POSTER SUMMARY

integrated micro-chromatography systems

A summary of "LC-MS/MS Method for the Determination of Tricyclic and Tetracyclic Antidepressants in Human Urine"

Overview:

Antidepressants are regularly prescribed to treat depression as well as a variety of other disorders and diseases, and they can also be abused. Because of the widespread usage of antidepressants, patient monitoring is necessary for improved clinical healthcare. Tricyclic antidepressants are heavily glucuronidated when metabolized by the human body, resulting in the need for a hydrolysis step during sample preparation. A new and efficient method for the quantitation of eight cyclic antidepressants in human urine was developed and validated with the use of the genetically engineered β-glucuronidase IMCSzyme® coupled with fast LC-MS/MS for quick and accurate analysis.

Material and Methods:

Urine samples (100 µL) were incubated at 65°C for 30 minutes with 300 µL of a mix containing IMCSzyme®, Rapid Hydrolysis Buffer and deuterated internal standards. Hydrolyzed samples were transferred to a Oasis® WCX µElution solid-phase extraction plate after conditioning with methanol and water. The samples were washed with 10 mM ammonium acetate pH 6 buffer and methanol. Then the samples were eluted with 2% formic acid in 60/40 acetonitrile/methanol and diluted with water before injection onto LC-MS/MS.

Results:

The cyclic antidepressants tested were amitriptyline, desipramine, desmethyldoxepine, N-desmethylclomipramine, imipramine, mitrazapine, nortriptyline, and protriptyline. The calibration curve, with 5 points ranging from 10 – 100 ng/mL, yielded a correlation coefficient > 0.99. QC samples were run in sets over the course of 3 days, and % deviation was less than 11% and % RSD was less than 8% for all intra- and inter-batch runs.

Table 1. Validation Results Summary

Calibration Range:		10 - 100 ng/mL			
Correlation Coefficient (r^2)		≥ 0.991			
Precision and Accuracy					
QC	**Conc. (ng/mL)**	**Accuracy (%DEV)**		**Precision (% RSD)**	
		Intra (n=6)	Inter (n=8)	Intra (n=6)	Inter (n=8)
LLOQ	10	≤ 10	≤ 4	≤ 5.2	≤ 7.9
Low	12.5	≤ 11	≤ 3	≤ 4.7	≤ 7.1
Mid	50	≤ 6	≤ 2	≤ 4.0	≤ 4.8
High	75	≤ 5	≤ 1	≤ 2.9	≤ 3.5
Recovery:		62.8% - 126% (Mitrazapine: 4.8% - 9.6%)			
Stability:		**Condition**		**Accuracy (%Dev)**	
Freeze/Thaw:		3 cycles, < -20 °C		≤ 8.5	
Room Temperature:		24 hrs		≤ 2.6	
Autosampler Stability:		1 week, 15 °C		≤ 13.0	
Long - Term Storage Stability:		12 weeks, 5 °C		≤ 17.7	
		12 weeks, -20 °C		≤ 15.6	

Stability testing also showed excellent results even after freeze/thaw treatment and long-term storage. Internal standard – normalized matrix factors were determined for all analytes are were between 0.98 and 1.01 for all.

Conclusions:

The results of the tetracyclic and tricyclic antidepressants validation proved that the newly developed method is accurate. Since tricyclic antidepressants are heavily conjugated, IMCSzyme® was selected as the enzyme of choice for hydrolysis because of its quick and efficient performance.

By coupling IMCSzyme® with a new LC-MS/MS method, a rapid and accurate way of quantitating tetracyclic and tricyclic antidepressants in patient samples has been developed, which may lead to improved clinical treatments for these patients.

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A summary by IMCS from the poster "LC-MS/MS Method for the Determination of Tricyclic and Tetracyclic Antidepressants in Human Urine" presented by Amber F. Awad - Dominion Diagnostics at ASMS 2016