

Simple HRMS Data Review Using Workflows, Views, and Filters Within a Novel Integrated Scientific Information System

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TECHNOLOGY BENEFITS

- Accurate and simplified review of complex high resolution mass spectrometry (HRMS) data using workflows, filters, and views.
- Screening for a theoretical unlimited number of compounds in a single injection.
- Simultaneous collection of qualitative and quantitative unbiased data for either targeted or non-targeted analysis.
- Interrogation of data for the presence of unknown compounds of interest via filtering, binary compare, and statistical analysis.
- Structural elucidation of unknown compounds of significance.
- Historical data review performed using accurate mass precursor and fragment ion information.
- Utilization of collision cross section (CCS) values as an identification point for accurate mass screening in ion mobility enabled HRMS systems.

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KEY WORDS

Pesticides, residues, screening, food safety, high resolution mass spectrometry, HRMS, IM-MS, multi-analyte screening

INTRODUCTION

Multi-analyte screening methodologies are essential for monitoring food and environmental samples across the globe. The goal of these methods is to eliminate compliant samples and to identify non-compliant samples for subsequent confirmation and quantification. Sensitivity must be in line with the relevant regulatory limits in complex matrices. Also, a method must be validated in accordance with legislative requirements. This method would ideally be rapid, cost effective, and contain a streamlined process, from sample preparation to reporting results.

LC-MS/MS or GC-MS/MS tandem quadrupole technology meets the requirements above and currently exist as the de-facto technique used to perform these analyses. However, with a constantly increasing number of analytes being added to monitoring and watch lists, the scope of a typical screening method is being extended. In addition, requests to screen for compounds beyond a target list are becoming increasingly common. As a result, many laboratories are progressing towards High-Resolution Mass Spectrometry (HRMS) techniques that, in theory, can screen for an unlimited number of targets at the same time as providing information on unknown compounds or metabolites of interest.

In this technical note, we demonstrate the ease of use and efficacy of a non-targeted, data independent, analysis type (MS^E and HDMS^E), coupled with UNIFI, a state-of-the-art scientific information system, for multi-analyte screening in food and environmental samples. This technical note will also serve as a primer to a series of documents involving authentic sample analyses. Here, we focus on the introduction of a novel technique where a user in a routine environment can customize data review within the UNIFI Scientific Information System to establish a concise, rapid, simple, and consistent approach to reviewing HRMS data.

DISCUSSION

More food and environmental laboratories around the globe have a desire to expand the scope of their multi-residue screening methods to include an ever-increasing number of target compounds. There is also an increased interest in screening methods with the ability to discover and elucidate non-targeted masses of interest. Figure 1 summarizes the potential needs for a modern multi-residue screening method.

For laboratories focused exclusively on questions 1 and 2, a tandem quadrupole mass spectrometer, operating in multiple reaction monitoring (MRM) mode, is considered the gold standard for multi-residue screening. This technique is fast, reliable, and robust, and it is deemed to have an established, efficient data review process. The latest high resolution mass spectrometry (HRMS) systems can also be operated in fully targeted mode with acquisition types such as ToF MRM, SRM, and SIR with excellent quantification accuracy for detected compounds. However, the same duty cycle limitations exist when screening for a large list of compounds – they are fully targeted acquisitions with no information on unknown masses of interest, nor do they provide historical data review.

Laboratories wanting to answer all four questions in Figure 1 typically require the use of several MS techniques and software application managers to process the data. Although tandem quadrupole instruments have the ability to run in mixed target and non-target acquisition modes, the non-targeted modes suffer from a lack of sensitivity and selectivity afforded by a nominal mass full scan acquisition. The utilization of HRMS systems operated in “non-targeted screening”, “discovery”, “profile” or “unknown screening” modes enables the acquisition of data that can address all four questions in Figure 1.

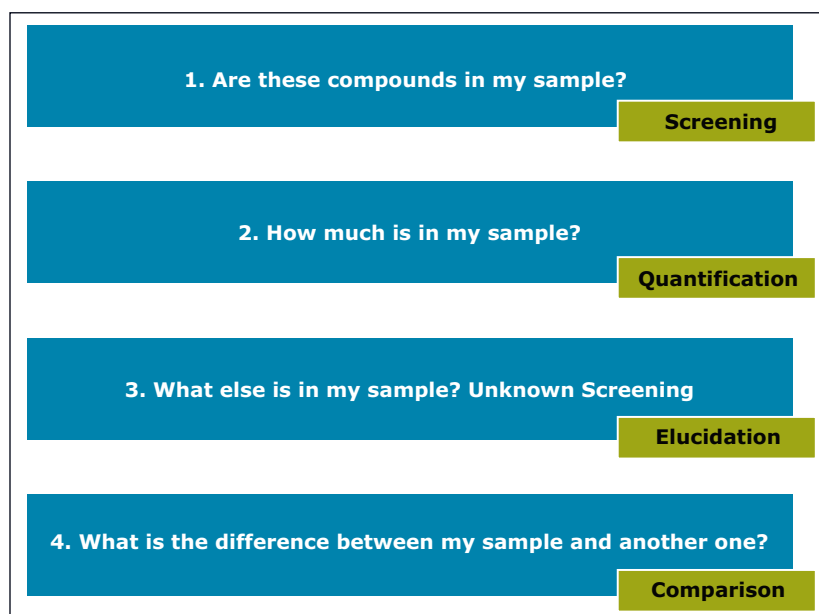


Figure 1. Fundamental questions for modern multi-residue screening methods.

There are two main types of non-targeted acquisition that are currently used with HRMS. Data Dependant, sometimes called Data Directed Analysis (DDA), or Independent Data Analysis (IDA), also referred to as Data Independent Analysis (DIA). The former uses a target list or criteria set around a detected response to switch on precursor masses of interest and collect MS/MS fragment data for confirmation. This offers good specificity for compounds that are included, but has serious limitations for non-targeted compounds. In the absence of target ions, the instrument may be set to switch to MS/MS for the most intense compounds within a typical scan. This assumes significant ions are more intense than non-relevant matrix ions. The constant switching between MS and MS/MS also introduces the possibility that compounds of interest may be missed, even when fast, modern instruments are used.

Independent data analyses collect precursor ion and fragment ion information for all masses in a chromatographic run, essentially by collecting two or more MS functions with low collision energy and elevated collision energy. Many variations of IDA are offered by different MS vendors such as non-targeted mode, MS^E, bbCID, DIA, all ions fragmentation, or, all ions MS/MS. However the principle stays the same in that no decisions are required and a user is able to collect a non-targeted dataset. Non-targeted data acquisition on Waters® Xevo QToF systems is achieved using MS^E.¹ This innovative acquisition mode, first introduced by Waters in 2004 on the Expression System, collects accurate mass precursor

ion data at low collision energy and accurate mass fragment ion data at elevated collision energy in alternate MS functions. Using MS^E a comprehensive and unbiased dataset is collected that can be interrogated for both target analytes and unknowns. High Definition Mass Spectrometry® (HDMS) or ion mobility mass spectrometry (IM-MS) data collection on Waters SYNAPT and Vion HDMS systems collect the same non-targeted data with the added benefits of achieving real time ion mobility separations orthogonal to the UPLC® separations.

Table 1 compares key attributes (normal text) and perceived weaknesses (italics text) of data acquired on a tandem quadrupole with that of a HRMS system operated in non-targeted mode. Together with the ability of modern HRMS instruments to perform quantification over several orders of magnitude and reach sensitivity levels governed by legislation, it is not difficult to see why more laboratories are gravitating to this technique. The wealth of information available from a non-targeted acquisition is staggering. Having all of this information allows more criteria to be used when matching compounds from a target list. This leads to a reduction in false detects, while reducing the time needed to review each sample injection. For non-targeted compounds of interest, the precursor and fragment ion information, in combination with advanced software tools, facilitates elucidation of these unknown masses.

QQQ	HRMS
2 nominal mass transitions	Accurate mass precursor
1 Ion Ratio	Accurate mass fragments
<i>Targeted acquisition</i>	Isotopic pattern scoring
<i>Limited by duty cycle</i>	Adduct presence
	Ion ratios
	Collision cross section (CCS)
	Historical LE/HE data review
Established data review	<i>Data review perceived complex</i>
	Unknown screening – What else is in my sample? MVA, discovery tools, or binary compare

Table 1. Key attributes and perceived weaknesses of tandem quadrupole versus non-targeted HRMS data acquisitions.

SAMPLE ANALYSIS AND DATA PROCESSING

Waters' scientists have been using non-targeted acquisition modes for over a decade. While data collection has remained the same, instrument hardware and software used to process, align, and deconvolute the non-targeted data have improved dramatically.

An MS^E or HDMS^E acquisition within UNIFI is able to answer all four questions in Figure 1 using a single LC-MS system with a single analysis method that contains all acquisition and processing information. This is made possible using data componentization, filters, views, and workflows. Figure 2 shows a schematic of the UNIFI data processing workflow.

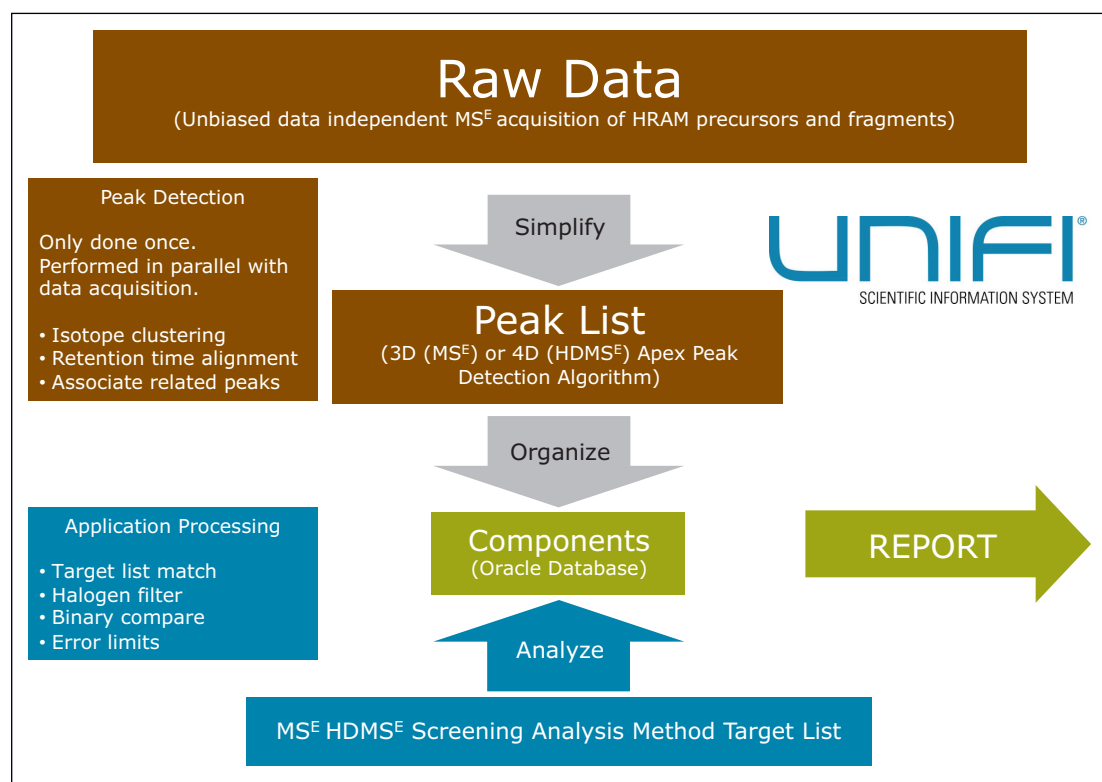


Figure 2. UNIFI data processing workflow for high resolution accurate mass (HRAM) data.

Componentization, in short, converts a raw data file into a spreadsheet of components. A component, in this case, is a series of masses related by a narrow retention time window around the apex of a three-dimensional chromatographic peak. A typical pesticide component, for example, may contain four isotopes, three adducts (H⁺, Na⁺, K⁺), and five associated high energy fragment ions. A target list within a UNIFI accurate mass screening experiment interrogates the component table and not the raw data. For more information, please refer to the componentization white paper.²

Most other HRMS software uses an Extracted Mass Chromatogram (EMC) or Extracted Ion Chromatogram (XIC or EIC) approach. Using that approach, spectral information is displayed, including noise and co-eluting peaks within the same retention time window as the extracted ion of interest. Figure 3 shows the difference between (A) an XIC, (B) a time-aligned Apex 3D componentized spectrum, and (C) a time-aligned and drift-aligned spectrum within UNIFI for carbendazim in a mandarin EU proficiency test sample. The mass spectrum of the time-aligned Apex 3D spectrum (B) is much cleaner than the XIC generated spectrum (A) because all of the ions present in the componentized spectrum must be within a small retention time window of the peak apex. The XIC method however, shows all ions extracted from the entire five second wide chromatographic peak. Ions of m/z 177 Da, 236 Da, 290 Da, and 305 Da shown in Figure 3A (highlighted by the arrow) do not have the same apex retention time as carbendazim, and are therefore not apparent in the componentized spectrum (3B). A further enhancement in specificity is observed with the IM-MS acquisition using ion mobility enabled systems, since a component will now consist of spectral ions that have to be both drift and time aligned.

Greater specificity and confidence in identifications is achieved with componentized data since a mass matched from the target list will contain the other analytical information with regards to the component such as isotopes, adducts, and accurate mass fragment ions within that particular Apex 3D narrowed retention time window. False positives will be reduced since false detections will be void of the confirmatory information used for the identification, and therefore can be easily excluded from the list of identified targets.

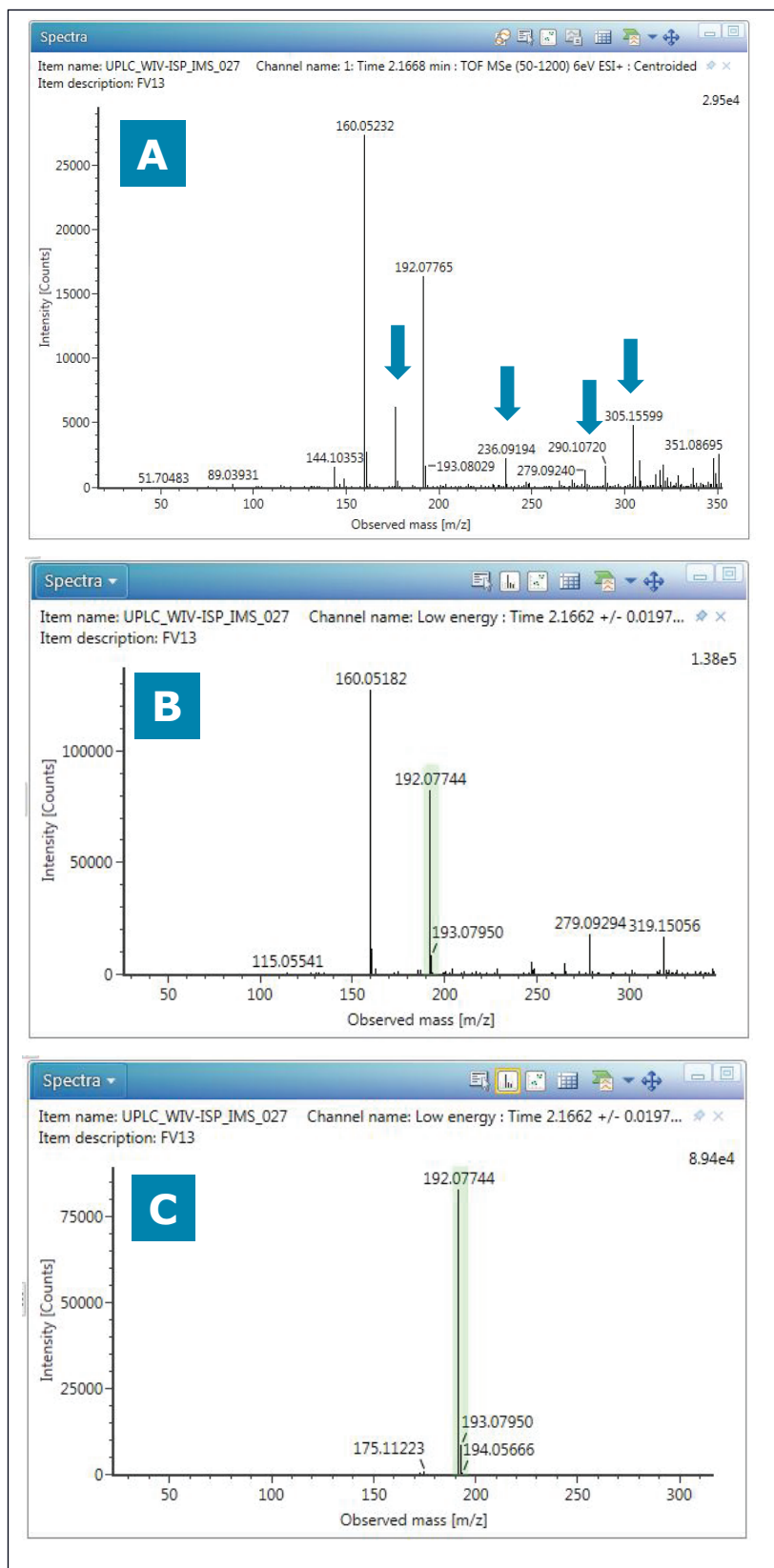


Figure 3. Comparison of spectra generated from: A. An extracted ion chromatogram from MS^E data; B. An Apex 3D time aligned componentized spectrum from MS^E data; and C. A drift- and time-aligned spectrum observed with IM-MS data.

DATA REVIEW

Interrogation of non-targeted, componentized MS^E or HDMS^E data in UNIFI is performed using filters, workflows, and views.

A filter is a question or a means to interrogate the componentized data generated in UNIFI. For example, “Show me the components identified with mass accuracy (± 5 ppm), retention time (± 0.2 mins), and the presence of a high energy accurate mass fragment ion.” A second example could come from an unknown screening perspective such as “Show me components with a high probability of containing a halogen atom.” Another example for interrogation of unknown compounds of interest would be “Show me all components with a common accurate mass fragment of 180.0634 Da.”

A view is the combination of plots, chromatograms, spectra, tables, and columns that are displayed together on the screen. The view visually provides all the information required to answer the question in a filter.

A workflow step is simply a saved view with a filter applied. A combination of these steps creates the workflow, which is designed to answer a series of targeted and/or unknown screening questions for each injection within an analysis.

The workflow allows a supervisor, for example, to determine what information to extract from a non-targeted acquisition and customize how the review process is implemented. This ensures that the time from injection to report is minimized and that all users review data in a consistent and concise manner.

Figure 4 illustrates the interaction of filters, workflows and views designed to facilitate getting a user from injection to report as fast as possible.

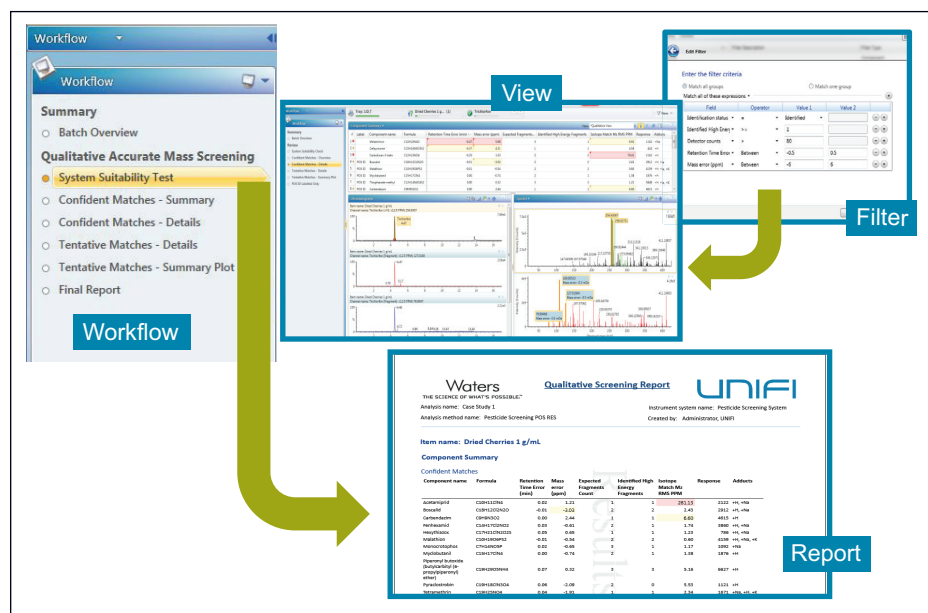


Figure 4. Streamlined injection to report process using filters, workflows, and views in UNIFI.

Multiple workflows can be designed and used with componentized data in order to cover each of the four questions in Figure 1. For reference, some example workflows are shown in Figure 5. A qualitative non-targeted screening analysis is depicted in Figure 5A, and includes a workflow step to look for halogenated (i.e. Cl and Br) species. The workflow in Figure 5B adds binary compare steps to review; for example, differences between a reference standard and authentic sample. The workflow shown in Figure 5C contains steps that enhance the review of both qualitative and quantitative analysis in a non-targeted screen. For the analysis of metabolites and biotransformations of residues, the workflow shown in Figure 5D would be appropriate.

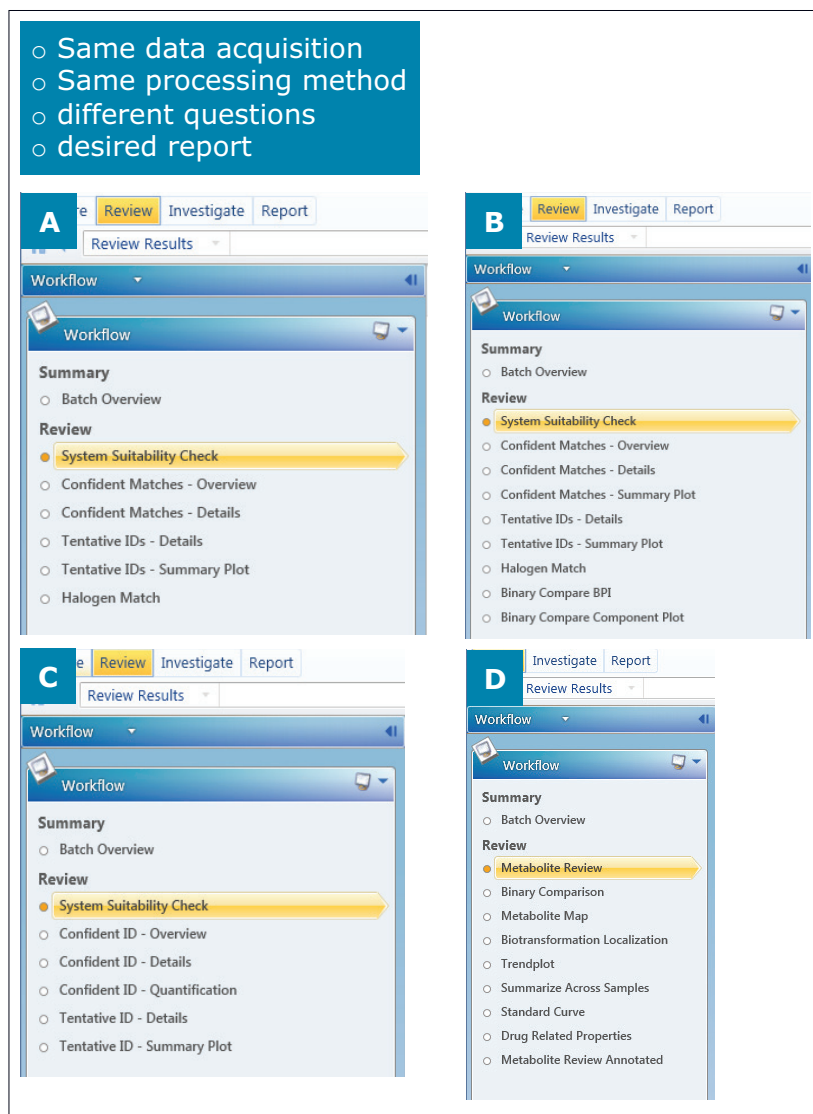


Figure 5. Example workflows used for A. Qualitative, non-targeted screening analysis; B. Unknown screening via binary compare; C. Qual-quant non-targeted screening analysis; and D. An unknown screening metabolite ID analysis.

CONCLUSIONS

- Non-targeted data acquisition in combination with a workflow-driven approach is an effective way to review complex screening data versus the typical extracted ion chromatogram approach.
- Data independent analysis using MS^E or HDMS^E combined with the UNIFI componentization approach organizes complex datasets for simple visualization and interrogation that fully utilizes all information generated.
- The use of filters, views, and workflows greatly increases the rate of data review and reduces time from injection to report.
- The use of broad one-time only processing parameters reduces the risk of false negatives.
- The use of customizable filters reduce false detects. This also provides rapid data review and a unique solution for reviewing all available data required to make a YES/NO decision fast.
- Storing all data, methods, and libraries within a relational database provides easily accessible and quickly searchable information.

References

1. An Overview of the Principles of MS^E, The Engine that Drives MS Performance. Waters white paper No. [720004036en](#), October 2011.
2. Waters UNIFI Scientific Information System Componentization. Waters white paper No. [720004597en](#), April 2013.

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