Rapid, Sensitive Screening for Analytes Implicated in Drug-Facilitated Crimes (DFC) using Exact Mass LC-oa-ToF.

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Abstract

Over the last few years, DFC *e.g.* sexual assault and robbery, have been increasing. The drugs implicated in DFC are wide-ranging and include illegal drugs, prescribed medications and 'over-the-counter' preparations. Owing to the diversity of the analytes, a variety of analytical techniques are usually required, including immunoassay, GC-MS, GC-FID and LC-UV. Our aim was to develop a simple, generic method to screen for these analytes using a single analytical technique based on LC-oa-ToF.

Where available, drug standards were used to create a reference library of retention time and spectral data. Analytes were separated using an ACQUITY™ HSS C18 column maintained at 30°C. Data was collected using two different voltages within the source region. Where reference material was unavailable, calculated theoretical monoisotopic masses were utilised.

Control urine and blood samples were spiked with mixed drug standards (1–500 ng/mL) and subjected to both qualitative and quantitative analysis using LC-oa-ToF. For the qualitative screen, data was processed using ChromaLynx™, a software program which automates chromatographic deconvolution followed by comparison of the spectral information with the reference library. Additional confidence was achieved using retention time and by measurement of the proximity of the actual acquired mass to the theoretical exact mass; a mass accuracy within 5 ppm was considered acceptable. Quantitative analysis was performed by spiking biological matrices with mixed standards. Following analysis, exact mass chromatograms were integrated.

We have developed a simple sensitive screening method for > 60 of the analytes; in most cases, limits of detection were better than 10 ng/mL and met the recommendations made by the Society of Forensic Toxicologists (SOFT) for the analysis of DFC drugs.

Introduction

Drug-facilitated crime (DFC) is a relatively new term for an old practice. According to legend 'Slipping a Mickey' relates to a practice made infamous by Chicago saloon owner Mickey Finn in the late 1890's. Finn secretly laced the drinks of his patrons, with drugs, in order to knock them unconscious. After which, he and his wife would strip them of their valuables. Victims would awaken later - remembering nothing !

Over the last few years DFC has been increasing. DFC includes robbery and assault (including sexual assault or drug-facilitated sexual assault: DFSA). A few years ago the Society of Forensic Toxicologists (SOFT) formed a committee

Figure 1: The age-old practice of 'Slipping a Mickey'; the surreptitious administration of a drug for criminal gain.

to address various aspects of DFSA and produced a list of compounds which have, or could be, implicated in DFSA in the United States[1]. They include illegal drugs, prescribed medications and 'over-the-counter' preparations.

Owing to the diversity of the analytes involved, a variety of analytical techniques are usually required, including immunoassay, GC-MS, GC-FID and LC-UV. Our aim was to develop a simple, generic method to screen for these analytes using a single analytical technique based on LC/ToF.

Methods

Reference library

A reference library was created for the compounds of interest which included those identified by the SOFT DFSA committee, metabolites and other analytes which are commonly prescribed in Europe. Where reference material was available (~70 compounds), standard exact mass spectra were acquired using a low aperture voltage to generate exact mass spectra and retention time (RT) information. Spectra were also acquired at a higher aperture voltage to generate fragment ions by collision-induced dissociation (CID) within the source. All spectra were quality checked using I-FIT software, which compares measured and theoretical isotope ratios[2].

Where reference material was unavailable (~30 compounds) theoretical spectra were added to the library using the elemental formula.

Chromatography

Acquity UPLC®	
Column:	Acquity HSS C_{18} (2.1 x 100mm, 1.88µm)
Column temp:	30°C
Injection vol:	10µL
Solvent A:	0.05% formic acid
Solvent B:	Methanol

Mass Spectrometry

Mass spectrometer:	Waters Micromass LCT, Premier
Ionisation mode:	Electrospray +ve
Capillary voltage:	3kV
Aperture 1:	10 and 50V
Mass range:	50—600Da
Resolution:	10,000 (W-mode)
Lockspray™ Leucine enkephalin reference:	$[M+H]^+$ = *m/z* 556.2771

Sample Preparation

Urine samples were subjected to liquid:liquid extraction (LLE) under acidic and basic conditions as described below.

Acidic: 125µL acetate buffer (pH 3.5) was added to 250 µL urine. After mixing, samples were extracted using 750µL solvent mix (DCM:ether:hexane containing 0.5% isoamyl alcohol). Samples were again mixed for 2 min. followed by centrifugation at 3000rpm for 5 min.

Basic: As above but using borate buffer (pH 9.5).

Supernatants from the acidic and basic extractions were pooled before drying, followed by reconstitution in mobile phase prior to LC-MS analysis.

Data processing

Data was processed automatically using ChromaLynx™ software (Waters).

Results and Discussion

Where reference material was available, pure standards were injected and mass spectra were acquired, in full scan mode, at low and high aperture voltages to create a library which included exact mass of the molecular ion, fragment ions and RT. Figure 2 shows the library data for MDMA which elutes at 2.7 min.

The chromatogram obtained on injection of a urine sample, spiked with morphine, hydromorphone and 7-aminoclonazepam (7-AC) is given in Figure 3. These data clearly demonstrate the benefits of exact mass and the ability to differentiate from other compounds of similar mass. Figure 3A shows the extracted TIC for the nominal mass *i.e. m/z* 286. All 3 compounds are clearly visible. Figure 3B shows the same data but with the extracted exact mass chromatogram for 7-AC ($C_{15}H_{12}N_3OCl$) *i.e. m/z* 286.0747 ± 5 mDa. The exact mass for both morphine and hydromorphone is *m/z* 286.1443 ($C_{17}H_{20}NO_3$).

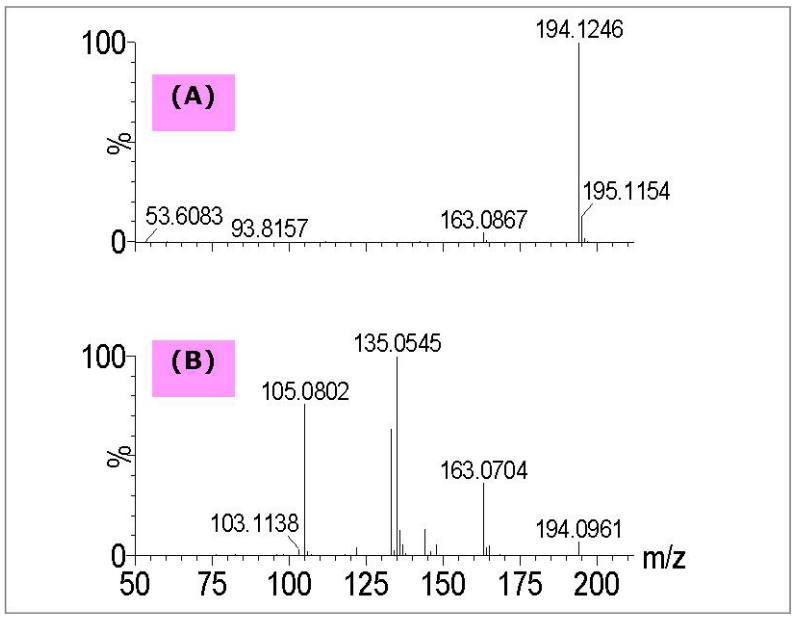


Figure 2: The library entries for MDMA (Ecstasy) at aperture settings of 10 (A) and 50V (B) respectively.

Data processing was achieved using ChromaLynx™ which automatically deconvolutes the chromatogram. The most intense ions (up to a max. of 8) are extracted using a defined mass window (either in mDa or ppm). The resultant data was then searched against the newly created DFC library.

Figure 4 shows a typical result for an authentic urine sample. Dihydrocodeine (DHC), acetaminophen and EDDP were identified by the new LC-oa-ToF screening method. These findings were consistent with the data obtained using the conventional screening methods *i.e.* HPLC-UV and immunoassay.

Library hits were also scored according to the agreement between measured exact masses in the acquired spectrum and the theoretical mass calculated from the elemental composition of the compounds in the library. Additional confidence of identified analytes was achieved using I-FIT (Figure 4B and C).

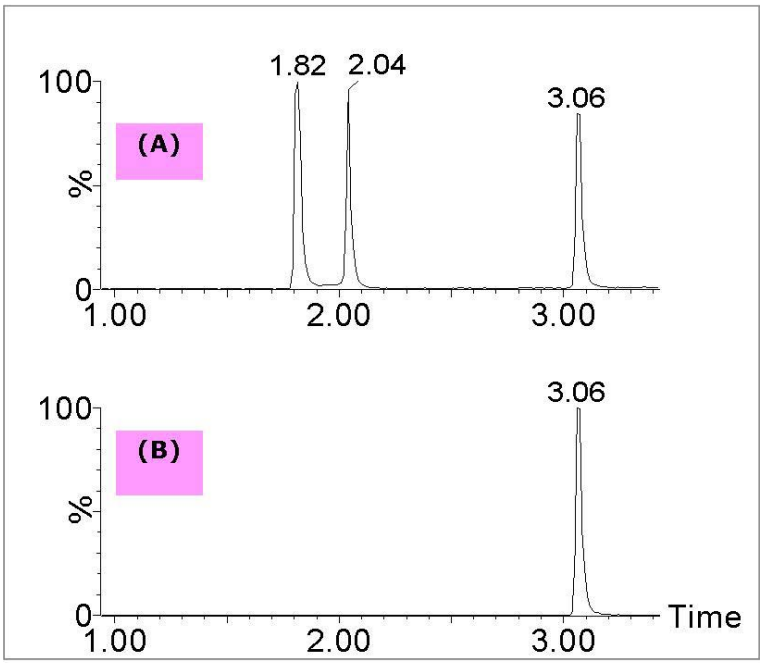


Figure 3: Extracted mass chromatograms for the nominal mass (Figure 3A) and exact mass of 7-AC (Figure 3B).

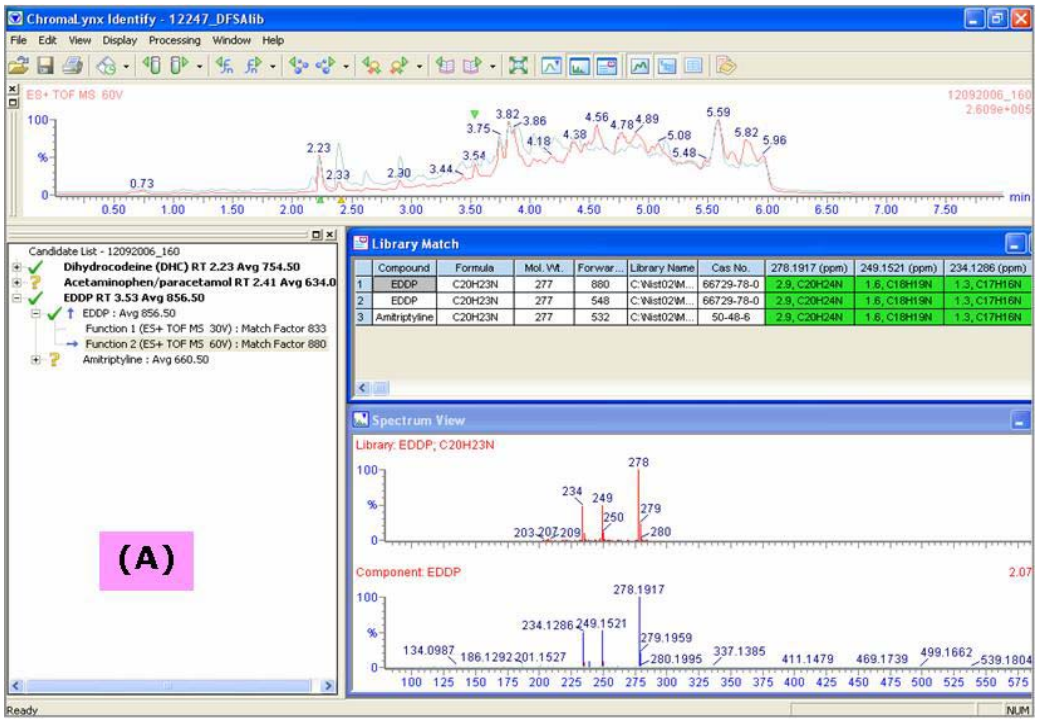


Figure 4A: Results for an authentic urine sample following ChromaLynxTM processing. Figure 4A shows the list of proposed candidates and the average match to the library. The spectrum view allows direct comparison of acquired spectra with library data.

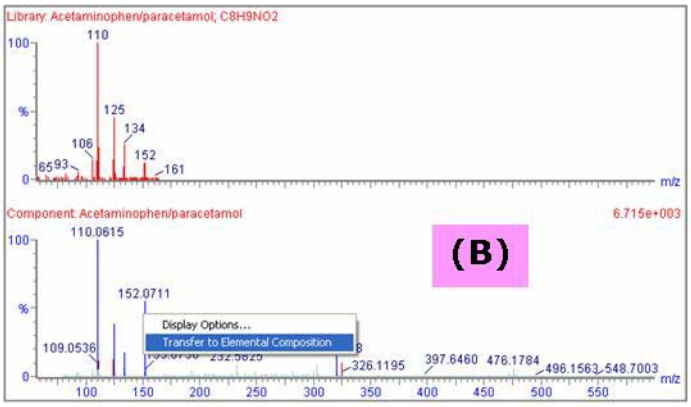
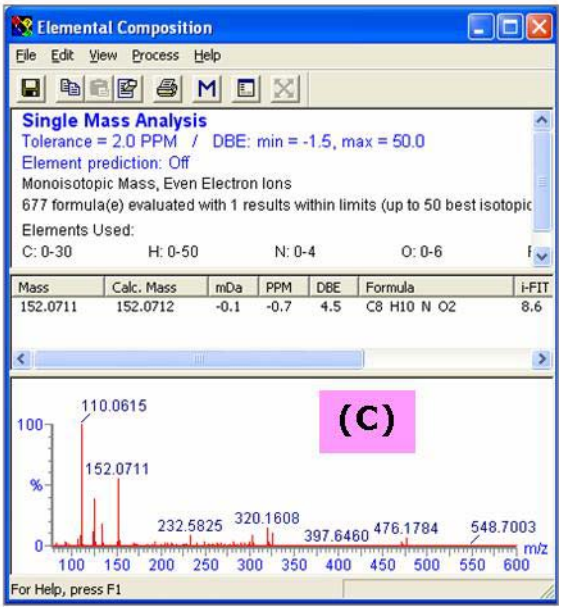


Figure 4B shows the spectral data for acetaminophen which was also identified in this sample. The results for the proposed candidate 'acetaminophen' was submitted for elemental composition. Figure 4C shows the proposed elemental composition for a m/z 152.0711; the result was $C_8H_{10}NO_2$ which corresponds to that of acetaminophen.



Conclusion

- A spectral library has been created for over 100 analytes which have been/may be implicated in DFC.
- The method uses LC/time-of-flight (ToF) mass spectrometry.
- Where reference material was available (~70 analytes) spectra were collected at low and high voltages to generate fragments.
- Where reference material was available identification was based on a combination of exact mass of the molecular ion, fragment ions and RT.
- Where reference material was unavailable (~30 analytes) identification was based on exact mass.

- Data was automatically processed using ChromaLynx^{TM} software. which effectively detects chromatographic peaks, even in complex matrices.
- The exact masses of proposed candidates were compared and scored against theoretical mass.
- Additional confidence of identification for any proposed candidates was achieved using I-FIT software.

References

[1] M LeBeau, W Andollo, WL Hearn, R Baselt, E Cone, B Finkle, D Fraser, A Jenkins, J Mayer, A Negrusz, A Poklis, HC Walls, L Raymon, M Robertson and J Saady. J Forensic Sci. 44: 227-230 (1999).

[2] K Hobby and B Leavens. Poster Presentation, IMSC, Prague 2006.

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