Overcoming Chiral Method Development Challenges: UPLC-MS/MS Method for Determination of Dextroamphetamine and Levoamphetamine in Human Plasma after Chiral Derivatization

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INTRODUCTION

Enantiomeric determination of dextro-/levo- (d-/l-) amphetamines is of great interest to both forensics (amphetamine and its analogues are the most abused drugs) and amphetamine-containing new drug development because the two enantiomers may behave very differently in pharmacokinetic and pharmacologic activities. Existing methods use GS/MS after derivatization to form diastereomers, or HPLC/MS using chiral stationary phase without derivatization or non-chiral stationary phase after chiral derivatization. These methods either require relatively long analysis time (> 10 mins) or can achieve LLOQ of only 1 ng/mL. This poster will outline the method development challenges and solutions applied for our UPLC-MS/MS method to reliably measure both d- and l-amphetamines of 0.1 to 100 ng/mL in human plasma with run time of 4.5 minutes.

METHOD

There were two major obstacles during the MD: 1. amphetamine is hydrophilic and was not well retained on chiral stationary phase under reversed phase LC conditions; 2. to overcome poor retention and poor enantioselectivity, we derivatized d/l-amphetamines with fluorenylmethyloxycarbonyl chloride (FMOC) and the derivatives remained chiral but more hydrophobic. With this approach, base-line enantiomeric separation was successfully achieved with run time of ~ 7 minutes using the AGP column as shown in below chromatogram Figure 1(a), however the sensitivity was not high enough to reach the LLOQ at 0.1 ng/mL due to broaden peaks. To overcome the difficulties, we first convert the enantiomers into diastereomers via the derivatization of (d-/l-) amphetamines with the chiral reagent, (S)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride [(S)-(+)-MTPA-Cl], and then utilize the separation power of UPLC to shorten the analysis time and to improve the sensitivity by sharpening the peaks as shown in Figure 1 (b), (c) and (e).

Table 1: Summary on Validation Parameters

Analyte Name	d-amphetamine	l-amphetamine
Internal Standard (IS)	(±)-amphetamine-d_5	
Sample Volume	200 µL	
QC Concentrations	0.1, 0.3, 4, 40, and 80 ng/mL	
Standard Concentrations	0.1, 0.2, 1, 3, 10, 30, 90, and 100 ng/mL	
Average Recovery of Analyte (%)	62.6/62.7	
Intraday Precision Range (%CV) (n=6)	0.9 to 7.4	0.8 to 9.6
Intraday Accuracy Range (%RE) (n=6)	-7.9 to 3.0	-7.5 to 5.3
Interday Precision (%CV) (n=18)	2.4 to 6.9	1.6 to 6.9
Interday Accuracy (%RE) (n=18)	-6.1 to 2.0	-5.7 to 3.8
Processed Sample Stability	173 Hours at 4°C	
Benchtop Stability in Plasma	24 Hours at Ambient Temperature	
Freeze/Thaw Stability in Plasma	5 Cycles at -20°C and -70°C	
Benchtop Stability in Whole Blood	2 Hours at Ambient Temperature	
Long-term Storage Stability in Plasma	77 Days at -20°C and -70ºC	
Dilution Integrity	1000 ng/mL diluted 20-fold	
Selectivity	≤ 20.0% LLOQ for analytes; ≤ 5.0% for IS	
Hemolyzed Effect Test	2% hemolyzed plasma has no impact on assay performance	

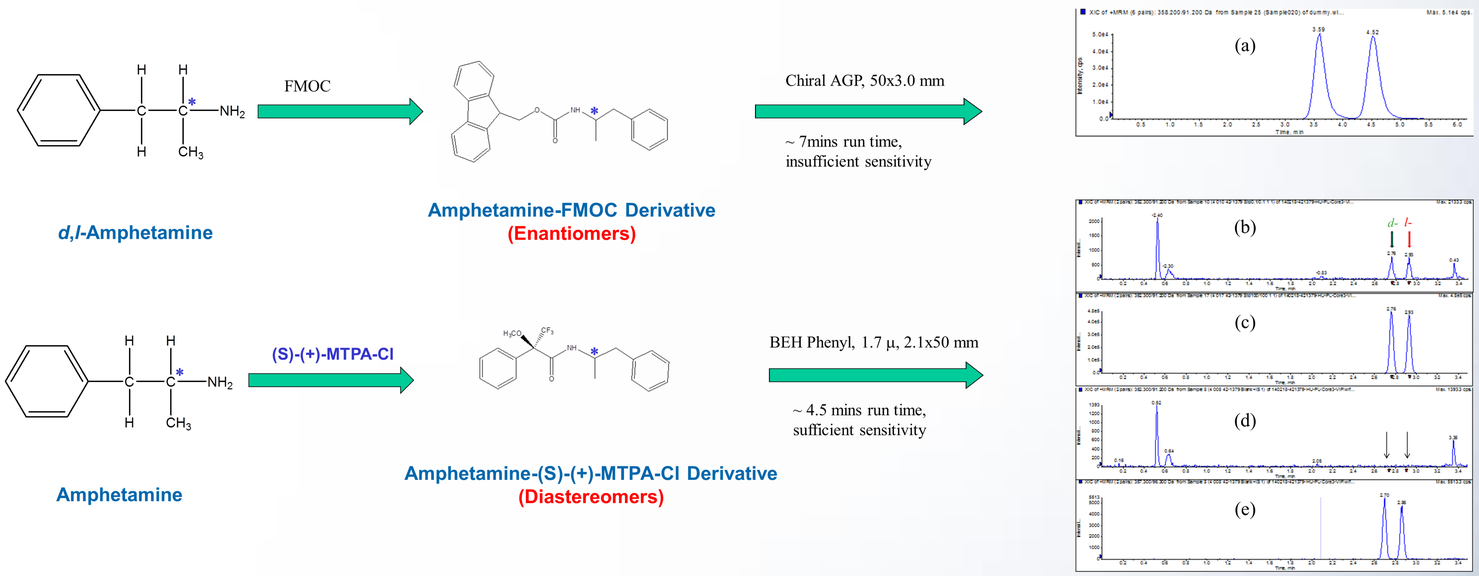
RESULTS & DISCUSSIONS

The chromatographic separation was achieved using an achiral stationary phase column (Waters ACQUITY UPLC® BEH Phenyl 1.7mm, 2.1x50 mm). Typical chromatograms are shown above: (b) @ 0.1 ng/mL (LLOQ); (c) @ 100 ng/mL (ULOQ); (d) processed blank plasma; and (e) stable isotope labeled internal standards. All parameters for the sample derivatization process and the liquid-liquid extraction procedures were optimized.

200 µL of plasma samples were mixed with 50 µL of internal standard working solution, then basified with 0.1 M sodium phosphate buffer at pH 10.0. The d-/l- amphetamines were then extracted using ethyl acetate prior to the derivatization.

Due to the volatility of amphetamines, the extracts were not dried down to avoid loss and cross-contamination, instead, the derivatization reagent was directly added into the ethyl acetate extract, then incubated in a 40 ºC water bath for 40 minutes to facilitate the reaction, and the derivatives were then extracted using hexanes as extracting solvent. The chromatograms (d) showed no interference peaks at the retention times of d-/l- amphetamines. A linear range of 0.1 to 100 ng/mL with correlation coefficient ~0.999 was shown for both enantiomers.

Figure 1 CHEMICAL STRUCTURES AND CHROMATOGRAGMS



SUMMARY

- Several approaches have been examined in order to achieve enantioseparation for d-/l- amphetamines; among them, UPLC separation of the diastereomers after chiral derivatization was found to be the most effective one;
- A sensitive and reliable UPLC-MS/MS method for enantiomeric determination of d-/l- amphetamines in human plasma has been developed using the chiral derivatization approach; The method was validated with key validation parameters summarized in Table 1.

REFERENCES

1. J. M. Plotka, C. Morrison and M. Biziuk, Trends in Analytical Chemistry, 30 (2011) 1139-1158.
2. D. J. Kuntz and M. J. Herrera; US Patent Application # 20130177994.
3. S. Chinaka, R. Iio, N. Takayama, S. Kodama, and K. Hayakawa, J. Health Sci. 52(2006) 649-654.
4. H. J. Leis, G. N. Rechberger, G. Fauler and W. Windischhofer, Rapid Commun. Mass Spectrom. 17 (2003) 569-575