Targeted and Non-Targeted Screening for Drugs with High Confidence based on High Resolution and Accurate Mass LC-MS/MS

PeakView™ Software with the XIC Manager

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Overview

The high resolution and accurate mass AB SCIEX TripleTOF™ 5600 LC/MS/MS system was used to screen for drugs from both forensic equine and forensic toxicology urine, and to quantify drug compounds with excellent accuracy and high reproducibility. Fast Information Dependent Acquisition (IDA) MS/MS spectra were used to additionally confirm the identity of detected compounds based on mass spectral library searching. The acquired full scan MS and MS/MS data can further be used to retrospectively mine data to identify non-targeted and unexpected compounds. An example is also given to demonstrate true unknown screening by the identification of the contents of an unknown pill.

PeakView™ software with the XIC Manager add-in was used for targeted and non-targeted data processing. The XIC Manger consists of a table for defining a list of masses or formula to generate extracted ion chromatograms (XIC), and the ability to review results is based upon retention times, accurate mass, isotopic pattern and MS/MS library searching.

Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of drugs. Triple quadrupole based mass analyzers operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results, but are limited to targeted screening only.

With an increasing demand for retrospective and non-targeted analyses, full scan mass analyzers are gaining popularity. The AB SCIEX TripleTOF™ 5600 LC/MS/MS system using the Accelerator TOF™ Analyzer and continuous recalibration for EasyMass™ accuracy allows the unmatched speed in acquisition of highly sensitive full scan MS spectra with high resolution and mass accuracy allowing accurate and reproducible quantification of targeted compounds. In addition, the unmatched speed of the TripleTOF™ 5600 LC/MS/MS

system allows 20-30 Information Dependent Acquisition (IDA) MS/MS spectra to be collected for compound identification based on MS/MS library searching. This allows the capability to perform targeted and non-targeted screening in a single LC-MS/MS run; reducing the possibility of missing the detection of compounds.

The complexity of such data requires powerful data mining tools. The XIC Manager can be used for target and non-targeted processing of high resolution MS and MS/MS data allowing for screening and identification with the highest confidence based on retention time, accurate mass molecular ion, isotopic pattern and automatic MS/MS library searching.

The acquired full scan MS data can further be used to retrospectively mine data for non-targeted compounds. The information of the accurate molecular ion, isotope pattern and detected fragment ions can be used to characterize the structure of unexpected compounds.

Here we present examples of using the AB SCIEX TripleTOF™ 5600 system for the screening and identification of drugs in urine. Features of the XIC Manager for targeted and non-

targeted screening are highlighted. Also presented is an example in which the TripleTOF™ 5600 LC/MS/MS system in combination with PeakView™ software and XIC Manager allows the identification of the contents of an unknown tablet.

Method Details

- Spiked equine urine samples underwent a β-glucuronidase hydrolysis, followed by a liquid/liquid extraction prior to LC-MS/MS analysis.
- Forensic toxicology samples were treated separately and were diluted 10 times with the aqueous mobile phase as a simplified means to aid in chromatographic peak shape optimization and reduce possible matrix interferences that may cause ion suppression effects.
- In the case of an unknown tablet, the pill was simply broken down in water, filtered and injected on to the LC-MS/MS.
- A Shimadzu UFLC$_{XR}$ system with a Restek 5 µm 60 Å, PFP Propyl Column, 50 x 2.1 mm was used, with a 7.5 min gradient of water and acetonitrile with ammonium formate buffer. A 17.5 min gradient was used for the forensic toxicology urine drug screening.
- The injection volume was set to 2 µL.
- The AB SCIEX TripleTOF™ 5600 system was operated with Turbo V™ source and Electrospray (ESI) probe.
- An IDA method was used containing a TOF-MS survey of 70 ms and up to 30 dependent TOF-MS/MS scans of 25 ms accumulation time; sufficient to acquire enough data points across the LC peak to allow reproducible and accurate quantitation. Collision Energy (CE) of 35 V and Collision Energy Spread (CES) of 15 V were used.

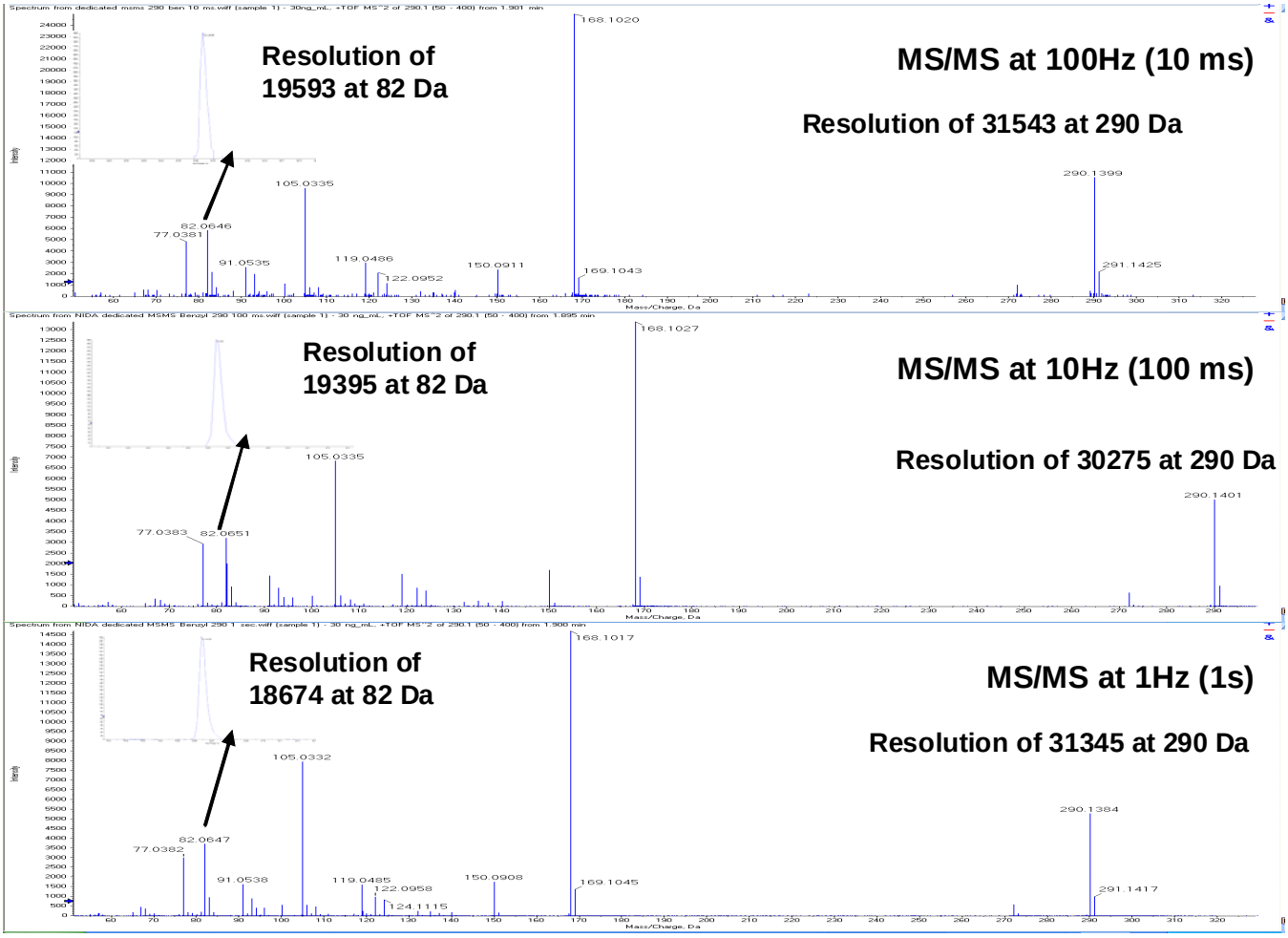

Figure 1. Highest resolution at every scan speed over the entire mass range with stable mass accuracy of ~ 1 ppm

Results and Discussion

The AB SCIEX TripleTOF™ 5600 system with Accelerator TOF™ Analyzer provides high resolution of up to 40,000, dependent on the mass detected, and stable mass accuracy of ~ 1 ppm at fastest acquisition speed in both MS and MS/MS mode. Comparisons of MS/MS resolution for mass 290 and 82 Da from product ion spectra of the $[M+H]^+$ ion at *m/z* 290 for benzoylecgonine, acquired using different scan speeds between 1 and 100Hz, are shown in Figure 1.

Defining an XIC List and Processing Options in the XIC Manager for Targeted Screening

After opening a data file in PeakView™ software the XIC Manager can be launched from the 'Show' menu.

The table contains a number of columns with values that can be edited, including name, formula, adduct/modification, retention time, width and more. To define an XIC, a mass must be entered. This can be done by: directly editing the cell, having the software calculate the value based on formula, isotope, and adduct provided, or by pasting values from a spreadsheet. The generated XIC list can be saved for future processing (Figure 2).

A number of processing and display settings can be adjusted in the 'Options' dialog. This includes intensity, signal-to-noise threshold, and confidence settings for mass error, retention time, isotope matching and library searching (Figure 3).

To start data processing, simply click the Show XIC button in the lower right of the table. The XIC Manager will automatically calculate XICs, perform compound identification and display results.

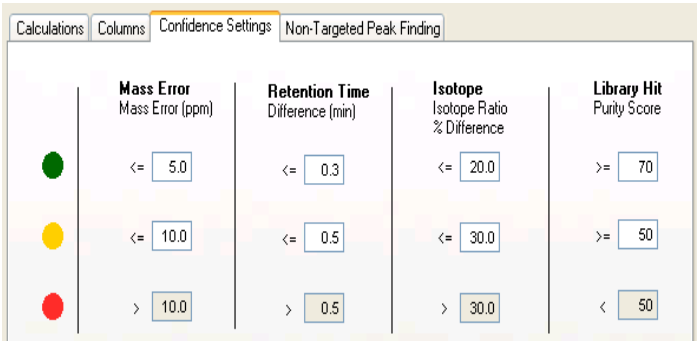

Figure 3. Confidence settings for compound identification

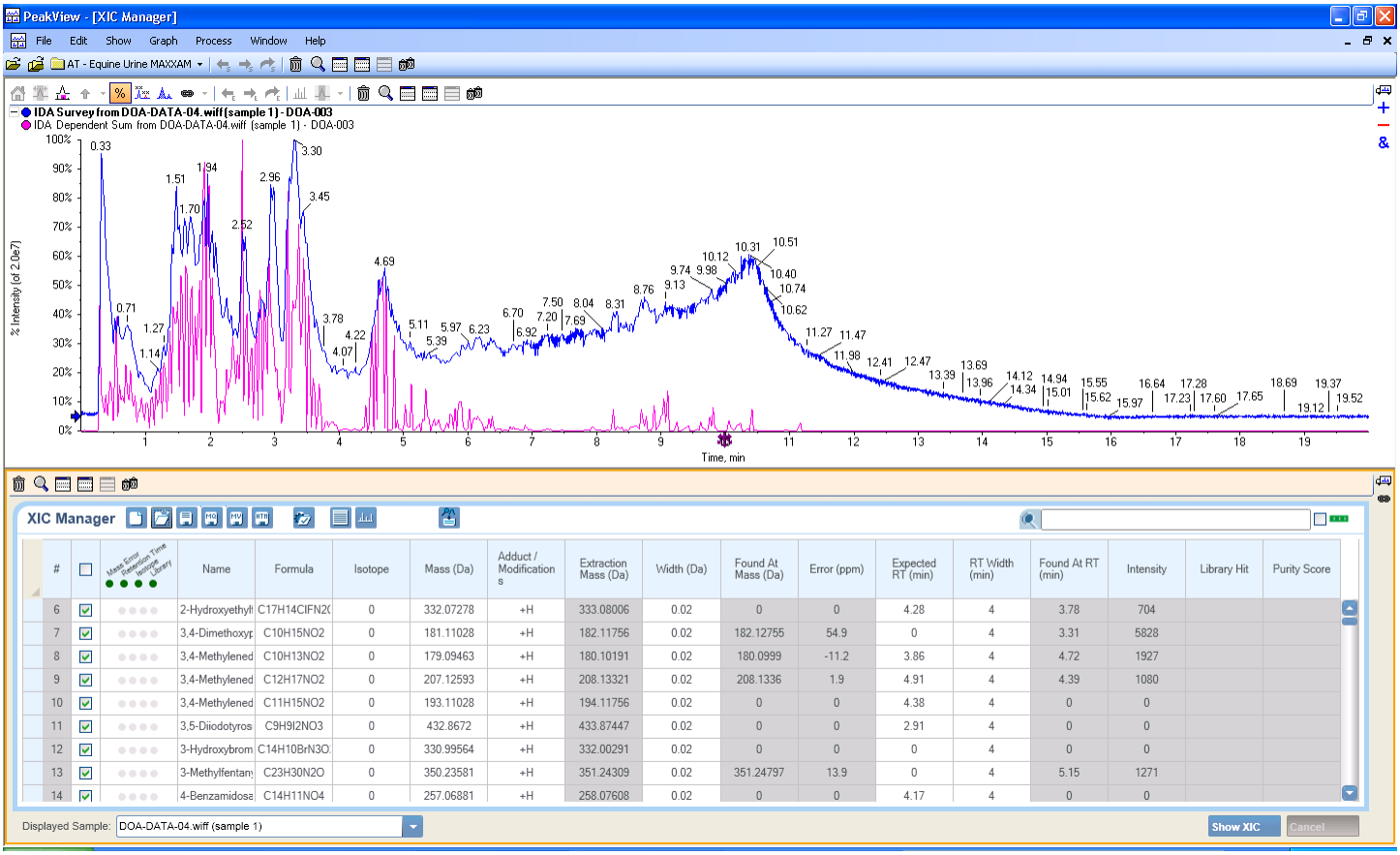

Figure 2. PeakView™ software with the XIC Manager add-in; the bottom pane shows an XIC table loaded for target drug screening and identification

Results Display in the XIC Manager

After processing, the results are displayed to show the mass error (ppm or mDa), found at retention time and library search results. XICs above a defined intensity threshold are highlighted in green and confidence data for compound identification is visualized using traffic lights. The example data presented in Figures 4, 5 and 6 shows the automatically generated XICs for each targeted analyte and compared against the user defined threshold.

Figure 4 shows XICs of drug compounds that were identified from a hydrolysed and liquid/liquid extracted spiked equine urine sample using information of the isotopic pattern of the detected molecular ion, retention time and accurate mass. Detection of other drugs that were in the forensic library was further confirmed using the MS/MS spectra searched against the MS/MS library. The IDA triggered accurate mass MS/MS spectrum of a drug compound is overlaid with the matched library spectrum as a visual indication of the closeness of the match. The bottom right panel of Figure 4 shows an example of the spectral overlay comparison of library versus equine urine sample of isoxsuprine. The sample spectrum is represented as the blue trace and the library is the grey trace.

Figure 5 represents another example of data processed using XIC Manager in PeakView™ Software for another spiked equine urine sample. In this example Atropine, Indapamide, Reserpine, Caffeine, Isoxsuprine, Morphine, Clenbuterol and Lidocaine were identified by isotopic pattern of the detected molecular ion, retention time and accurate mass as well as a library match. Other compounds not in the library were reliably identified by isotopic pattern of the detected molecular ion, retention time and accurate mass. All compounds were quantifiable from the same injection, Lidocaine shown as an example at 1.7 ng/mL..

The example data represented in Figure 6 is from a forensic toxicology sample diluted 10 fold prior to the LC-MS/MS analysis. The drugs Lidocaine, Diphenhydramine, Morphine and Norfentanyl were identified and quantified using retention time, accurate mass MS and MS/MS information.

Example calibration lines, generated using MultiQuant™ software, of two selected drug compounds are represented in Figure 7.

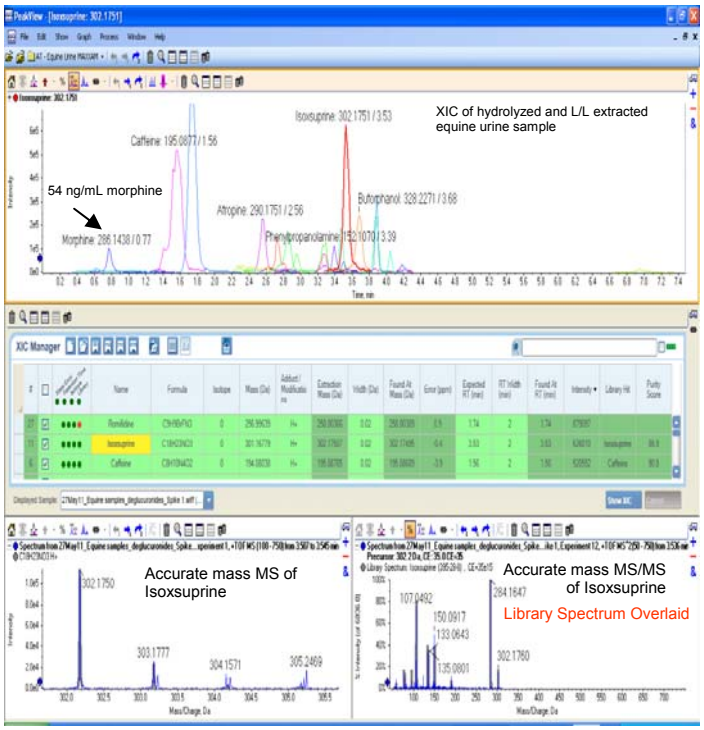

Figure 4. The drugs Atropine, Caffeine, Isoxsuprine, Morphine, Indapamide, Diclofenac, Meclofenamic Acid and Clenbuterol were identified using retention time, mass error, isotope pattern and confirmed with MS/MS library matches. Other spiked drugs that were not in the library were identified by retention time, mass error and isotope pattern. All drugs were also quantified; the example given is morphine (54 ng/mL).

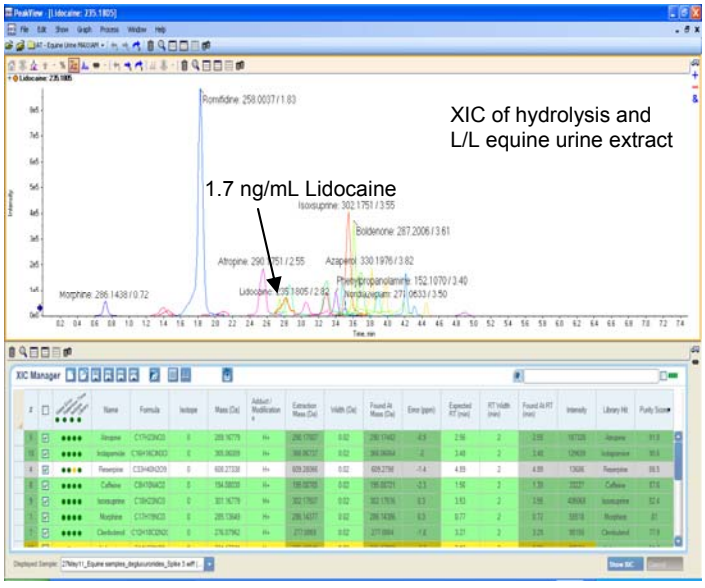

Figure 5. The drugs Atropine, Indapamide, Reserpine, Caffeine, Isoxsuprine, Morphine, Clenbuterol and Lidocaine were identified using retention time, mass error, isotope pattern and confirmed with MS/MS library matches. Other spiked drugs that were not in the library were identified by retention time, mass error and isotope pattern. All drugs were also quantified; the example given is lidocaine (1.7 ng/mL).

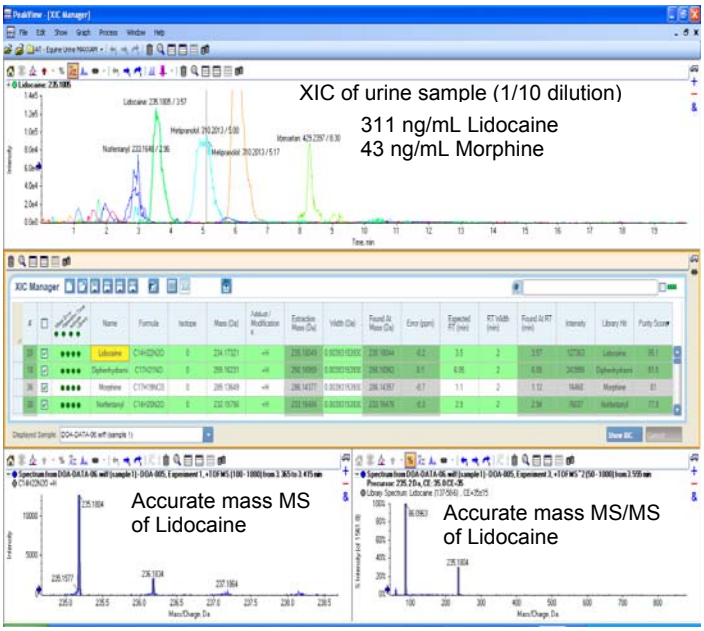

Figure 6. The drugs Lidocaine, (Diphenhydramine, Morphine and Norfentanyl) were identified and quantified using retention time, accurate mass MS and MS/MS information, in a forensic toxicology urine sample.

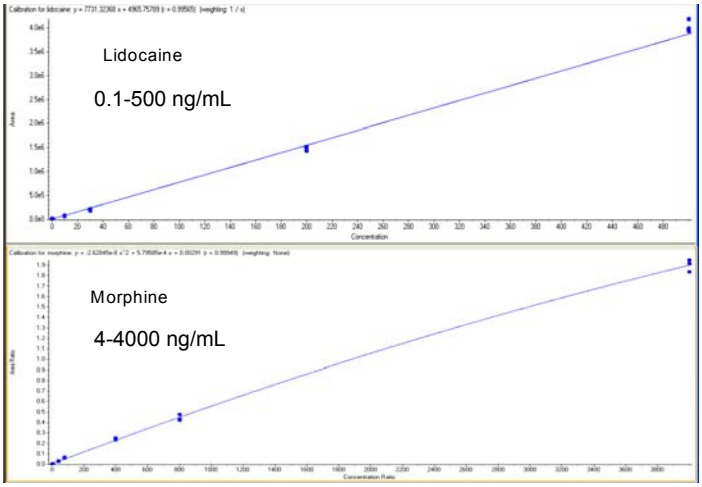

Figure 7. Example calibration lines of two selected drug compounds

Retrospective Data Mining

The acquisition of full scan MS data enables retrospective data mining of initially non-targeted analytes. For example the urine sample in Figure 8 was tested positive for 5 drugs, cocaine, ecgoninemethylester, benzoylecgonine, codeine and citalopram. However, 5 additional compounds were also identified using the orthogonal technique that was not included in the original targeted screening using LC-MS/MS. The XIC manager was used to retrospectively screen for paracetamol, theobromine, secbutabarbital, cotinine and codeine, providing confirmation based on the presence of an accurate mass molecular ion and the characteristic fragment ions in MS/MS mode.

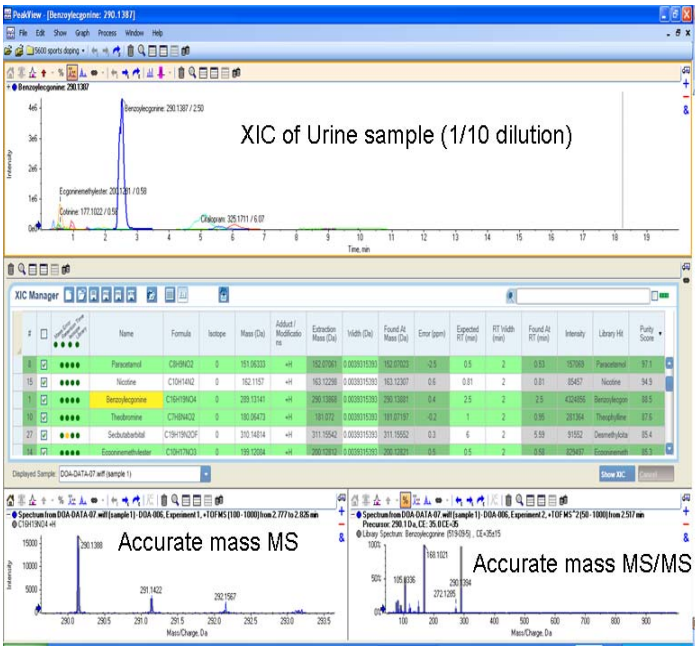

Figure 8. The drugs Benzoylecgonine, Cociane, Ecgoninemethylester, Codeine and Citalopram were identified using retention time, accurate mass MS and MS/MS information, in a urine sample. Paracetamol, Theobromine, Secbutabarbital, Cotinine and Serotonin were retrospectively confirmed.

For true unknown screening, the 'Enhance Peak Find'  feature of the XIC manager can be used to extract the most intense peaks and Formula Finder is used to identify elemental compositions that match the accurate MS data. This work flow is represented in Figure 9 in which an unknown tablet was broken down in water, filtered and injected on to the LC-MS/MS. The Enhance Peak Find listed an accurate mass of 296. Formula finder was used to determine elemental compositions that match the accurate mass and this aided in the determination of the tablet's main constituent being diclofenac. A library match also aided in the confident identification.

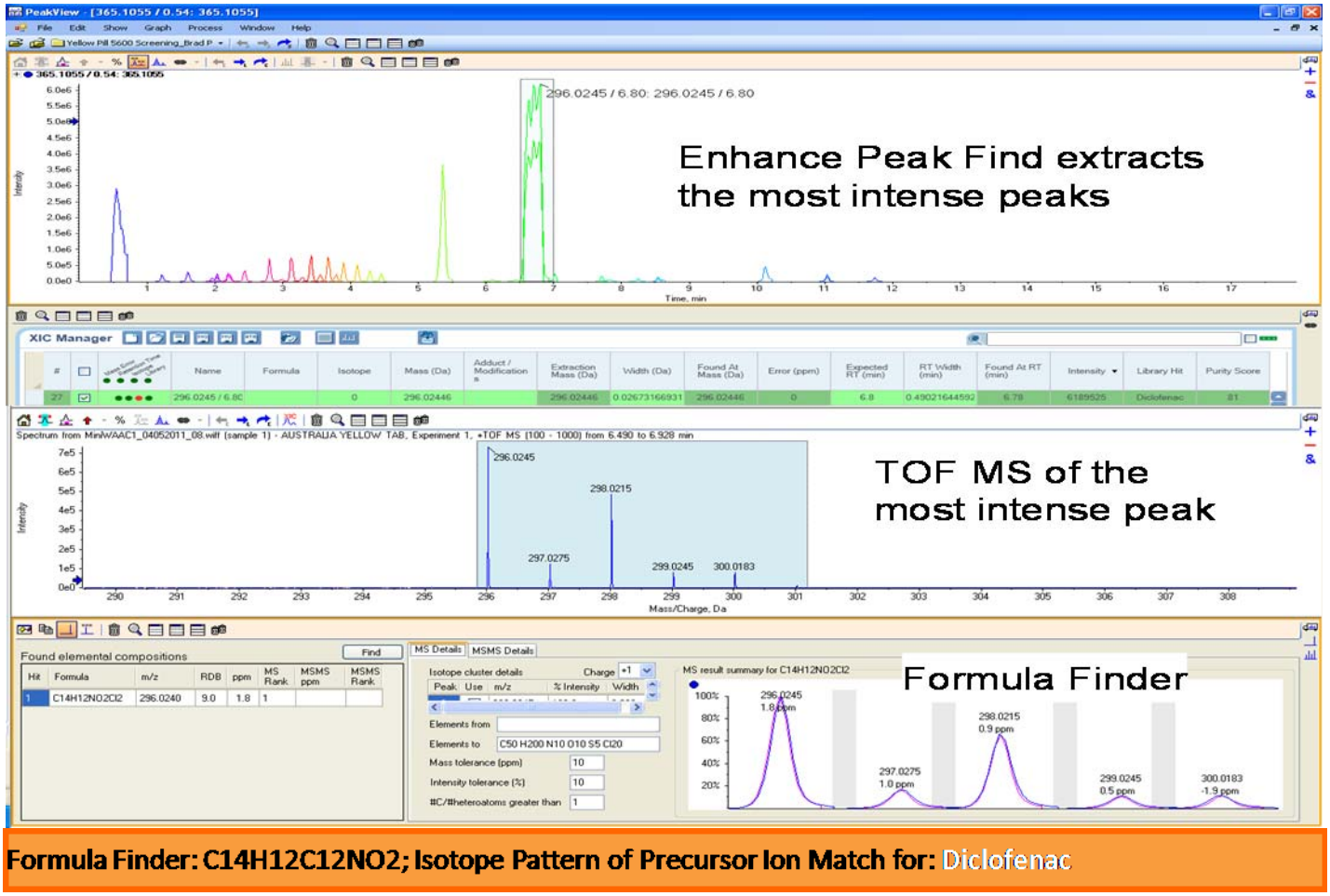

Figure 9. Identification of an unknown pill. Enhance Peak Find generated a list of most intense peaks and formula finder aided in matching elemental compositions to the accurate mass.

Conclusion

- The AB SCIEX TripleTOF™ 5600 LC/MS/MS System was used to screen for, quantify and identify drugs in urine.
- The high sensitivity, resolution and unmatched scan speed of the Accelerator TOF™ analyzer enables reproducible and accurate quantitation.
- Accurate mass MS/MS spectra were searched against an existing LC-MS/MS library of drugs to confirm the identity of quantified analytes.
- The acquired MS and MS/MS data can be used to retrospectively identify unexpected and non-targeted compounds.
- The TripleTOF™ 5600 LC/MS/MS system in combination with PeakView™ and XIC Manager software allows the identification of unknowns.

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