TECHNICAL NOTE

imcs integrated micro-chromatography systems

MicroSep Biological Science

A summary of "Fast Simple, and Accurate Method for Urine Drugs of Abuse Screening and Quantitation Using Liquid Chromatography with Time of Flight (TOF) Mass"

Overview:

Many clinical laboratories processing large number of samples in a limited time frame require minimal preparative steps to reduce time and cost. In a poster at SOFT 2015 presented by a pain-medication monitoring laboratory, the researchers reported a LC-QTOF MS method with minimal sample processing steps. A critical component of this dilute and shoot method was the genetically modified -glucuronidase, IMCSzyme®, which has higher purity than other commercially available -glucuronidases, which translates to no interferences identified on the

Material and Methods:

Urine samples were prepared using 50 L of urine, 25 L of IMCSzyme®, 50 L of acetate buffer (pH = 7) and 50 L of internal standard solution. Samples were then transferred to a 96-well plate and heated at 60 °C for one hour. An additional 50 L of mobile phase A was added to the hydrolyzed urine and centrifuged at 3,000 RPM for 5 minutes prior to injection.

The mass spectrometer instrument, Compact™ Q-TOF (Bruker Daltonics) was calibrated with sodium formate clusters at the beginning of every injection. Two linear gradients were implemented using Shimadzu LC-20 AD on a Perkin Elmer analytical column (C18, 2.1 x 100 mm, 2.7 um) heated to 40 °C with a flow rate of 0.4 mL/min for separation of 35 analytes (Table 1). The initial conditions were 95% A with first gradient to 70% A from 1.5 minutes to 3.5 minutes and a second gradient to 10% A from 3.5 minutes to 9.5 minutes. The column was re-equilibrated to initial conditions for 3 minutes.

Table 1. *Validation Study Statistics; Concentrations Given in ng/mL. %CV is Measured at the Cutoff. Linear Concentration Ranges Were Chosen to Approximate Expected Range of Drug/Metabolites Present in a Urine Sample.*

Benzodiazepines	LOQ	ULOL	Cutoff	CV%
Alprazolam	5	200	20	4.4
a-OH-Alprazolam	10	200	20	3.6
Clonazepam	10	200	20	2.6
7 - Amino Clonazepam	5	200	20	3.8
Diazepam	100	2000	100	2.3
Lorazepam	100	2000	100	1.6
Nordiazepam	50	2000	100	0.7
Oxazepam	50	2000	100	3.9
Temazepam	50	2000	100	3.9
Opiates	**LOQ**	**ULOL**	**Cutoff**	**CV%**
Codeine	50	2000	100	1.1
Morphine	50	2000	100	0.3
6-MAM	5	200	10	2.5
Hydrocodone	100	2000	100	1.1
Hydromorphone	50	2000	100	6.4
Oxycodone	50	2000	100	2.9
Oxymorphone	50	2000	100	0.9
Methadone	200	1000	200	0.8
EDDP	200	1000	200	0.7
Fentanyl	2	50	2	10.3
Norfentanyl	2	50	2	5
Buprenorphine	50	2000	100	3.9
Tramadol	50	2000	100	3.9
Tapentadol	50	1000	50	1.7
Antidepressants	**LOQ**	**ULOL**	**Cutoff**	**CV%**
Amitriplyline	100	5000	100	8.9
Notriptyline	100	5000	100	2.4
Imipramine	100	5000	100	5.9
Despramine	100	5000	100	2.2
Doxepin	100	5000	100	2.3
Other drugs/ metabolites	**LOQ**	**ULOL**	**Cutoff**	**CV%**
Amphetamine	50	2000	100	1.6
Methamphetamine	50	2000	100	1.2
Benzoylecgonine	50	2000	100	4.3
MDMA	50	2000	100	1.2
PCP	5	200	20	0.9
Zolpidem	50	2000	50	4.3

Conclusion

The calibration curves of peak area ratio plotted against target concentrations were linear with a correlation coefficient greater than 0.99 for all 35 analytes. Limit of quantitation (LOQ) and upper limit of linearity (ULOL) were determined from 5 replicates of each concentration within 20% of target concentration with majority of the analytes having a CV below 5% at LOQ or cutoff. This poster highlights the application of a LC-QTOF MS as a sensitive, selective and reproducible solution for quantitation of drugs in urine samples that was achieved using the genetically modified -glucuronidase, IMCSzyme®.

This information was summarized by IMCS from the technical poster "Fast Simple, and Accurate Method for Urine Drugs of Abuse Screening and Quantitation Using Liquid Chromatography with Time of Flight (TOF) Mass" presented by E. Howard Taylor - Addiction Labs of America at SOFT 2015

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