

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):

What is in my Sample?

Implementing Targeted Compound Databases and MS/MS Libraries for Identification

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Overview

Toxicology 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 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 first article (Step 1 of 3), we describe how PeakView[®] software with the XIC Manager can be used to process TripleTOF[®] data files to screen and identify compounds using targeted compound databases and MS/MS libraries.

Later articles will describe other workflows, including comparative screening and general unknown screening to search data for possible suspicious unexpected compounds.^{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 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 targeted compound database and library (XIC table) and processing parameters

Open your data file(s) in PeakView® software

Start a new session in the 'XIC Manager' menu

Build and edit your XIC table

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

- The XIC table defines a list of masses or formulas to generate extracted ion chromatograms (XIC) for targeted compounds.
- The XIC table allows a list of compounds and masses to be modified based on the compounds of interest to your laboratory.
- The columns in the table, including compound name, formula, isotope, adduct, retention time (RT), width, and others, can be edited based on your methods and needs (Figure 1).
- Note that a mass must be entered in the 'Extraction Mass' column in order for the compound to be searchable in your database.
- The 'Extraction Mass' can be entered manually or is calculated automatically based on the formula, isotope, and adduct provided.
- The generated XIC table can be saved for future processing.
- Confidence settings for compound identification based on mass error, retention time error, isotope matching, and library searching purity scores can be adjusted based on your laboratories' quality control criteria (Figure 2).
- Select your mass spectral library for automatic MS/MS library searching in the 'Options' dialog box.

Set-up your processing options in the 'Options' dialog box

Select your MS/MS library

Figure 2. Confidence settings for compound identification

2. Analyzing your data using the XIC Manager

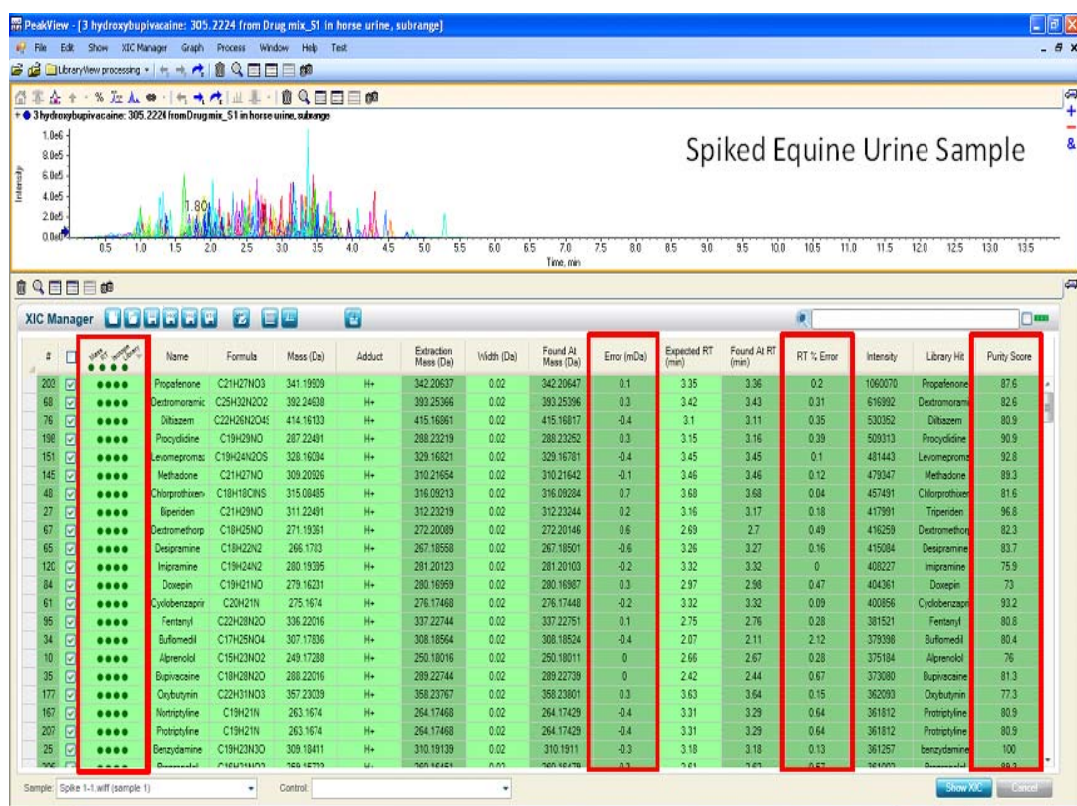
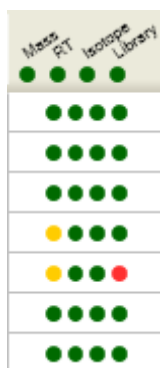


Figure 3. The result display of the XIC Manager: the 259 spiked drugs were identified with high confidence in spiked equine urine sample

- XICs of all targeted compounds are displayed in the chromatogram pane (Figure 3 top).
- XICs above a user defined intensity threshold are highlighted in green.
- 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 4. The result display of the XIC Manager: the drugs Levomepromazine and Promazine were identified with high confidence in an unknown equine urine sample, based on accurate mass, isotope pattern, retention time and MS/MS library match.

- 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 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 3 and 4 are examples of quick screening and identification of drugs and metabolites using the XIC Manager. Different drugs were identified in equine urine extracts with high confidence.

Summary

High resolution and accurate mass LC-MS/MS was used to screen for and identify drugs of abuse and metabolites in 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 for screening and identification with highest confidence by automatically processing LC-MS/MS data using information on retention times, accurate mass, isotopic pattern and MS/MS library searching. 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 2 of 3)' Application Note AB SCIEX (2012) #6930213-01
- 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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- 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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