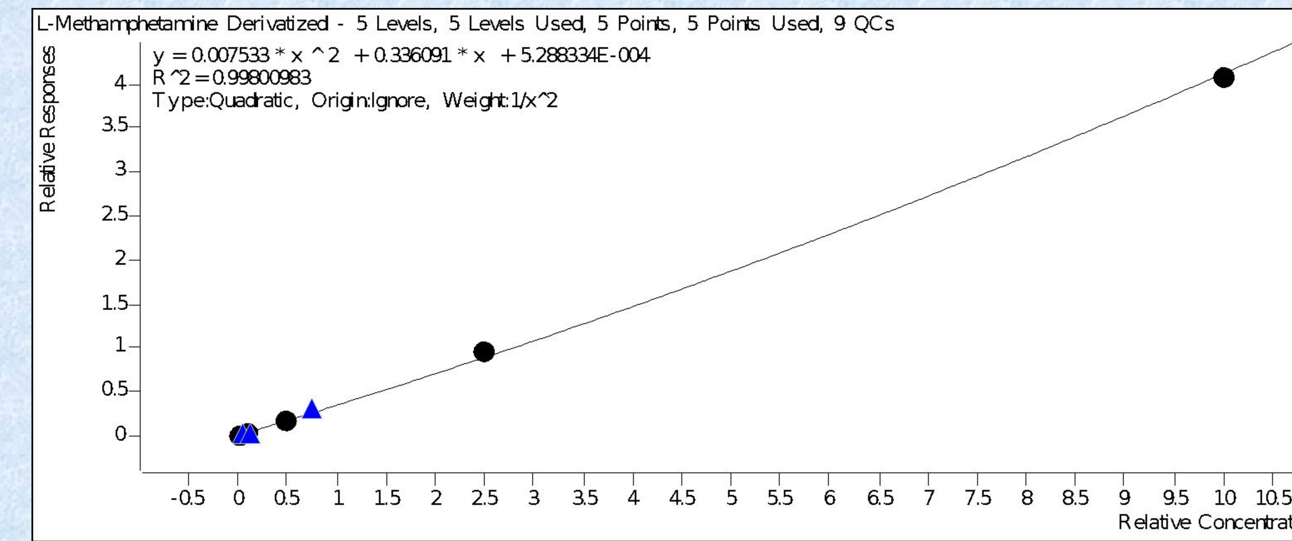
Sample Preparation

The required amount of hair that was used and analyzed throughout this study was 10 mg. The hair was first decontaminated to remove any external contaminates including hair care products, sebum, and potential external drug analytes. The hair was aliquoted into a glass tubes and first cleaned with 3 mL of methanol for 5 minutes. The methanol was aspirated and discarded. The second and third washes consisted of adding 3 mL of sodium phosphate buffer at pH 6.0 for 5 minutes and aspirated after each wash. To the calibration and QC materials, 1 mL of the previously made calibrations/QC in hair extraction buffer, were added to each tube. All other samples, including authentic donor samples had 1 mL of blank hair extraction buffer added to their respective tubes. Fifty microliters (50uL) of methamphetamine-d5 internal standard was added and specimens were incubated at 75 °C for two hours. One (1 mL) of sodium phosphate buffer was then added to the samples. Specimens were transferred to Cerex Trace-B solid phase extraction (SPE) cartridges and allowed to pass through the columns. Columns were subsequently washed with deionized water, 0.1M acetic acid, and 25% methanol. Methamphetamine was eluted from the SPE cartridges with 1.5 mL of dichloromethane : isopropyl alcohol : ammonium hydroxide (70:26:4), acidified with dilute sulfuric acid, and evaporated to dryness under a stream of nitrogen gas at 40°C. Extracts were reconstituted with sodium bicarbonate buffer (pH 9.0) and derivatized with 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide, also known as Marfey's reagent, for 1 hour at 56°C. Finally, extracts were neutralized with dilute hydrochloric acid prior to LC/MSMS analysis.

LC-MS/MS Conditions

Chromatographic separation was achieved using an Agilent Technologies 1290 liquid chromatograph equipped with Zorbax Eclipse Plus C18 columns (2.1mmx50mm 1.8um). Mobile phases consisted of 0.1% formic acid (A) and 100% methanol (B). The chromatographic run time was 6 minutes. Positive identification was made using an Agilent 6460 triple quadrupole mass spectrometer with a Jetstream source operating in positive ion mode with the following common parameters: drying gas temperature 350°C, sheath gas temperature 400°C, drying gas flow 10 L/min, sheath gas flow 11 L/min, nebulizer pressure 50 psi, capillary voltage 4000 V, and nozzle voltage 1000 V. A multiple reaction monitoring (MRM) method monitored for all ion transitions. One MRM transition served as a quantifier transition and a second MRM transition served as a qualifier transition for all drugs. One MRM transition was used to monitor internal standards. All qualifier ion ratios were determined to be within +/- 20% of calibrator qualifier ion ratios. The calibration range of *d/l*-MAMP was 25 – 10,000 ng/mL (Fig.1,2).

Results & Discussion



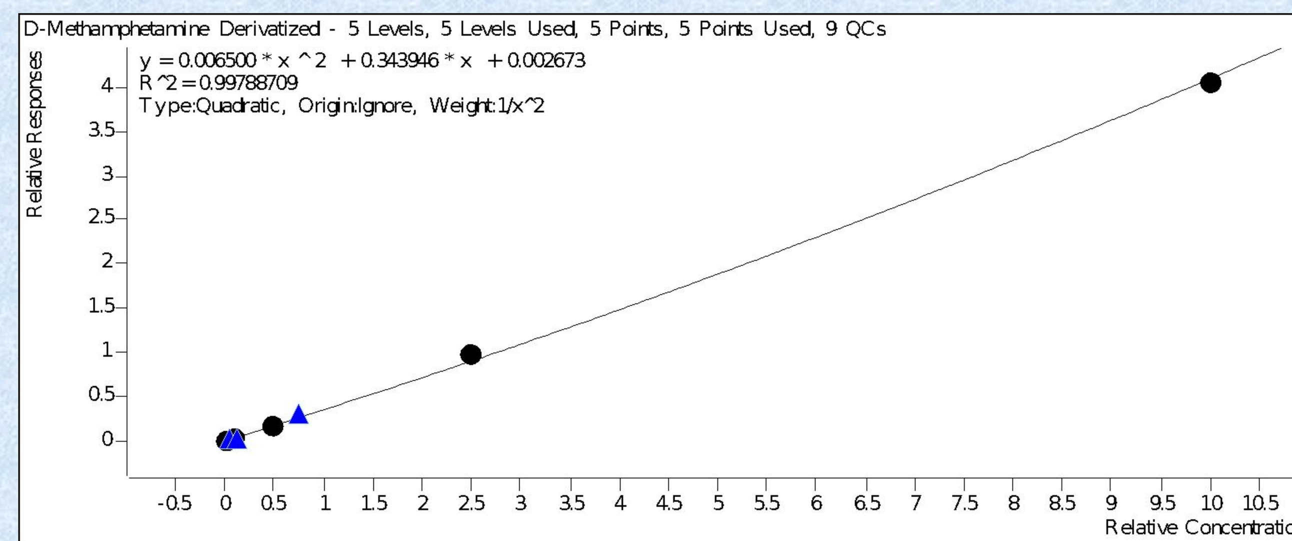
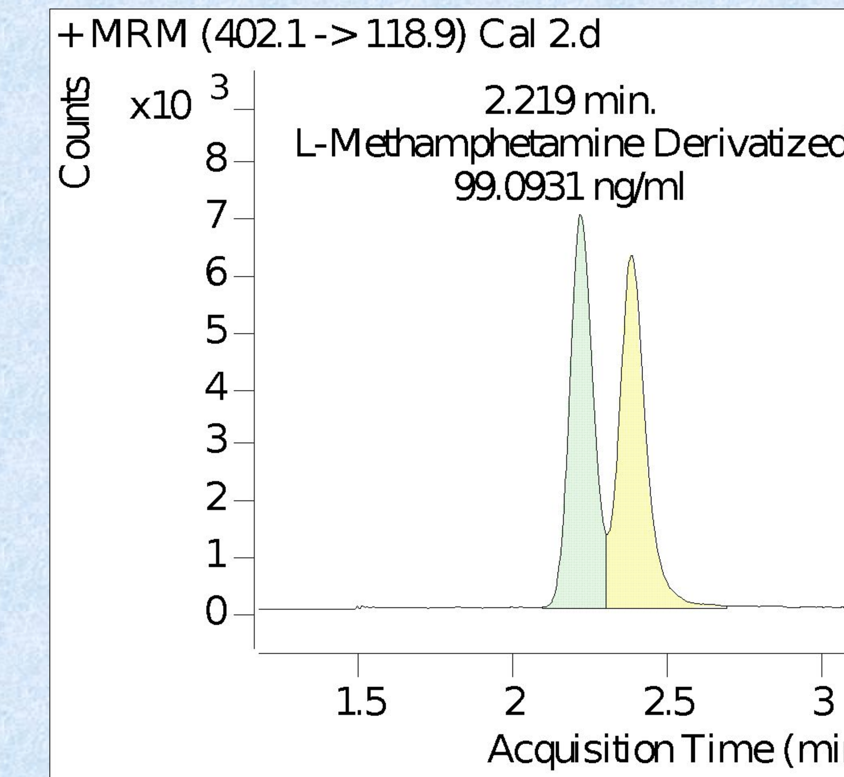


Fig.1 Calibration Data



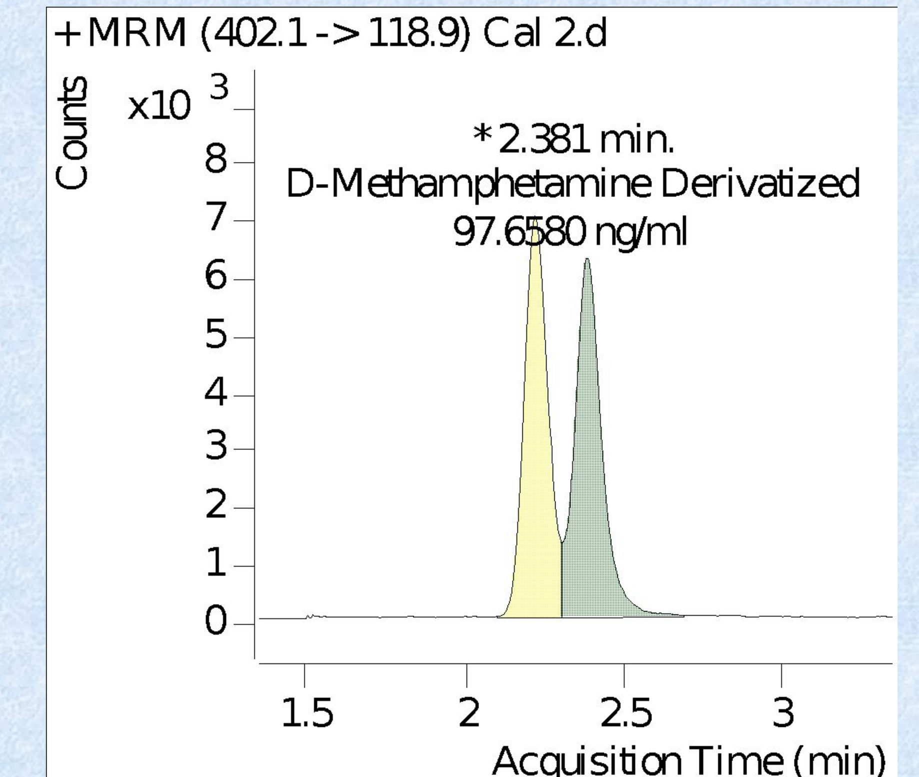


Fig.2 Limit of Quantitation

Method Validation Procedures

Linearity, accuracy, and precision were determined in triplicate over five separate days. Selectivity, matrix effect, recovery and process efficiency were evaluated in specimens from fifteen hair samples. Additional validation studies included carryover, dilution integrity, processed sample stability, and a positivity case study. Linearity, accuracy and precision, recovery, matrix effects, and specificity of the method were all within acceptable criteria. Interday (N=15) accuracy of quality control samples ranged from 91-101%, while precision (RSD) ranged from 2.5-5.9%. The recovery of target analytes from spiked hair samples averaged 102% and 99% for *d/l*-MAMP respectively (Fig.3). The method was evaluated for potential interferences from other sympathomimetic amines such as ephedrine, pseudoephedrine, phenylpropanolamine, MDMA, MDA, and MDEA, and other drugs of abuse such as benzodiazepines, opiates. No interferences were identified at concentrations up to 10,000 ng/mL. Finally, having previously tested positive for methamphetamine using non-chiral analysis, 20 de-identified authentic hair samples were analyzed using this validated method. 98% of all samples tested positive for *d*-MAMP at greater than 20% (Fig.4). Sample 1 represents 100% d-MAMP, Sample 2 represents 7.05% *l*-MAMP and 92.95%.

Analyte Name	Matrix Effect	Recovery	Process Efficiency
L-Methamphetamine	133% (66%-199%)	102% (64%-199%)	128% (90%-156%)
D-Methamphetamine	145% (62%-208%)	99% (59%-214%)	133% (96%-167%)

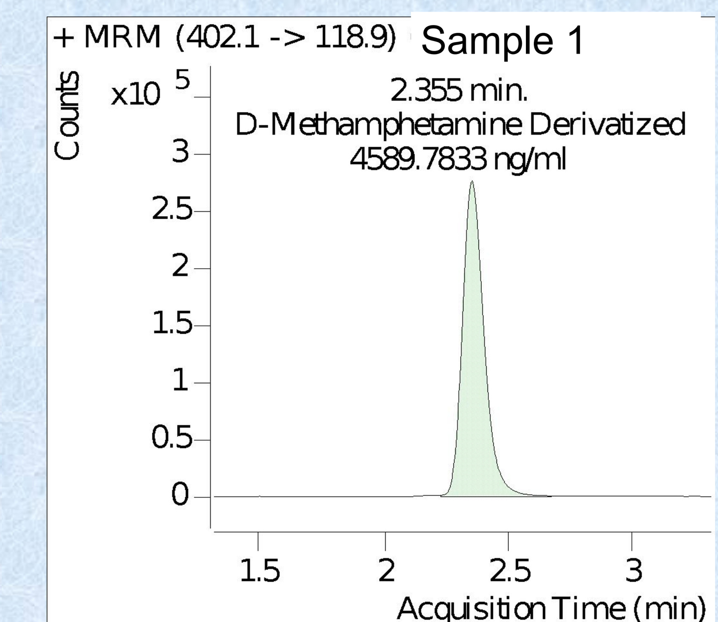
d-Methamphetamine (n=15)				
QC Sample	Average	% Error	SD	RSD
PC1	37.8	-5.4	2.1	5.5
PC2	112.4	-10.1	5.8	5.2
PC3	747.3	-0.4	44.3	5.9

l-Methamphetamine (n=15)				
QC Sample	Average	% Error	SD	RSD
PC1	38.1	-4.6	1.6	4.3
PC2	114.0	-8.8	2.8	2.5
PC3	754.3	0.6	32.5	4.3

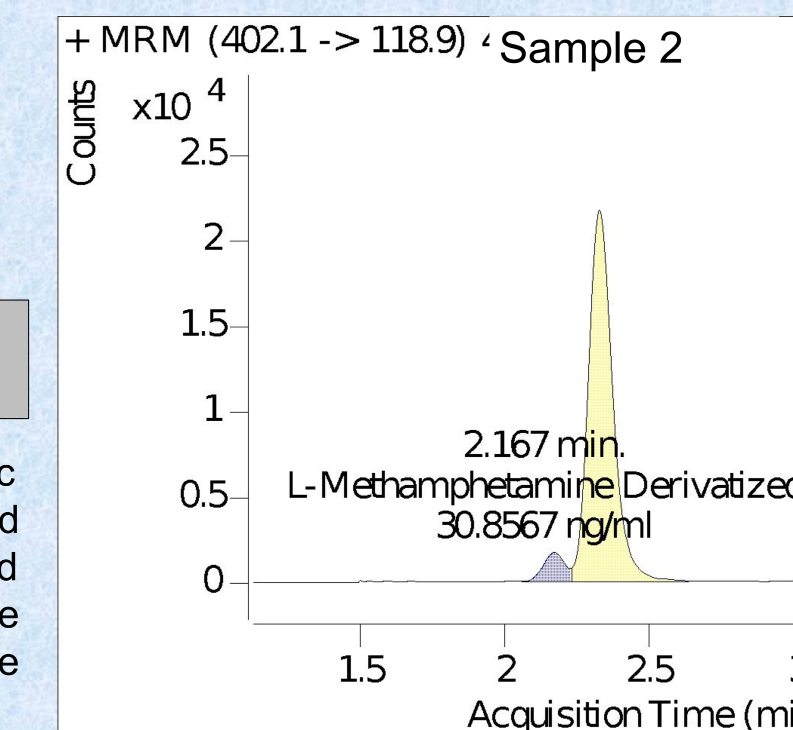
Fig.3 Validation Data

Authentic Hair Samples (n=20)		
Result	Total	Percent
>20% *d*-Methamphetamine	20	100%
<20% *d*-Methamphetamine	0	0%

Fig.4 Authentic Hair Samples



Sample 1 - 100% *d*-Methamphetamine



+MRM (402.1 -> 118.9) Sample 2
Counts
x10 4
*2.323 min.
D-Methamphetamine Derivatized
407.1382 ng/ml
Acquisition Time (min)

Sample 2 – 7.05% *l*-Methamphetamine 92.95% *d*-MAMP

Conclusions

This study concludes that *d/l*-MAMP can be distinguished from their enantiomeric forms through chiral analysis and traditional LC/MSMS. This method was developed and validated using authentic hair samples. Authentic hair samples (n=20) determined that 100% contained d-MAMP with only 1 sample contained *l*-MAMP at 7.05% (Sample 2). This method will provide laboratories with a means to accurately assess the enantiomeric composition of MAMP positive hair samples.

For any questions, please email me at kristen.payes@jjay.cuny.edu