Delivering the Right Results

Drug Screening in Non-Derivatized Urine by Automated Solid Phase Microextraction (SPME) and Comprehensive Multidimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

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OBJECTIVE

☑ Demonstrate the detectability of non-derivatized drugs in complex sample matrices such as urine by automated SPME combined with GCxGC-TOFMS.

☑ Show calibration linearity capabilities over the range of 10 to 1000 ng/mL for the forensic application of drug screening in urine.

☑ Illustrate the feasibility of this analysis for non-derivatized drugs in urine by SPME-GCxGC-TOFMS describing the advantages of multi-dimensional chromatography (GCxGC) and time-of-flight mass spectrometry (TOFMS).

INTRODUCTION

Comprehensive multi-dimensional gas chromatography in combination with time-of-flight mass spectrometry detection (GCxGC-TOFMS) was used for this drugs of abuse analysis in urine without time consuming sample derivatization. Methamphetamine, cocaine, diacetylmorphine, codeine, oxycodone, ecstasy, acetylcodeine, monoacetylmorphine, hydrocodone, and LSD were identified in this research.

This poster presents experimental data from the forensic application conducted by automated solid phase microextraction (SPME) – GCxGC-TOFMS. Non-derivatized, 8 mL aliquots of urine were spiked with a multiple drug standard mixture prepared from Sigma–Aldrich standards. Hexachlorobenzene (HCB) was added as an internal standard at a concentration of 500 ng/mL. Automated SPME sample preparation and injection was performed with a Gerstel MPS2 auto sampler equipped with a SPME prep station. GCxGC-TOFMS analysis was conducted with a LECO Pegasus® 4D mass spectrometer coupled with an Agilent 7890 gas chromatograph fitted with a LECO secondary GC oven and quad jet dual stage thermal modulator.

This poster illustrates the benefits of enhanced chromatographic separation and analyte detectability with emphasis on the increased peak capacity and resolution gained by multi-dimensional gas chromatography. The advantage of time–of–flight mass spectrometry (TOFMS) to acquire full mass range spectral data at fast acquisition rates, which are necessary when using multi-dimensional chromatography, will be shown in the drugs in urine research. TOFMS provides simultaneous, non-skewed mass spectral information which is required for accurate deconvolution of overlapping peaks, and the data density needed to allow Deconvolution algorithms to correctly identify poorly resolved chromatographic peaks which are buried in heavy sample matrices. Examples of deconvolution are illustrated along with the experimental research data from the non-derivatized drugs of abuse screening in urine analysis.

The data from this research will show the identifications of target analytes in very complex sample matrices. The use of automated SPME applied to non-derivatized urine samples coupled with comprehensive multi-dimensional chromatography and time–of–flight mass spectrometry detection demonstrates this as a favorable technique for qualitative and quantitative analysis for drug screening in urine.

GCxGC-TOFMS

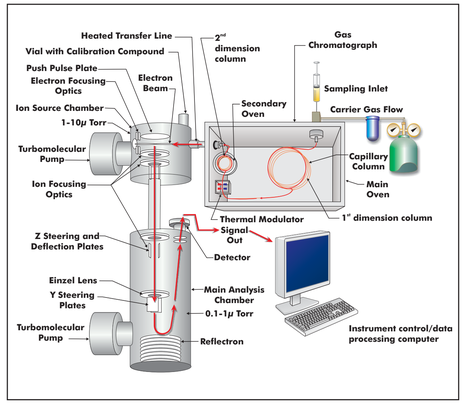


Figure 1. The diagram above shows a schematic of a GCxGC-TOFMS system. This system can acquire 500 full range mass spectra/second (500 Hz). TOFMS provides the acquisition rates and non-skewed mass spectra necessary to provide accurate mass spectral identifications for complex samples separated by comprehensive multi-dimensional gas chromatography. The diagram above illustrates the flow path of a comprehensive multi-dimensional chromatographic (GCxGC) system. Samples are injected and volatilized into the gas phase. Components are swept by a carrier gas into the primary 1st dimension capillary column and separated commonly by a non-polar stationary phase. Separated components enter a quad jet dual stage thermal modulator and are refocused prior to introduction into a smaller diameter secondary column, usually a polar stationary phase. An orthogonal separation in the 2nd dimension is accomplished before time-of-flight mass spectrometer (TOFMS) detection.

EXPERIMENTAL

Sample Preparation: Automated SPME Method Parameters

Auto Sampler: Single rail CTC CombiPAL equipped with SPME sample agitator/prep station and SPME fiber conditioning station

SPME Fiber: 50/30 μm DVB/Carboxen™/PDMS Stable Flex

Agitator Speed: 200 rpm
Extraction Temperature: 37°C

Extraction Time: 30 minutes
Injection desorption time: 2 minutes

Fiber Conditioning Station Temperature: 270°C
Fiber Bake Out (conditioning) Time: 40 minutes

Instrumental Method: GCxGC-TOFMS Analysis

Mass spectrometer: LECO Pegasus 4D

GC: Agilent 7890 equipped with a GERSTEL MPS2 Auto Sampler
GC Primary Column: 30 m x 0.25 mm id x 0.25 μm film thickness, Rxi-5MS (Restek Corp.)
GC Secondary Column: 1.5 m x 0.18mm id x 0.20μm film thickness, Rtx-200 (Restek Corp.)
Carrier Gas: Helium @ 1.5mL/min constant flow
Injection Port Temperature: 260°C
Injection Mode: Splitless
Transfer Line Temperature: 260°C

Primary Column GC Temperature Program: Initial temperature 40°C for 2.0 min ramped @ 6°C/min. to 290°C hold for 10.0 min

Secondary Oven GC Temperature Program: Initial temperature 50°C for 2.0 min ramped @ 6°C/min. to 300°C hold for 10.0 min

GCxGC Parameters: Modulator enabled
Modulator Temperature Offset: 25°C
Column offset: 10°C
Modulation Period (2nd dimension separation time): 5 sec.
Hot Pulse Time: 0.80 sec.
Cool Time: 1.70 sec.

Mass Spectrometer parameters:
Mass Range: m/z = 45 – 550
Acquisition Rate: 200 spectra/s
Detector Voltage: 1650 V
Electron Energy: -70eV
Ion Source Temperature: 230°C

AUTO SPME GCxGC-TOFMS RESULTS

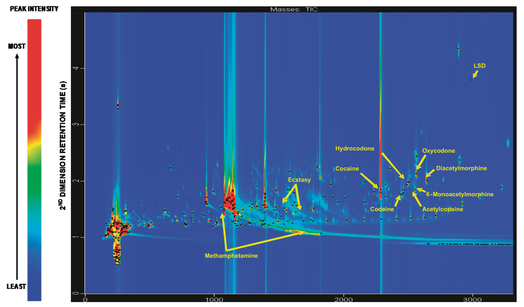
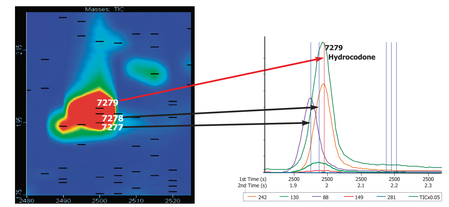


Figure 2. The total ion chromatogram above shows a two-dimensional contour plot of the 250 ng/mL drug mix standard spiked into an 8 mL aliquot of urine analyzed by automated solid phase microextraction (SPME) followed by comprehensive multi-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS). The contour plot is labeled with the ten non-derivatized drugs identified with NIST library matches greater than 75%. Over 9000 peaks were identified with a S/N ratio of 50 or more for this analysis.

True Signal Deconvolution®

Example of True Signal Deconvolution Possible with Time–of–Flight Mass Spectrometry



Peak #	Name	R.T. (s)	UniqueMass	Similarity
7277	Cyclohexaneacetic acid, a-ethyl-	2500 , 1.950	88	511
7278	Undeca-2,4,6,8,10-pentaenal, 11-(2-furyl)-, oxime	2500 , 1.975	130	622
7279	Hydrocodone	2500 , 1.990	242	893

Figure 3. The illustration above shows the benefits of time–of–flight mass spectrometry to allow fast acquisition rates which provide the data density and non-skewed mass spectra required to facilitate Deconvolution algorithms that successfully identify trace components, even in heavy sample matrices. Hydrocodone (Peak 7279) is identified with a similarity of 893 along with two other components in approximately 40 milliseconds.

CALIBRATION LINEARITY

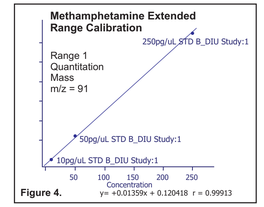


Figure 4.

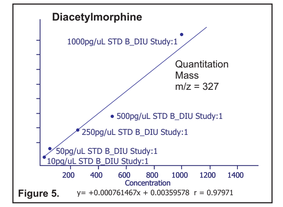


Figure 5.

Name	Absolute R.T. (sec , sec)	Range, Concentration	Min Valid Concentration	Max Valid Concentration	Masses	Quantitate Mode	Equation	Correlation Coefficients	Curve Order	Type
Methamphetamine	1070 , 1.740	2	250	1500	134	Equation	y= +0.00116133x + 0.091255	0.99483	1	Analyte
Ecstasy	1555 , 1.490	1	5	1500	135	Equation	y= +0.00152614x - 0.0262993	0.91266	1	Analyte
Hexachlorobenzene	1765 , 1.520	1	250	750	284	Equation	NA	NA	1	ISTD
Cocaine	2285 , 1.800	2	250	1500	303	Equation	y= +0.00750837x + 0.0476363	0.90428	1	Analyte
Codeine	2445 , 1.769	1	5	1500	162	Equation	y= +0.00072016x + 0.00415785	0.93206	1	Analyte
Hydrocodone	2500 , 1.990	1	5	1500	242	Equation	y= +0.00075027 4x - 0.00944874	0.96399	1	Analyte
6-Monoacetylmorphine	2555 , 1.855	1	5	1500	268	Equation	y= +0.00035705 7x - 0.0028296	0.94953	1	Analyte
Oxycodone	2560 , 2.140	1	5	1500	315	Equation	y= +0.00169896x - 0.0195396	0.98705	1	Analyte
Diacetylmorphine	2640 , 2.015	1	5	1500	327	Equation	y= +0.00076146 7x - 0.00359578	0.97971	1	Analyte

Table 1.

MASS SPECTRAL DATA

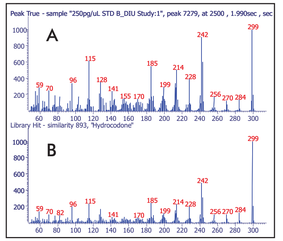


Figure 6. (A) above shows the deconvoluted mass spectra for peak 7279. The mass spectrum (B) above shows the library match for the drug Hydrocodone with a match similarity of 893. TOFMS is able to acquire high quality deconvoluted mass spectral data in a 40 millisecond time frame even when buried in heavy sample matrix with coeluted compounds as shown in Figure 3.

CONCLUSIONS

The analysis of non-derivatized drugs in urine by automated SPME-GCxGC-TOFMS shows that trace levels of drugs (ppb) from various classes can be detected in urine. The study achieved linearity of 90% or greater for nine drugs over the concentration range from 10 to 1000 ng/mL. The results of this study indicate that trace level screening of drugs in urine can be performed without sample derivatization by automated solid phase microextraction (SPME) coupled with GCxGC-TOFMS. This research demonstrates that the integration of an automated sampling method (SPME) without sample derivatization coupled with GCxGC-TOFMS analysis provides an effective and sensitive method for drug screening. Study results prove that GCxGC-TOFMS achieves the resolution, sensitivity, and mass spectral integrity that is necessary to accurately identify trace levels of drugs in complex sample matrices. Future research will include experimental designs to meet acceptance criteria guidelines set by most forensic toxicology laboratories.