Targeted and Non-Targeted Screening for Drugs with High Confidence using the AB SCIEX TripleTOF™ 4600 System and Intuitive Data Processing tools

PeakView® Software with the XIC Manager

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Overview

The high resolution and accurate mass AB SCIEX TripleTOF™ 4600 LC/MS/MS system was used to screen for drugs from forensic equine 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 in a general unknown comparative screening workflow in which a sample-control comparison can be made for easy, fast and reliable identification of compounds present in the sample.

PeakView® software with the XIC Manager add-in was used for targeted and non-targeted data processing. The XIC Manager manages large lists of compounds and performs extracted ion chromatogram (XIC) calculations, both targeted and non-targeted peak finding operations and library searching. The XIC Manager allows the ability to review results based upon retention times, accurate mass, isotopic pattern and MS/MS library searching. The PeakView® software can further be used to aid in the formula and structural elucidation, elemental composition determination and fragmentation interpretation to confidently identify unknowns.

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™ 4600 LC/MS/MS system is a hybrid quadrupole/ time-of-flight (QqTOF) instrument with 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™ 4600 LC/MS/MS system allows 20-30 Information Dependent Acquisition (IDA) MS/MS spectra to be collected for confident compound identification based on MS/MS library searching and/or structural elucidation determinations. 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.

Figure 1 AB SCIEX TripleTOF™ 4600 System

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 mass molecular ion, isotope pattern and detected fragment ions can be used to characterize the structure of unexpected compounds using features of the PeakView® software. Formula Finder can be used to determine the empirical formula and Fragment Ion Prediction for determining the proposed structure.

Here we present examples of using the AB SCIEX TripleTOF™ 4600 system for the screening and identification of drugs in equine urine. Features of the XIC Manager for targeted and non-targeted screening are highlighted.

Method Details

- A Shimadzu UFLC$_{XR}$ system with a Phenomenex Kinetex 2.6 µm C18 Column, 100 Å, 50 x 2.1 mm was used, with a 15.5 min gradient of water and acetonitrile with ammonium formate buffer.
- 20 µL 5 times diluted equine urines spiked with 10, 20, 40 and 50 ng/mL of a 259 drug mixture were injected on to the LC-MS/MS system.
- The AB SCIEX TripleTOF™ 4600 system was operated with DuoSpray™ source and Electrospray (ESI) probe.
- An IDA method was used containing a TOF-MS survey of 70 ms and up to 20 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.

Results and Discussion

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

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

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.

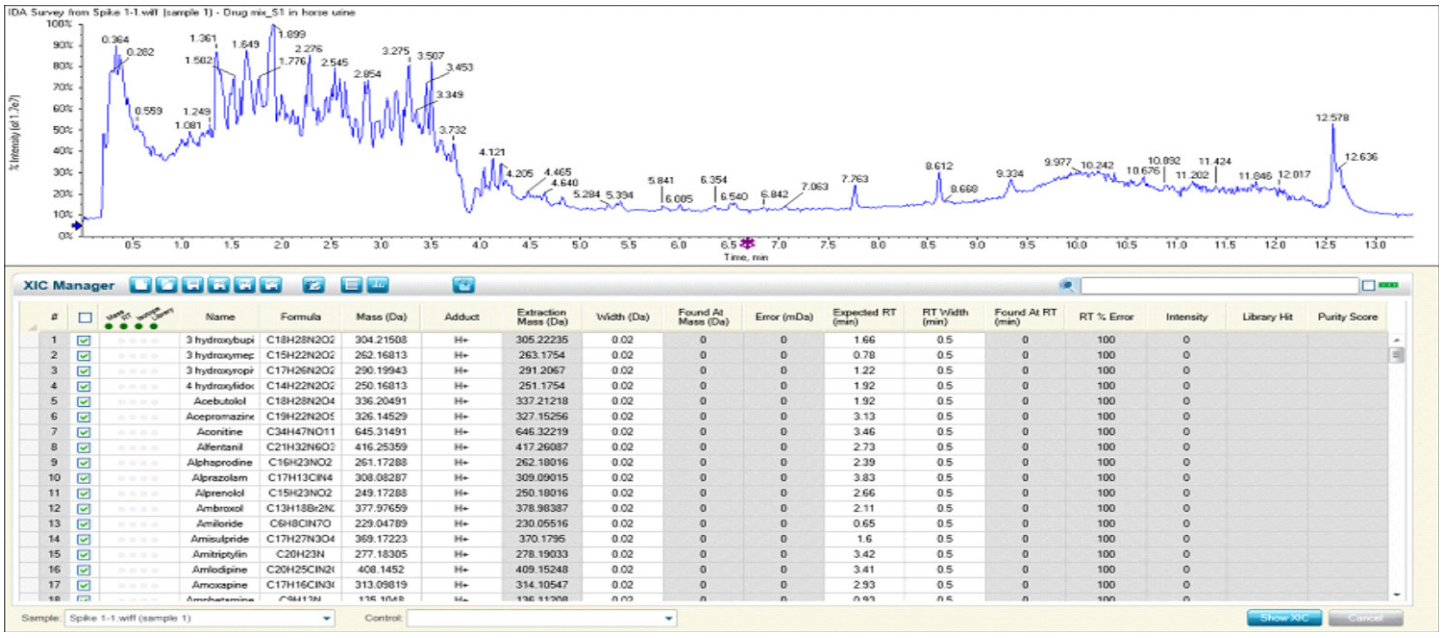

Figure 2. PeakView® software with the XIC Manager add-in; the bottom pane shows an XIC table loaded for target drug screening and identification

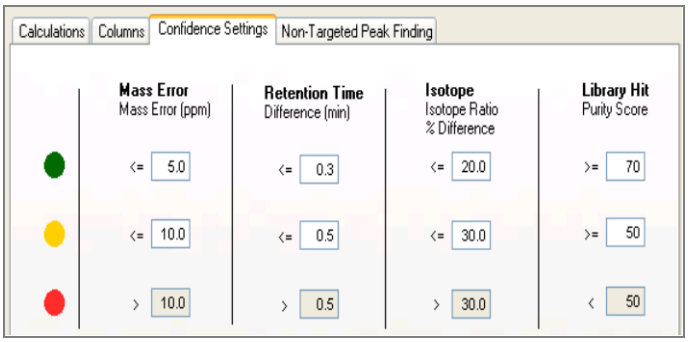

Figure 3. Confidence settings for compound identification

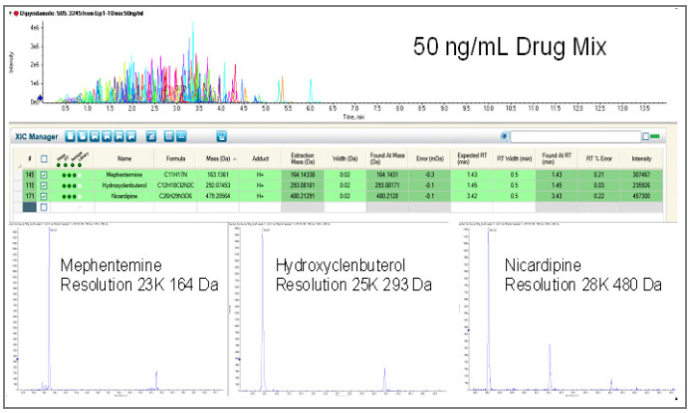

Figure 4. Resolution and mass accuracy across the mass range for selected drug compounds

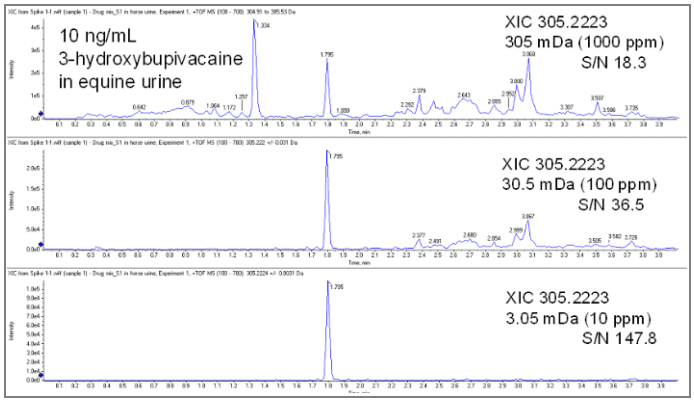

Figure 5. Increasing selectivity and S/N using narrow extracted ion chromatograms (XICs)

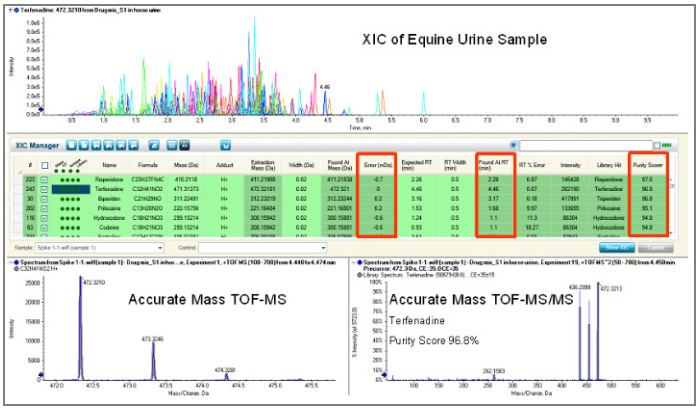

Figure 6. 259 drugs were detected and identified using retention time, mass error, isotope pattern and confirmed with MS/MS library matches.

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 and 6 shows the automatically generated XICs for each targeted analyte and compared against the user defined threshold. High resolution and accurate mass LC-MS/MS chromatograms contain comprehensive information of all molecules present in the sample that are amenable to the ionization techniques and polarity used. Narrow XICs can be generated to selectively screen for targeted compounds. The new AB SCIEX TripleTOF™ 4600 system provides high resolution of up to 35,000 dependent on the mass detected (Figure 4) and stable mass accuracy of ~2 ppm at fast acquisition speed in MS and MS/MS mode. This allows the generation of narrow XICs to achieve both selectivity and increased S/N when screening for a large set of targeted drug compounds in complex samples (Figure 5).

Figure 6 shows XICs of 259 drug compounds that were identified from a spiked equine urine sample using information of the isotopic pattern of the detected molecular ion, retention time accurate mass and MS/MS spectral searching against a forensic dug 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 6 shows an example of the spectral overlay comparison of library versus equine urine sample of terfenadine. The sample spectrum is represented as the blue trace and the library is the grey trace.

All the drugs identified are quantifiable using the MultiQuant™ software, an example is represented in Figure 7.

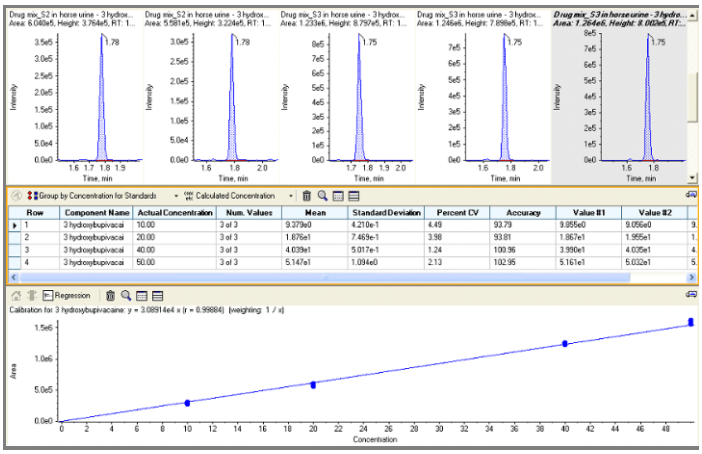

Figure 7. Example quantification of a selected drug compound using MultiQuant™ software.

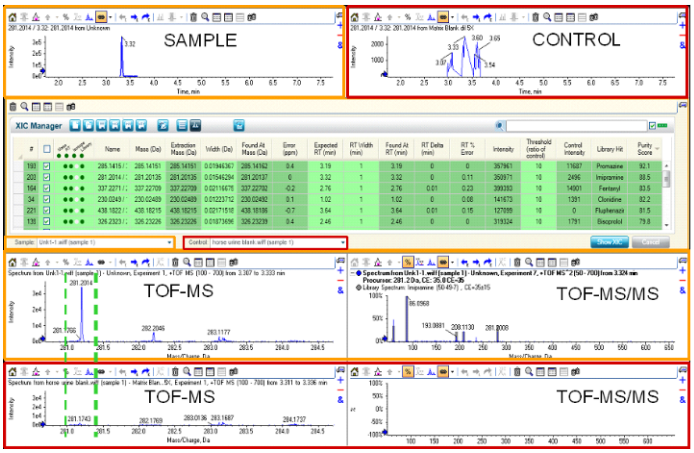

Figure 8. Sample-Control Comparison in a General Unknown Comparative Screening. The drugs Pramazine, Imipramine, Fentanyl, Clonidine, Fluphenazine and Bisprolol were identified using retention time, mass error, isotope pattern and confirmed with MS/MS library matches in a sample-control comparison workflow. The spectral comparisons between the sample and control are shown for Imipramine

Non-Targeted Screening and Unknown Identification

Figure 8 represents another example of data processed using XIC Manager in PeakView® Software using a Sample-Control comparison workflow and the 'Enhance Peak Find' feature of the XIC manager for non-target screening. This workflow simplifies the identification of unknowns in a sample by reducing the number of identifiable features by comparing them to a control, in this case an equine urine control. The General Unknown Comparative Screening determines any features that differ from that found in the control and are automatically extracted. The XIC Manager displays the results by comparing the TOF MS and MS/MS spectra from both the unknown sample and control. In the example shown in Figure 8, all ions with 10 times higher intensity in the sample then in the control are reported and automatically searched against the iMethod™ forensic LC/MS/MS library. The identifiable features were narrowed down to 6 compounds of significance. Pramazine, Imipramine, Fentanyl, Clonidine, Fluphenazine and Bisprolol were successfully identified by isotopic pattern of the detected molecular ion, retention time and accurate mass as well as a library match. Figure 5 shows a comparison of the TOF MS and MS/MS spectra from the control and the sample for Imipramine, demonstrating the absence of the feature from the control.

The sample-comparison workflow can be combined with further steps in unknown identification using the new Formula Finder in PeakView® software version 1.2 which allows the determination of molecular formula and structure of the unknown compound. Figure 9 shows a sample in which the "Enhance Peak Find" and sample-control comparative screening has identified a feature present in the sample that is absent from the control.

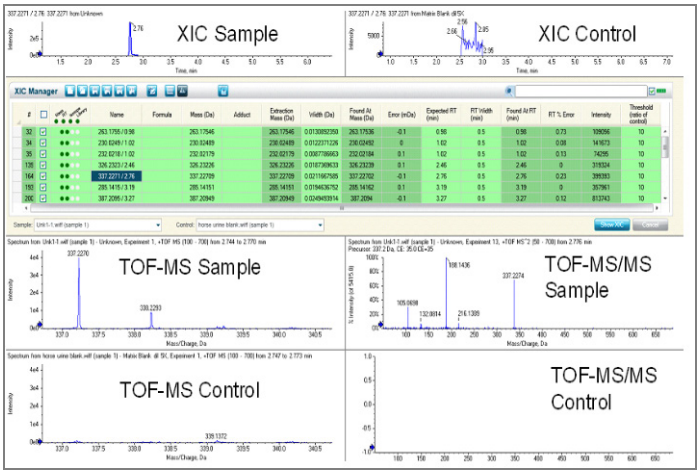

Figure 9. Non-targeted peak finding followed by comparison of identified features of sample injection to control matrix injection, aids in narrowing down the search for true unknown compounds.

The Formula Finder uses high resolution accurate mass information of the molecular ion, adducts, isotopic pattern and fragment ion information to empirically calculate potential molecular formula which can only be achieved by combining all available MS and MS/MS data. Figure 10 shows the calculated formula as C22H28N2O, derived from both MS and MS/MS information for the feature identified in Figure 9.

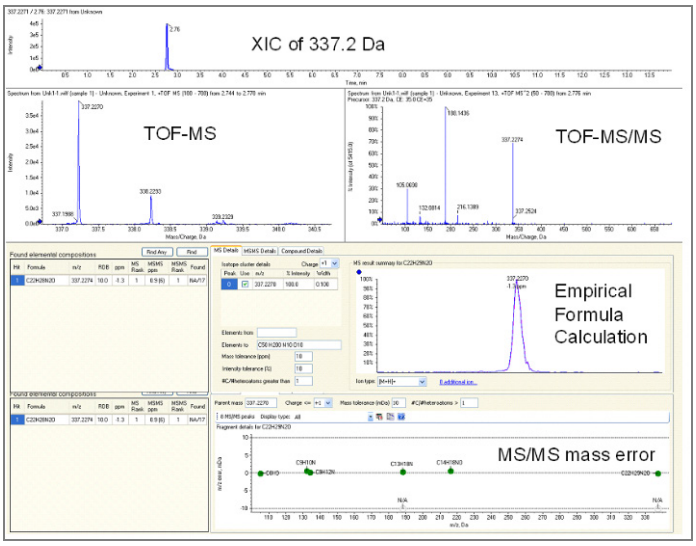

Figure 10. Identification of an unknown. Enhance Peak Find generated a list of most intense peaks and Formula Finder aided in matching elemental compositions to the accurate mass using MS and MS/MS data

The calculated formula was then automatically searched against ChemSpider to find matching structures. Figure 11 shows 17 possible hits.

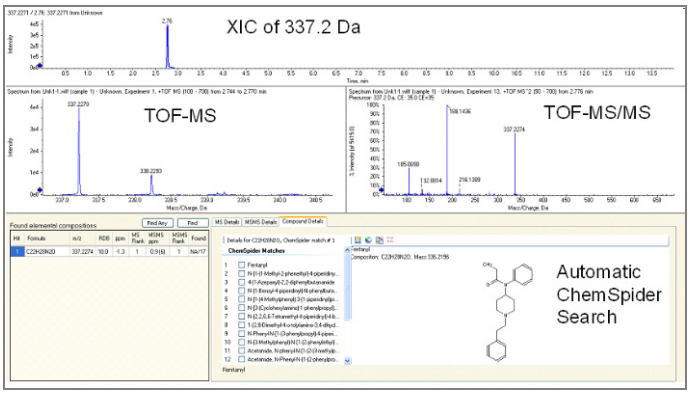

Figure 11. Combined empirical formula calculator and automatic ChemSpider search in PeakView® software. Accurate mass, isotopic pattern and MS/MS data were used to calculate the molecular formula; automatically searched against online databases. This resulted in 1 possible molecular formula and 17 potential structures.

Once a potential structure is identified the proposed structure can be imported into the fragment prediction tool of PeakView® software (Figure 12). This tool automatically compares the MS/MS pattern with potential fragmentations of the imported structure. In this example 100% of the fragment ions were explained by the structure of Fentanyl.

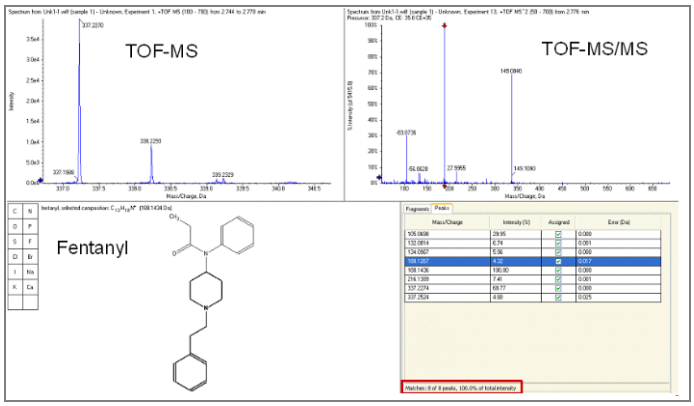

Figure 12. Proposed structure is confirmed using fragment ion prediction in PeakView® software. Eight out of eight peaks matched with 100% explainable MS/MS fragment ions for the Fentanyl structure

Conclusion

- The AB SCIEX TripleTOF™ 4600 LC/MS/MS System was used to screen for, quantify and confidently 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 in a General Unknown Comparative Screening in an easy, fast and reliable workflow to identify unexpected and non-targeted compounds.
- The TripleTOF™ 4600 LC/MS/MS system in combination with PeakView® and XIC Manager Software, with Formula Finder and Fragment Ion Predictor, allows the confident identification of unknowns.

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