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):

How much is in my Sample?
Comparative Screening for Identification of Targeted Compounds above a Specific Concentration

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Overview

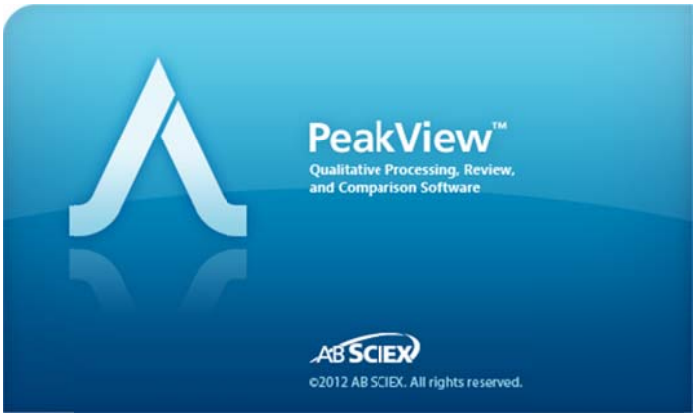
Drug testing labs are always looking for ways to improve their methods and workflows. Getting to the answers - What is in my sample? How much? How sure am I of this result? etc. - must be a quick process, and as painless as possible for the laboratory analysts. In this series of articles, we will discuss 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 in extracts of 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 drugs and metabolites 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 second article (Step 2 of 3), we describe how PeakView® software with the XIC Manager can be used to process TripleTOF® data files to quantitatively compare samples to identify compounds above a specific concentration.

Other articles describe other workflows, including targeted screening and identification and general unknown screening to search data for possible suspicious unexpected drugs.[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 toxicological and forensic 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 comparative screening

Open two or more data files to compare in PeakView® software

Start a new session in the 'XIC Manager' menu

Build or open your XIC Manager table

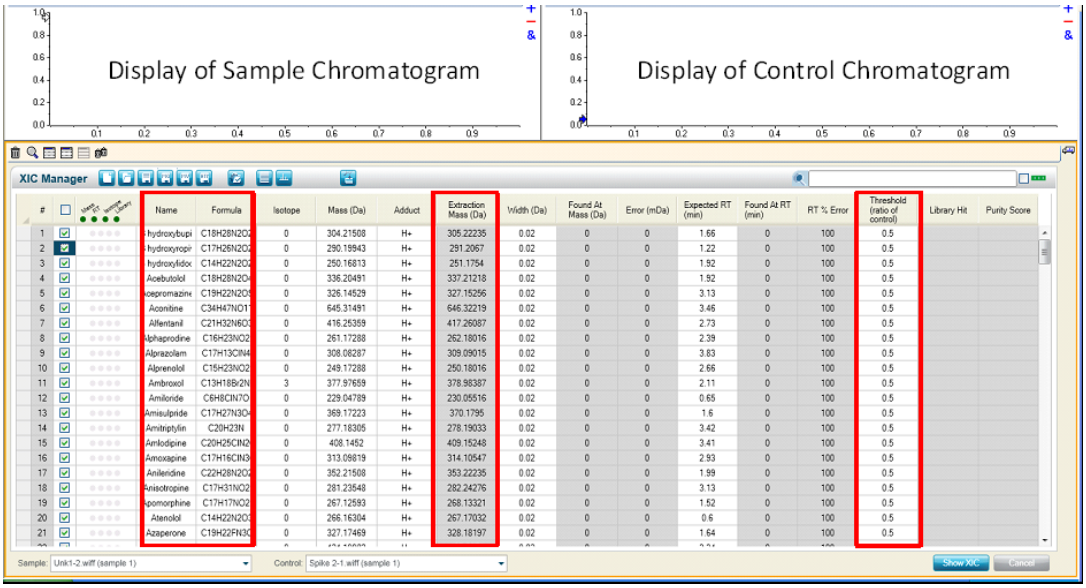


Figure 1. The XIC Manager with an XIC table loaded for comparative screening and identification

- 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]
- Processing of LC-MS/MS data is performed through extracted ion chromatograms (XIC) using a defined list of masses or formulas (XIC table).
- Define your confidence settings for compound identification and select the mass spectral library for automatic MS/MS library searching in the 'Options' dialog box.
- Automatic identification with highest confidence is based on retention times, accurate mass, isotopic pattern and MS/MS library searching.
- The XIC Manager can also quantitatively compare samples to highlight identified compounds above a target concentration.
- Select unknown sample(s) using the drop-down menu below the XIC table.
- Select control, like solvent standard or matrix sample spiked with all targeted compounds at relevant concentration (i.e. cutoff levels).

Select 'control' and unknown sample(s)

Define "threshold' (ratio sample and control)

- Define threshold for comparative screening (ratio sample and control).
- A threshold of 1 will highlight all compounds in the XIC table which are present in unknown samples at a concentration higher than in the control sample, a threshold of 0.5 will highlight all compounds having at least 50% of the signal in comparison to the control sample.
- The generated XIC table can be saved for future processing.

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

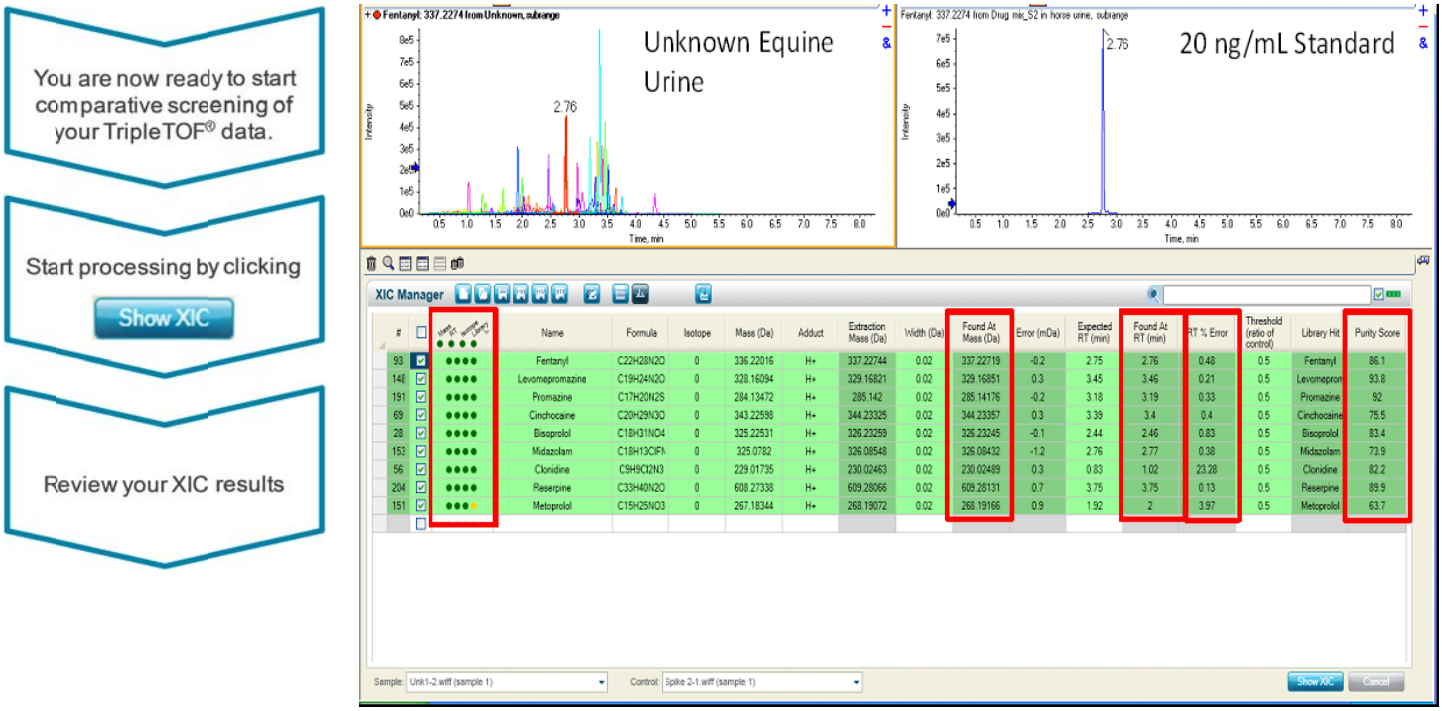
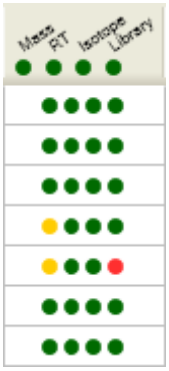


Figure 2. The result display of the XIC Manager: The XIC is extracted for fentanyl from the 20 ng/mL drug standard (right chromatogram). The corresponding peak in the unknown is highlighted at 2.76 min (left chromatogram) and has been extracted because the concentration of fentanyl in the unknown is above 10 ng/mL (0.5 threshold). A number of other drugs were identified with confidence in the unknown sample at a concentration above the 10 ng/mL cutoff.

- XICs of all targeted compounds in the unknown sample selected are displayed in the chromatogram pane (Figure 2 top left).
- Selecting a compound of interest in the table will display the XIC of this compound in the control sample for visual comparison (top right).
- XICs with a higher signal in the unknown sample than in the control are highlighted in green.
- The threshold (ratio sample and control) can be adjusted at any time during processing.
- Compound identification is based on chromatographic and mass spectrometric information, including RT error (min or %), mass error (ppm or mDa), isotope matching, and library search results (middle).
- Identification criteria are visualized using ‘traffic lights’.



3. Reviewing your results using the ‘traffic lights’ and the spectra display

Figure 3. The result display of the XIC Manager: the drugs Fentanyl, Levopromazine, Promazine, Cinchocaine and Bisoprolol were identified with high confidence in an equine sample at a concentration above 10 ng/mL* 0.5 threshold),

- The ‘traffic lights’ visualize the confidence of compound identification in your sample(s) for each processing parameter.
- Note that the XIC results are shown relative to the processing options you set-up.
- Review accurate mass MS and MS/MS spectra for both the unknown and control sample by clicking the ‘Show MS and MS/MS button’.
- The acquired spectra are displayed in blue.
- The theoretical isotopic pattern of the molecular ion and the theoretical MS/MS spectra for the compound are displayed in gray.
- Combining chromatographic, accurate mass MS and MS/MS information results in highest confidence of compound identification.
- The XIC result table can be saved for future review and processing.

The results shown in Figures 2 and 3 are examples of comparative screening and identification of drugs of abuse using the XIC Manager. Different drugs of abuse were identified in equine urine extracts with high confidence.

Drugs above the target concentration of 10 ng/mL were highlighted in the XIC table. This procedure can be used to automatically flag drug cutoff level violations.

Summary

High resolution and accurate mass LC-MS/MS was used to screen for and identify drugs of abuse in forensic 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 quantitatively compare samples to identify compounds above a specific concentration. Compound identification is based on information on retention times, accurate mass, isotopic pattern and MS/MS library searching, resulting in unmatched confidence. This data processing workflow enables high throughput data analysis.

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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?

Discovering and identifying non-targeted or unknown contaminants

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 3 of 3)' Application Note AB SCIEX (2012) #6930313-01

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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