Using High Performance GC-TOFMS to Effectively Monitor Patients for Opioids and other Drug Classes

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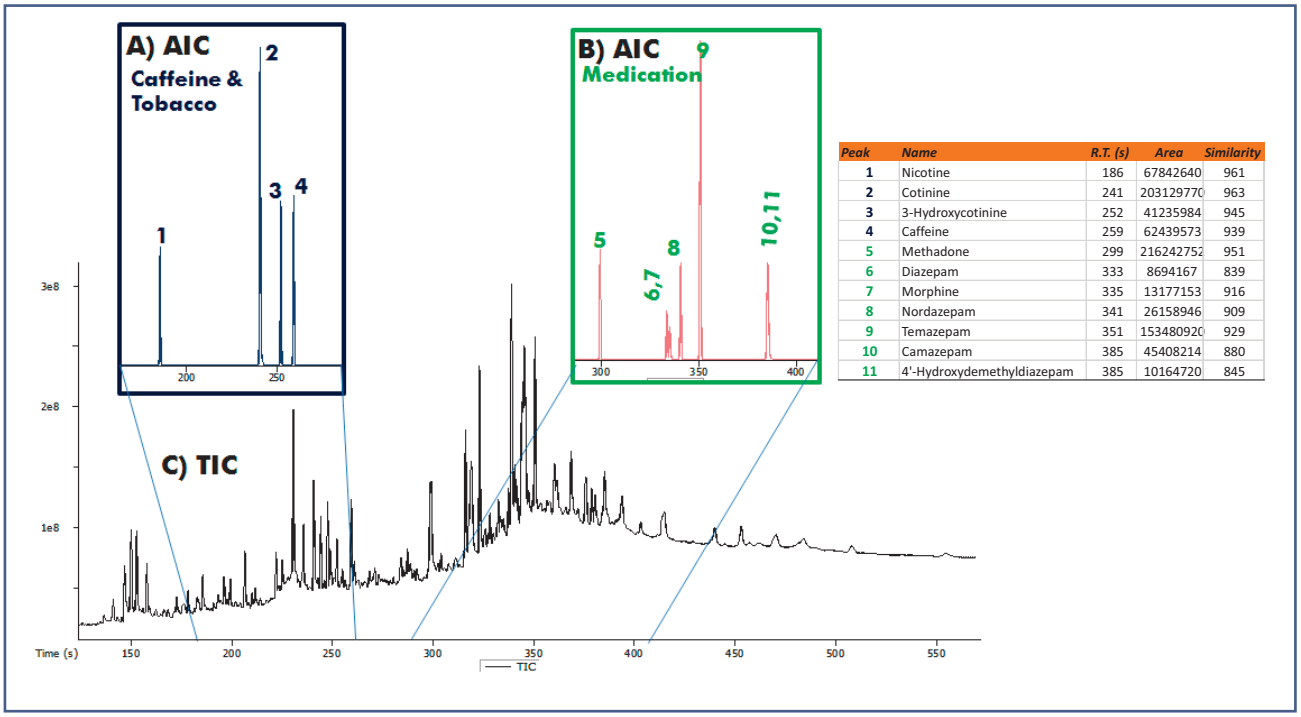
Key Words: Drugs of Abuse, High Throughput, GC-TOFMS

1. Introduction

During the past decade drug abuse has increased to epidemic levels. The advent of high performance gas chromatography time-of-flight mass spectrometers (GC-TOFMS) has greatly improved monitoring of these drugs and their metabolites in complex matrices such as saliva, plasma, and urine. The ability to acquire and process data at very rapid rates has facilitated throughput and, combined with modern software tools, streamlined comprehensive profiling of drug dependent subjects. These individuals use prescription opiates to control pain, and often supplement these opiates with additional non-prescribed medications and illicit drugs. Complete and regular "snapshots" of urine samples (Figure 1) can result in better patient monitoring, but more importantly, lead to more effective medical treatment. It is clear from Figure 1 that patient A has consumed tobacco related products, and a variety of therapeutic drugs. In this study, we take advantage of the speed, resolution, and robustness of a new high-performance, benchtop GC-TOFMS to increase complex sample throughput, provide confidence in drug analysis results, and afford accurate information on patient drug use.



Peak	Name	R.T. (s)	Area	Similarity
1	Nicotine	186	67842640	961
2	Cotinine	241	203129770	963
3	3-Hydroxycotinine	252	41235984	945
4	Caffeine	259	62439573	939
5	Methadone	299	216242752	951
6	Diazepam	333	8694167	839
7	Morphine	335	13177153	916
8	Nordazepam	341	26158946	909
9	Temazepam	351	153480920	929
10	Camazepam	385	45408214	880
11	4'-Hydroxydemethyldiazepam	385	10164720	845

Figure 1. Analytical Ion Chromatograms (AICs) and TIC showing tobacco related compounds and medication found in a urine sample (Patient A).

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2. Experimental

Urine samples were obtained from anonymous donors participating in an outpatient drug monitoring program. Samples (1.5 mL) were mixed with acetate buffer and β-glucuronidase and incubated at 56 °C for 1 hr to hydrolyze conjugates. They were then eluted through solid phase extraction cartridges (HyperSep Verify-CX) with 3% NH_3/MeOH (2 x 0.5 mL). A steady stream of nitrogen gas was used to remove the solvents, and the dry residue was reconstituted in 50 mL of methanol, and transferred to 2 mL GC vials with 250 µL inserts for GC-TOFMS analysis using the conditions shown below in Table 1.

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 0.18 µm coating (Restek, Bellefonte, PA, USA)
Oven Program	40 °C (2 min), to 250 °C @ 20 °C/min (2 min)
Transfer Line	300 °C
Mass Spectrometer	**LECO Pegasus BT**
Ion Source Temperature	250 °C
Mass Range	45-650 m/z
Acquisition Rate	20 spectra/s

3. Results and Discussion

The Pegasus BT analysis of underivatized patient B urine resulted in the identification of aromatics, terpenes, terpenoids, fatty acids, phenols, nitrogen containing heterocycles, and sterols (Figure 2). Table 2 lists the retention times, formulas, peak areas, and spectral similarity values for 48 representative compounds in the sample. Comparison of the Peak True (Deconvoluted) spectra produced using the instrument with commercially available mass spectral databases resulted in an average spectral similarity value of 899/1000 for these analytes.

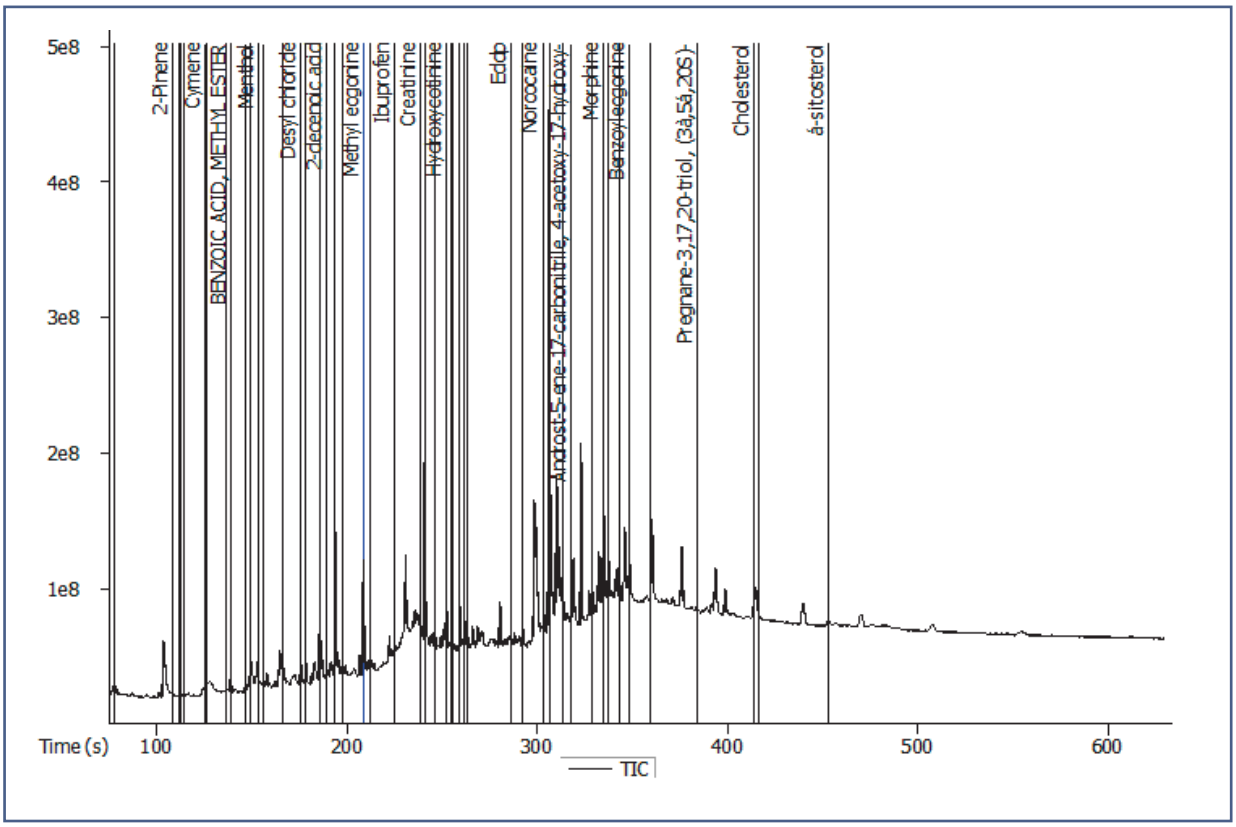

Figure 2. TIC with peak markers showing representative compounds in urine (Patient B).

Table 2. Representative Compounds in Patient B's Urine

Name	R.T. (s)	Formula	Area	Similarity	Name	R.T. (s)	Formula	Area	Similarity
Toluene	78	C_7H_8	68742699	936	Creatinine	239	$C_4H_7N_3O$	96967382	954
2-Pinene	108	$C_{10}H_{16}$	2592584	896	Cotinine	241	$C_{10}H_{12}N_2O$	365969168	961
Pentanoic Acid	112	$C_5H_{10}O_2$	4115553	857	Meconine	246	$C_{10}H_{10}O_4$	8497754	885
Benzaldehyde	113	C_7H_6O	4458659	868	Hydroxycotinine	252	$C_{10}H_{12}N_2O_2$	35993330	904
Phenol	114	C_6H_6O	7062856	864	Indole-3-acetic acid, methyl ester	255	$C_{11}H_{11}NO_2$	14451785	861
Cymene	125	$C_{10}H_{14}$	3955255	911	Caffeine	259	$C_8H_{10}N_4O_2$	24191268	908
Urea	126	CH_4N_2O	115905220	957	Theobromine	262	$C_7H_8N_4O_2$	11089591	890
4-Pyridinol	137	C_5H_5NO	32685450	864	1,7-Dimethylxanthine	263	$C_7H_8N_4O_2$	49432675	936
Benzoic Acid, methyl ester	139	$C_8H_8O_2$	24545334	924	Eddp	287	$C_{20}H_{23}N$	3257428	869
3-Pyridinol, 6-methyl-	147	C_6H_7NO	13960804	896	Octadecanoic Acid	292	$C_{18}H_{36}O_2$	3610529	813
Benzoic Acid	149	$C_7H_6O_2$	102650759	938	Norcocaine	303	$C_{16}H_{19}NO_4$	11355052	917
Menthol	154	$C_{10}H_{20}O$	2713470	859	Cocaine	306	$C_{17}H_{21}NO_4$	666636532	959
2-Octenoic acid	156	$C_8H_{14}O_2$	5898784	808	Androst-2-en-17-one, (5à)-	313	$C_{19}H_{28}O$	11565504	877
Nonanoic acid	166	$C_9H_{18}O_2$	6468336	893	Androst-5-ene-17-carbonitrile, 4-acetoxy-17-hydroxy-	318	$C_{22}H_{31}NO_3$	4077387	802
Desyl chloride	176	$C_{14}H_{11}ClO$	8986827	931	Codeine	329	$C_{18}H_{21}NO_3$	12045347	914
Indole	176	C_8H_7N	42665204	895	Morphine	335	$C_{17}H_{19}NO_3$	56614133	942
2-Methoxy-4-vinylphenol	178	$C_9H_{10}O_2$	24244271	893	Androstan-17-one, 3-hydroxy-, (3α,5β)-	337	$C_{19}H_{30}O_2$	11859491	915
Nicotine	186	$C_{10}H_{14}N_2$	150462709	963	6-Monoacetylmorphine	343	$C_{19}H_{21}NO_4$	14366371	937
2-Decenoic Acid	190	$C_{10}H_{18}O_2$	6294634	907	Benzoylecgonine	348	$C_{16}H_{19}NO_4$	40341171	919
Ecgonidine, methyl ester	194	$C_{10}H_{15}NO_2$	166369883	951	Allopregnane-3α,20α-diol	359	$C_{21}H_{36}O_2$	1426345	820
(R,S)-Nornicotine	197	$C_9H_{12}N_2$	21850579	888	Pregnane-3,17,20-triol, (3σ,5β,20S)-	384	$C_{21}H_{36}O_3$	2146321	848
Methyl ecgonine	208	$C_{10}H_{17}NO_3$	104114603	958	Cholesterol	414	$C_{27}H_{46}O$	4121245	912
Benzoic acid, 4-ethoxy-, ethyl ester	212	$C_{11}H_{14}O_3$	14434304	860	τ-Tocotrienol	416	$C_{28}H_{42}O_2$	5595302	886
Ibuprofen	225	$C_{13}H_{18}O_2$	7687914	890	β-sitosterol	453	$C_{29}H_{50}O$	3449016	901

The relatively short acquisition times (~10 min.) for these complex urine samples resulted in many chromatographic peak coelutions. Time of flight MS provided fast acquisition rates with no spectral skewing, which allowed optimal performance from ChromaTOF software's deconvolution algorithms. This deconvolution produced high quality, Peak True spectra for comparisons with large, well-established mass spectral databases, and resulted in quick, automated and confident identification of compounds. Examples of this extra dimension of separation via spectral deconvolution are illustrated in Figures 3 and 4. Figure 3 displays an Analytical Ion Chromatogram (AIC) and eXtracted Ion Chromatograms (XICs) for coeluting compounds camazepam and 4'-hydroxydemethyldiazepam from patient A, which were separated through deconvolution with excellent mass spectral similarity scores (880 and 845/1000). Figure 4 shows the coeluting compounds desyl chloride and indole separated through deconvolution with excellent spectral similarity scores (931 and 895/1000).

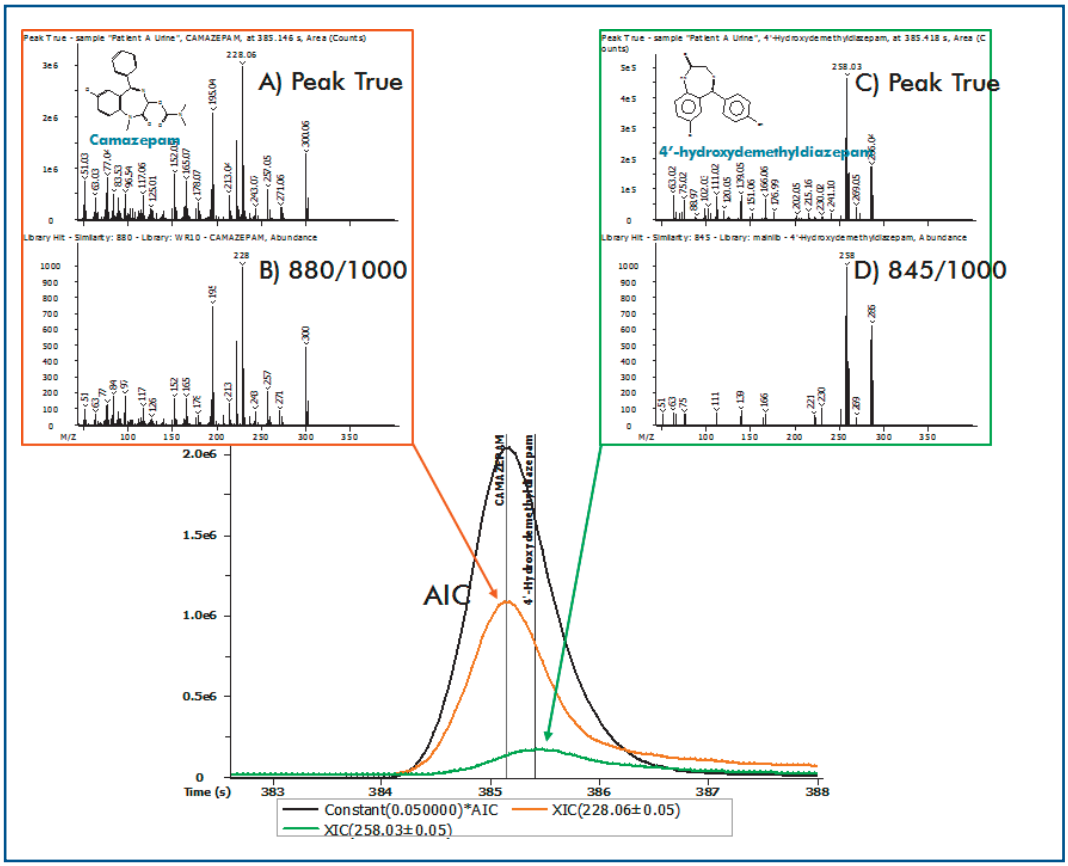

Figure 3. AIC showing coeluting compounds in patient A urine. Peak True and library mass spectral data for Camazepam (A, B) and 4'-hydroxydemethyldiazepam C, D).

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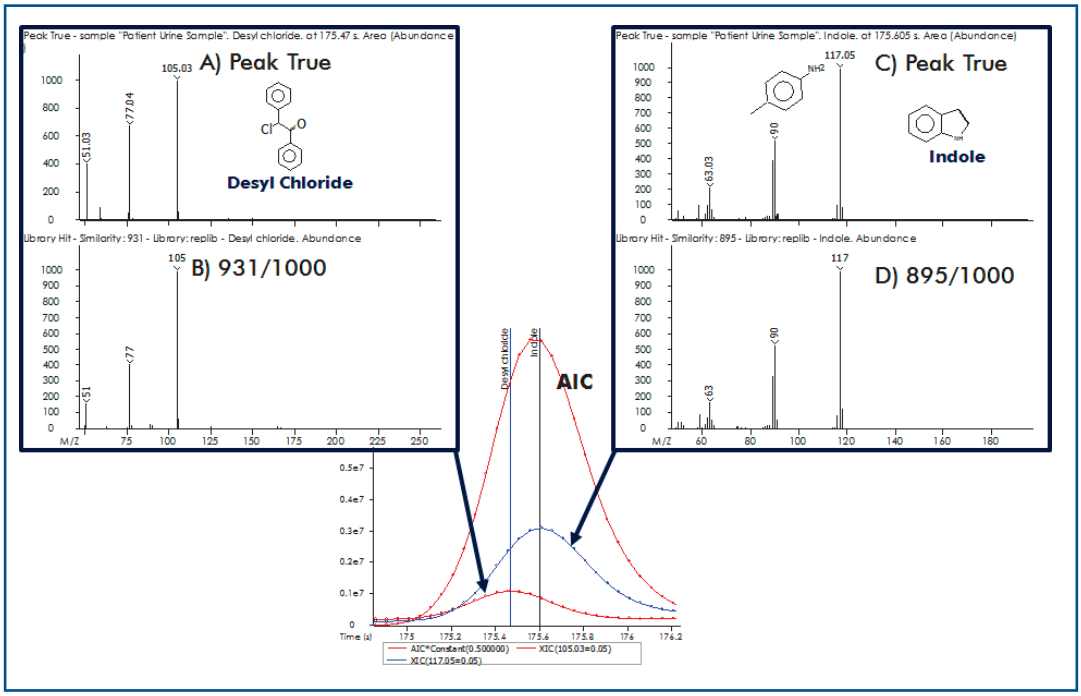
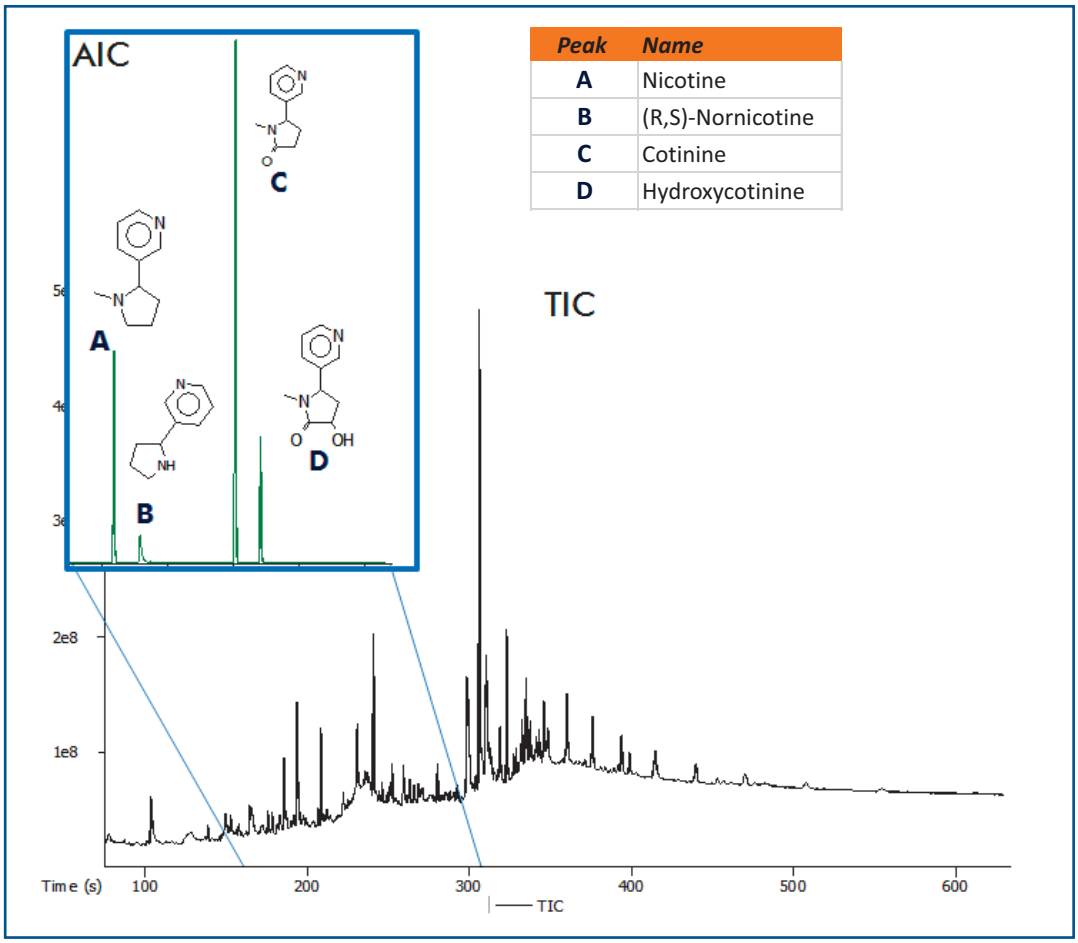


Figure 4. AIC showing coeluting compounds in patient B urine. Peak True and library mass spectral data for desyl chloride (A, B) and indole (C, D).

Patient B consumed tobacco products, as evident by the nicotine and related compounds found in the urine sample (Figure 5). In addition, numerous medications such as ibuprofen, morphine, and codeine as well as the illicit drug cocaine were detected in this sample (Figure 6).



Peak	Name
A	Nicotine
B	(R,S)-Nornicotine
C	Cotinine
D	Hydroxycotinine

Figure 5. TIC and AIC (above) showing tobacco products in patient B's urine.

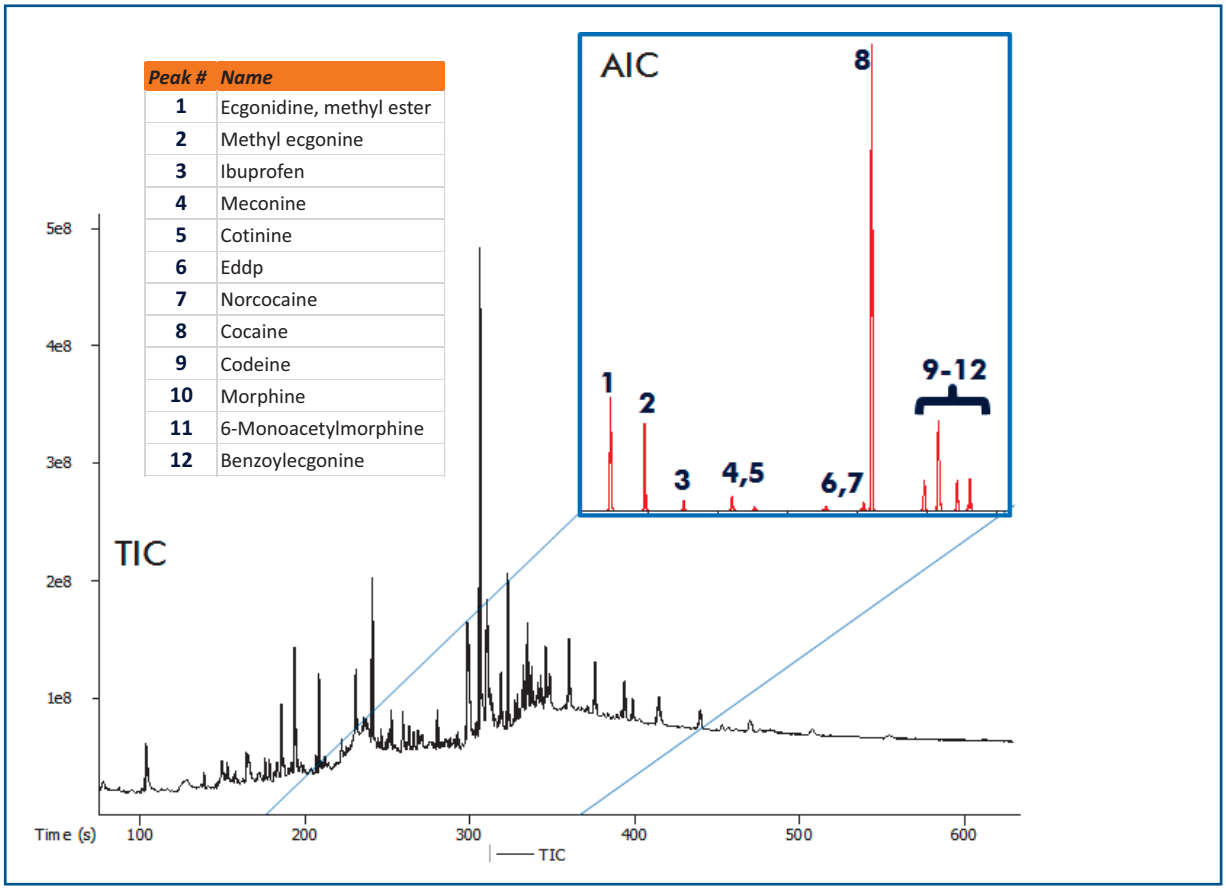


Figure 6. TIC and AIC (above) showing medications and illicit drugs in patient B's urine.

Examples of the high quality spectra produced by the Pegasus BT are displayed for codeine and morphine in Figure 7 (Spectral similarity values = 914 and 942/1000). Mass delta values of -0.01 Da resulted in an increased level of confidence for identification of cocaine and Eddp, a methadone metabolite in the patient's urine (Figure 8).

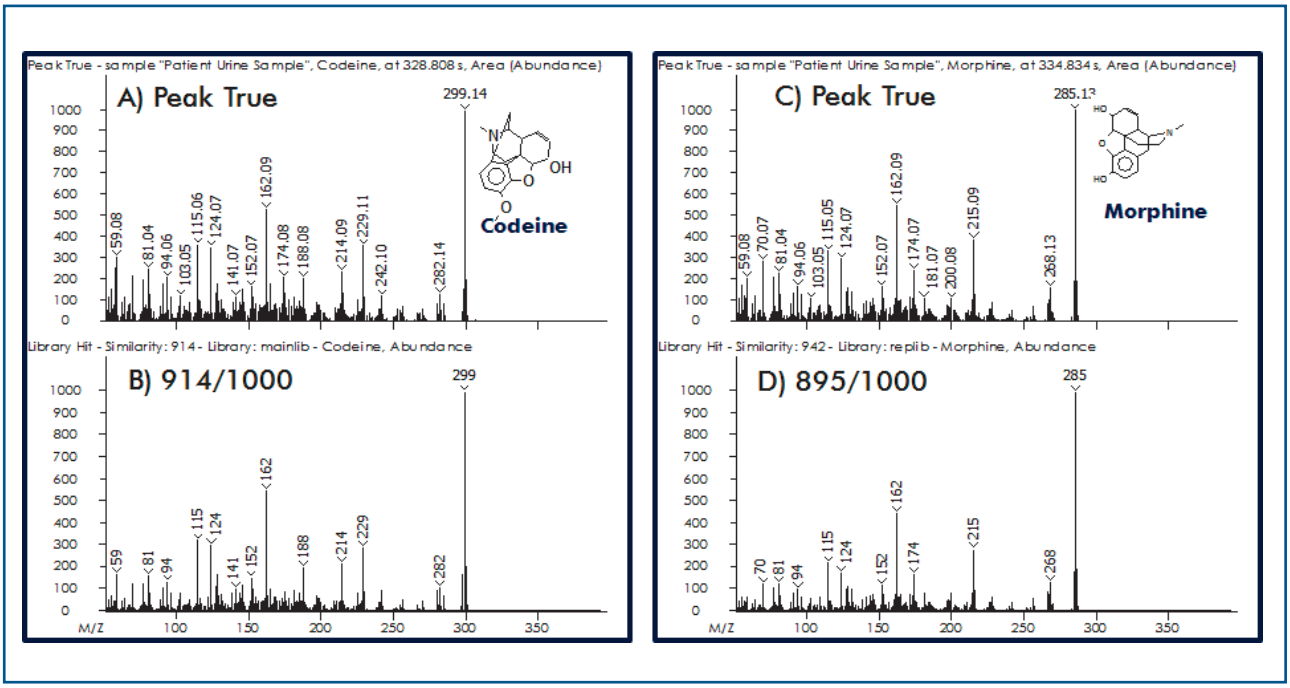


Figure 7. Peak True and library mass spectral data for codeine (A, B) and morphine (C, D).

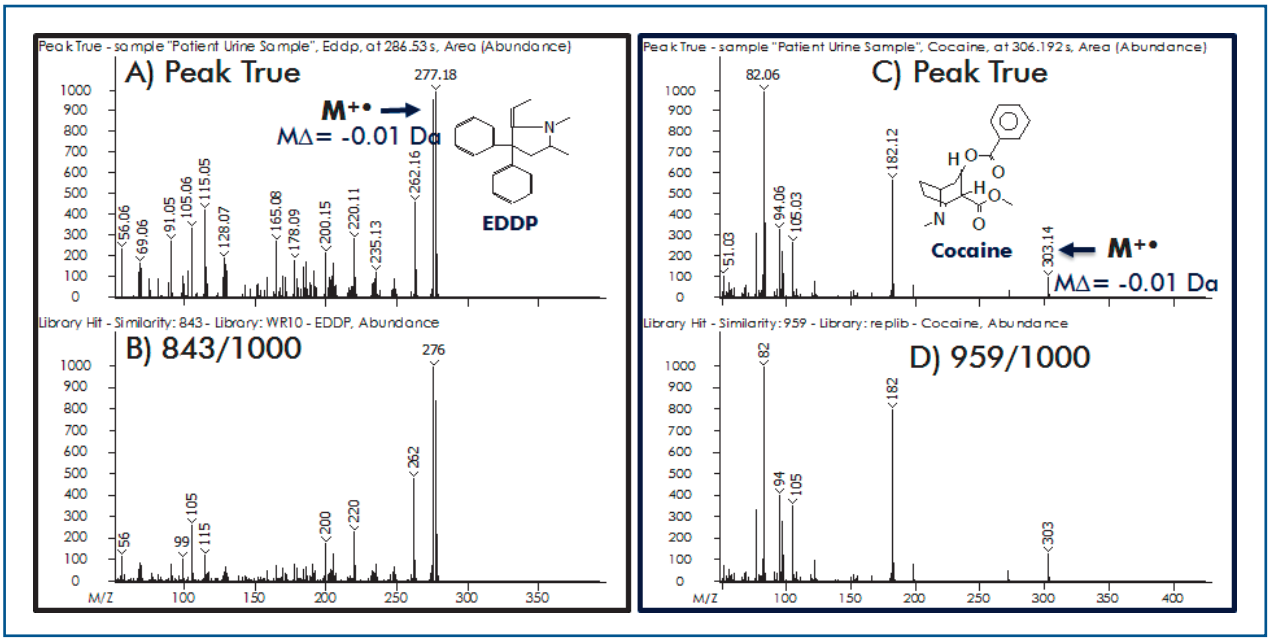


Figure 8. Peak True and library mass spectral data for EDDP (A, B) and cocaine (C, D) with the added confidence of low mass delta values.

4. Conclusion

The widespread prevalence of drug abuse in society has mandated the development of effective methods for patient monitoring. These methods should provide a complete picture of prescribed opioids, additional prescription medications, and illicit drugs used by patients. High performance GC-TOFMS is preferred over other analytical methods (e.g., LC-MS) because of its robust chromatography, universal ionization, increased sensitivity, and dynamic range, and production of reproducible high quality spectral data. The utilization of powerful software tools that include deconvolution, and rapid data mining through comparison to large, well-established databases resulted in confident identification of drugs and other substances in a single run.

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