Fast Screening for Selected Doping Substances in Urine Samples Using GC-TOFMS

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1. Introduction

Screening for relevant drug components is an important tool for doping analysis, forensic toxicology, and for drug abuse screening. As normal mass spectrometry equipment is only sensitive in selective ion techniques, extensive analyses have to be performed to detect and quantify all drugs of interest.

Time-of-flight mass spectrometry offers new perspectives for such purposes. This technology allows full scan screening with an extraordinarily high acquisition speed (up to 500 spectra/second) and sensitivities that scanning instruments can only achieve using single ion monitoring mode. Additionally, intelligent software algorithms perform automatic data processing, looking for all present components in a given chromatogram while mathematically separating overlapping spectra of peaks within coelutions. From this, analytes masked by matrix components can easily be identified and accurately quantified.

In this note, a drug screening method for the determination of several beta-blockers and opiates in multiple urine samples is presented. The run time of the GC separation was less than 9 minutes.

In parallel, other compounds not present in a target list but present in the sample can generally be identified by standard library search of the deconvoluted spectra. Thus, a target analysis is always combined with a sensitive full scan screening.

2. Experimental Conditions

GC-Parameters: Agilent 6890
Column:
XTI-5; 20 m x 0.18 mm x 0.20 µm
Injector temperature: 250°C
Injection: 1 µl, split ratio 5:1
Oven Program:
150°C for 0 minute, to 320°C at 30°C/minute, hold for 2.5 minutes
Flow Rate:
1.4 mL/minute Helium at constant flow

MS Parameters: Pegasus® GC-TOFMS
Mass Range: 60 to 600 amu
Acquisition Rate: 20 spectra/second
Ion Source Temperature: 170°C
Total Acquisition Time: 500 seconds

Sample Preparation

The sample extracts were obtained after enzymatic hydrolysis by solid phase extraction of 2.5 mL urine, elution with CHCl3:2-Propanol 80:20, containing 2% ammonia, evaporation to dryness and derivatization by addition of 100 µL of MSTFA, and a reaction time of 30 minutes at 60°C.

3. Results

A standard mixture containing 35 beta-blockers, amphetamines and opiates was taken. A chromatogram of a standard mixture at 2 µg/mL is shown in Figure 1.

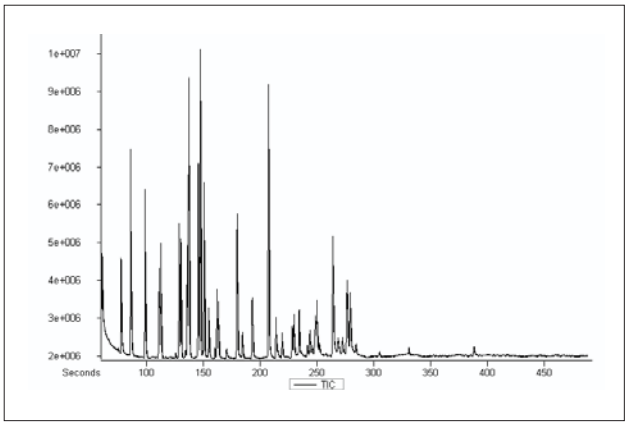

Figure 1. Total Ion Chromatogram (TIC) of silylated standard mixture at 2 µg/mL.

From this, 14 analytes were taken in order to check for general sensitivity of the method. These 14 analytes were put into a reference table and the compounds out of this list were then automatically searched, identified, and semi-quantified using the Sample Comparison algorithm of the Pegasus software. The reference table is shown in Table 1.

Table 1. Reference table of 14 beta-blockers and opiates.

#	Name	M/Z	R.T.	Area	Height
1	Penbutolol mono-TMS	363	209.23	285760	18404
2	Propranolol TMS	72	215.7	13086000	766530
3	Metoprolol bis-TMS	144	231.58	3077600	226150
4	Timolol TMS	86	236.11	11611000	659580
5	Betaxolol mono-TMS	263	247.81	41600	2478
6	Pindolol bis-TMS	205	249.93	3231900	165300
7	Propranolol bis-TMS	144	250.52	2613200	166460
8	Bunolol bis-TMS	234	259.87	129100	6896
9	Sotalol tris-TMS	250	265.81	80509	4801
10	Nadolol tris-TMS	510	266.05	72944	2984
11	Codeine TMS	178	270.4	293880	15912
12	Morphine bis-TMS	236	278.05	429340	22152
13	Pindolol tris-TMS	277	280.99	497520	33123
14	Strychnine	334	391.17	322230	11743

The target detection limit of the method was 0.4 µg/mL out of a urine sample. To prove the sensitivity of the method, a spiked urine sample at this concentration level was analyzed and automatically processed using the reference table. The obtained peak list showed all the expected compounds at signal-to-noise (S/N) levels of at least 100:1, even when using the less intense characteristic high mass ion traces. Thus, the system exceeds the necessary sensitivity. In total, the obtained peak table contained 270 compounds with a S/N ratio better than 30, thus showing that other relevant drug or doping agents can be detected if present. The chromatogram is shown in the following figure.

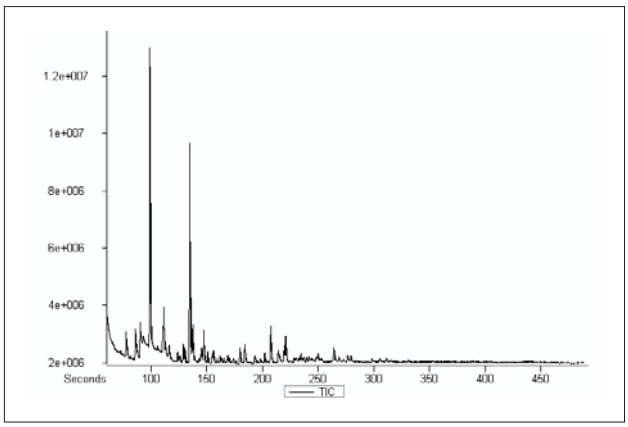

Figure 2. TIC of a urine sample spiked with 0.4 µg/mL.

As always, when applying fast GC conditions to samples with high matrix content, many sample components coelute and data processing is usually difficult and time consuming. The automatic Peak Find and Deconvolution algorithms within the Pegasus software package accomplish these difficult tasks in only a couple of minutes.

Figure 3 shows a coelution of two beta-blockers together with other matrix compounds.

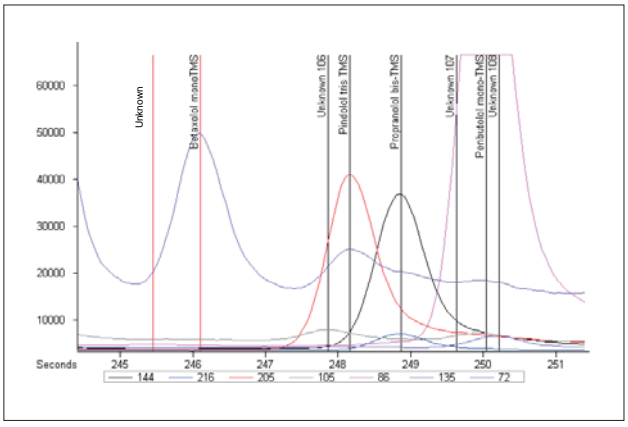

Figure 3. Coeluting beta-blocker peaks (Peaks are detected even below the baseline of the TIC).

The deconvoluted spectra of the coeluting Pindolol and Propranolol together with their respective library spectra are presented in the following figure.

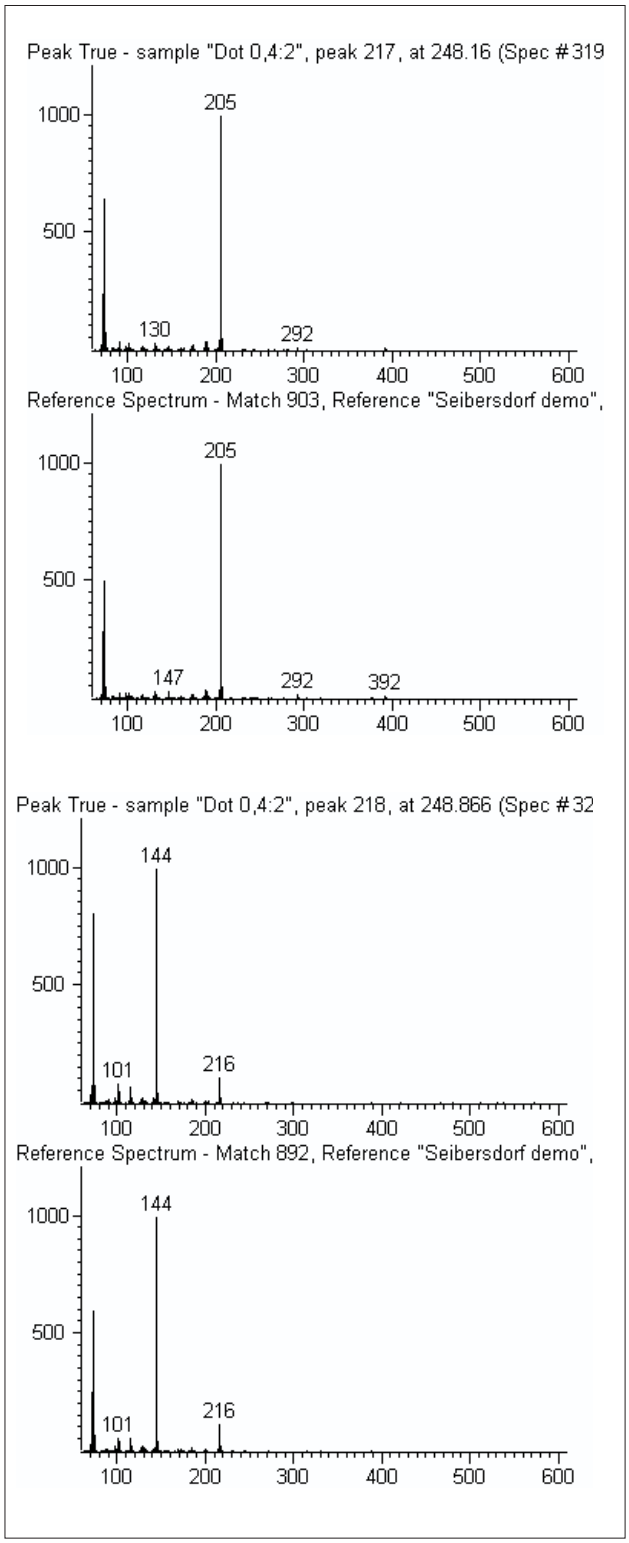

Figure 4. Mass spectra of the coeluting betablockers Pindolol and Propranolol.

4. Conclusions

The described work demonstrates the use of GC-TOFMS and SPE in screening and identifying drugs (that are used for doping or other abusive purposes) out of complex matrices such as urine. The use of a Time-of-Flight mass spectrometer in this work is an innovative approach, which demonstrates a number of advantages over other types of mass spectrometers.

The strength of the Pegasus Time-of-Flight detector for the analysis of these complex mixtures lies in its automated data handling capabilities. Peak finding, spectral determination (deconvolution), library searching, and quantification are all automatic, even when peaks are below the baseline of the TIC. This is all possible due to the high degree of spectral continuity generated as well as the large data density allowed by the Pegasus GC-TOFMS system—up to 500 full mass spectra/second.

5. Acknowledgements

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