Rapid Screening Analysis for Barbiturates in Human Urine by GC-TOFMS with the Pegasus® BT

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Key Words: Pegasus BT, GC-TOFMS, Rapid Drug Screening, Drug of Abuse

1. Introduction

Illicit drug use in the United States has significantly increased over the last few years. Unfortunately, during the same period of time, forensic laboratories have experienced larger workloads and diminishing resources. Routine drug analysis methods typically target specific drugs using SIM and/or MS/MS protocols. These methods can provide faulty results if the analyses are carried out in complex matrices due to coelutions of targeted compounds. Data acquisition using gas chromatography coupled with mass spectrometry (GC-MS) is still regarded as a "gold standard" for forensic analyses. GC-MS combines two different analytical processes—physical separation (GC) and positive identification of the individual components separated (MS)—providing more accurate results. Challenges associated with matrix interferences are overcome through a combination of well-developed GC methods and robust deconvolution algorithms.

This note demonstrates superior technology for analysis of barbiturates in human urine. Sample preparation and analysis times were reduced without sacrificing the thorough characterization of samples. Simple extraction techniques were used to prepare non-derivatized human urine samples, which were analyzed under optimized conditions with a runtime of under 9 minutes. Data processing methods included automated NonTarget Deconvolution™ and Target Analyte Finding for both qualitative analysis of sample data and quantitation. Positive compound identifications were made via spectral similarity searches against large, well-established databases.

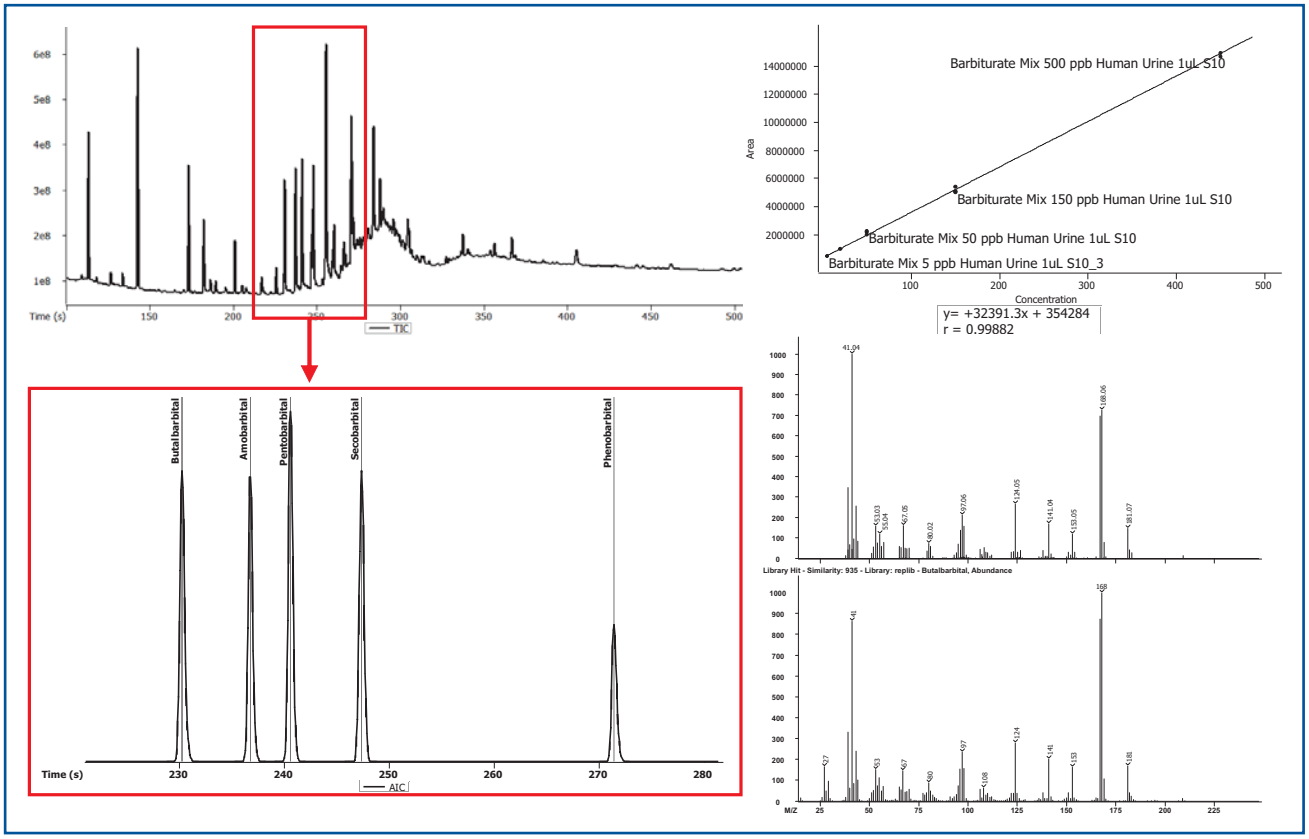

Figure 1. Total Ion Chromatogram (TIC) shows the summed intensity of all spectral peaks. Below the TIC, an expanded Analytical Ion Chromatogram (AIC) demonstrates the ability to selectively determine analytes of interest in a complex human urine extract. A calibration curve and library similarity score for butalbarbital is also shown.

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2. Experimental

Urine standards used for analysis were obtained from the National Institute of Standards and Technology (NIST). Barbiturate Mixture-5 standard was purchased from Cerilliant Corporation. The sample mixtures consisted of human urine and five drug standards (Butalbarbital, Amobarbital, Pentobarbital, Secobarbital, and Phenobarbital). Calibration curves ranging from 5 to 500 ng/mL were generated for these prescription drugs.

Urine Extraction and Standard Addition

Urease (100 mg) was added to 2 mL of urine sample which was then incubated at 37°C for 15 minutes. The mixture was treated with 2 mL of 5M NaOH and extracted with 7 mL of CH_2Cl_2:1) vortex for 1 minute, 2) centrifuge at 12,000 rpm for 5 minutes, and 3) separate the layers. The organic layer was dried with anhydrous $CaCl_2$ and barbiturates (Butalbarbital, Amobarbital, Secobarbital, and Pentobarbital) were spiked into the solution at the following levels: 5, 10, 50, 100, and 500 ng/mL. The CH_2Cl_2 was removed using N_2(g) and the residue was reconstituted in $CHCl_3$.

Table 1. GC-TOFMS (Pegasus BT) Conditions

Gas Chromatograph	**Agilent 7890 with MPS2 Autosampler**
Injection	1 µL, split 10:1 @ 260°C
Carrier Gas	He @ 1.4 mL/min, Constant Flow
Column	Rxi-5ms, 20 m x 0.18 mm i.d. x 0.18µm coating (Restek, Bellefonte, PA, USA)
Oven Program	50°C (0.1 min), to 320°C @ 50°C/min (5 min)
Transfer Line	300°C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250°C
Mass Range	35-650 m/z
Acquisition Rate	20 spectra/s

3. Results and Discussion

This table summarizes detection limit information for barbiturates using LECO's *Pegasus* BT. Data showed that the instrument is capable of detecting well below the minimum cutoff level required by the Federal mandatory guidelines.

Table 2. Detection Limits of Barbiturates in Human Urine and Government Cutoff Levels[1,2]

Compound names	Detection limits (ng/mL)	Government's cutoff level (ng/mL)
Amobarbital	0.5	200
Phenobarbital	5	200
Butalbarbital	1	200
Secobarbital	5	200
Pentobarbital	0.5	200

The extracted urine and barbiturates standards were analyzed using conditions described in Table 1. The Analytical Ion Chromatogram (AIC) shown in Figure 2 displays the chromatographic peaks for the spiked barbiturates. GC-TOFMS analysis resulted in rapid and automated identification of barbiturates in the urine sample.

A list of the barbiturates along with their retention times, similarity scores, and CAS numbers are listed in Table 3. Identifications are based on similarity score from library search results, using the NIST 2014 library, as well as retention time matching with neat standards for the barbiturates.

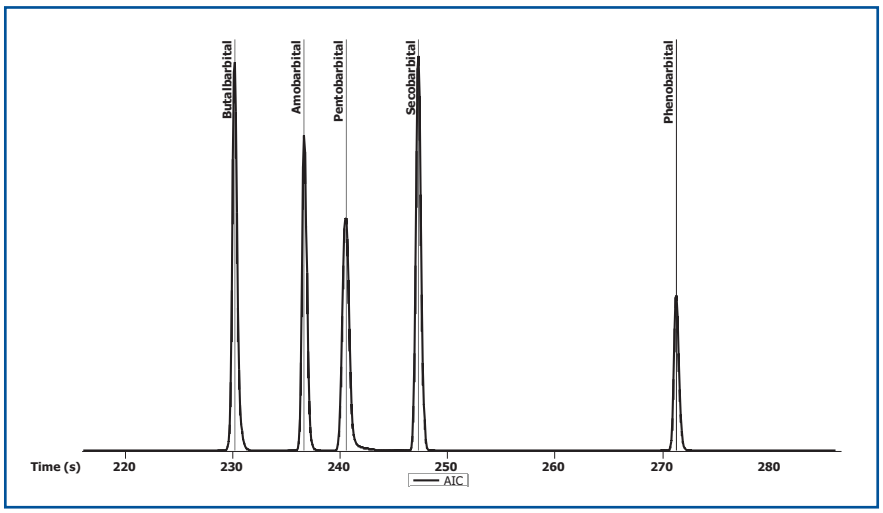

Figure 2. Analytical Ion Chromatogram (AIC) showing 5 barbiturates in human urine separated in under 5 minutes with an average similarity value 927/1000.

Table 3: Peak Table for Barbiturates in Human Urine Sample

Peak #	Names	R.T(s)	Similarity	CAS
1	Butalbarbital	230.669	932	77-26-9
2	Amobarbital	237.163	960	57-43-2
3	Pentobarbital	241.062	938	76-74-4
4	Secobarbital	247.825	941	76-73-3
5	Phenobarbital	271.667	866	50-06-6

The combination of the novel *Pegasus* BT GC-TOFMS and next-generation software tools allowed for drug identification through the acquisition of non-skewed mass spectral data. Data processing methods were programmed to carry out comprehensive detection of the chemical constituents of the multi-component barbiturates mixture in urine. The Peak Finding methods efficiently processed large volumes of GC-MS data resulting in high quality, Peak True (deconvoluted) mass spectral data like that shown in Figure 3 for an analyte of interest, secobarbital. Comparison to the NIST library database resulted in a similarity score of 941/1000.

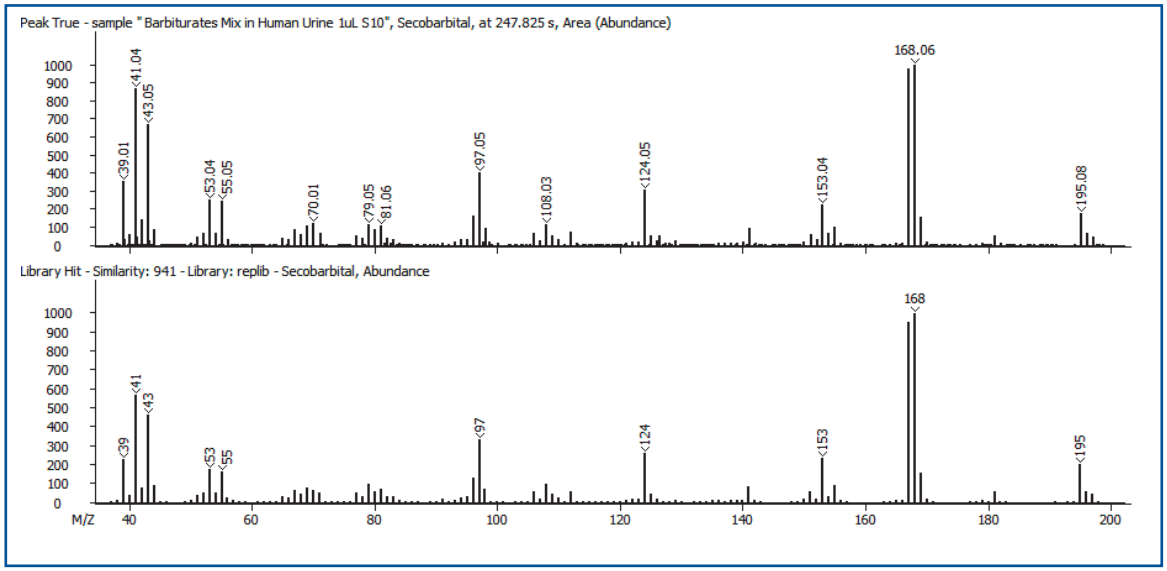

Figure 3. The Peak True (deconvoluted) mass spectrum and library hit mass spectrum for Secobarbital with similarity score of 941/1000, eluting at 247.825 seconds.

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Good calibration linearity for barbiturates was demonstrated over the concentration range of 5 ng/mL to 500 ng/mL. The average correlation coefficient for the butalbarbital, amobarbital, secobarbital, and pentobarbital curves was 0.998 (Figure 4).

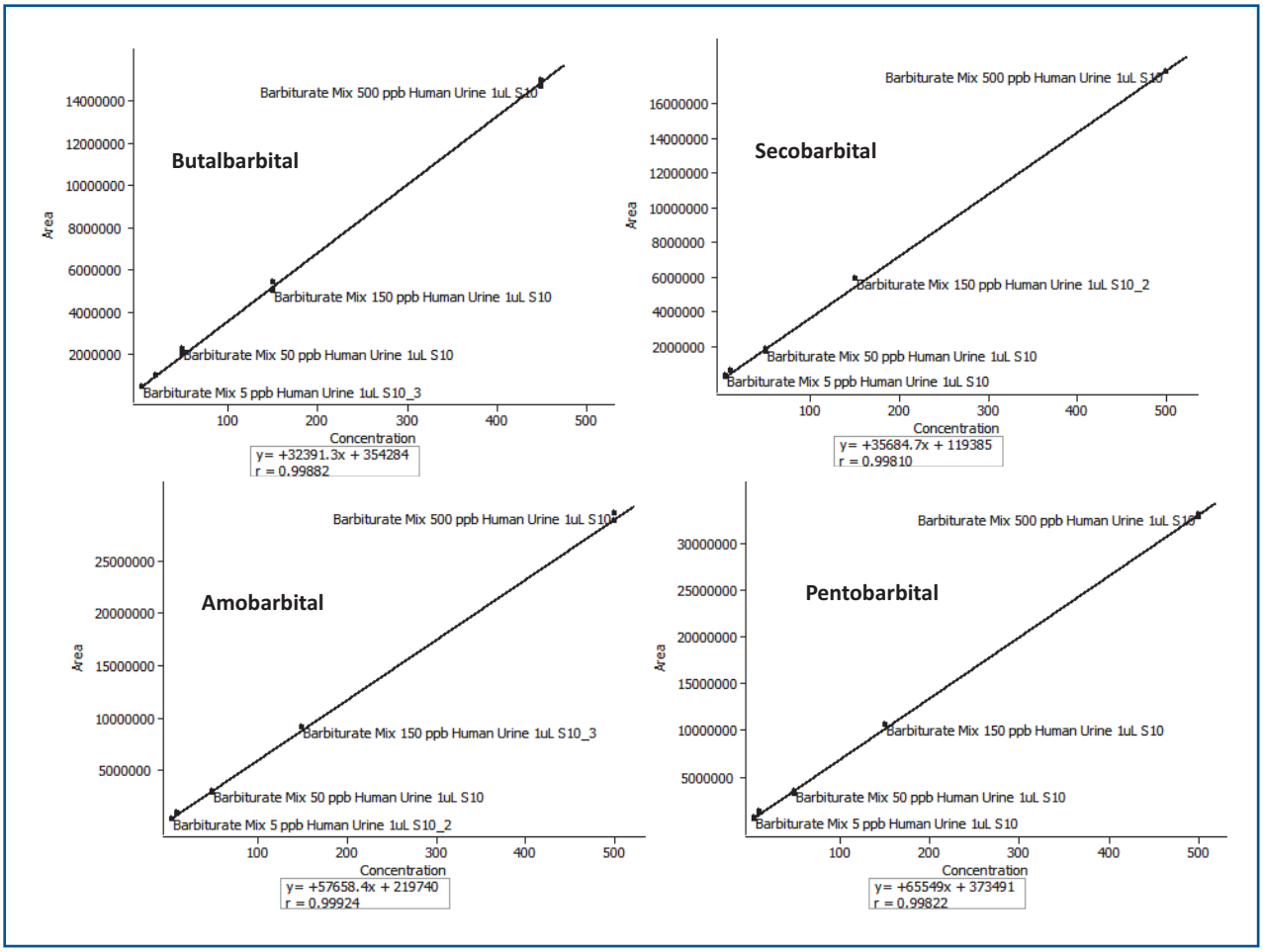

Figure 4. Calibration curves for Pentobarbital, Butabarbital, Secobarbital, and Amobarbital (5 ng/mL to 500 ng/mL).

4. Conclusion

High performance GC-TOFMS analysis facilitated drug identification through the acquisition of full range, non-skewed mass spectral data. The detection limits of non-derivatized barbiturates in human urine were well below the values stated by the U.S. Nuclear Regulatory Commission, U.S. Department of Transportation, and Substance Abuse and Mental Health Services Administration. Excellent calibration linearity was demonstrated for barbiturates in the urine extracts. With each analysis completed in less than nine minutes, the *Pegasus* BT GC-TOFMS is a clear solution for forensic labs in need of quality data with fast results.

5. Reference

[1]"26.163 Cutoff Levels for Drugs and Drug Metabolites." *U.S. Nuclear Regulatory Commission*. N.p., 2 Dec. 2015. Web. 11 Aug. 2016.

[2]"Clinical Drug Testing in Primary Care-TAP 32 Technical Assistance Publication Series." *Substance Abuse and Mental Health Services Administration*. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, Dec. 2012. Web. 11 Aug. 2015.

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