

Unique Software Tools to Enable Quick Screening and Identification of Drugs of Abuse and Metabolites in Forensic Samples using Accurate Mass LC-MS/MS

Using PeakView[®] Software with the XIC Manager to Get the Answers (Step 3 of 3):

Is anything else in my sample?

Discovering and identifying non-targeted or unknown contaminants

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Overview

Drug testing labs are always looking for ways to improve their methods and workflows. Getting to the answers - What is in my sample? How much? How sure am I of this result? etc. - must be a quick process, and as painless as possible for the laboratory analysts. In this series of articles, we will discuss workflows that laboratories can use to enhance their methods for improved performance, accuracy, and throughput.

For these workflows, an AB SCIEX TripleTOF[®] LC/MS/MS system was used to screen for and identify drugs of abuse in extracts equine urine samples. TripleTOF[®] technology enables the acquisition of high resolution and accurate mass MS and MS/MS information on all peaks observed in any given sample. These information-rich data files can offer insight into the many residues and contaminants present in a given sample.

The strength of the workflow lies in the software, where PeakView[®] software with the XIC Manager add-in enables high throughput data analysis, allowing the information rich data files to be quickly screened for both targeted and non-targeted compounds.

In this third article (Step 3 of 3), we describe how PeakView[®] software with the XIC Manager can be used to process TripleTOF[®] data files to screen for suspicious unexpected drug compounds.

Earlier articles described other workflows, including targeted and comparative screening with identification based chromatographic and accurate mass MS and MS/MS information.^{1,2}



Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of drugs of abuse and metabolites. 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 of forensic and toxicological samples, full scan mass analyzers are gaining popularity. The AB SCIEX TripleTOF[®] systems allow the acquisition of highly sensitive full scan MS spectra with high resolution and mass accuracy. In addition, Information Dependent Acquisition (IDA) can be used to collect MS/MS spectra with unmatched speed for accurate and reliable compound identification using MS/MS library searching.^{3,4}

The complexity of such data requires powerful data mining tools. The XIC Manager can be used for targeted and non-targeted processing of high resolution MS and MS/MS data.⁵

The Data Analysis Workflow

1. Setting-up your XIC table and processing parameters for unknown screening

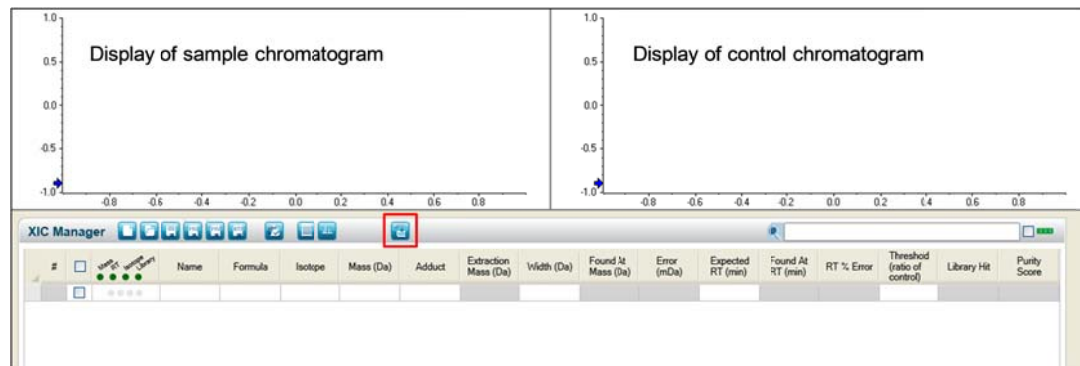
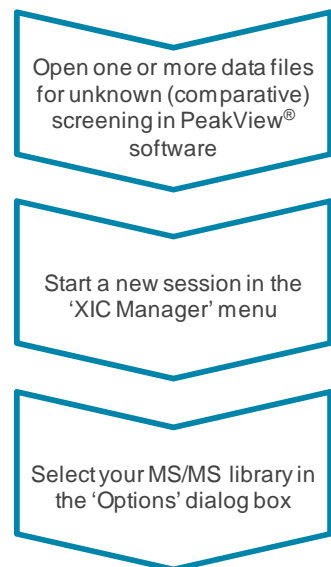


Figure 1. The XIC Manager with two samples loaded and ready for comparative unknown screening. To start the non-targeted peak finding algorithm simply click the highlighted bottom.

- The XIC Manager can be used for screening and identification of targeted drugs of abuse and metabolites using compound databases and MS/MS libraries.¹
- The XIC Manager can also be used for comparative screening to identify compounds above a target concentration.²
- Processing of LC-MS/MS data is performed through extracted ion chromatograms (XIC) using a defined list of masses or formulas (XIC table).
- Automatic identification with highest confidence is based on retention times, accurate mass, isotopic pattern and MS/MS library searching.
- The XIC Manager can also be used to explore chromatograms using a non-targeted peak finding algorithm to find and identify unexpected drugs.
- Open a single data file for unknown screening or two data files for comparative unknown screening (i.e. sample-control-comparison).
- Select the suspicious sample using the drop-down menu below the XIC table.
- Select the control, like an uncontaminated and unadulterated sample.
- Note that the XIC table is left blank. No information on expected masses or retention times is required.



Sample: Control:

2. Performing comparative unknown screening on your data using the XIC Manager

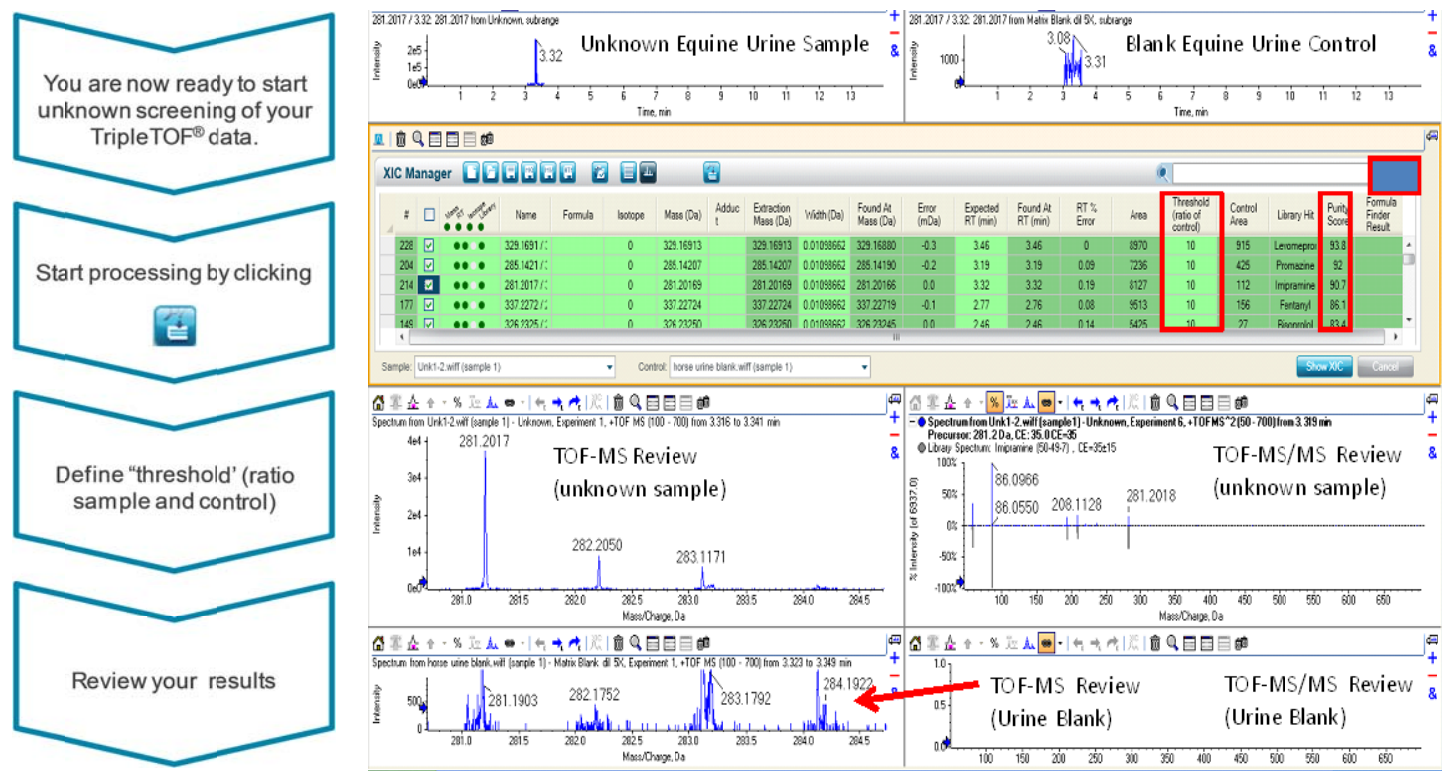


Figure 2. The result display of the XIC Manager: The drugs Levomepromazine, Promazine, Impipazine, Fentanyl, Bisoprolol were identified using MS/MS library searching after non-targeted peak finding and automatic comparison of an unknown equine urine with a blank equine urine control.

- Start the non-target peak finding algorithm by simply clicking 'Build New List...' button on top of the XIC table (Figure 1).
- Once the processing is completed, define a threshold for comparison (ratio sample and control).
- A threshold of 10 will highlight all compounds in the XIC table which are present in the suspicious sample with a signal at least 10x higher than in the clean control sample.
- XICs with a higher signal in the unknown sample than in the control are highlighted in green. Click the 'Display Highlighted XIC Only' button in the top right of the XIC table to display only these XICs.
- XICs of all found compounds in the two samples selected are displayed in the chromatogram pane (Figure 2 top).
- MS/MS spectra of all XICs found are searched automatically against the selected MS/MS library.
- The AB SCIEX iMethod™ meta library is the largest commercially available LC-MS/MS library containing spectra of over 2500 compounds.
- Library search results are listed in the XIC table (middle).
- Spectra can be reviewed in the MS and MS/MS pane (bottom).
- Spectra of non-identified compounds can be further processed in PeakView® software.

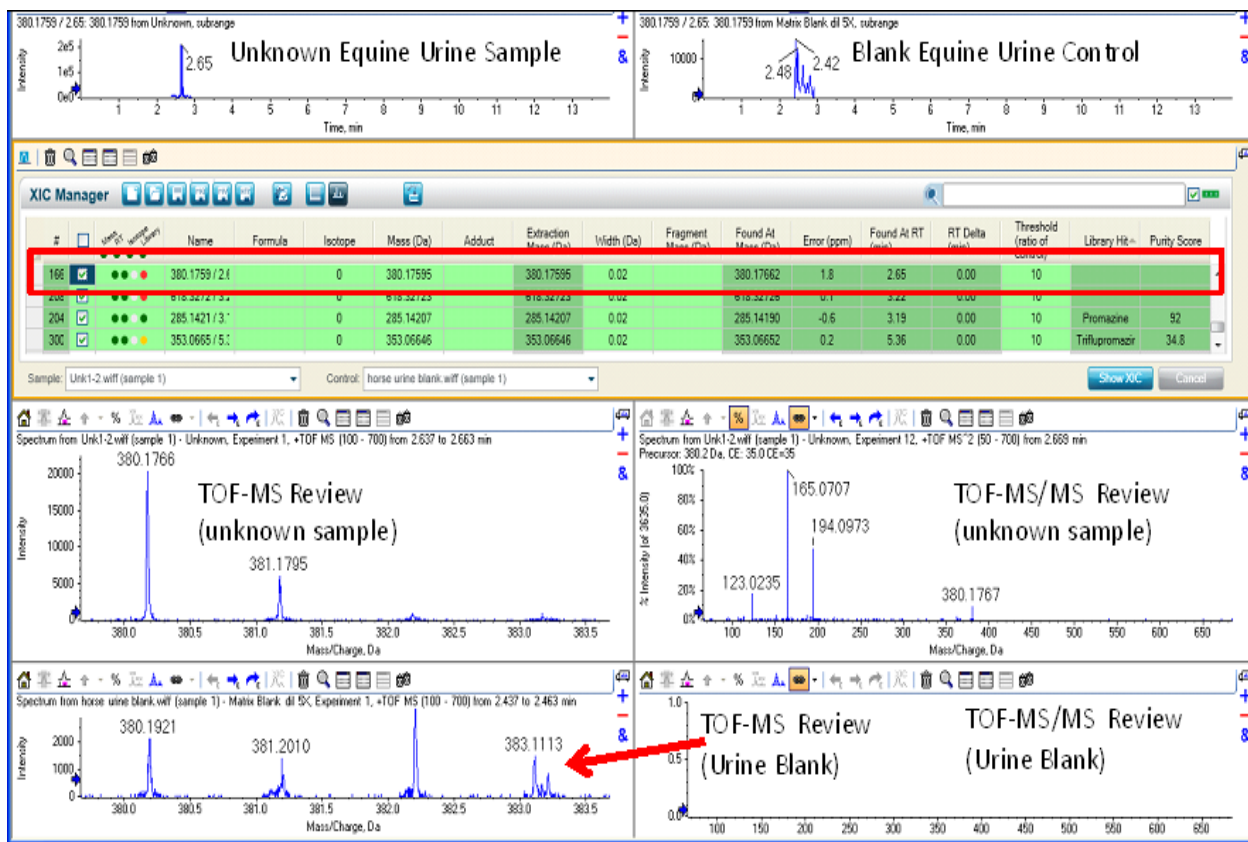


Figure 3. The result display of the XIC Manager: The drugs Levomepromazine, Promazine, Impazine, Fentanyl, Bisoprolol were identified using MS/MS library searching after non-targeted peak finding and automatic comparison of an unknown equine urine with a blank equine urine control (Figure 2). A number of peaks were identified that did not have a library match, the example shown is at m/z 380 and an MS/MS was acquired for it. This non-identified compound can be further processed in PeakView® software.

3. Identifying unknown compounds using empirical formula finding and automatic ChemSpider search

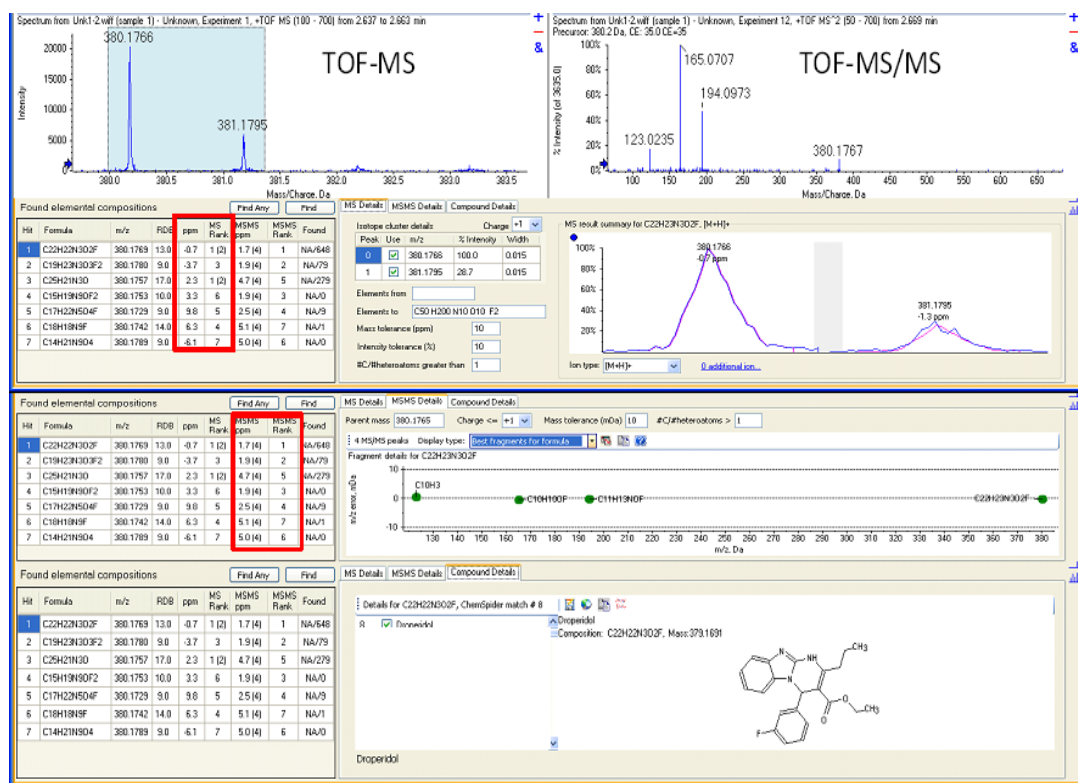
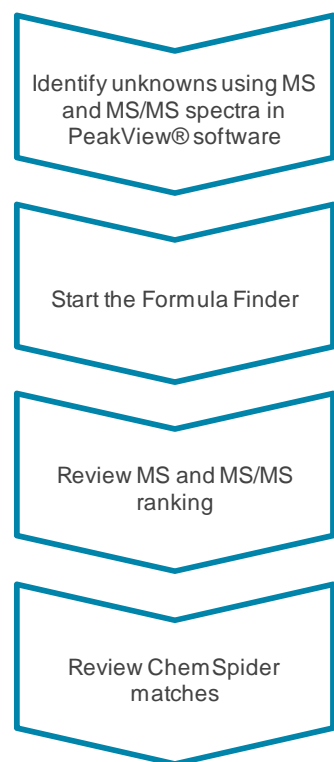


Figure 4. Unknown identification using PeakView® software: The drug Droperidol was tentatively identified after empirical formula calculation and automatic ChemSpider search

- Highlight the isotopic pattern of the compound of interest in the MS spectrum and start the Formula Finder in the 'Show' menu of PeakView® software.
- Drag the MS icon or MS/MS icon of the formula finder pane to select another spectrum for processing.
- The Formula Finder automatically uses accurate mass information of the molecular ion, adducts, isotopic pattern, and fragment ion information to empirically calculate potential molecular formulas for the detected compound.
- Review the MS and MS/MS ranking. The 'MS Details' tab shows an overlay of acquired and theoretical isotopic pattern (Figure 4 top) and the MS/MS Details' tab shows the mass accuracy of predicted fragment ions (middle).
- The Formula Finder also performs an automatic ChemSpider search returning possible structures for all calculated formulas (bottom).
- Note that you need to register in ChemSpider to activate this feature.

- You can further evaluate structures using the 'Fragments Pane' in PeakView® software. This feature compares the structure with known fragmentation pathways and reports how many fragments of the MS/MS spectrum match.⁴

The results shown in Figures 2, 3 and 4 are examples of using the comparative screening function using a non-targeted peak finding algorithm to find unknown compounds in complex forensic samples.

A simple MS/MS library search against the iMethod™ meta library can identify unexpected compounds using this workflow. The Formula Finder and automatic ChemSpider search can be used for identification if the library search does not return a hit for a compound of interest.

Summary

High resolution and accurate mass LC-MS/MS was used to screen for and identify drugs of abuse in extracts from equine urine samples. The AB SCIEX TripleTOF[®] system was operated in IDA mode to acquire MS and MS/MS information simultaneously.

The XIC Manager add-in of PeakView[®] software was used to compare samples using a non-targeted peak finding algorithm to identify unexpected compounds in suspicious samples. Compound identification is based on MS/MS library searching. The Formula Finder and automatic ChemSpider search is a useful and easy-to-use tool for identification if the library search does not return a hit for a compound of interest.

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The Complete Series of PeakView[®] with XIC Manager Software Workflow Demos:

Step 1 of 3:

What is in my sample?

Implementing targeted compound databases and libraries for identification

Step 2 of 3:

How much is in my sample?

Comparative screening for identification of targeted compounds above a specific concentration

Step 3 of 3:

Is anything else in my sample?

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References

- 1 A. Taylor and F. Le Floch: 'Unique Software Tools to Enable Quick Screening and Identification of Drugs of Abuse and Metabolites in Forensic Samples using Accurate Mass LC-MS/MS - Using PeakView[®] Software with the XIC Manager to Get the Answers (Step 1 of 3)' Application Note AB SCIEX (2012) #6930113-01
- 2 A. Taylor and F. Le Floch: 'Unique Software Tools to Enable Quick Screening and Identification of Drugs of Abuse and Metabolites in Forensic Samples using Accurate Mass LC-MS/MS - Using PeakView[®] Software with the XIC Manager to Get the Answers (Step 2 of 3)' Application Note AB SCIEX (2012) #6930213-01
- 3 A. Schreiber and C. Borton: 'Target and Non-Target Screening for Pesticide Residues in Food Samples using the AB SCIEX TripleTOF[®] 5600 System' Application Note AB SCIEX (2010) #0460110-02
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- 5 A. Taylor, M. Jarvis and B. Patterson: 'Targeted and Non-Targeted Screening for Drugs with High Confidence based on High Resolution and Accurate Mass LC-MS/MS' Application Note AB SCIEX (2011) #3550111-01

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