Forensic Toxicology Analysis of Non-derivatized Drugs in Urine by Automated Solid Phase Microextraction (SPME) GCxGC-TOFMS

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Key Words: Non-Derivatized Drugs, Automated SPME, GCxGC-TOFMS

1. Introduction

Comprehensive two dimensional gas chromatography in combination with time-of-flight mass spectrometry detection (GCxGC-TOFMS) was used for 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 application presents experimental data from the forensics analysis conducted by automated solid phase microextraction (SPME)-GCxGC-TOFMS. A multiple drug standard mixture prepared from Sigma-Aldrich standards was spiked at concentrations from 10 to 1000 ppb into 8mL aliquots of urine. Hexachlorobenzene (HCB) was added as an internal standard at a concentration of 500 ng/mL. Automated solid phase microextraction (SPME) sample preparation was conducted on the non-derivatized spiked urine standards using a Gerstel MPS2 autosampler equipped with a SPME prepstation followed by thermal desorption of the extracted sample in the GC injection port.

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

2. Application Objectives

- 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 multidimensional chromatography (GCxGC) and time-of-flight mass spectrometry (TOFMS).

3. Experimental Conditions

Aliquots of urine spiked with a drug standard mixture at concentrations from 10, 50, 250, 500, and 1000 ng/mL were prepared and analyzed without derivatization. Hexachlorobenzene was added to each sample as an internal standard (ISTD) at 500 ng/mL prior to extraction. Each sample was placed in a 10 mL glass SPME autosampler vial and sealed. Automated SPME extraction was conducted using the Gerstel MPS2 Prepstation.

GCxGC-TOFMS results were generated with a LECO Pegasus® 4D GCxGC-TOFMS equipped with a Gerstel MPS2 autosampler and a SPME prepstation. The Pegasus 4D instrument was equipped with an Agilent 7890 gas chromatograph featuring a LECO quad jet dual stage thermal modulator and secondary oven. LECO ChromaTOF® software was used for all acquisition control and data processing. The autosampler was a single rail CTC Combi Pal equipped with SPME sample agitator/prepstation and SPME fiber conditioning station. Automated sample extraction was performed using a 50/30 µm DVB/Carboxen™/PDMS Stable Flex SPME fiber. The SPME method was developed in the ChromaTOF software autosampler methods section using the CTC Combi PAL option. The sample agitator was set to ON at a speed of 200 rpm and extraction temperature of 37°C. The solid phase microextraction time was set for 30 minutes and sample desorption time in the GC injection port was 2 minutes. The fiber was then conditioned in the fiber bakeout station at 270°C for 40 minutes prior to concurrent sample extraction for the next analysis.

A 30 m x 0.25 mm x 0.25 µm film thickness, Rxi-5ms, (Restek Corp) GC capillary column was used as the primary column for the analysis. In the GCxGC configuration, a second column 1.5 m x 0.18 mm id. x 0.18 µm film thickness Rtx-200, (Restek Corp) was placed inside the LECO secondary GC oven after the thermal modulator. Helium carrier gas flow rate was set to 1.5 mL/min at a corrected constant flow via pressure ramps. The primary column was programmed with an initial temperature of 40°C for 2 minutes and ramped at 6°C/minute to 290°C for 10 minutes. The secondary column temperature program was set to an initial temperature of 50°C for 2 minutes and then ramped at 6°C/minute to 300°C with a 10 minute hold time. The thermal modulator was set to +25°C relative to the primary oven and a modulation time of 5 seconds was used. The MS mass range was 45-550 m/z with an acquisition rate of 200 spectra per second. The ion source chamber was set to 230°C and the detector voltage was 1650V with an electron energy of -70eV.

4. Results and Discussion

Three major points will be discussed including the detectability of non-derivatized drugs in the complex sample matrix urine. The calibration linearity over the range of 10 to 1000 ng/mL will be shown along with the feasibility of this analysis for non-derivatized drugs in urine by SPME-GCxGC-TOFMS.

Ten drugs of abuse and metabolites were detected in this analysis of non-derivatized drugs in urine. Methamphetamine, cocaine, diacetylmorphine, codeine, oxycodone, ecstasy, acetylcodeine, monoacetylmorphine, hydrocodone, and LSD were identified by comparison match to the NIST 05 mass spectral library. This analysis illustrates the capabilites of comprehensive two dimensional chromatography coupled with time-of-flight mass spectrometry to provide maximum chromatographic separation and resolution along with the deconvolution algorithms which accurately identify trace ppb levels of drugs of abuse in urine without time consuming sample derivatization. On average, 9000 peaks were identified per sample with a S/N ratio of 50 confirming the complexity of the sample matrix and difficulty of this type of analysis.

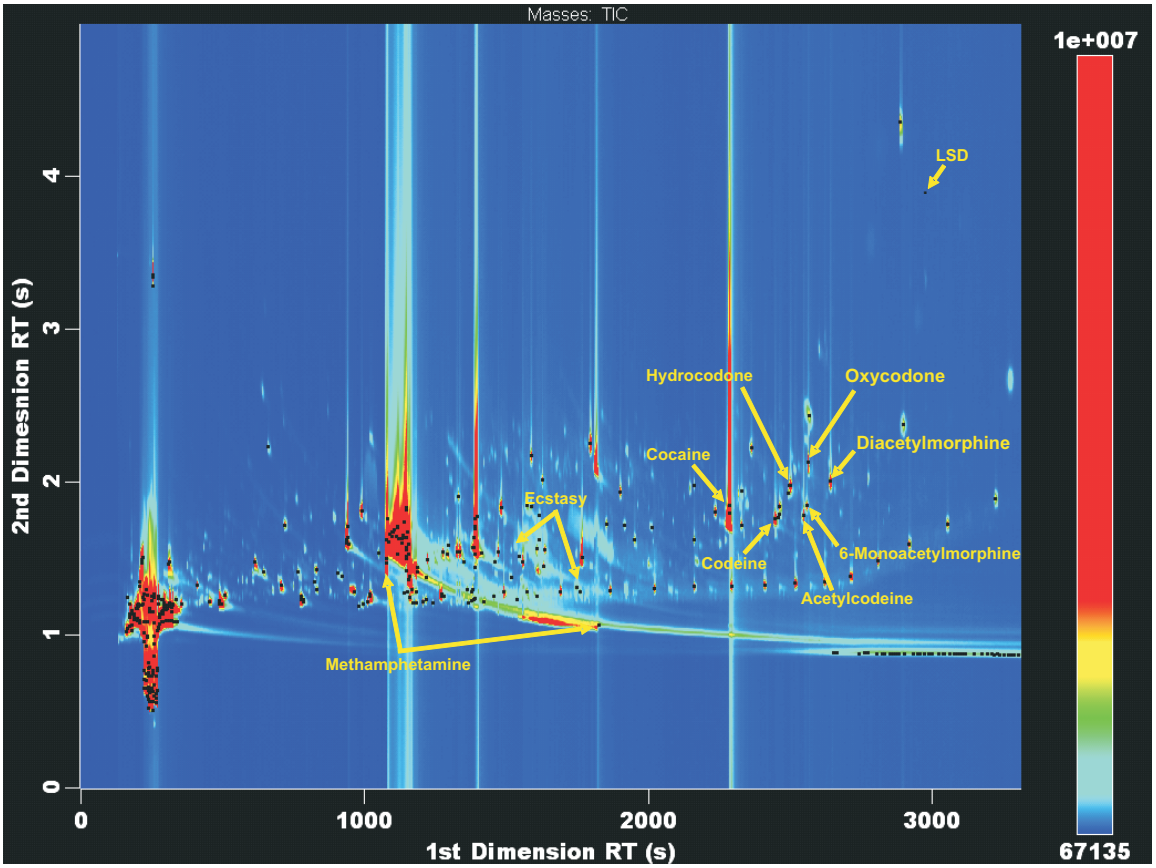


Figure 1. The total ion chromatogram above shows a two dimensional contour plot of the drug mix standard spiked into an 8 mL aliquot of urine at 250 ng/mL analyzed by automated SPME-GCxGC-TOFMS. The contour plot is labeled with the ten non-derivatized drugs identified with NIST library matches greater than 75%. Over 9000 peaks were identified with a S/N ratio of 50 or more for this analysis.

Detectability of Non-derivatized Drugs in Urine by Automated SPME-GCXGC-TOFMS

The minimum detectable concentrations were based on the data collected from samples spiked with the drug standard mixture in the range of 10 to 1000 ppb. Relative limits of detection were calculated for seven drugs by extrapolation from the ratio of concentration versus signal to noise. The relative limits of detection indicate that drug screening in urine without sample derivatization is sensitive to very low ppb or even ppt levels by SPME-GCxGC-TOFMS analysis.

Table 1. The peak table below lists the relative minimum detection limits of seven non-derivatized drugs spiked in urine at part per billion (ppb) levels. *The relative limits of detection were calculated as an extrapolation from a ratio of concentration versus signal to noise.

Name	*Concentration	R.T. (s)	Similarity	UniqueMass	Quant Masses	Quant S/N	Area
Methamphetamine	0.212 ng/mL	1160 , 1.445	820	72	91	11786	408058871
Ecstasy	0.145 ng/mL	1555 , 1.490	917	135	135	17286	13151439
Cocaine	0.038 ng/mL	2285 , 1.820	909	82	182	65374	260235677
Codeine	0.065 ng/mL	2445 , 1.770	948	162	162	38418	10242005
Oxycodone	0.277 ng/mL	2565 , 2.090	926	315	315	9009	2699361
Heroin	0.082 ng/mL	2640 , 2.015	920	327	327	30427	7137490
LSD 25	9.830 ng/mL	3095 , 3.700	796	221	221	254	71671

Calibration Linearity for Nine Non-derivatized Drugs of Abuse and Metabolites

Calibration curves were developed from 10 to 1000 ng/mL for nine non-derivatized drugs of abuse in this study. Methamphetamine and ecstasy are known to give poor chromatographic performance and exhibit significant peak tailing. The peak combine feature in the ChromaTOF® software was used to define accurate peak areas for methamphetamine and ecstasy. Extended range capability in the calibration feature of ChromaTOF software can designate different quantitation masses for two calibration ranges for the same analyte. Extended range calibration was used for methamphetamine and cocaine to compensate for ion fragment mass signal saturation from a particular fragment ion in the mass spectrum. ChromaTOF software allows the user to define a second quantitation mass spectral ion fragment that is not signal saturated, thereby allowing for greater dynamic range of concentration for a specific analyte in a single calibration. The non-derivatized drugs in urine analysis achieved linearity of 95% or greater for six out of the nine drugs identified in this research. All of the components achieved at least 90% linearity for the range from 10 to 1000 ng/mL.

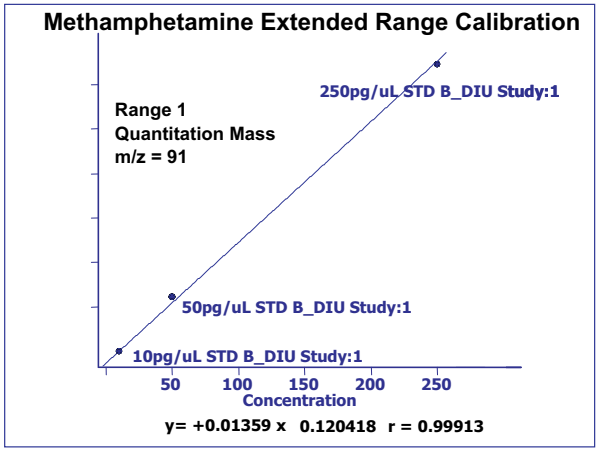


Figure 2. Figure 2 above shows the extended range (1) calibration curve for methamphetamine from 10 – 250pg/µL with a correlation coefficient value of 0.9991.

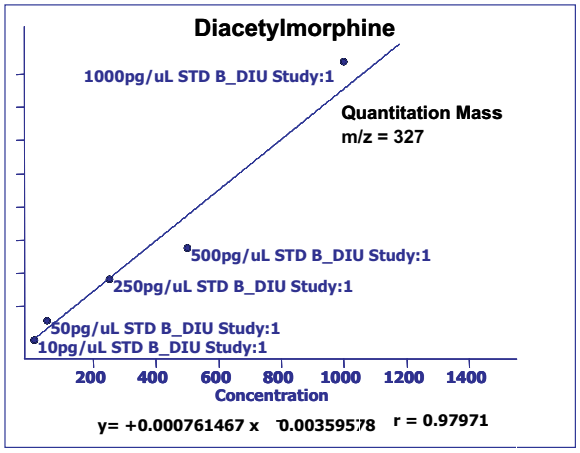
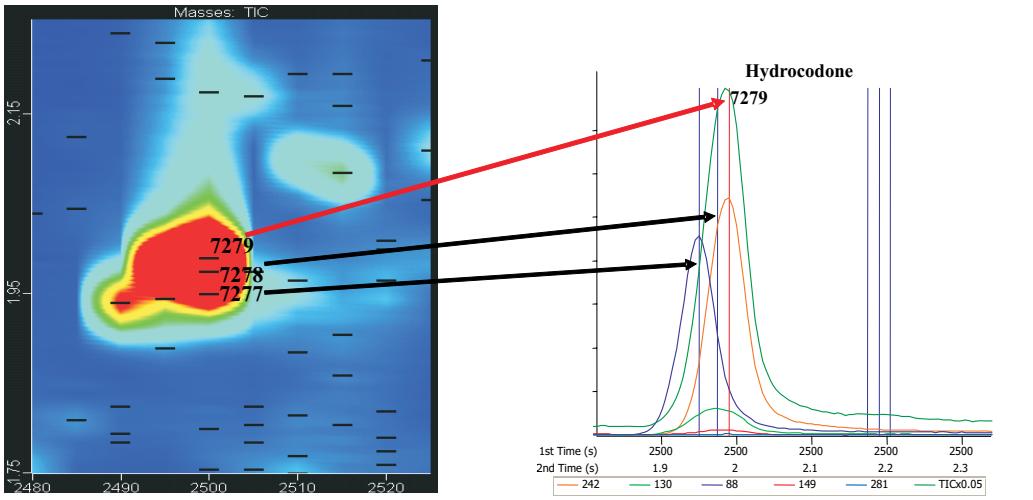


Figure 3. Figure 3 above shows the calibration curve for Diacetylmorphine from 10 – 1000pg/µL with a correlation coefficient value of 0.9797.

Table 2. This table shows a partial table used to develop calibration curves in the ChromaTOF software. The "Range, Concentration" column allows the user to define either range 1 or 2 with extended range capabilities. The "Masses" column defines the ion fragment mass used as the quantitation mass for each analyte and calibration range. Six of the nine components listed achieved correlation coefficient values of 0.9500 or greater. All components had correlation coefficient values greater than 0.9000.

Name	Absolute R.T. (sec , sec)	Range, Conc.	Min Valid Conc.	Max Valid Conc.	Masses	Correlation Coefficients	Curve Order	Type
Methamphetamine	1070 , 1.740	2	250	1500	134	0.99483	1	Analyte
Ecstasy	1555 , 1.490	1	5	1500	135	0.91266	1	Analyte
Hexachlorobenzene (INTERNAL STANDARD)	1765 , 1.520	1	250	750	284	NA	1	ISTD
Cocaine	2285 , 1.805	2	250	1500	303	0.90428	1	Analyte
Codeine	2445 , 1.769	1	5	1500	162	0.95206	1	Analyte
Hydrocodone	2500 , 1.990	1	5	1500	242	0.96399	1	Analyte
6-Monoacetylmorphine	2555 , 1.855	1	5	1500	268	0.94953	1	Analyte
Oxycodone	2560 , 2.140	1	5	1500	315	0.98705	1	Analyte
Diacetylmorphine	2640 , 2.015	1	5	1500	327	0.97971	1	Analyte

Example of True Signal Deconvolution® Possible with Time-of-Flight Mass Spectrometry



Peak #	Name	R.T. (s)	UniqueMass	Similarity
7277	Cyclohexaneacetic acid, à-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 4. 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 the deconvolution algorithms that successfully identify trace components even in heavy sample matrices. Hydrocodone (Peak 7279) is identified with a similarity of 893 as well as two other components in approximately 40 milliseconds.

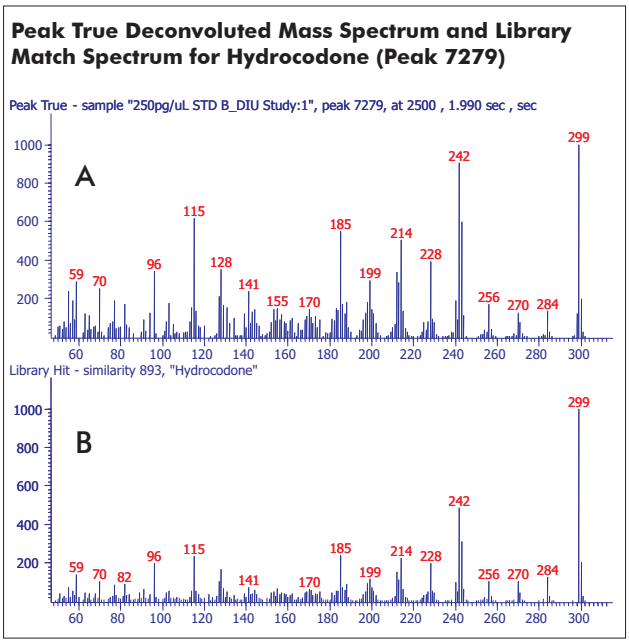


Figure 5. The mass spectrum labeled (A) above shows the deconvoluted mass spectra for peak 7279. The mass spectrum labeled (B) above shows the library match for the drug Hydrocodone with a match similarity of 893. This example illustrates the capability of TOFMS to acquire high quality deconvoluted mass spectral data in a 40 millisecond time frame even when buried in heavy sample matrix with coeluted compounds as shown in Figure 4.

5. Conclusions

Automated SPME-GCxGC-TOFMS analysis of non-derivatized drugs in urine results show that trace (ppb) levels of drugs from various chemical classes can be successfully detected in urine. This research application achieved calibration linearity of 90% or greater for nine drugs with a 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 providing accurate identifications 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 to accurately identify trace ppb levels of drugs in complex sample matrices without sample derivatization.

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