Delivering the Right Results

Utilization of GC-TOFMS and Automated Sample Derivatization for High-Throughput Workplace Urine Drug Testing by SAMHSA Guidelines

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OVERVIEW

- The Substance Abuse and Mental Health Services Administration's (SAMHSA) mandated guidelines for workplace drug testing were used to analyze the cocaine metabolite benzoylecgonine, and the heroin metabolite 6-mono-acetylmorphine in urine.
- SPE (Solid Phase Extraction) was performed using UCT Cleanscreen® SPE tubes (UCT Inc. Bristol, PA) with a Supelco SPE vacuum manifold (Supelco Inc./Sigma Aldrich, Bellefonte, PA).
- GC-TOFMS analysis was conducted with the LECO TruTOF® HT (GC-TOFMS) for the analysis of the two drug metabolites in urine.
- Automated derivatization was completed post SPE using a GERSTEL dual rail MPS2 multipurpose autosampler and prepstation.
- Calibration curves were developed with a linearity of 99% for both drug metabolites.
- Ion ratios for specified qualifier ions were used to confirm analyte verification along with full mass spectral library search match identification.

INTRODUCTION

Workplace drugs of abuse screening guidelines and procedures are set by regulation of the Substance Abuse and Mental Health Services Administration (SAMHSA). Conventional methods for urine drug testing rely heavily upon selected ion monitoring (SIM) analysis with single quadrupole GCMS in order to achieve the sensitivity required. The benefits of GC-TOFMS for drug confirmation in urine include the ability to acquire full range non-skewed mass spectra at fast acquisition rates essential for high-throughput analysis in a single injection. TOFMS provides optimum sensitivity and the data density necessary to allow processing by deconvolution software algorithms which can successfully identify drugs with full range mass spectra even in complex samples that contain severely overlapping peaks.

This research was performed on two drug classes using benzoylecgonine and 6-mono-acetylmorphine spiked into 5 mL aliquots of urine. The samples were pH adjusted prior to extraction and derivatization. Automated sample derivatization using N,O-bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane (BSTFA + 1% TMCS) was conducted using a GERSTEL dual rail multipurpose autosampler. Each sample was then immediately analyzed by GC-TOFMS. Results show drug identification using ion-ratios, full mass spectral library search, and quantitative data that meet mandatory SAMHSA cut-off concentration limits. Analyte detection is shown in the parts-per-billion range far below the cut-off limits set by SAMHSA. The data illustrates trace level identification of drugs in urine using the LECO TruTOF HT. This research demonstrates the practical applicability of GC-TOFMS analysis with automated derivatization for workplace drug screening using SAMHSA mandated guidelines.

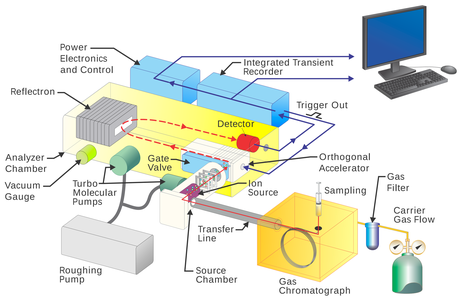

Figure 1. LECO TruTOF HT Flowpath.

EXPERIMENTAL METHODS

Standards of drug metabolites were purchased from Sigma-Aldrich in solution at 1 mg/mL

Drug Target Analytes

- Benzoylecgonine (metabolite of cocaine)
- 6-mono-acetylmorphine (metabolite of heroin)

Sample Preparation (Solid Phase Extraction)

- SPE (UCT CleanScreen) tubes were preconditioned with methanol, DI water, and 100 mM phosphate buffer.
- 5 mL aliquots of urine were placed in the SPE tube.
- The drug metabolites (benzoylecgonine and 6-mono-acetylmorphine) with deuterated internal standards were spiked into the urine.
- Phosphate buffer was added (pH 6) prior to SPE.
- The urine was passed through the column in a slow dropwise manner.
- The SPE column was washed with water, 100 mM acetic acid, and methanol.
- Analytes were eluted into a clean silanized glass test tube with 3mL of (methylene chloride: isopropanol: ammonium hydroxide mixture) composition (78:20:2).
- Extracts were concentrated to dryness with nitrogen.
- Extracts were reconstituted in 200 µL of methylene chloride and centrifuged for 3 min at 3000 rpm.
- Extracts were transferred to a 2 mL autosampler vial with silanized insert.
- The samples were evaporated to dryness in a heating block at 55ºC.
- The prepared sample vials were capped and placed in the sample tray of the GERSTEL MPS2 autosampler prior to automated trimethylsilyl derivatization and subsequent GC-TOFMS analysis.

Automated Trimethylsilyl Sample Derivatization with the GERSTEL Dual Rail MPS2 Prepstation

- A prep ahead bounded sequence was made in GERSTEL MAESTRO software.
- 50 µL of ethyl acetate was added to the extracted sample vial.
- 50 µL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) was added to the sample vial.
- The vial was moved by the MPS2 rail to the sample agitator/heater.
- The vial was mixed by the agitator for 20 minutes at 70ºC.
- The vial was moved back to the sample tray and a 1 µL injection of the sample was made into the GC-TOFMS for analysis.

GC-TOFMS Analysis Parameters

- Gas Chromatograph: Agilent 6890 equipped with a GERSTEL dual rail MPS2 autosampler and prepstation
- GC Column: 30 m x 0.25 mm id. x 0.25 µm film thickness Rxi-5ms (Restek Corp., Bellefonte, PA)
- Carrier Gas: Helium set at 1.5 mL/min
- Injection Mode: splitless
- Injection Volume: 1 µL
- Inlet Temperature: 275°C
- Primary Column Temperature Program: Initial temperature 90ºC for 1.0 min ramped @ 10°C/min to 320ºC held for 1.0 min
- Total Run Time: 25.0 min

Mass Spectrometer: LECO TruTOF HT

- Mass Range: 45 to 750 m/z
- Acquisition Rate: 10 spectra/s
- Ion Source Temperature: 250°C
- Detector Voltage: 2950 V
- Acquisition Delay: 180 s

GC-TOFMS RESULTS

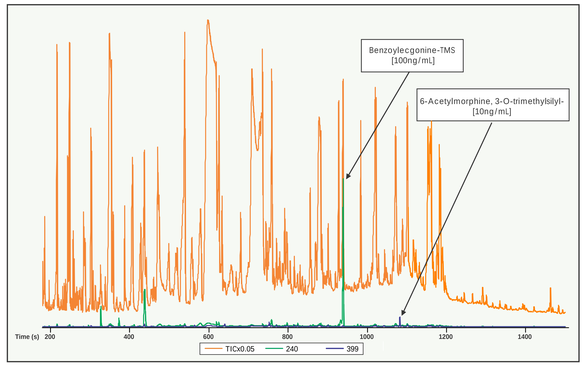

Figure 2. This figure shows the total ion chromatogram with the quant mass ions for benzoylecgonine and 6-mono-acetylmorphine at the SAMHSA minimum allowable cut-off level concentrations. This GC-TOFMS analysis illustrates that both metabolites are easily detected at the minimum cut-off level concentrations set by SAMHSA.

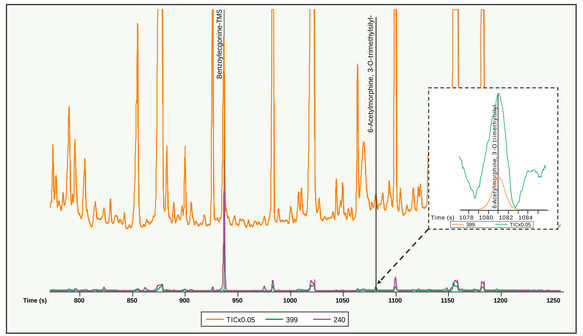

Figure 3. This figure shows a zoomed-in portion of the GC-TOFMS analysis in 5 mL of urine for the concentrations of benzoylecgonine and 6-mono-acetylmorphine at ½ the minimum cut-off level concentrations set by SAMHSA.

Peak #	Name	R.T. (s)	Area	Height	Conc. ng/mL	Quant Masses	Similarity	Quant S/N	Library
30	Benzoylecgonine-TMS	937	22518511	1538364	50	240	999	5013.5	DRUG SCREEN
161	6-Acetylmorphine, 3-O-trimethylsilyl- II	1081	1096675	70594	5	399	999	492.77	DRUG SCREEN

Table 1. The peak table above illustrates that both analytes are detected with S/N ratios of 5013 and 493, respectively. The match similarities for the library searches are 99.9% for both metabolites.

CALIBRATION LINEARITY

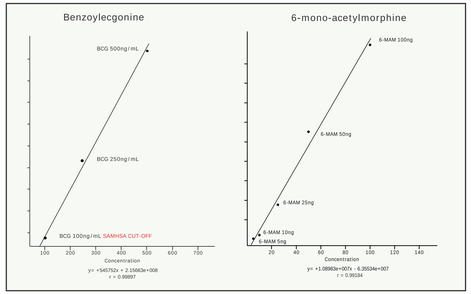

Figure 4. This figure shows the calibration curve development for benzoylecgonine using SAMHSA guidelines with solid phase extraction in 5 mL of urine and auto-derivatization. The calibration low-level concentration for benzoylecgonine begins at the SAMHSA cut-off limits. The calibration curve for 6-monoacetylmorphine was conducted with BSTFA to develop a 5 point calibration. In this experiment five multi-level standards were prepared without extractions to eliminate recovery and sampling errors. Injections were made so that the on-column concentration range was from 5 to 100 ng. Linearity for both calibration development studies of greater than 99% was achieved.

TOF-MASS SPECTRAL DETECTION at ½ SAMHSA CUT-OFF LIMITS

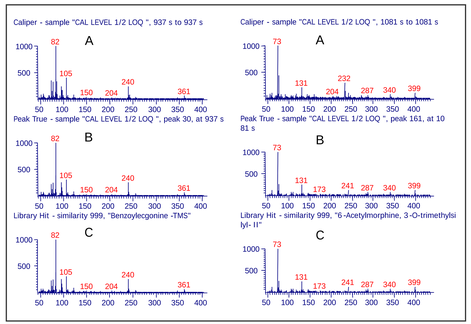

Figure 5. This figure shows the mass spectral results at ½ the minimum SAMHSA cut-off concentration levels. It is important to note that the mass spectra identifications are made with the full range mass spectra for all concentrations as well as additional confirmation using selected ion ratios. The mass spectra labeled as (A) are the total ion (Caliper) mass spectra. The mass spectra labeled (B) are the (Peak True) deconvoluted mass spectra from each caliper mass spectra. The mass spectra labeled (C) are the library search matches for each peak. Excellent mass spectral matches are achieved at ppb levels for both drug metabolites.

ChromaTOF® CUSTOM FORENSIC REPORT

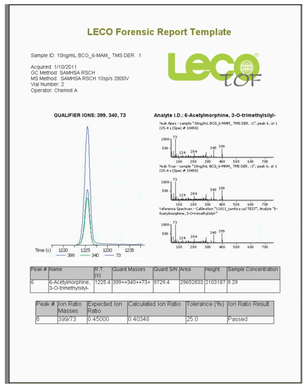

Figure 6. The report in this figure was developed with the drug testing laboratory in mind. The ChromaTOF Reports feature allows the user to develop a customized report. This example of a forensic workplace drug screen report was designed to provide the needed information required for identification and quantification of each drug tested. The report header was designed to show the pertinent analytical tracking information. Each drug is assigned qualifier ions. A chromatogram of these extracted qualifier ions is shown for each drug. The qualifier ions used for 6-mono-acetylmorphine were m/z 399, 340, and 73. Mass spectra are provided showing the peak apex ms, the deconvoluted peak true ms, and the library search match mass spectrum. The first table shows the name of the drug identified, the retention time, the quant masses used, S/N, peak area, peak height, and the calculated concentration. The second table shows the ion ratio analyte verification. The masses used for ion ratio are shown, followed by the expected ion ratio and the actual calculated ion ratio. The tolerance percent is listed at 25%. The drug test result is listed as Pass or Fail. This customized forensic report provides the user with an automated drug screen report.

CONCLUSIONS

In conclusion this research accomplished the goals set for this research, The LECO TruTOF HT proved that it is a valuable tool for forensic workplace drug screening. The mandated guidelines set by the Department of Health and Human Services were followed and achieved. Low level drug metabolite detection was demonstrated below the SAMHSA minimum cut-off concentrations. Calibration linearity of greater than 99.9% was achieved. An automated derivatization and sample analysis was verified that improves sample turn-around time. A customized forensic report using the ChromaTOF Reports feature was illustrated to show quantification, full range mass spectral library searchable identification, and analyte confirmation using qualifier ion ratios by the SAMHSA criteria. This work presents the advantages and benefits of utilizing GC-TOFMS with automated sample derivatization as a practical solution for workplace drug testing.

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