

Delivering the Right Results

Analysis of Drug Facilitated Sexual Assault (DFSA) Drugs by Gas Chromatography-Time-of-Flight Mass Spectrometry

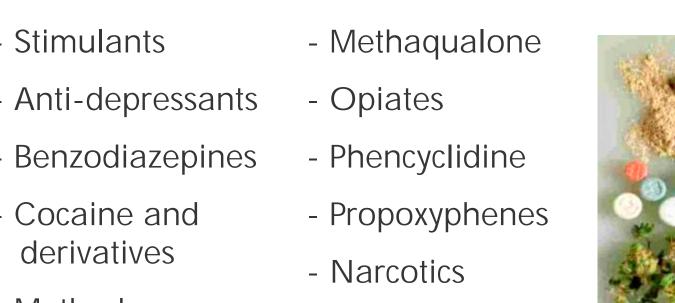
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OVERVIEW

- Anonymous case study urine samples collected from victims of alleged sexual assault were provided by the University of Miami's Miller School of Medicine. These samples were screened for potential drug facilitated sexual assault (DFSA) drugs by Gas Chromatography Time of Flight Mass Spectrometry (GC-TOFMS).
- The purpose of this research was to show that GC-TOFMS and mass spectral deconvolution can significantly reduce the data analysis time burden placed on analysts at forensic crime laboratories.
- Standards of known DFSA drugs were used to create a reference within the ChromaTOF® software. The reference was then incorporated into the data processing method and used to expedite the data analysis time for actual case study urine samples.
- The results of this study showed the ability of GC-TOFMS to provide fast and accurate screening of DFSA case study samples.
- Utilizing automated peak find algorithms, the data processing time for each sample was approximately 30 seconds. This is a significant time savings over existing methods of manual data analysis which often take an hour per sample.

Positive and negative control urine extracts along with twenty-four anonymous case study samples were received and screened for potential DFSA drugs. Urine samples were prepared at The University of Miami's Miller School of Medicine. Preparation included liquid-liquid extraction after addition of 4 internal standards. After extraction, the organic solvent was evaporated to dryness. Dried extracts were received and 50 µL of reconstitution solvent (70% isooctane: 20% dichloromethane: 10% ethanol) was added to each test tube prior to analysis. The extraction procedure above is utilized for basic drugs.

Some of the drug classes that are known to have been used to facilitate sexual assault are listed below.













EXPERIMENTAL

SAMPLE INTRODUCTION Agilent 7683B Autosampler Injection: 1µL

GC: Agilent 7890 Gas Chromatograph Column: 10 m x 0.18 mm x 0.18 µm Phenomenex ZB-DRG1

Inlet: Splitless, 60 s purge time, 20 mL/min @ 280°C Carrier Gas: He, Ramped Flow, 0.43 mL/min (hold 2 min) to 2 mL/min, ramped @10 mL/min GC Oven: 40°C (2 min hold), 50°C/min to 280°C, hold 10 min

MS Transfer Line: 280°C Run Length: 16.8 minutes (all analytes eluted in ~13 min)

MS: LECO TruTOF® HT

Ion Source: El at -70 eV Ion Source Temperature: 300°C Spectral Acquisition Rate: 20 spectra/s Mass Range: 30-500 m/z

Instrument Control and Data Review: ChromaTOF version 4.24



EXPERIMENTAL WORKFLOW

- Positive control urine standards were analyzed by GC-TOFMS. DFSA related drug compounds were identified using the NIST 2008 mass spectral library.
- A Reference was created using the ChromaTOF software. DFSA drug compounds detected in control standards were added to the reference. Retention time deviation and mass spectral match criteria were set to 3 seconds and 700, respectively.
- Case study samples were analyzed by GC-TOFMS.
- The Automated Peak Find capabilities of the ChromaTOF software were used to process data from twentyfour case study samples. The reference feature of the software was utilized to expedite analysis time.

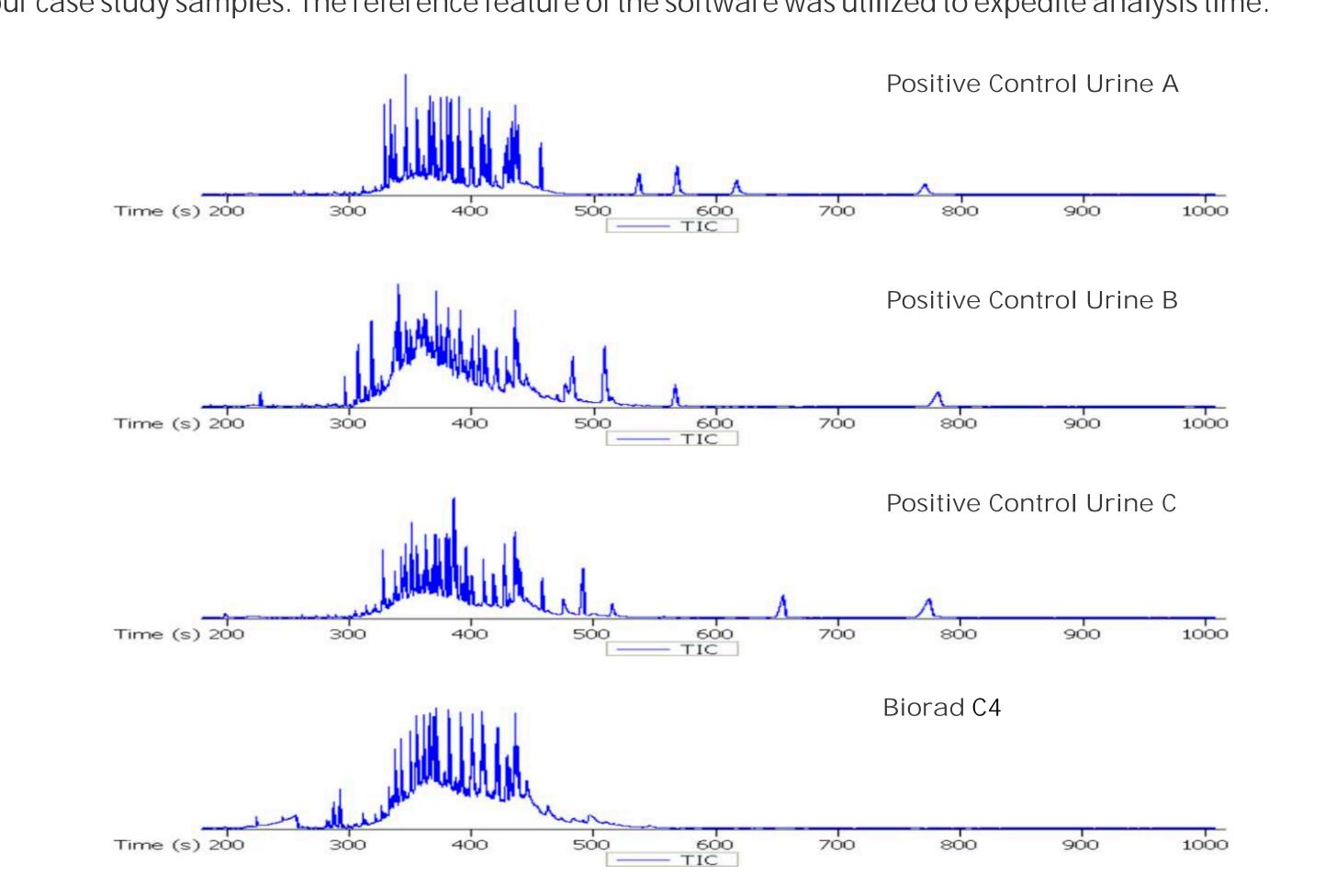


Figure 1. Stacked total ion chromatograms (TIC) for positive control urine standards. Notice the complexity of the urine sample matrix.

MS DECONVOLUTION EXAMPLE

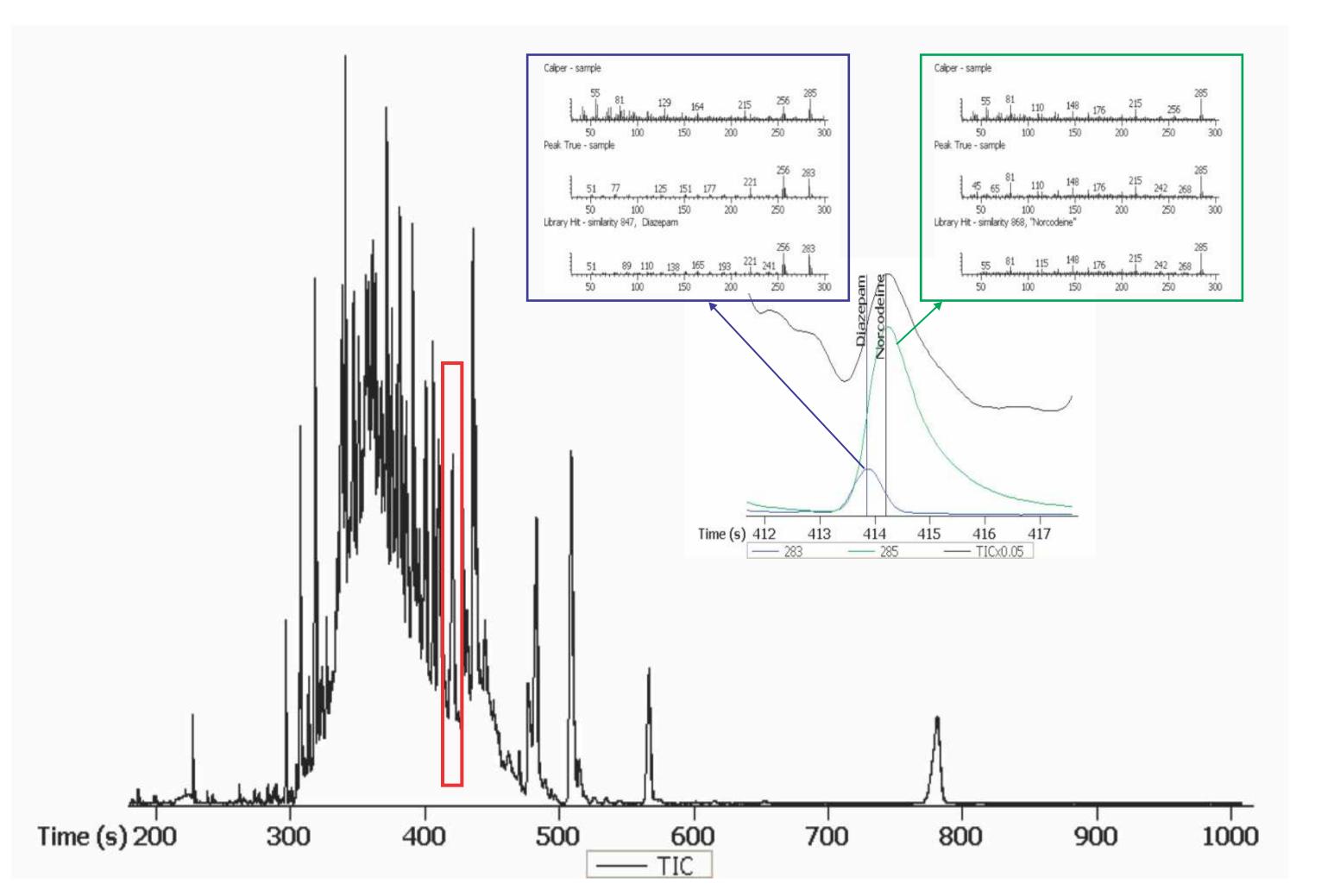


Figure 2. TIC for positive control urine Sample B. An expanded view of the region contained within the red rectangle is highlighted. This region of the chromatogram shows the ability of mass spectral deconvolution to successfully detect and identify coeluting analytes (diazepam and norcodeine) in the presence of heavy matrix interference. The Caliper (raw), Peak True (deconvoluted), and library match spectra are also shown in this figure.

Amphetamine	Tripelennamine	Cyclobenzaprine	Oxycodone	
Methamphetamine	Eddp	Desipramine	Nordiazepam	
n-propylamphetamine	Cyclizine	Desmethyldoxepin	Midazolam	
Phenmetrazine	Metoprolol	Bupivacaine	Prazepam	
Mda	Venlafaxine	Mirtazapine	Amoxapine	
Mdma	Carbinoxamine	Benztropine	Olanzapine	
MDEA	Methadone	2-(6-Methoxynaphthyl)propionamide	Promazine	
Amfebutamone	Methylephedrine	Naproxen	Hydroxyzine	
Demarol	Phenothiazine	Sertraline	Quinidine	
Ritalin	Propoxyphene	Desmethylsertraline	Zolpidem	
Pheniramine	Bromdiphenhydramine	Dehydroabietate	Clozapine	
Norfluoxetine	Dextromethorphan	Methylcodeine	Diltiazem	
Fluoxetine	Norvenlafaxine	Codeine	Desmethylclozapine	
Diphenhydramine	Amitriptyline	Ethylmorphine	Alprazolam	
Phencyclidine	Dextropropranolol	Valium	Verapamil	
Clonitazene	Doxepin	Hydrocodone	Noverapamil	
Lidocaine	Imipramine	Desalkylflurazepam	Strychnine	
Ketamine	Nortriptyline	Oripavine	Trazodone	
Doxylamine	Triphenidyl	Norpropoxypheneamide	Thioridazine	
Tramadol	Cocaine	Paroxetine		
Iminobibenzyl	Methaqualone	Loxapine		

Table I. DFSA drug compounds that were detected and identified in the positive control urine standards.

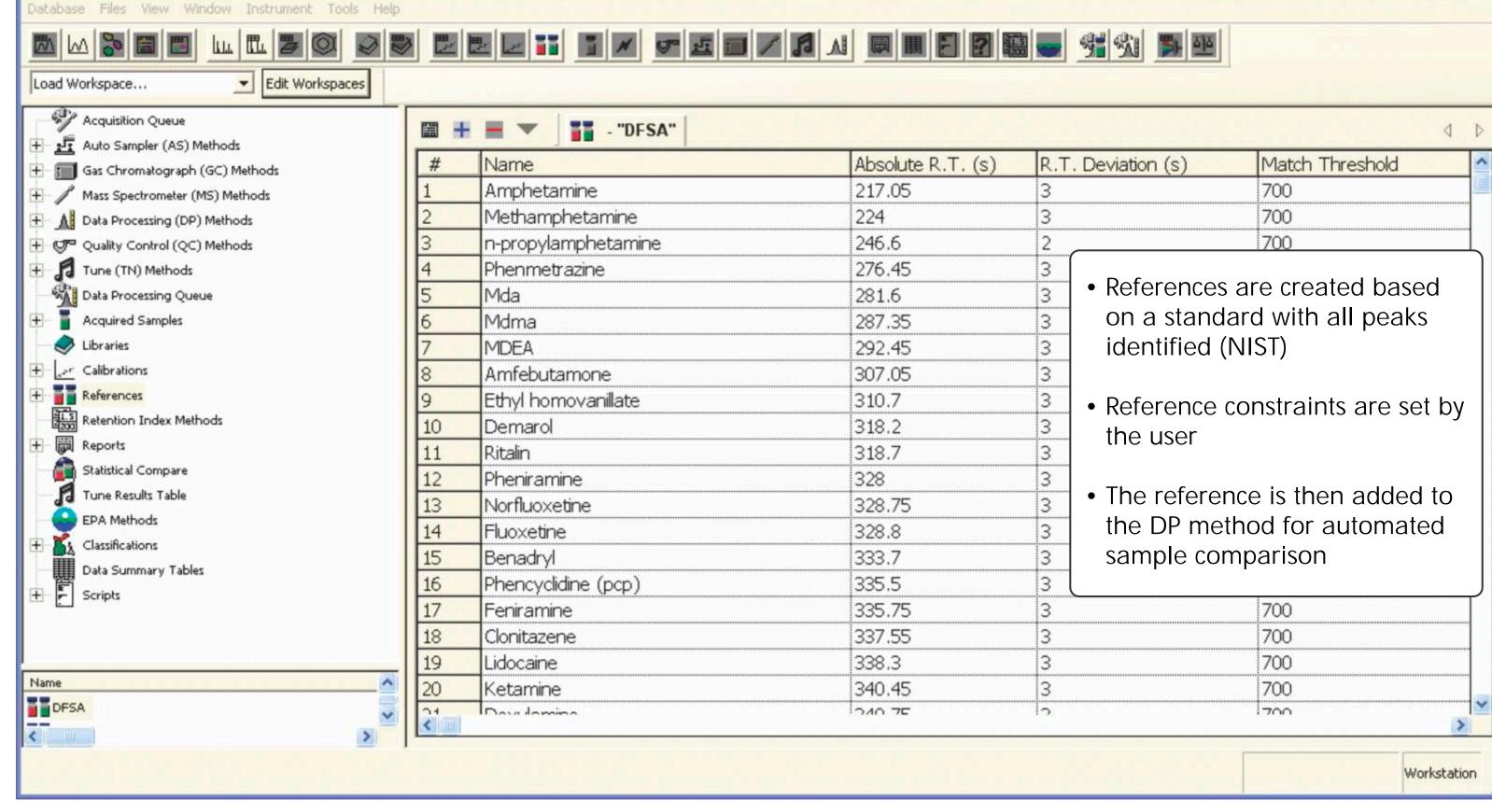


Figure 3. This figure shows an example of the Reference table within the ChromaTOF software.

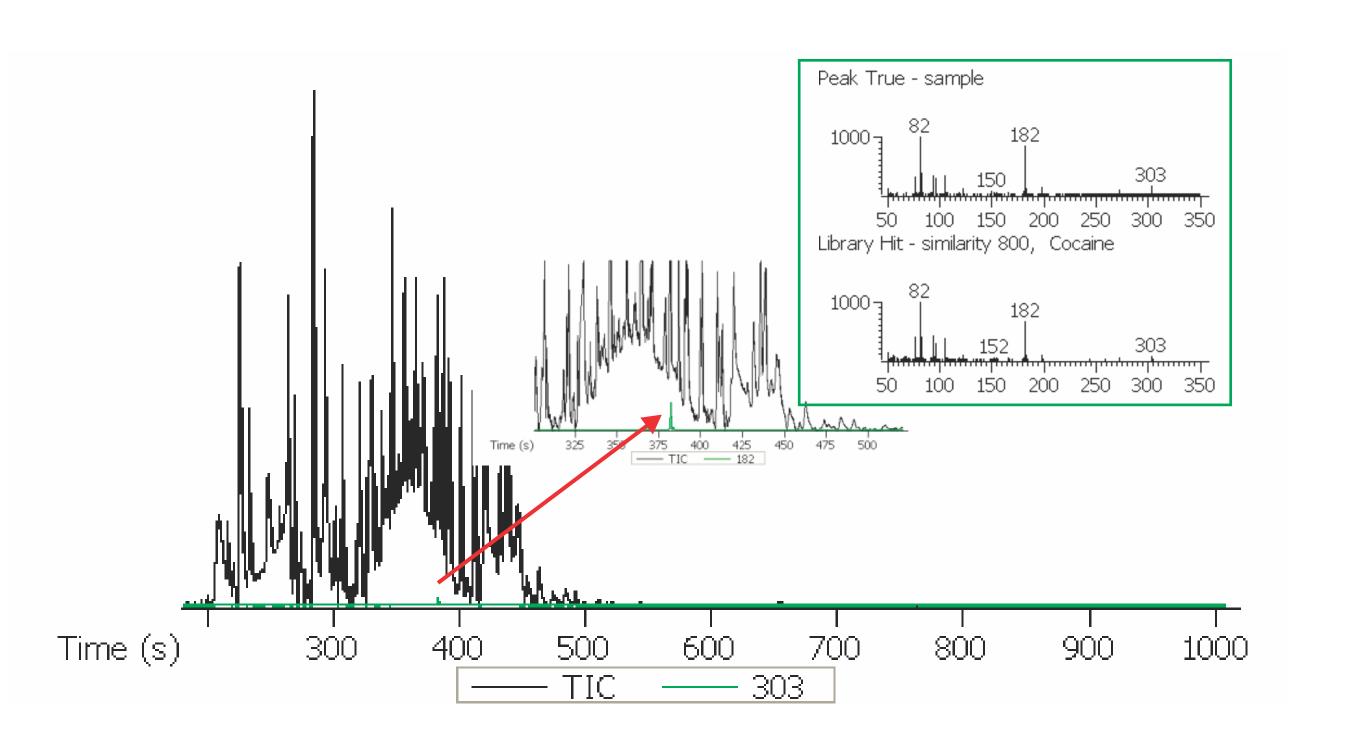


Figure 4. TIC for case study Sample 18. An expanded view of the region near 380 seconds shows the ability of Automated Peak Find algorithms to detect and identify cocaine in the presence of high urine matrix interference. The Peak True (deconvoluted) and library match spectra showing a successful NIST library match are also included in this figure.

RESULTS CONTINUED

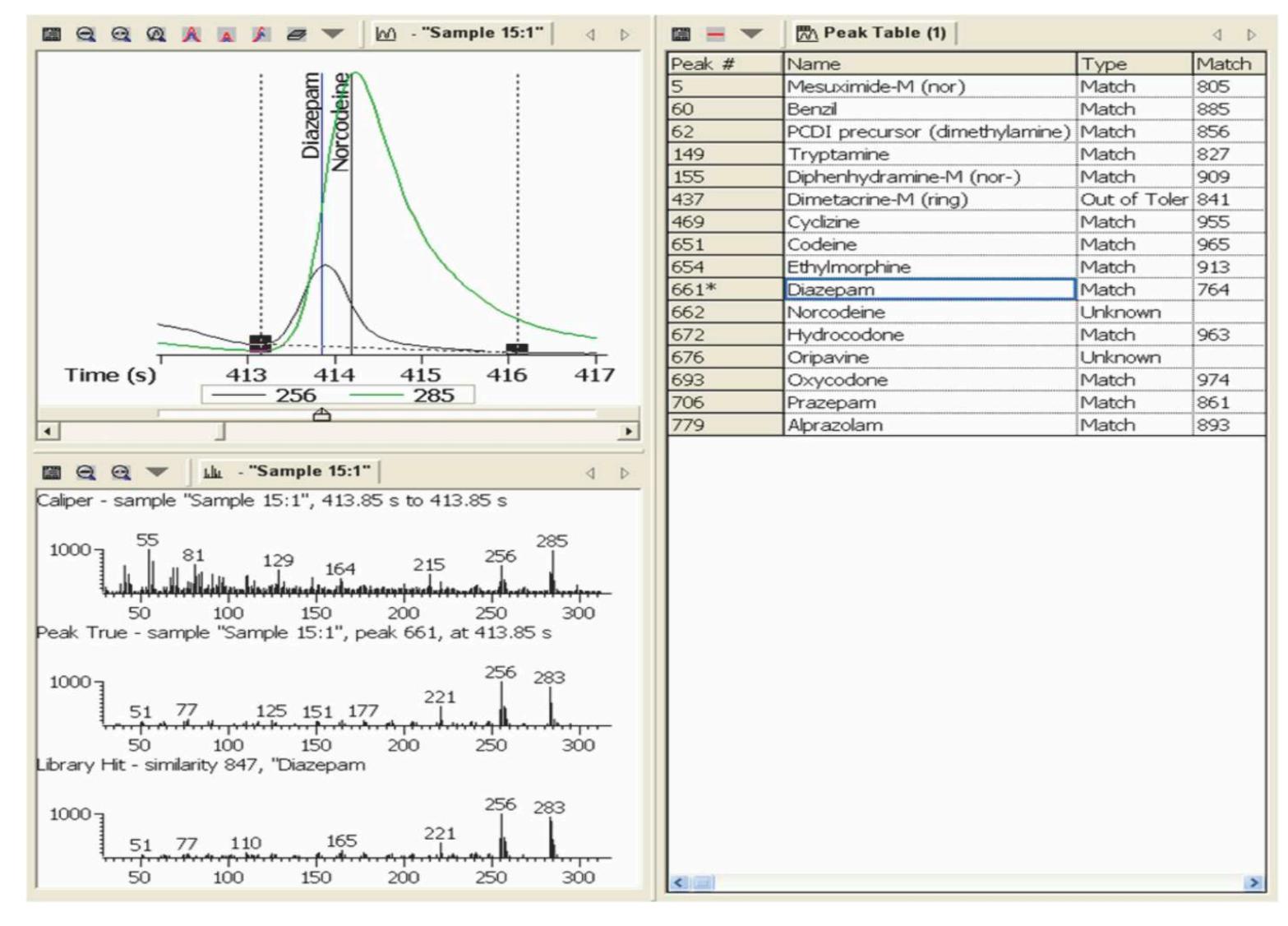


Figure 5. This figure shows an example of results obtained using references within the ChromaTOF software. The example shows the detection and identification of both targeted (diazepam) and non-targeted (norcodeine) compounds. Compounds that were identified in both the sample and standards are designated as a "Match" in the peak table while compounds only present in the sample are designated "Unknown".

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
*NDD	Paroxetine	*NDD	Quinine	Quinine	*NDD
Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
*NDD	Cocaine	Quinine	*NDD	Alprazolam	Mda
-	-	-	-	-	Mdma
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18
Oxycodone	Lidocaine	Diphenhydramine	Cocaine	Amfebutamone	Amphetamine
Alprazolam	Cocaine	Codeine	Ecgonine ME	Cocaine	Methamphetamine
Oxymorphone	Ecgonine ME	Diazepam	Levamisole	Diltiazem	Cocaine
Diltiazem	Cinnamoylcocaine	Hydrocodone	Benzocaine	Ecgonine ME	MDMA
-	Hydrocodone	Oripavine	Alprazolam	Trazadone	Ecgonine ME
-	Oxycodone	Oxycodone	Tetracaine artifact	Levamisole	Levamisole
-	Cocaethylene	Oxydiazepam	-	Valpromide	CocaineMetabolite
-	Oxymorphone	Alprazolam	-	Alprazolam	Methylphenidate
-	Levamisole	oxymorphone	-	Cocaine Metabolites	-
-	-	-	-	Lidocaine artifact	-
Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24
Diphenhydramine	Quinine	Diphenhydramine	*NDD	Diphenhydramine	*NDD
Cocaine	Chlorpheniramine	Dimetacrine artifact	-	-	-
Diltiazem	-	-	-	-	-
Ecgonine ME	-	-	-	-	-
Benzylecgonine	-	-	-	-	-
Levamisole	-	-	-	-	-
Cocaine Metabolites	-	-	-	-	-
		*NDD = No [Drugs Detected		

Table II. The table shows the results for the analysis of the twenty-four case study urine samples. Compounds identified that could be potentially used as DFSA drugs were added to this table. Many other compounds were also detected in the urine samples, but were not reported in this table. Drugs from several classes including benzodiazepines, antidepressants, antihistamines, hallucinogens, and opioids were among those detected in a portion of the

DISCUSSION

- There are several benefits to using time-of-flight mass spectrometry (TOFMS) for applications such as DFSA drug screening. These include, but are not limited to:
- The ability to collect full mass range spectra without sacrificing speed or sensitivity can be beneficial for screening samples that contain both target and non-target analytes. As the list of drugs being used to facilitate sexual crimes continues to grow, the analyst must have the ability to effectively detect and identify non-targeted compounds.
- Low pg detection limits are possible with full mass range data acquisition. This is important since many of the DFSA drug compounds have a short half-life. In addition, victims of sexual assault often wait several days before coming forward. Therefore, these compounds can be present in urine samples at trace levels.
- Unlike scanning mass analyzers, the spectral continuity provided by TOFMS allows for optimal performance of mass spectral deconvolution algorithms. Deconvolution provides the ability to detect and identify coeluting compounds that are often in the presence of matrix interference.

CONCLUSIONS

- The results of this research displayed the ability of GC-TOFMS to effectively screen case-study urine samples for the presence of potential DFSA drugs.
- The use of mass spectral deconvolution algorithms can increase sample throughput by reducing the need for total chromatographic resolution which allows shorter run times.
- Of the 24 samples analyzed, 16 contained drugs that would be considered potential DFSA drugs. This represents 66.7% of the samples analyzed and correlates well with the literature. The literature cited states that approximately two-thirds of samples submitted test positive for DFSA type compounds.
- ChromaTOF's reference function, which allows both spectral match and retention time criteria to be set, can reduce the likelihood of false positives and/or misidentifications.
- The use of Automated Peak Find algorithms and mass spectral deconvolution can dramatically decrease the time burden placed on analysts charged with data review for such samples. The current manual data processing protocol performed by the university requires approximately one hour per sample. The use of Automated Peak Find and mass spectral deconvolution provided data analysis times of approximately 30 seconds per sample.

- Chip Walls and Dr. Lisa Reidy at The University of Miami's Miller School of Medicine
- Phenomenex Corporation for providing the capillary GC column used for these analyses
- 2010 ASMS Organizing Committee

REFERENCES

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