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

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Novel Aspect (limit 20 words):
A quick, sensitive and reliable UPLC-MS/MS method for Enantiomeric Determination of Dextroamphetamine and Levoamphetamine in Human Plasma after Chiral Derivatization

Introduction (limit 120 words):
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 within run time of ~4.5 minutes.

Method (limit 120 words):
There were several obstacles during the MD: 1. amphetamine is hydrophilic and was not well retained on chiral stationary phase under reversed phase LC conditions. Attempts with chiral stationary phase (e.g., protein based AGP column) under reverse phase conditions ended with poor enantio-selectivity and poor sensitivity; 2. to overcome poor retention and poor enatio-selectivity, we derivatized d/l-amphetamines with FMOC and successfully achieved base-line enantiomeric separationwith run time of ~ 7 minutes using the afore-mentioned AGP column, but the LLOQ reached could not satisfy the LLOQ requirement of ~ 0.1 ng/mL; 3. However, derivatization with a pure chiral reagent, d-MTPA, not only provided the diastereomeric separation using a Waters BEH Phenyl column, but also achieved the required LLOQ of 0.1 ng/mL.

Preliminary data(limit 300 words):
d-/l- amphetamines were successfully separated (R~1.5) after derivatization with a pure chiral compound d-MTPA**,** and 0.1 ng/mL of LLOQ was achieved for both enantiomers using 200 µL of human plasma samples. All parameters for the sample derivatization process and the liquid-liquid extraction procedures were optimized. With two liquid-liquid extractions, one prior to the derivatization using ethyl acetate as extracting solvent and the other one after the derivatization using mixture of ethyl acetate and hexanes as extracting solvent, the chromatograms 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.