Multi-Fold Reduction in Drugs of Abuse Detection Limits Using Full Scan GCMS with a High Efficiency Source

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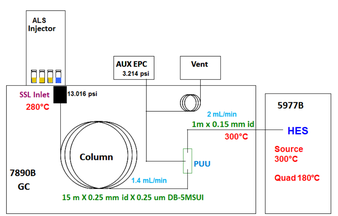
Introduction

Full-spectrum identification is required for broad range, forensic drug screening in biological samples. Since GCMS screening detection is limited by the sensitivity of the system in full scan mode, an EI source with high ionization efficiency benefits the analysis by producing significantly more ions, which results in better precision and thus improved detection limits. A forensic toxicology analyzer utilizing deconvolution reporting software, a forensic toxicology database library and a GCMS equipped with the High Efficiency Source (HES) is employed to detect a greater number of underivatized drugs screened at significantly lower concentrations.

Method

Negative human serum, 2 mL, was extracted using the Bond Elut Certify general drug screen method M2721. Fractions were pooled and evaporated, reconstituted in 0.1 mL methanol, and spiked with a standard of mixed drugs to yield 10 – 1000 ng/mL (in vial) standards. Spiked extract was injected (1 µL) into the instrument, which was set up as a GC/MS Forensic Toxicology Analyzer with the High Efficiency Source (HES) and equipped with a 15 m x 0.25 mm i.d. x 0.25 µm DB-5ms Ultra Inert column (5% phenyl phase), 1 m x 0.15 mm x 0 µm i.d. restrictor and column backflush. Backflushing was accomplished using a Purged Ultimate Union (PUU) controlled by an Aux EPC module. The oven was ramped from 90°C to 325°C over a 15 minute run. The split-splitless inlet was operated in pulsed splitless mode at a temperature of 280°C. The source temperature was 300°C and the quadrupole was 180°C. The instrument was autotuned.

The HES was exchanged with a standard extractor source (autotuned using etune.u) and the injections repeated. Data files were processed using Deconvolution Reporting Software (DRS), which utilizes AMDIS, and a forensic toxicology library in order to generate a table of hits with associated AMDIS and NIST library match scores.



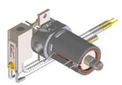
Method, cont.

New 5977B High Efficiency Source

5977B High Efficiency Source, magnet removed

More intense electron beam x Longer path length for electron beam/effluent interaction

= Up to 20x More Ions Produced



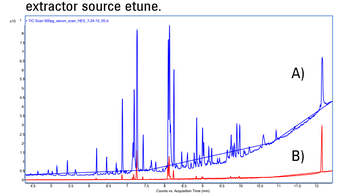
5977A Extractor Source

Spectral deconvolution was by Deconvolution Reporting Software (DRS) which utilizes AMDIS. A Minimum Match Factor of 75 was set for searches against a custom library, and NIST hits (reverse match) were reported as well.

Results

The HES maximizes the number of ions that are created in the source and transferred into the quadrupole analyzer, which equates to more signal, and thus better sensitivity. This increase in response translates into more drug targets found during the screening process with good library matches.

Overlay of TICs of 2.5 ng/mL (500 pg injected) standard in serum using A) HES autotune, and B) extractor source etune.



Significant Improvement in Forensic Toxicology Analysis

Improved detection levels in serum (ng/mL) using DRS/AMDIS with 75 as the Minimum Match factor

Improved screening capability with the HES was demonstrated using human serum, as seen in the table below. The table shows minimum concentrations of underivatized drugs detected with the HES and extractor source based on identification using DRS/AMDIS with a Minimum Match Factor of 75. Drug target compounds screened in serum, such as methadone, cocaine, hydrocodone, THC and others, can now be positively identified using full-scan mode at lower concentration (for example, 5 ng/mL for hydrocodone). The HES allows for classical spectra which are easily searched within custom in-house libraries or those from NIST. When a component is identified in AMDIS based on how well individual mass ion profiles match up according to exact retention time, S/N, peak shape, and component width setting or resolution, a spectrum is reconstructed for that component. This spectrum, designated as an extracted spectrum, is then searched against a library such as a custom, in-house library or that from NIST.

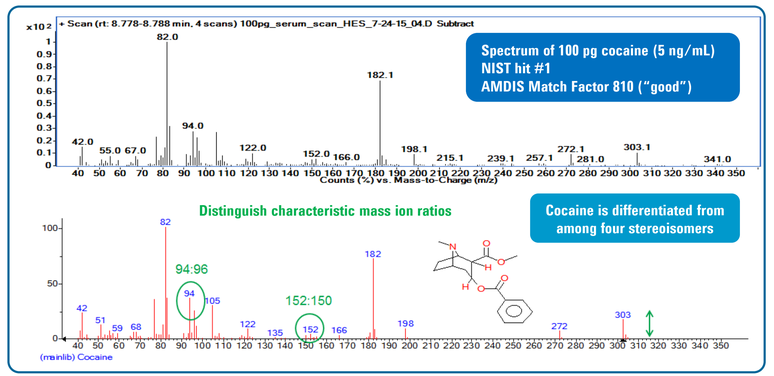
		5977B HES		*5977A Extractor source	
Fold increase in detection		Concentration in serum (ng/mL)	AMDIS Score	Concentration in serum (ng/mL)	AMDIS Score
	Amphetamine	25	94	25	75
	Nicotine	2.5	92	2.5	81
	MDA	25	77	25	76
	MDMA	25	85	25	83
50 X	MDEA	0.5	76	25	97
5	Meperidine	0.5	85	2.5	85
10	Phencyclidine	2.5	83	25	90
10	Methadone	2.5	87	25	89
10	Cocaine	2.5	77	25	94
2	SKF-525a	2.5	77	5	81
5	Codeine	5	88	25	90
	Diazepam	2.5	90	2.5	81
5	Hydrocodone	5	91	25	90
2	Tetrahydrocannabinol	2.5	75	5	78
10	Oxycodone	2.5	80	25	83
	Flunitrazepam	25	88	25	75
10	Diacetylmorphine	5	79	50	83
	Fentanyl	2.5	85	2.5	77
10	Alprazolam	5	76	50	85
10	Verapamil	2.5	84	25	90
	Strychnine	25	86	25	77

* Autotuned using etune.u

Drugs identified at lower concentrations using the HES versus the extractor source are highlighted in blue.
Concentrations assume 100% recovery from a 2 mL serum sample, reconstitution of extract in 0.1 mL and 1 µL injected.
This is a screen and derivatization was not used; some drugs are known to require derivatization for optimal analysis.
The benzodiazepines oxazepam, lorazepam, temazepam, nitrazepam, and clonazepam were not identified at the highest concentrated tested of 50 ng/mL.

Strict Spectral Integrity Allows for Isomer Differentiation

The HES allows for classical spectra which are easily searched within custom in-house libraries or those from NIST. The figure shows the mass spectrum of 100 pg cocaine spiked in serum (A) compared with NIST spectrum (B). Cocaine is the first hit in NIST. The match factor is 810 (good), and is differentiated from pseudococaine, which has a match factor of 788 (fair). The equivalent concentration is 5 ng/mL based on complete recovery. Excellent NIST library matches (≥900) for cocaine as a first hit are returned at concentrations above 5 ng/mL. Cocaine was differentiated among four stereoisomers based on characteristic ratios that were discernable due to spectral integrity.



Conclusions

The High Efficiency Source of the Agilent 5977B GC/MSD greatly enhances the signal of drug targets. Resulting spectra are classical and NIST searchable. When combined with Deconvolution Reporting Software, detection levels during screen analysis approach those using SIM mode with derivatization.

References

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3. Schlesinger, H. L. Topics in the chemistry of cocaine. B. Narcotics 1985, 1, 63-78. United Nations Office on Drugs and Crime, Vienna, Austria. https://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1985-01-01_1_page006.html