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Quantitation of Designer Synthetic Amphetamines Using Ultra-Fast Liquid Chromatography / Mass Spectrometry

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1. Introduction

New synthetic designer drugs of abuse are being produced as readily available consumer products such as bath salts and incense. The uncontrolled and unregulated synthesis of these drugs causes numerous variations in formula, structure, and stereochemistry. These variations cause the analysis of the compounds of interest to be very difficult and extremely time consuming. In this study we describe a new simple and efficient method by which these new synthetic drugs of abuse can be rapidly identified and quantified.

2. Materials and Method

This assay tests for the presence of five amphetamines in human urine, water and synthetic urine with no other sample pretreatment or derivatization beyond dilution. All matrix samples were spiked with the synthetic amphetamines Methiopropamine (MPA), 5,6-Methylenedioxy-2-aminoindane (MDAI), Dimethyamyllamine Methylhexanamine (DMAA), Methylenedioxypyrovalerone (MDPV), and Methoxetamine (MXE). A direct injection method was used for analysis.

Sample Preparation:

Stock standard solutions of 1mg/mL of each the five analytes were prepared in methanol / acetonitrile. Working solutions of the analytes mixed together were prepared in three different matrices: drug-free urine, Mobile Phase A and Surine Negative Urine (Cerilliant, USA). A five point calibration curve was used for quantitative analysis in the range from 2,000 ng/mL to 50 ng/mL. For the urine samples, vials were spiked, centrifuged, and diluted four-fold with mobile phase A. [1]

Chromatography:

Instrument: Shimadzu Nexera UHPLC system
Column: Shim-pack XR-ODS III (2.0 mm ID x 50 mm ; 1.6µm)
Mobile Phase A: 95% Water, 5% Methanol, 0.1% Formic Acid
Mobile Phase B: 95% Methanol, 5% Water, 0.1% Formic Acid
(All mobile phases were purchased from Burdick & Jackson)
Gradient Program:
5% B (0-0.2min) – 95% B (0.75-1.00 min) – 5% B (1.01 – 3 min).
Flow Rate: 0.5 mL/min
Column Temperature: 50 ° C
Injection Volume: 1 µL

Mass Spectrometry:

Instrument: Shimadzu LCMS-8030 Triple Quadrupole Mass Spectrometer
Ionization: ESI
Polarity: Positive
Scan Mode: MRM & Product Ion Scan

The differences in responses of all the analytes in the five different matrices studied was found to be within ±15% of the expected value. The value of ±15% was proposed as an acceptable limit for matrix effects variability in 2007. [2]

3. Results

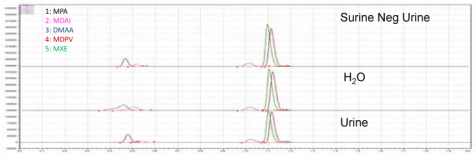

Figure 1. Total Ion Chromatogram of selected transitions obtained from the analysis of 1,000 ng/mL standard mix in three different matrices.

	Estimated Limit of detection (ng/mL, S/N 3:1)			Estimated Limit of quantitation (ng/mL, S/N 10:1)		
Analyte	Urine	Water	Surine Neg Urine	Urine	Water	Surine Neg Urine
MPA	0.7	2.6	13.7	2.3	8.0	41.6
MDAI	1.5	4.6	18.5	3.7	8.9	56.0
DMAA	1.6	3.0	13.5	5.0	9.0	40.8
MDPV	0.3	1.1	4.4	1.0	3.4	13.3
MEX	0.4	1.1	4.2	1.1	3.5	12.7

Table 1. Limit of detection and limit of quantitation calculated as three times the S/N (LOD) and ten times the S/N (LOQ) following the analysis of the 500 ng/mL standard prepared in the three different matrices.

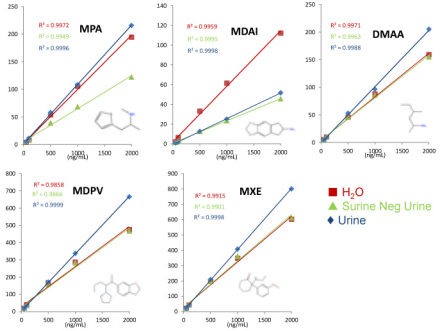

Figure 2. Calibration curves for each of the compounds (n=5) at 4 levels in water, Surine Neg Urine, and Urine.

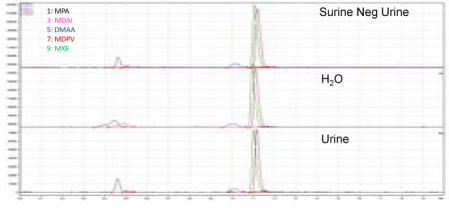

Figure 3. Total Ion Chromatogram of MRM transitions acquired with Survey Event selected to obtain a product ion scan from the 1,000 ng/mL standard mix in three different matrices. A relative high concentration of 1,000 ng/mL was chosen because it correlates to the currently employed immunoassay screening methods.[1]

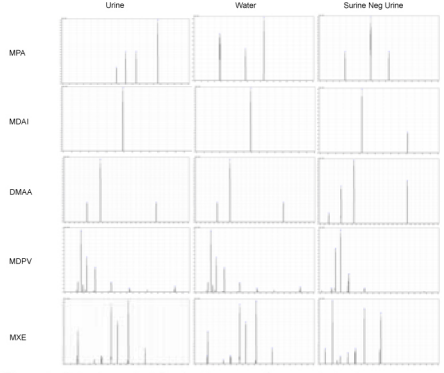

Figure 4 . Data dependent Product ion scans from amphetamine compounds

4. Conclusions

A direct analysis method has been demonstrated to be robust and reliable for the confirmatory analysis of the amphetamines.

The short run-time, combined with a simple sample preparation procedure, allows the method to be adapted for routine drug testing in high sample throughput laboratories.

5. References

1. Charlotte B. A. et al. Wiley Online Library: 11 July 2011
2. 2007 American Association of Pharmaceutical Scientists (AAPS)/ Food and Drug Administration (FDA) conference report: Quantitative Bioanalytica/ Methods Validation and implementation: Best Practices for Chromatographic and
Ligand Binding Assays.
3. Nguyen, H.P. et al Hydrophilic Interaction Chromatography – A Guide for Practitioners. Wiley, Hoboken NJ. 2012. (Accepted; In-Press).
4. Ariane W. Et al. Anal Bioanal Chem (2010) 396:2403–2414