Development of a Screening Analysis by LC Time-Of-Flight MS for Drugs of Abuse

Application

Forensic Toxicology

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Abstract

The most common method of screening for drugs of abuse in human samples is a straight-forward immunoassay technique, which although allowing for a rapid turnaround of screening samples, involves a slower confirmatory test of derivatization and detection by gas chromatography/mass spectrometry (GC/MS).

This application note presents the potential for the Agilent Time-of-Flight Mass Spectrometer (LC/MSD TOF) for use as both a screening and a confirmation tool in one analytical run of 30 minutes.

Introduction

Many drugs of choice are derived directly from natural substances. One of the most common of these is *cannabis*. An extensive review of the illicit drug market in 25 major U.S. cities is pro-vided in the Office of National Drug Control Policy Document "Pulse Check" [1].

Interest in the analysis of drugs of abuse covers many areas, all with different concerns in the results obtained. Some of the areas of significance include:

- Workplace screening
- Forensic pathology
- Accident investigation
- Crime scene investigation

Today, screening of drugs of abuse is performed through a variety of methods, with the most common lab-based technique being an Enzyme Multiple Immunoassay Test (EMIT), with a confirmatory analysis by GC/MS, if required. This immunoassay technique allows for screening to be performed and reported in as little as 2 hours, yet more commonly a 36–48 hour turnaround time is required. A further disadvantage of the EMIT technique is that it lacks the specificity to identify anything more than the class of drug detected.

The current analytical confirmatory technique of GC/MS was developed in order to achieve the sensitivity and specificity required to accurately determine the exact type and level of the drug compound, within the class indicated by the immunoassay technique. In order to achieve this detection, many of the drugs require derivatization to ensure adequate volatility and/or thermal stability required for GC analysis. See Table 1.

Table 1. National Institute of Drug Analysis Compound Class and Detection Limit Summary

Compound class	Detection limits (ng/mL)	Confirmation
Amphetamines	1000	EMIT/GC/MS
Barbiturates	300–3000	EMIT/GC/MS
Cocaine	300	EMIT/GC/MS
Methadone	300	EMIT/GC/MS
Opiates	300	EMIT/GC/MS
Phencyclidine	25	EMIT/GC/MS
Propoxyphene	300	EMIT/GC/MS
Benzodiazepines	300	EMIT/GC/MS
Methaqualone	300	EMIT/GC/MS
Cannabinoids	50	EMIT/GC/MS

Recently published Agilent application notes have shown the potential of LC/MS for the screening analysis and therapeutic monitoring of drugs of abuse using a single quadrupole instrument [2, 3]. Numerous other publications discuss selected drugs of abuse, or drug classes, illustrating the potential for the technique to one day replace GC/MS as either the confirmatory tool or as both the screening and confirmatory tool in one analysis.

Accurate mass measurement, such as that provided by the Agilent LC/MSD TOF, greatly increases the confidence of identification because it inherently limits the possible number of candidate compounds. The better the precision and accuracy of the mass measurement, the fewer the number of compounds theoretically possible for a given accurate mass. This is particularly useful for the analysis of samples from a variety of sources, each with their own potential interferences, such as those encountered with explosives residue analysis.

This application note provides an overview of the power of the Agilent TOF mass spectrometer for the screening and confirmation analysis of drugs of abuse. The TOF mass spectrometer provides accurate mass determinations (<3 ppm) with good linearity, proving its use as an excellent tool for the detection, confirmation, and quantitation of different drug classes. The method used here is not intended to represent one that will determine the lowest possible level of any one particular analyte or class of analytes, but rather is a procedure that could be expanded to cover a wider range of components used in screening analyses.

The compounds studied and their molecular formulas are shown in Table 2.

Table 2. Compounds Included in Study

Compound	Molecular formula	Drug class
α-hydroxyalprazolam	$C_{17}H_{13}N_4OCl$	Benzodiazepine
7-Aminoclonazepam	$C_{15}H_{12}N_3OCl$	Benzodiazepine
Diazapam	$C_{16}H_{13}N_2OCl$	Benzodiazepine
Oxazepam	$C_{15}H_{11}N_2O_2Cl$	Benzodiazepine
Temazepam	$C_{16}H_{13}N_2O_2Cl$	Benzodiazepine
7-Aminoflunitrazepam	$C_{16}H_{14}N_3OF$	Benzodiazepine
7-Aminonitrazepam	$C_{15}H_{13}N_3O$	Benzodiazepine
dl-11-nor-9-carboxy-δ-9-THC	$C_{21}H_{28}O_4$	Cannabinoid
Codeine	$C_{18}H_{21}NO_3$	Opiate
Morphine 3β-d-glucuronide	$C_{23}H_{27}NO_9$	Opiate
6-acetylmorphine	$C_{19}H_{21}NO_4$	Opiate
EDDP perchlorate	$C_{20}H_{24}NO_4Cl$	Opiate
(+)-ephedrine	$C_{10}H_{15}NO$	Stimulant
Fenfluramine	$C_{12}H_{16}NF_3$	Stimulant
dl-MBDB:HCL	$C_{12}H_{18}NO_2Cl$	Stimulant
(±) BDB Hydrochloride	$C_{11}H_{16}NO_2Cl$	Stimulant
dl-MDEA	$C_{12}H_{17}NO_2$	Stimulant
dl-MDA	$C_{10}H_{13}NO_2$	Stimulant
dl-MDMA	$C_{11}H_{15}NO_2$	Stimulant
dl-Methamphetamine	$C_{10}H_{15}N$	Stimulant
dl-Amphetamine	$C_9H_{13}N$	Stimulant
Phentermine	$C_{10}H_{15}N$	Stimulant
(+)-Psuedoephedrine	$C_{10}H_{15}NO$	Stimulant
(–)-Cotinine	$C_{10}H_{12}N_2O$	Other
4'-Hydroxynordiazepam	$C_{15}H_{11}ClN_2O_2$	Benzodiazepine
Nordiazepam	$C_{15}H_{11}N_2OCl$	Benzodiazepine
Flunitrazepam	$C_{16}H_{12}N_3O_3F$	Benzodiazepine
Flurazepam	$C_{21}H_{23}N_3OClF$	Benzodiazepine
Desalkylflurazepam	$C_{15}H_{10}N_2OClF$	Benzodiazepine
(–)-δ-9-THC	$C_{21}H_{30}O_2$	Cannabinoid
(±)-11-hydroxy-δ-9-THC	$C_{21}H_{30}O_3$	Cannabinoid
Cocaine	$C_{17}H_{21}NO_4$	Cocaine
Benzoylecgonine	$C_{16}H_{19}NO_4$	Cocaine
Buprenorphine	$C_{29}H_{41}NO_4$	Opiate
Morphine	$C_{17}H_{19}NO_3$	Opiate
Normorphine	$C_{16}H_{17}NO_3$	Opiate
Meperidine	$C_{15}H_{21}NO_2$	Opiate
Normeperidine	$C_{14}H_{19}NO_2$	Opiate
dl-Methadone	$C_{21}H_{27}NO$	Opiate
EMPD	$C_{19}H_{21}N$	Opiate
Naloxone	$C_{19}H_{21}NO_4$	Opiate
Oxycodone	$C_{18}H_{21}NO_4$	Opiate
LSD	$C_{20}H_{25}N_3O$	Hallucinogen
Iso-LSD	$C_{20}H_{25}N_3O$	Hallucinogen
(±)-phenylpropanolamine:HCL	$C_9H_{13}NO$:HCl	Stimulant
Fluoxetine:HCL	$C_{17}H_{18}F_3NO$:HCl	Prosac
GHB	$C_4H_7O_3Na$	Other
(–)-Nicotine	$C_{10}H_{14}N_2$	Other

Methodology

The work undertaken in this study was performed on an Agilent 1100 system consisting of:

Binary pump
Standard auto-sampler
Thermostated column compartment
Diode Array Detector (DAD)
G1969 LC/MSD TOF.

Instrument Conditions

Pump

Time (min)	% Water (0.1% formic acid)	% Methanol (0.1% formic acid)	Flow rate (mL/min)
0	90	10	0.4
4	90	10	0.4
22	0	100	0.4
29	0	100	0.4
29.6	90	10	0.4
30	90	10	0.4

Post time:	5 minutes
Total run time:	35 minutes
Injection volume:	10 µL, with needle wash
Column temperature:	30 °C
Column:	ZORBAX SB-AQ, 150 mm × 2.1 mm × 3.5 µm

MS Detection

Ionization	**ESI Positive**
Gas temp	350 °C
Drying gas	10 L/min
Nebulizer pressure	40 psig
Capillary V (+ve)	3500 V

MS Conditions

Scan *m/z* range	100–1000
Fragmentor	125 V
Storage mode	Profile
Skimmer	60 V
Oct RF	200 V

Reference Mass Introduction with LC-TOF

The Agilent TOF MS uses a reference mass in the generation of reliable high level accurate mass. The electro-spray source for the TOF is a unique dual spray assembly that allows the simultaneous constant introduction of a reference mass component.

The reference mix 1 used in these experiments consists of 2 mL of purine and 0.8 mL of HP-0921. This mixture was prepared in 1 L of 90:10 methanol:water to better represent the mobile phase.

The control software enables the use of the following reference masses:

Positive Ion Detection
121.050873
922.009798

Analysis of Drugs of Abuse by LC-TOF MS

An overwhelming advantage of using TOF MS for the trace level detection of any component is the confirmatory information that is provided through accurate mass. An example of this mass accuracy is shown in Table 3, where the observed masses for each component are detected, and their deviation from the theoretical masses for the adduct are shown.

The ability to closely match the expected mass and the observed mass provides the analyst with a higher level of confidence in the assignment given to a chromatographic peak. In the screening for components such as drugs, which may have a significant impact on the life of a person, this additional confidence is of great importance. This capability also allows the possibility of using this technique as a screening tool for a wide range of components.

Table 3. Theoretical Accurate Mass, Observed Mass and Mass Error

Compound	Monoisotopic mass	Retention time	Adduct	Observed mass	Adduct accurate mass	Mass error (ppm)
α-Hydroxyalprazolam	324.0778	17.76	$[M+H]^+$	325.0852	325.0850	0.41
7-Aminoclonazepam	285.0669	13.32	$[M+H]^+$	286.0739	286.0741	–0.93
Diazapam	284.0716	19.15	$[M+H]^+$	285.0796	285.0789	2.39
Oxazepam	286.0509	17.4	$[M+H]^+$	287.0579	287.0581	–0.98
Temazepam	300.0666	18.2	$[M+H]^+$	301.0741	301.0738	0.89
7-Aminoflunitrazepam	283.1121	15.3	$[M+H]^+$	284.1191	284.1093	–0.94
7-Aminonitrazepam	251.1059	8.82	$[M+H]^+$	252.1134	251.1131	1.04
dl-11-nor-9-carboxy-δ-9-THC	344.1988	21.38	$[M+H]^+$	345.2061	345.2060	0.18
Codeine	299.1521	5.5	$[M+H]^+$	300.1592	300.1594	–0.73
Morphine 3 β-d-glucuronide	461.1686	1.7	$[M+H]^+$	462.1764	462.1758	1.17
6-Acetylmorphine	327.1471	8.9	$[M+H]^+$	328.1542	328.1543	–0.41
EDDP perchlorate	377.1394	15.42	$[M-O_4Cl]^+$	278.1909	278.1903	2.06
(+)-Ephedrine	165.1154	2.46	$[M+H]^+$	166.1225	166.1226	–0.85
Fenfluramine	231.1235	12.9	$[M+H]^+$	232.1303	232.1307	–1.98
dl-MBDB:HCL	243.1026	10.63	$[M-Cl]^+$	208.1337	208.1332	2.37
(±) BDB hydrochloride	229.087	9.5	$[M-Cl]^+$	194.1181	194.1175	2.81
dl-MDEA	207.1259	9.6	$[M+H]^+$	208.1332	208.1332	–0.03
dl-MDA	179.0946	4.9	$[M+H]^+$	180.1019	180.1191	–0.03
dl-MDMA	193.1103	6.4	$[M+H]^+$	194.1174	194.1175	–0.08
dl-Methamphetamine	149.1204	3.85	$[M+H]^+$	150.1281	150.1277	2.49
dl-Amphetamine	135.1048	3.05	$[M+H]^+$	136.1125	136.112	3.11
Phentermine	149.1204	5.34	$[M+H]^+$	150.1278	150.1277	0.49
(+)-Psuedoephedrine	165.1154	2.76	$[M+H]^+$	166.1231	166.1226	2.76
(–)-Cotinine	176.095	2.56	$[M+H]^+$	177.1023	177.1022	0.34
4'-Hydroxynordiazepam	286.0509	14.23	$[M+H]^+$	287.0582	287.0581	0.06
Nordiazepam	270.056	18.1	$[M+H]^+$	271.0634	271.0632	0.49
Flunitrazepam	313.0863	18.1	$[M+H]^+$	314.0924	314.0935	–3.65
Flurazepam	387.1514	15.23	$[M+H]^+$	388.1591	388.1586	1.17
Desalkylflurazepam	288.0466	18.2	$[M+H]^+$	289.0535	289.0538	–1.19
(–)-δ-9-THC	314.2246	22.31	$[M+H]^+$	315.2328	315.2318	2.99
(±)-11-hydroxy-δ-9-THC	330.2195	21.07	$[M+H]^+$	331.2267	331.2267	–0.22
Cocaine	303.1471	12.6	$[M+H]^+$	304.1545	304.1543	0.54
Benzoylecgonine	289.1314	12.1	$[M+H]^+$	290.1386	290.1386	–0.29
Buprenorphine	467.3036	16.11	$[M+H]^+$	468.3107	468.3108	–0.29
Morphine	285.1365	2.2	$[M+H]^+$	286.1438	286.1437	–0.1
Normorphine	271.1208	2	$[M+H]^+$	272.1286	272.1281	1.7
Meperidine	247.1572	12.4	$[M+H]^+$	248.1652	248.1645	2.8
Normeperidine	233.1416	12.5	$[M+H]^+$	234.1493	234.1488	1.9
dl-Methadone	309.2093	16.72	$[M+H]^+$	310.2166	310.2165	0.19
EMPD	263.1674	16.4	$[M+H]^+$	264.1754	264.1746	2.7
Naloxone	327.1471	5.74	$[M+H]^+$	328.1541	328.1543	–0.72
Oxycodone	315.1471	7.2	$[M+H]^+$	316.1547	316.1543	1.15
LSD	323.1998	14.7	$[M+H]^+$	324.2073	324.207	0.8
Iso-LSD	323.1998	14.55	$[M+H]^+$	324.2078	324.207	2.34
(±)-Phenylpropanolamine:HCL	187.0764	1.95	$[M-Cl]^+$	152.1069	152.1069	–0.59
Fluoxetine:HCL	345.1107	16.3	$[M-Cl]^+$	310.1412	310.1413	1.85
GHB	126.0293	1.72	$[M+H]^+$	127.0369	127.0365	2.63
(–)-Nicotine	162.1157	1.6	$[M+H]^+$	163.1233	163.1229	1.99
2-oxo-3-hydroxy-LSD	355.1896	n.d.			Not detected in +veESI	

A greater than two-fold increase in sensitivity for many components is seen with the narrowing of the mass-extraction window. Figure 1 shows the reduction in noise that is observed with the extraction of a smaller mass range for flunitrazepam, commonly known as Rohypnol, a date-rape drug. The ability of TOF-MS to accurately determine the presence of components such as Rohypnol at low levels may assist with investigations into reported abuse of the illicit substance, and prove to be a critical factor in confirmation when dealing with complex matrices.

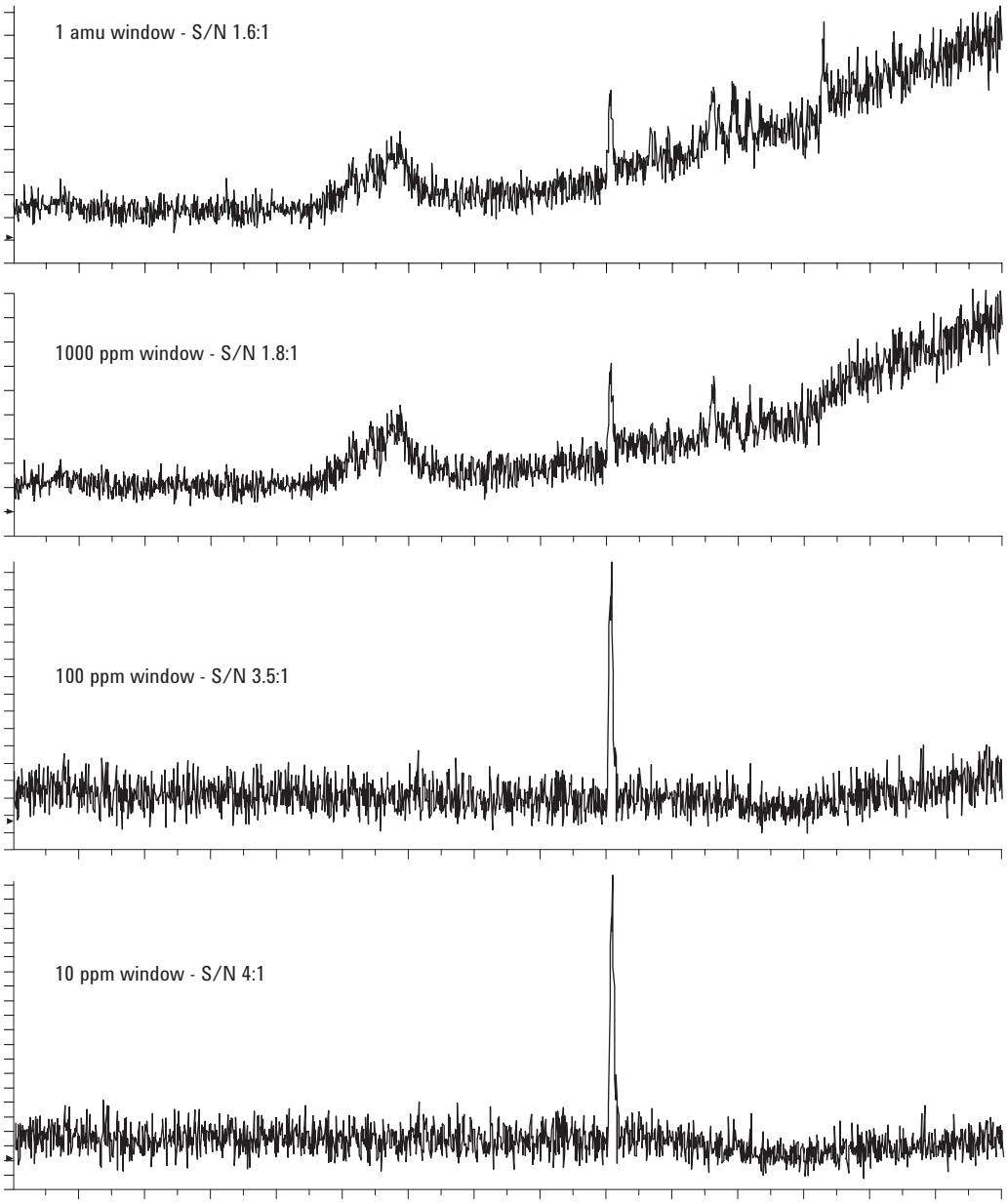


Figure 1. Effect of extracted ion range on noise – 1 ng/mL flunitrazepam.

TOF Linearity

TOF-MS has traditionally been considered as unsuitable for quantitation due to the use of time-to-digital conversion of data. The Agilent TOF MS uses analog-to-digital conversion, allowing for far better quantitative data than the alternative technology of time-to-digital conversion. Several of the components analyzed by TOF were tested for linearity as part of this study. Figures 2–5 show the linearity of four selected components, most displaying linearity over three orders of magnitude from 1 ng/mL to 1000 ng/mL. However, some components, such as δ-9-THC and fluoxetine, only exhibit a narrower linear range, a result of their ionization behavior (Figures 6 and 7). Nevertheless, regression values of over 0.999 were seen for each of these components.

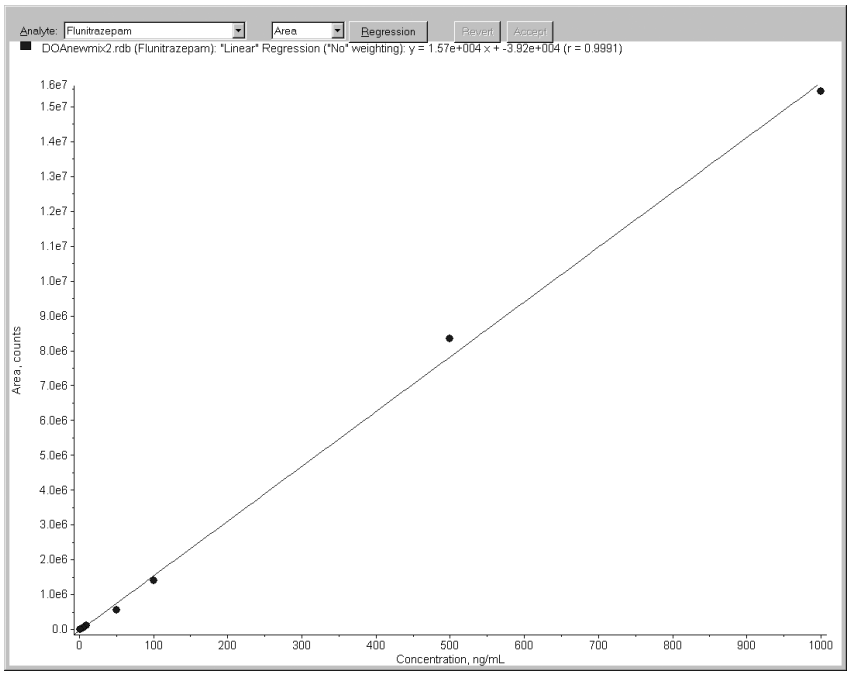


Figure 2. Calibration curve for flunitrazepam from 1 ng/mL to 1000 ng/mL with TOF-MS.

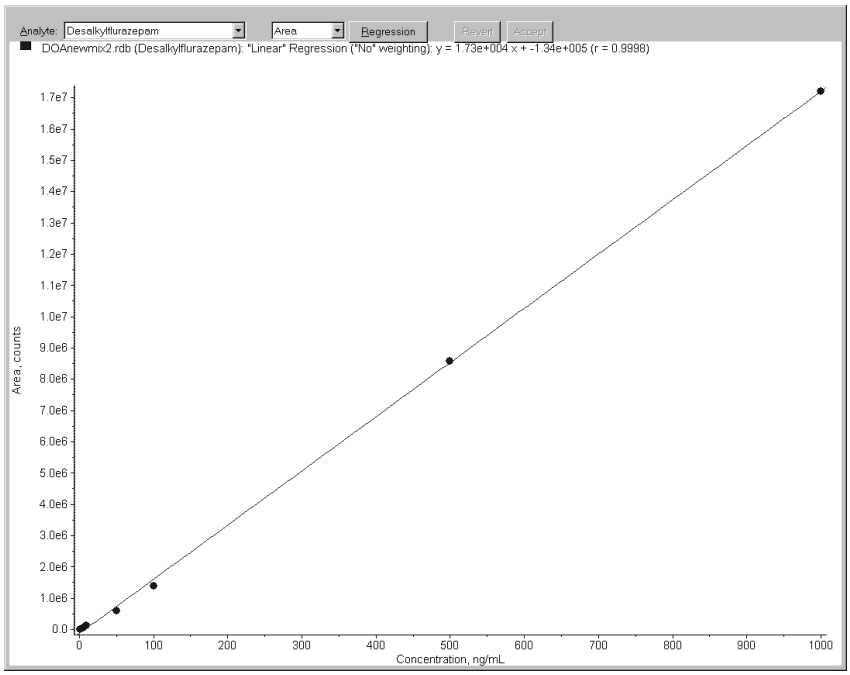


Figure 3. Calibration curve for desalkylflurazepam from 1 ng/mL to 1000 ng/mL with TOF-MS.

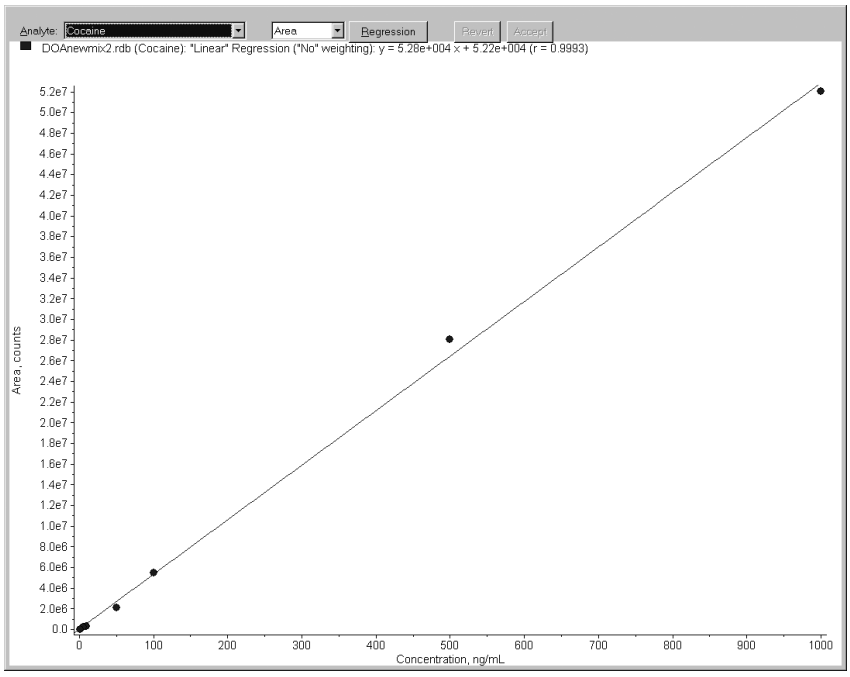


Figure 4. Calibration curve for cocaine from 1 ng/mL to 1000 ng/mL with TOF-MS.

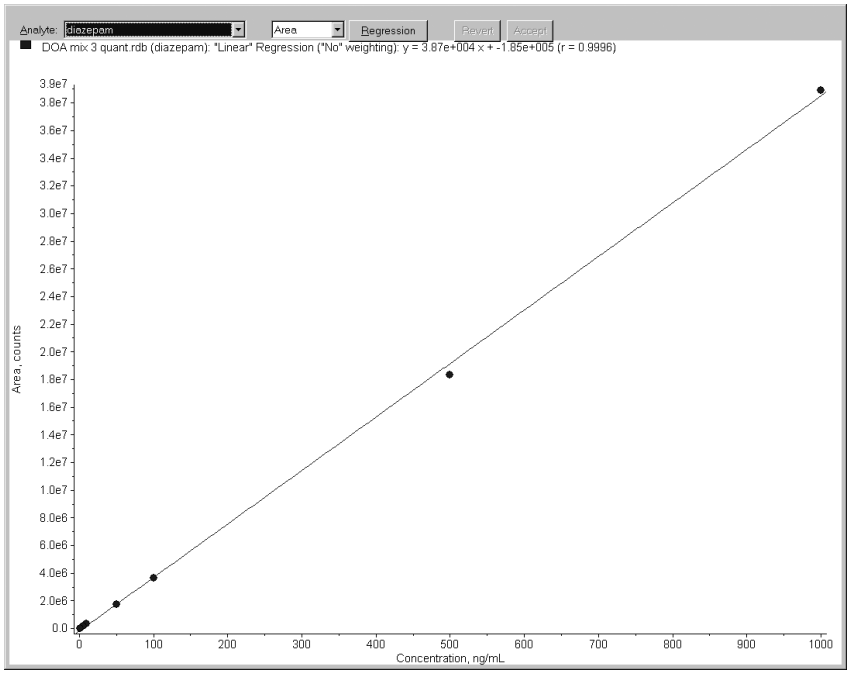


Figure 5. Calibration curve for diazepam from 1 ng/mL to 1000 ng/mL with TOF-MS.

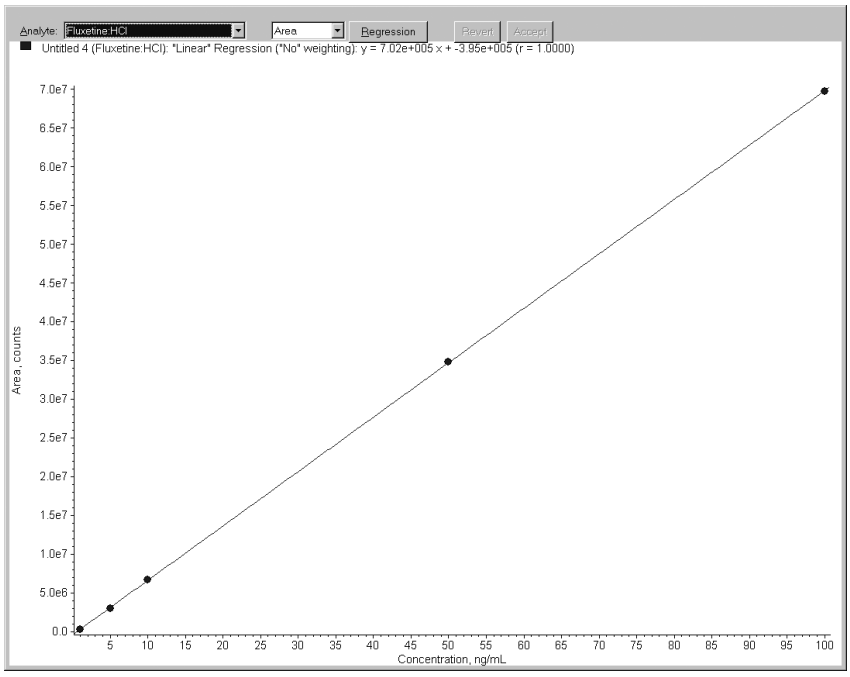


Figure 6. Calibration curve for (–)-δ-9-THC from 1 ng/mL to 100 ng/mL with TOF-MS.

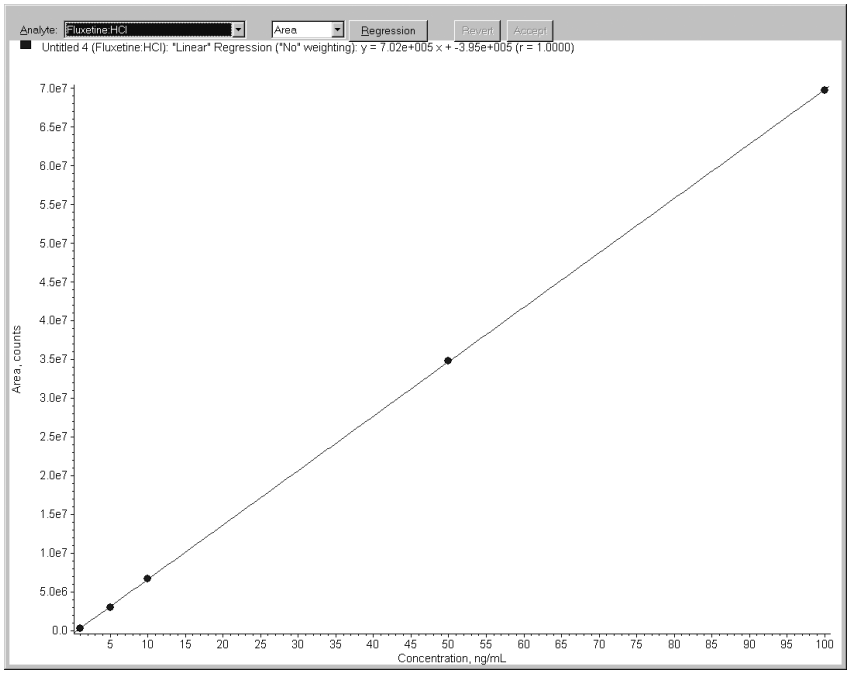
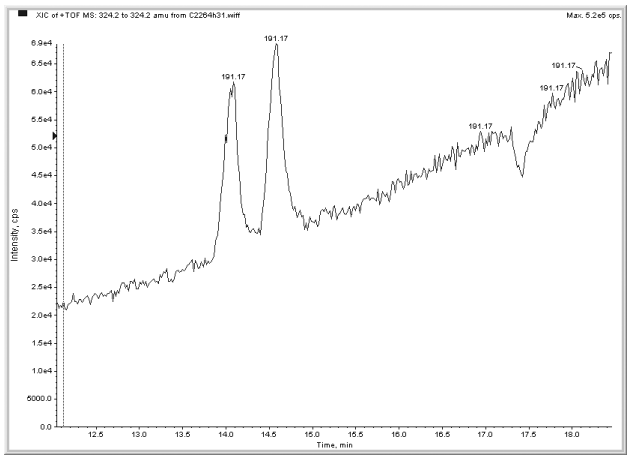
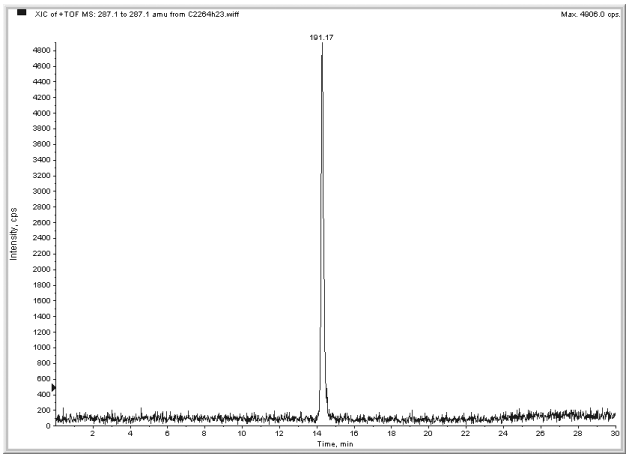


Figure 7. Calibration curve for fluoxetine from 1 ng/mL to 100 ng/mL with TOF-MS.

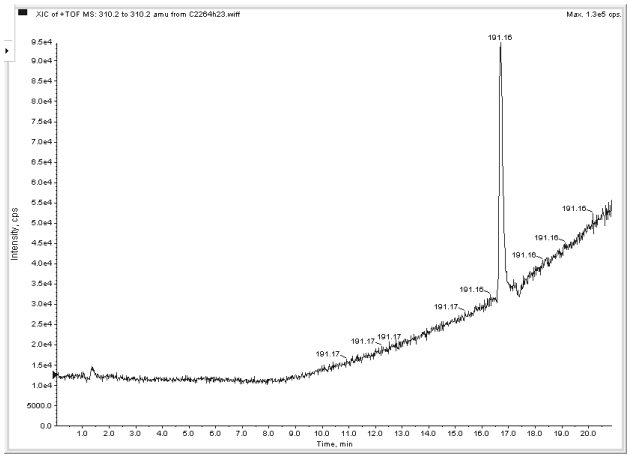
Chromatogram examples for four components at 1 ng/mL are shown below in Figure 8 with 10 ppm extraction windows.



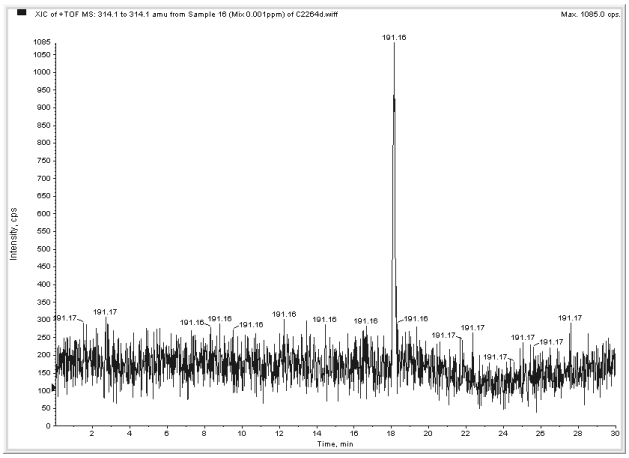
1 ng/mL iso-LSD and LSD (respectively)



1 ng/mL 4′-hydroxynordiazepam



1 ng/mL dl methadone



1 ng/mL flunitrazepam (Rohypnol)

Figure 8. Ten ppm extraction window of 1 ng/mL solutions of four drugs of abuse.

Table 4 summarizes the limits of detection (LOD) for each of the components analyzed under this method. Note that while the method has not been optimized for any one component, it is designed to provide a broad screening tool for the analysis of drugs of abuse.

Table 4. LOD for Components by LC/MSD TOF

Component	Accurate mass	LOD (ng/mL)
α-Hydroxyalprazolam	325.085	5
7-Aminoclonazepam	286.0741	1
Diazapam	285.0789	1
Oxazepam	287.0581	5
Temazepam	301.0738	5
7-Aminoflunitrazepam	284.1093	5
7-Aminonitrazepam	251.1131	1
dl-11-nor-9-carboxy-δ-9-THC	345.206	5
Codeine	300.1594	50
Morphine 3β-d-glucuronide	462.1758	5
6-Acetylmorphine	328.1543	10
EDDP perchlorate	278.1903	1
(+)-Ephedrine	166.1226	20
Fenfluramine	232.1307	1
dl-MBDB:HCL	208.1332	5
(±) BDB Hydrochloride	194.1175	10
dl-MDEA	208.1332	10
dl-MDA	180.1191	50
dl-MDMA	194.1175	50
dl-Methamphetamine	150.1277	20
dl-Amphetamine	136.112	50
Phentermine	150.1277	20
(+)-Psuedoephedrine	166.1226	20
(–)-Cotinine	177.1022	20
4'-Hydroxynordiazepam	287.0581	0.5
Nordiazepam	271.0632	0.5
Flunitrazepam	314.0935	1
Flurazepam	388.1586	1
Desalkylflurazepam	289.0538	1
(–)-δ-9-THC	315.2318	1
(±)-11-Hydroxy-δ-9-THC	331.2267	1
Cocaine	304.1543	1
Benzoylecgonine	290.1386	5
Buprenorphine	468.3108	1
Morphine	286.1437	10
Normorphine	272.1281	10
Meperidine	248.1645	1
Normeperidine	234.1488	1
dl-Methadone	310.2165	1
EMPD	264.1746	1
Naloxone	328.1543	5
Oxycodone	316.1543	20
LSD	324.207	1
Iso-LSD	324.207	1
(±)-Phenylpropanolamine:HCL	152.1069	1
Fluoxetine:HCL	310.1413	1
GHB	127.0365	20
(–)-Nicotine	163.1229	5
2-oxo-3-hydroxy-LSD	Not detected in +veESI	ND

Detection of Drugs of Abuse in Bodily Fluids

Urine is the matrix of choice for the detection of drugs of abuse and was therefore chosen to evaluate the LC/TOF MS method developed. To present a "worst case scenario", neat urine was spiked for this analysis and run directly with no sample clean-up. This would not normally be done; however, it was used as an illustration of the method's ability to provide a quick screening result without cleanup.

A further discussion of a solid phase extraction (SPE) sample preparation method that may be considered can be found in Agilent Technologies application note 5989-2260EN [4].

In the first instance, neat and spiked urine was scanned for both cocaine and benzoylegonine, a metabolite of cocaine (Figures 9 and 10). Recoveries of the spiked samples (100 ng/mL – representing a level lower than the traditional EMIT screen) were both approximately 100%.

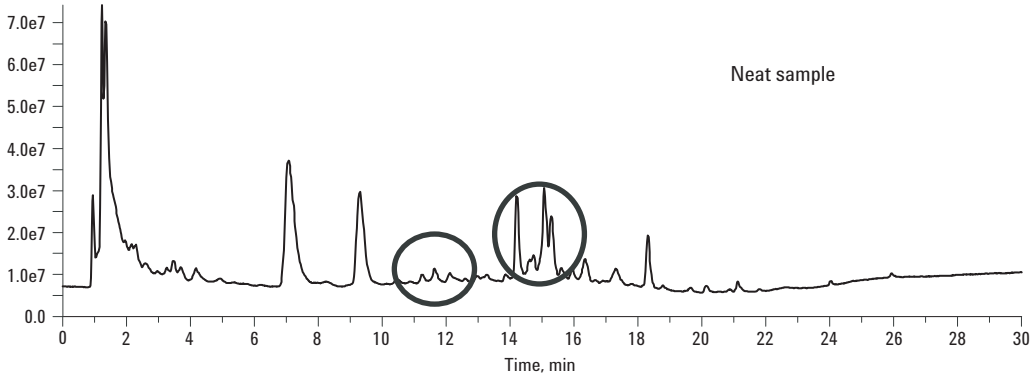


Figure 9. TIC of Blank Urine – Injected neat – circle shows expected retention time of analytes of interest.

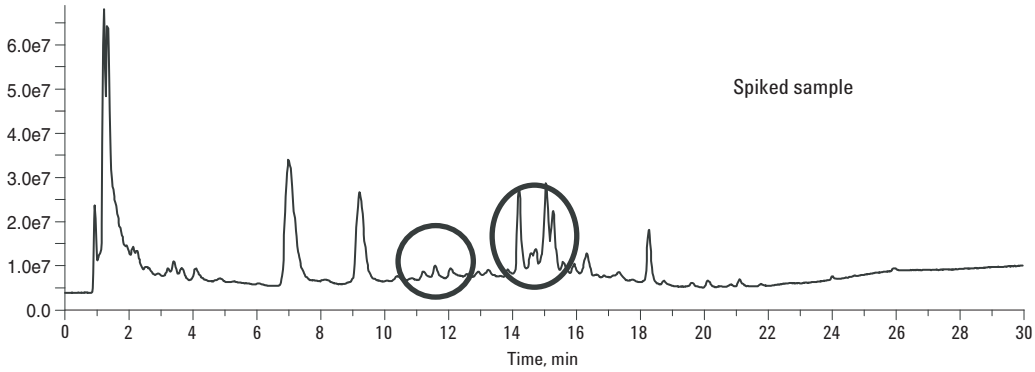


Figure 10. TIC of Neat Urine with 100 ng/mL spike of cocaine, benzoylecognine, flunitrazepam, and 7-aminoflunitrazepam.

It can be seen from Figures 11 and 12 that the ability to narrow the mass extraction window greatly reduces the noise for a given mass, and with retention time information can provide a high level of confidence in the assignment of a component.

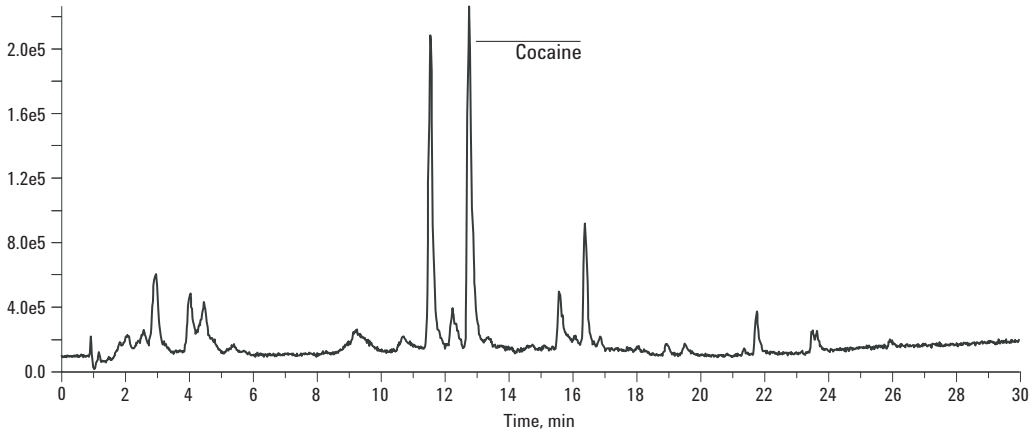


Figure 11. One amu extraction window of scanned target mass 304.1543 – cocaine.

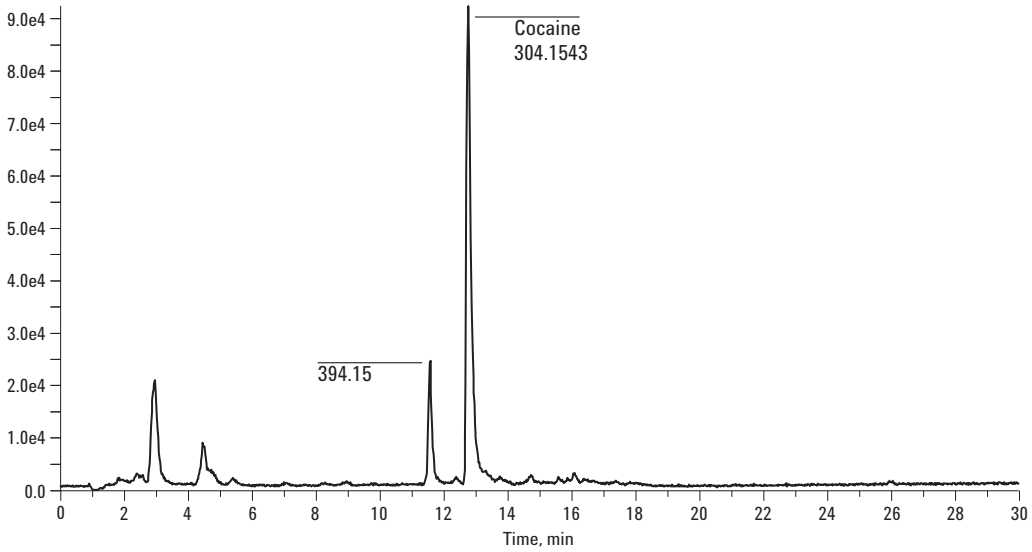


Figure 12. Ten ppm extraction window of scanned target mass 304.1543 – cocaine.

In the instance with Figure 12, the larger peak at approximately 12.9 minutes shows an excellent match with cocaine (mass error of ~0.2 ppm), while the earlier peak at 11.5 minutes has a mass of 394.15 and a fragmentation product in the extraction window (Figures 13 and 14).

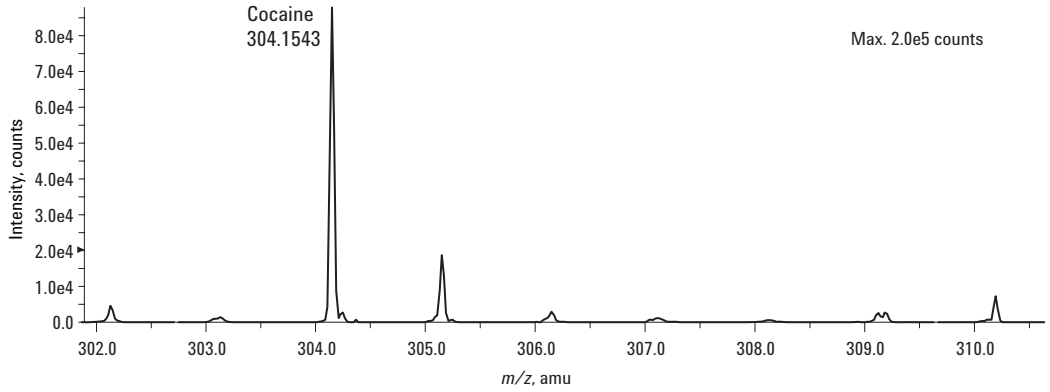


Figure 13. Confirmation of mass of cocaine.

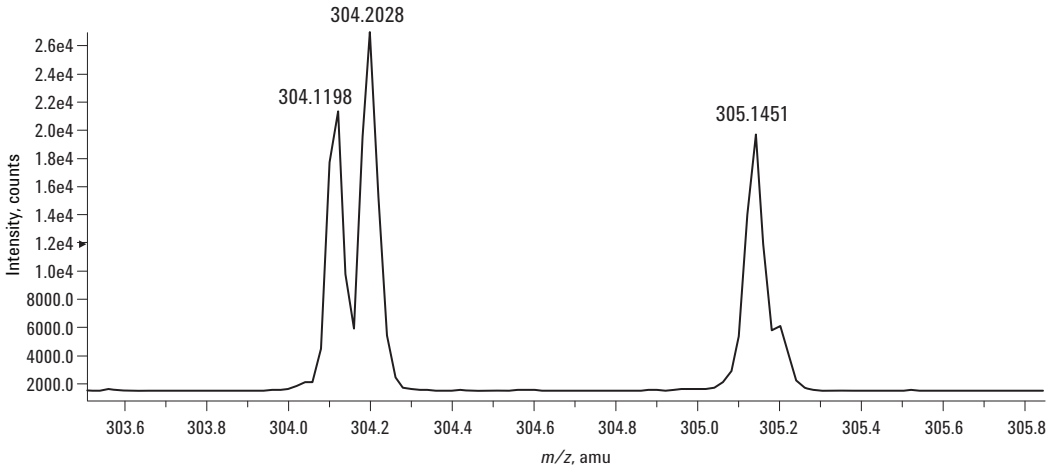


Figure 14. Mass spectrum 304 – 305 amu for peak at 11.9 minutes – no match of mass for cocaine.

A second drug that was of particular interest was flunitrazepam, (Rohypnol), and one of its metabolites, 7-aminoflunitrazepam. Again, these components were spiked at 100 ng/mL into neat urine and injected directly. The recoveries achieved for these two components were approximately 40%, which is likely a result of ion suppression in the source. However, due to the excellent detection limits pos-sible, even with up to 60% suppression of the signal, a clear peak can be seen for both com-pounds at 100 ng/mL (a level well below the EMIT screening reporting limits) when extracting a narrow mass window (Figures 15 and 16).

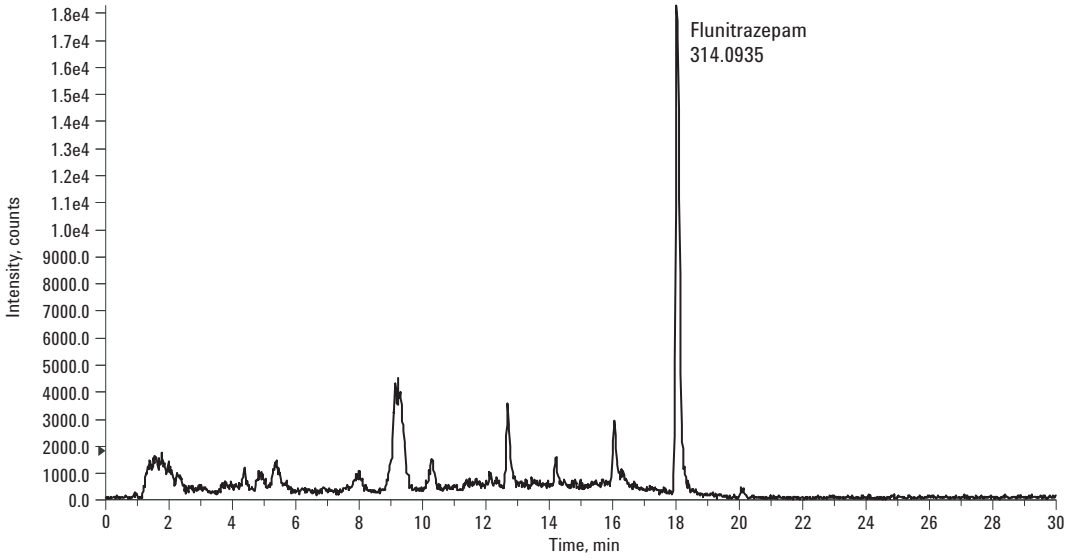


Figure 15. Ten ppm extraction window of scanned target mass 314.0935, flunitrazepam in neat urine. Retention time helps confirm presence at 18.1 minutes.

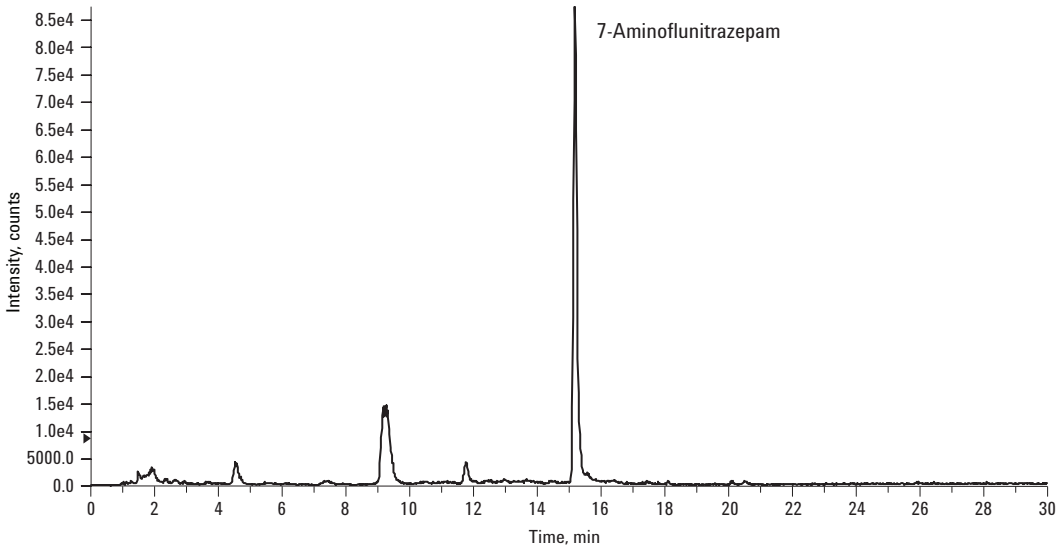


Figure 16. Ten ppm extraction window of scanned target mass 284.1191, 7-aminoflunitrazepam in neat urine. Retention time helps confirm presence at 15.3 minutes.

The second common matrix encountered in the screening of drugs of abuse is blood and plasma. To test the method when analyzing plasma, a sample was spiked with desalkylflurazepam. Sample preparation was again kept to a minimum, with a simple acetonitrile precipitation performed on the sample prior to injection. The effect of the mass extraction window on the detection of peaks, seen in Figures 17 to 20, shows the removal of interferences from the spiked plasma sample.

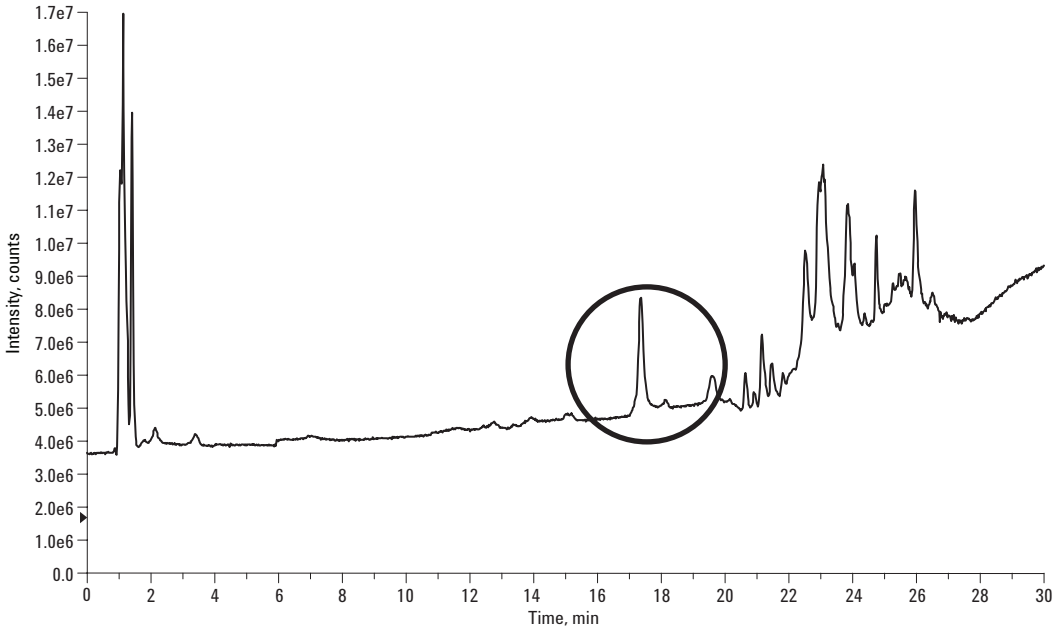


Figure 17. TIC of unspiked plasma.

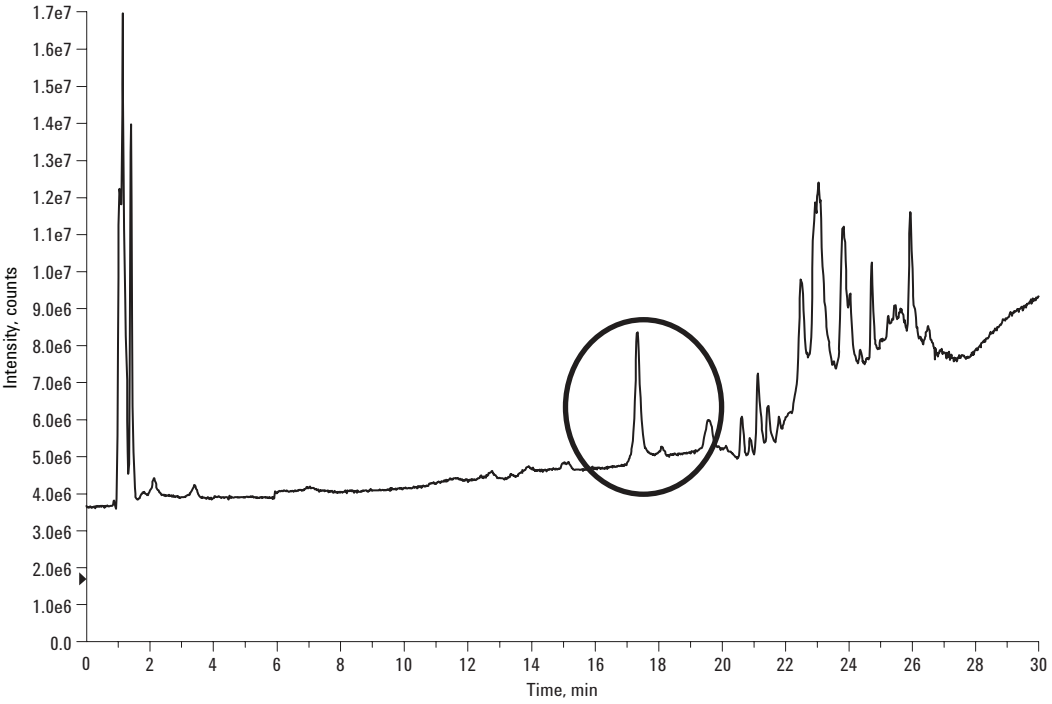


Figure 18. TIC of plasma spiked with 200 ng/mL of desalkylflurazepam.

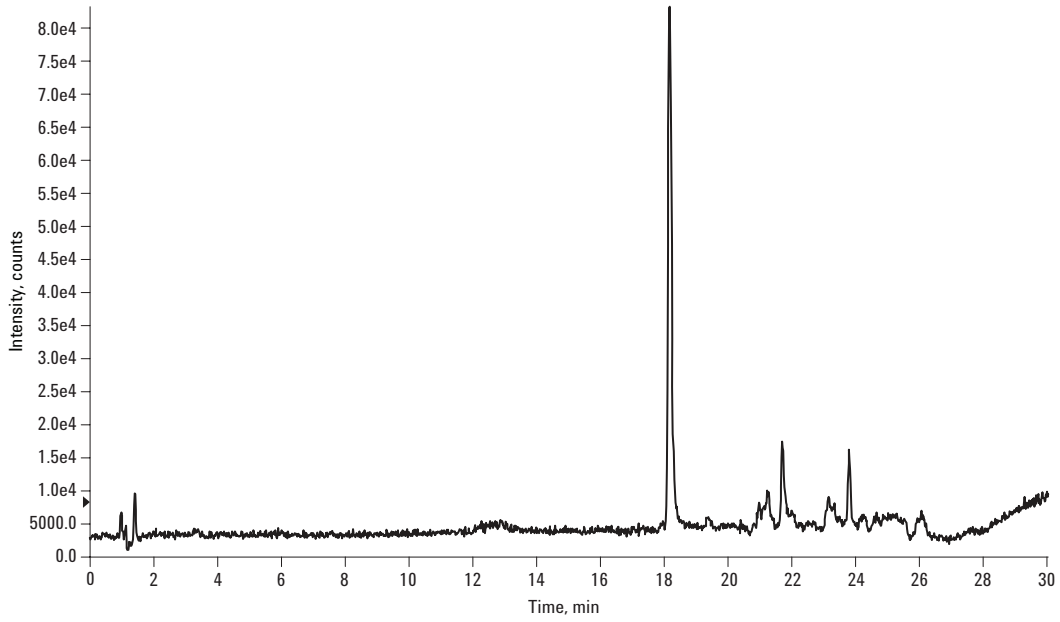


Figure 19. Mass extraction window (0.1 amu) of spiked plasma sample (346 ppm mass window).

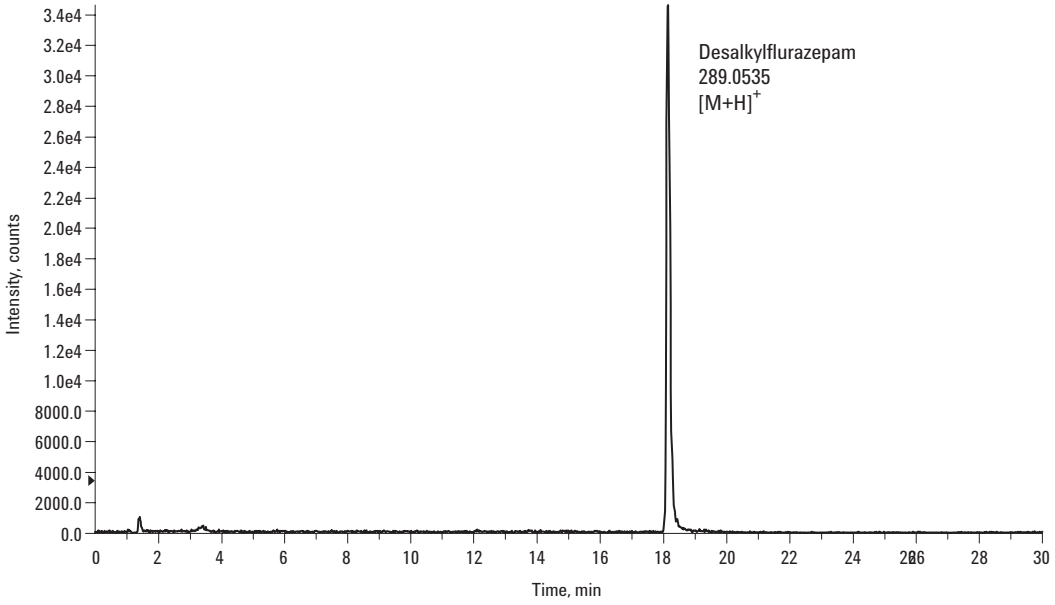


Figure 20. Ten ppm (0.003 amu) mass extraction window of spike plasma – note minimal noise.

Analysis of Coronial Samples – Using a Screener Database

The instrumentation and software provides the user the ability to create a screener database for all components they wish to automatically screen for. The minimum requirement for this database is the empirical formula and name for the component of interest, although the inclusion of a retention time will assist with confidence in the confirmation and reduce analysis time.

Several samples were acquired from the local coronial office to test the procedure that was developed. These samples were provided as butyl chloride extracts of blood samples obtained from deceased persons, for screening using the developed method.

The coronial samples supplied were screened using a database created from the 48 components analyzed under this method.

Sample Preparation

Samples were obtained from 1-mL blood volumes, liquid-liquid extracted with 6–8 mL of butyl chloride following centrifugation. Organic layer evaporated to dryness and then reconstituted in 100-µL mobile phase for a final 10-fold concentration.

Sample 1

Sample 1 was known to contain amphetamine, codeine, diazepam, and nordiazepam from the previous analysis performed at the coronial office. In addition to the four previously reported components, the screen also indicated the presence of

- Nicotine
- Cotinine
- Acetylmorphine
- Ephedrine
- Methamphetamine
- Pseudoephedrine

The total ion chromatogram (TIC) for this sample is shown in Figure 21.

An excerpt of the screen report is shown in Figure 22 with the details for cotinine. For each component included in the compound database, the screening report displays the extracted ion chromatogram, spectra of detected peak, and enlarged spectra of the target mass. This is accompanied by a summary table with the mass and retention time error. In this instance, an excellent match is seen for both retention time and mass.

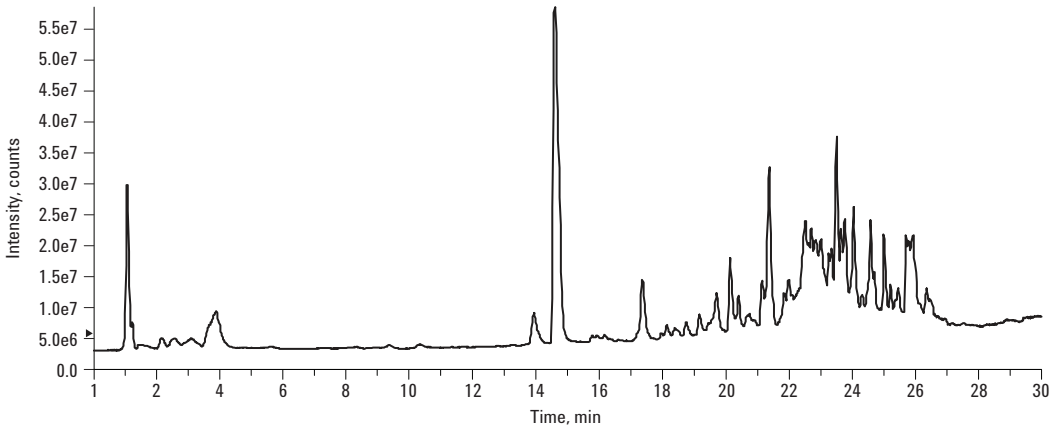
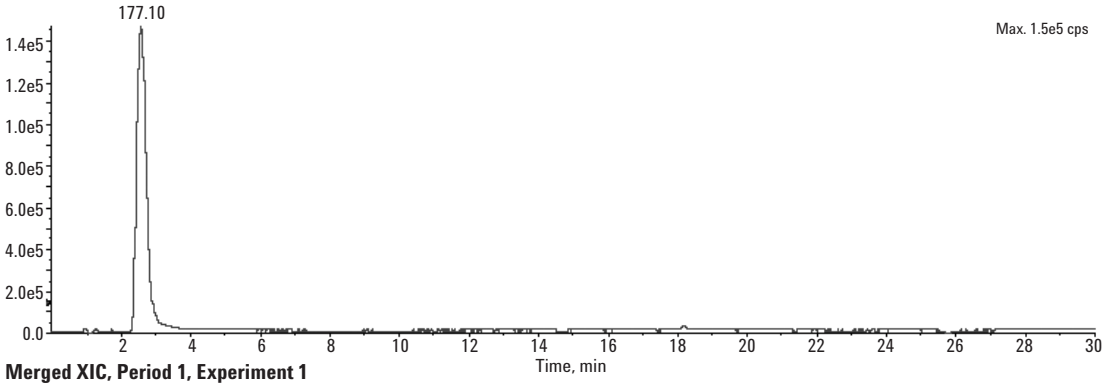
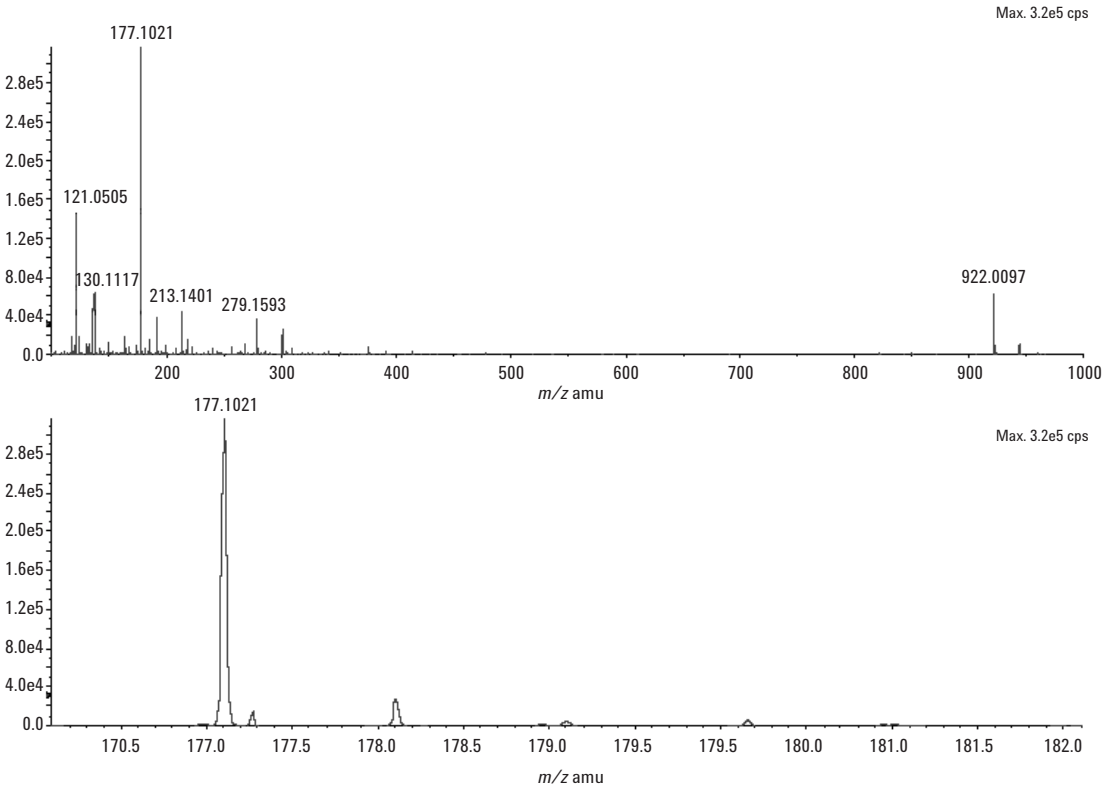


Figure 21. TIC of Coronial Sample 1.



Merged XIC, Period 1, Experiment 1



Formula	Compound name	Mass	Peak RT (min)	Peak area	Description	
$C_{10}H_{12}N_2O$	(-)-colinine	176.09496	2.57	2.78259 E7	_	
Species	**Abundance (counts)**	**Ion mass**	**Measured mass**	**Error (mDa)**	**Error (ppm)**	**Ret. time error (min)**
$[M + H]^+$	316885.14	177.10224	177.10215	-0.00009	-0.51	0.01

Figure 22. Excerpt from screener report of coronial Sample 1 showing confirmation of the presence of cotinine.

The results obtained from the analysis of Sample 1 suggest the deceased was a smoker, with the presence of both the nicotine and cotinine in the sample.

Sample 2

Sample 2 was known to contain citralopram, codeine, doxylamine, and tramadol from the previous analysis at the coronial office. In addition to the four previously reported compounds, screening with the database further showed the presence of

- Diazepam
- 6-acetylmorphine
- MDMA
- Methampethamine
- Cotinine
- Meperidine
- nicotine

A TIC of the sample is shown in Figure 23. It could again be inferred that this patient was a smoker.

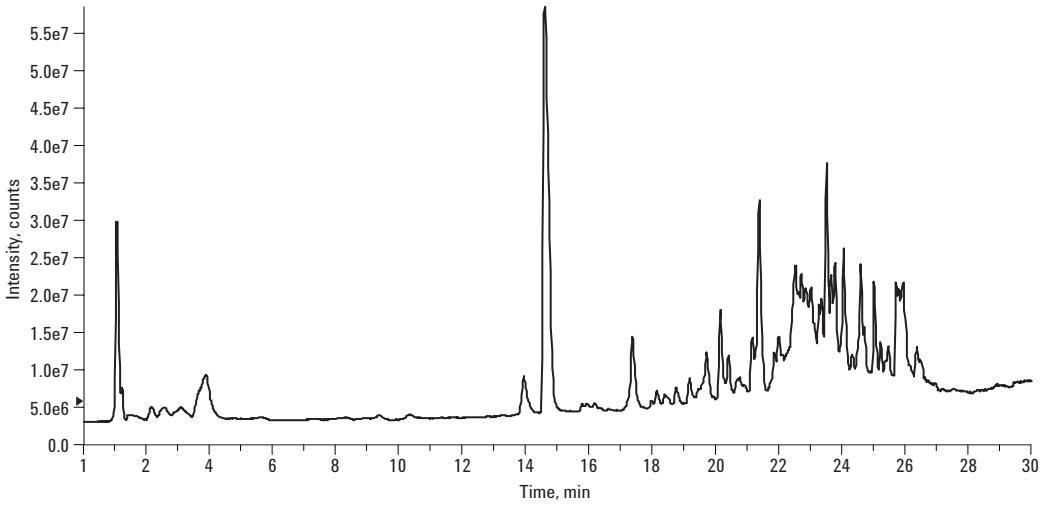
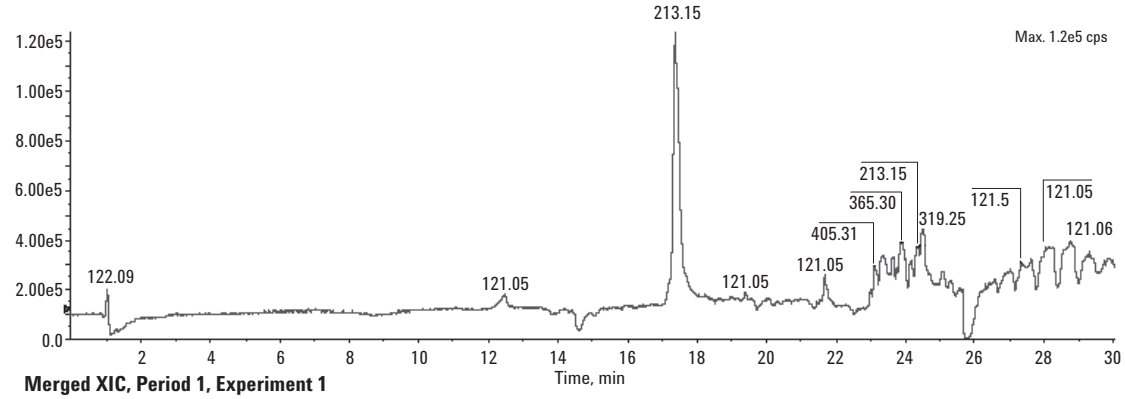
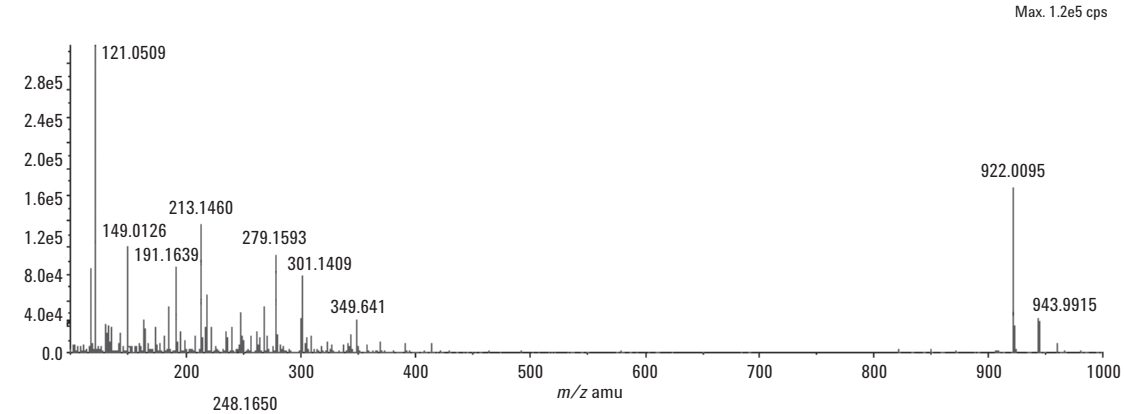
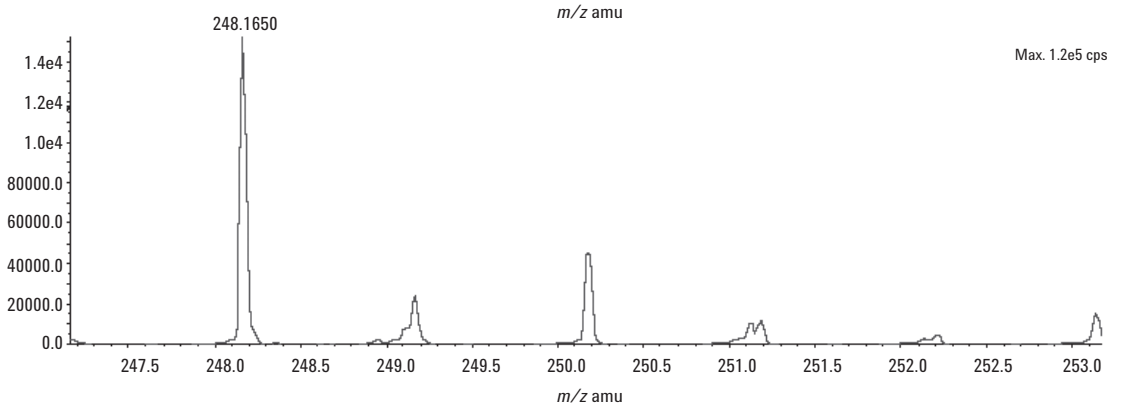


Figure 23. TIC of coronial Sample 2.

An excerpt of the screener report, in this instance for the confirmation of meperidine, is shown in Figure 24. Again, an excellent match to both retention time and mass can be seen.







Formula	Compound name	Mass	Peak RT (min)	Peak area	Description	
$C_{15}H_{21}NO_2$	Meperidine	247.15723	12.47	4.20401 E5	_	
Species	**Abundance (counts)**	**Ion mass**	**Measured mass**	**Error (mDa)**	**Error (ppm)**	**Ret. time error (min)**
$[M + H]^+$	15313.85	248.16451	248.16498	0.00047	1.90	0.07

Figure 24. Excerpt from screener report indicating presence of meperidine.

Conclusions

The analysis of drugs of abuse is important in many different areas of our society, for example law enforcement. Current ana-lytical techniques use a two-step screening and confirmation procedure to achieve the required specificity and sensitivity required. This applica-tion note has investigated 48 of the more common drugs of abuse and their applicability for determi-nation through LC-TOF MS. It is not intended to be a comprehensive study of all possible components, but provides an excellent launching pad for the inclusion of the full gamut of possibilities

This application note shows the potential of the Agilent LC-TOF-MS as a single tool for both screening and confirmatory analysis, with quantitative information, often at levels below those currently analyzed for today. As a final example of the power of this technique, real-life coronial samples were evaluated under a screening protocol, with an additional seven components other than those previously reported by the coronial office detected.

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