

Advanced Automated Library Searching for Compound Identification in Forensic Toxicology Samples

LibraryView™ Software 1.0

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Introduction

LC-MS/MS utilization in forensic toxicology screening for drugs and drug metabolites has become increasingly popular due to the selectivity, sensitivity and the speed of LC-MS/MS analysis. MS/MS confirmation with automated searching against available spectral libraries has proven to add a superior level of confidence to the compound identification.

One of the key factors of the complete solution for forensic toxicology screening is automation of the library searching with the advanced capability to dynamically review the acquired data. Solutions need to be accurate and robust. The ability to search multiple libraries, create subsets of libraries, adjust and refine search parameters as well as re-search acquired data provides the user with substantial flexibility. Setting mass tolerance, intensity thresholds and searching multiple collision energies enhance the data under revision. The ability to search or re-search entire data files or a specific mass spectrum with different parameters can improve overall data quality and throughput. Additionally, reporting tools allow the information to be disseminated to the end user.

Strategies for Compound Screening

A number of LC-MS/MS compound screening workflows have been established; each one having its own strengths and weaknesses making them complimentary to one another. The choice as to which one to use depends ultimately upon the end need. A common part of each of the different LC-MS/MS screening workflow includes generating MS/MS spectra that can be used to search against MS/MS spectral libraries as an added degree of confident compound identification.

MULTI-TARGETED SCREENING

Targeted Screening is a directed screening approach that analyses samples for the specific list of drugs. This method is often referred to as “multi-targeted screening” (MTS) and currently constitutes the majority of the screening tests



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performed. The types of drugs used or abused are often limited to a few hundred compounds; therefore most MTS methods are focused on detecting a subset of the most commonly used drugs. Restricting the analysis in this way allows the use of sensitive and selective workflows, providing detection of low concentrations of drugs in complex biological matrices. Since this approach detects only those compounds selected, a priori, it will not reveal the presence of a compound not included in the target drug list.

GENERAL UNKNOWN SCREENING

While the majority of screening tests are targeted, interest in general unknown screening (GUS) is continuing to grow. GUS does not use a target analyte list, so the analysis allows the ability to detect unexpected compounds and metabolites. The tradeoff for GUS is a compromise in the level of detection and selectivity; the benefit of identifying unpredicted analytes, however, far outweighs this limitation in many applications.

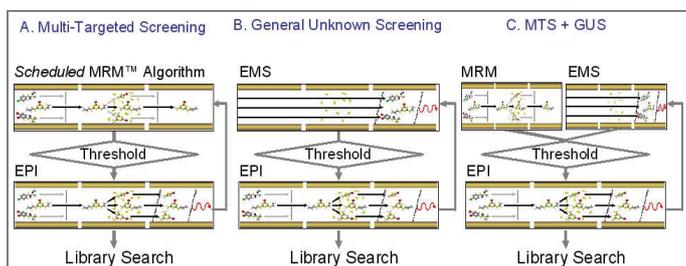
MULTI-TARGETED SCREENING + GENERAL UNKNOWN SCREENING

The 3200 Q TRAP® instrument is a hybrid triple quadrupole/linear ion trap mass spectrometer – a unique, flexible LC/MS/MS system that can accommodate a wide variety of both quantitative and qualitative workflows. The instrument is based

on a triple quadrupole platform, using Q1 and Q3 as mass selective filters and Q2 as a collision cell for fragmentation. Because it is a triple quadrupole, true Multiple Reaction Monitoring experiments (the gold standard in highly selective and sensitive quantitative analysis) can be performed as part of a MTS workflow (Figure 1A). In addition to triple quadrupole functionality, the Q TRAP® instrument incorporates linear ion trap capabilities in Q3, allowing ion accumulation for full scan MS or MS/MS analysis, which provides high sensitivity qualitative spectra. In full scan MS mode an Enhanced MS (EMS) scan can be used for a GUS workflow procedure (Figure 1B) allowing the user to interrogate the data for additional “unknowns” at a later date. Ion trap enhanced product ion (EPI), or full scan MS/MS, spectra can be acquired after triggering off either an MRM (Figure 1A) or EMS (Figure 1B) survey scan after a set threshold is reached, and searched against a spectral library for compound identification and/or structural information. It is the ability to use both triple quadrupole and linear ion trap scan functions on a single platform – and even within a single LC/MS/MS run – that makes the QTRAP® LC/MS/MS system adaptable to a wide variety of both screening and quantitative tests.

For a comprehensive drug screening both MTS and GUS are required. A comparison of MTS, GUS and MTS+GUS for detection and identification of drugs in plasma using a Q TRAP® LC/MS/MS system is presented. We present concurrent analysis of MTS and GUS (MTS+GUS) for the comprehensive screening of forensic toxicological analysis in which EPI spectra are acquired for “target” and “unknown” compounds (Figure 1C). The EPI spectra were submitted for library searching against an AB SCIEX iMethod™ Meta MS/MS spectral library which contains MS/MS spectra for over 2400 compounds including pesticides, mycotoxins, veterinary drugs, pharmaceuticals and drugs of abuse, to confirm compound identifications.

Figure 1. Screening Workflows



A. Multi-Targeted Screening (MTS); high sensitive and selective detection. B. General Unknown Screening (GUS); comprehensive detection. C. Combined MTS and GUS; efficient and effective MS/MS spectrum collection from both targeted and unpredicted compounds

UNAMBIGUOUS CONFIRMATION AND IDENTIFICATION OF COMPOUNDS BY MS/MS SPECTRAL LIBRARY SEARCHING

Compound identification using triple quadrupole based mass analysers is typically performed by monitoring of two MRM transitions and calculating the area ratio of the quantifier and qualifier ion. This MRM approach is limited to targeted screening and quantitation and the potential risk of false positive and negative results can be minimized by acquiring EPI spectra, using the Q TRAP®, triggered from an MRM or EMS. An EPI is an MS/MS spectrum that contains the complete chemical fingerprint of a molecule that can be matched to a library spectrum and thus providing an added degree of confidence for unambiguous confirmation of a known target or identification of an unknown compound. Software is an important part of this library matching process and confidence in identification of the compounds; to allow the user to effectively search compound libraries.

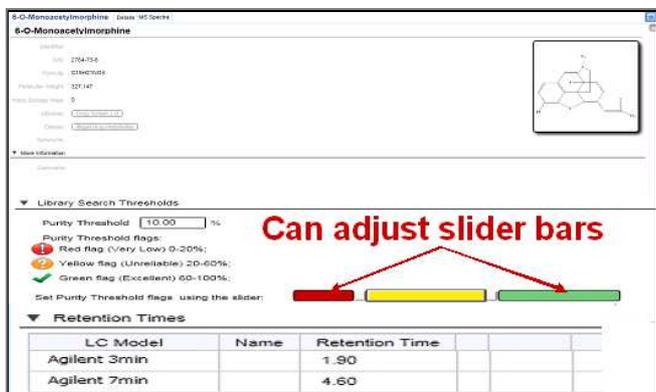
The new LibraryView™ Software is able to do this by allowing the user to mine compound libraries using unknown spectrum for compound identification. LibraryView™ Software is an easy-to-use software solution for fast and effective identification and confirmation of the presence of unknown compounds in a sample analysed using AB SCIEX Q TRAP® or TripleTOF® mass spectrometers. The LibraryView™ Software interface provides an intuitive venue to create, modify and customize compound libraries to include only the compounds you need to search, with the ability to add multiple MS/MS libraries and spectral data for dynamic and robust library searching. Compounds can be easily added and deleted from these libraries.

LibraryView™ Software

- Create and maintain full scan MS/MS spectral libraries
- Interactively search library spectra with acquired data
- Leverage available MS/MS libraries as well as creating new libraries to meet user applications
- Compatible with Wiley's accurate mass library for forensic applications
- Export user created libraries or purchased libraries
- Create custom reports using existing Reporter software
- Create analytical methods from the compound database

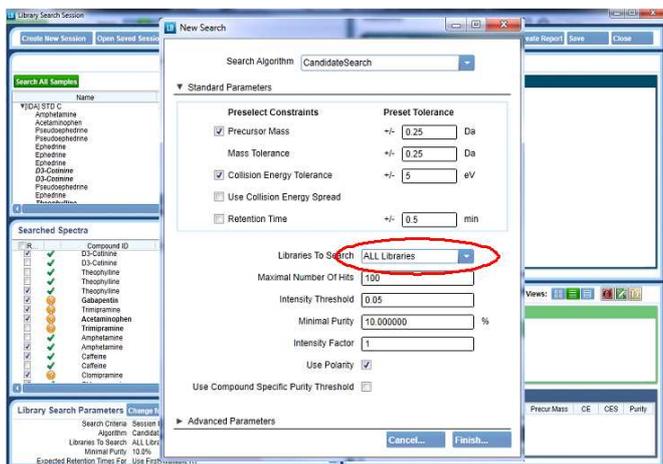
LibraryView™ Software allows compound specific library search thresholds (Figure 2) to be used as well as uses user-configurable search parameters (Figure 3).

Figure 2. Compound Specific Library Search Thresholds



Modifying and customizing compound libraries

Figure 3. User-Configurable Search Parameters

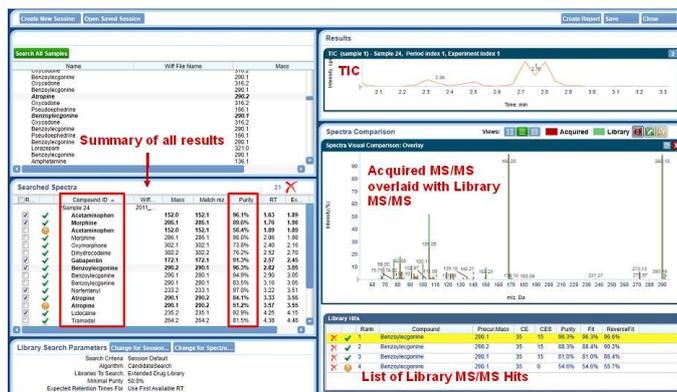


Adjusting individual search parameters to fit the needs of the session or the individual spectra. Search all the libraries or just the library you need.

The automated library searching provides fast mining of compound libraries for compound identification of unknown spectrum and displays the results in a user friendly, interactive library search results software pane (Figure 4).

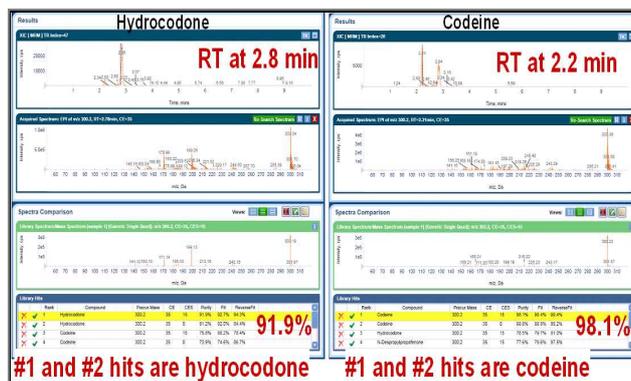
Figure 5 shows how the ability to interact with the software adds confidence in your library hits and compound identifications by being able to search through the list of library MS/MS hits for a particular acquired spectrum. In this example shown in Figure 5 we confidently identify the correct isobaric when comparing hits for hydrocodone and codeine. The acquired spectrum on the right has hits for both codeine and hydrocodone but the highest hits are for hydrocodone and the ability to visually look through the list of hits gives confidence that this acquired spectrum is hydrocodone and not codeine. On the other hand the acquired spectrum on the right has been confidently identified as corresponding to codeine and not hydrocodone.

Figure 4. Interactive Library Search Results



Library search window allows full interaction with the data, compare purity scores for multiple hits, change search parameters or re-search different libraries

Figure 5. Identifying the Correct Isobaric



Comparing purity scores and spectral matches for multiple hits and confidently identifying the correct isobaric compound

We present here the effectiveness of using LibraryView™ Software to confidently identify compounds from MS/MS data collected from the 3TR drug screening strategies described above. A test sample containing 7 psychotropics drugs (mimicking conceivable compounds) and 2 pesticides (mimicking unpredicted compounds) were analysed by each of the screening strategies and the LibraryView™ Software automated library searching results were compared.

Experimental

A mixture of nine compounds [seven psychotropics (PS) and two pesticides (PE)], (Table 1), were spiked into plasma at 1 µg/mL and de-proteinized by adding acetonitrile, then diluted in water to 100 ng/mL.

Table 1. Correspondence Table of Each Chemical in the Mixture (PS: Psychotropics, PE: Pesticides)

| | Compound Name | Category |
|---|-----------------|----------|
| 1 | Promethazine | PS |
| 2 | Amitriptyline | PS |
| 3 | Amoxapine | PS |
| 4 | Haloperidol | PS |
| 5 | Trazodone | PS |
| 6 | Carbamazepine | PS |
| 7 | Diphenhydramine | PS |
| 8 | EPN | PE |
| 9 | Dichlorvos | PE |

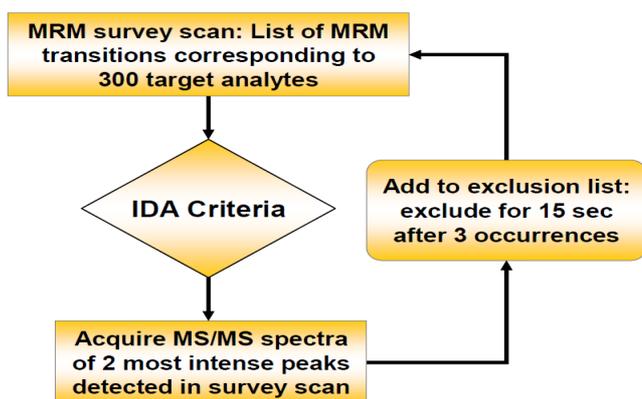
Spiked as conceivable drugs; Spiked to represent unexpected compounds

The mixture was analysed using a 3200 Q TRAP® mass spectrometer. Both targeted, general unknown screening and MTS+GUS experiments were performed on the mixture.

MTS

For MTS experiments, independent data acquisition (IDA) using an MRM survey scan and two dependent EPI scans were utilized. The experiment is outlined in Figure 6.

Figure 6. MRM-IDA-EPI Workflow



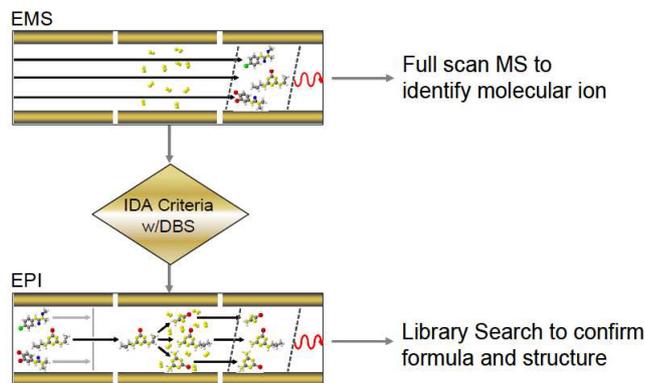
Multiple Reaction Monitoring-Information Dependent Acquisition-Enhanced Product Ion (MRM-IDA-EPI) workflow used for multi-target screening (MTS)

A survey scan consisting of 300 targeted MRM transitions was used to identify potential drugs. When a signal in an MRM transition is detected, the precursor ion is submitted for an enhanced product ion scan (linear ion trap full scan MS/MS).

GUS

GUS experiments were based on the use of a full scan single enhanced MS survey scan to detect the major peaks. This enhanced MS (EMS) survey scan is acquired in linear ion trap mode for optimal sensitivity. Pesticides, mycotoxins, veterinary drugs, designer drugs, pharmaceuticals, drug metabolites and drugs of abuse can all be detected with GUS based workflows. Dependent EPI spectra were collected on the two most intense peaks in the EMS survey scan. Figure 7 shows the outline of the experiment. When using single MS as a survey scan, matrix/background interferences can be present at high levels. To maximize the likelihood that dependent EPI spectra are acquired of potential exogenous drugs, dynamic background subtraction (DBS) is used. DBS is an algorithm that subtracts background signals in real time to minimize acquisition of dependent EPI spectra of background signals.

Figure 7. EMS-IDA-EPI Workflow

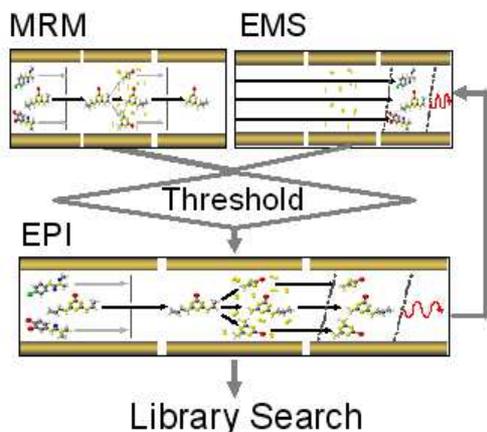


Enhanced Mass Spectrum-Information Dependent Acquisition-Enhanced Product Ion (EMS-IDA-EPI) workflow used for General Unknown Screening (GUS)

MTS+GUS

Targeted and general unknown screening were combined in a single method by having two survey scans; both an EMS and an MRM survey scan with an IDA criteria to trigger EPI dependent scans for “target” and “unknown” compounds that can be used in library searching. The workflow is shown in Figure 8.

Figure 8. MRM+EMS-IDA-EPI Workflow



Multiple Reaction Monitoring +Enhanced Mass Spectrum-Information Dependent Acquisition-Enhanced Product Ion workflow used for combined Multi-Targeted and General Unknown Screening (MTS+GUS)

LibraryView™ Software was used to automatically search all IDA triggered EPI spectra for library match based on purity score and compound confirmation, searching against the AB SCIEX iMethod™ Meta MS/MS spectral library database, that had been imported into the software. The software allows easy visualization of library searching results and reporting (Figure 10).

Results

As expected, the Multi-Targeted Screening workflow detected and returned library hits for all seven spiked psychotropics with, on average, the highest purity scores when compared to the other screening workflows (Table 2). Figure 9 shows a screen shot of the interactive library search results, showing the compounds identified and their respective purity score library matches. An example of one of the drugs found is shown where both the acquired spectrum and its top library spectral match score are displayed as a visual comparison. The time in the chromatogram where this compound eluted is also shown.

General Unknown Screening workflow also detected and returned library hits for the conceivable seven psychotropics with comparable purity scores to the MTS but in addition also found two pesticides with greater than 90% purity score library hits (Table 2). A total of nine compounds were detected and confidently identified using the GUS workflow and LibraryView™ Software, including the unexpected compounds EPN and Dichlorvos that the MTS workflow missed. Figure 10 shows a screen shot of the interactive library search results, showing the compounds identified and their respective purity score library matches. An example of one of the unexpected pesticides found is shown where both the acquired spectrum and its top library

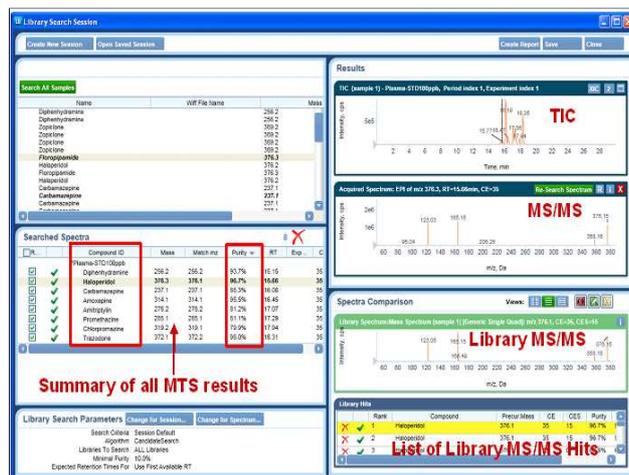
spectral match score are displayed as a visual comparison, in this case as an UpDown view. Figure 10 also shows a report that has been generated, showing a summary of the results of the MS/MS library search. Custom reports can be generated using existing Reporter software; creating reports with custom templates in Word or PDF format that contains all the analysis information you need.

Table 2. LibraryView™ Software Library Match % Purity Score Results after Searching the iMethod™ Meta MS/MS Library with IDA triggered EPIs Generated from Three Screening Workflows

| | Compound Name | MTS | GUS | MTS+GUS |
|---|-----------------|-----|-----|---------|
| 1 | Promethazine | 81 | 76 | 70 |
| 2 | Amitriptyline | 81 | 84 | 78 |
| 3 | Amoxapine | 95 | 96 | 89 |
| 4 | Haloperidol | 97 | 98 | 98 |
| 5 | Trazodone | 98 | 98 | 97 |
| 6 | Carbamazepine | 88 | 85 | 84 |
| 7 | Diphenhydramine | 93 | 91 | 86 |
| 8 | EPN | × | 94 | 94 |
| 9 | Dichlorvos | × | 94 | 86 |

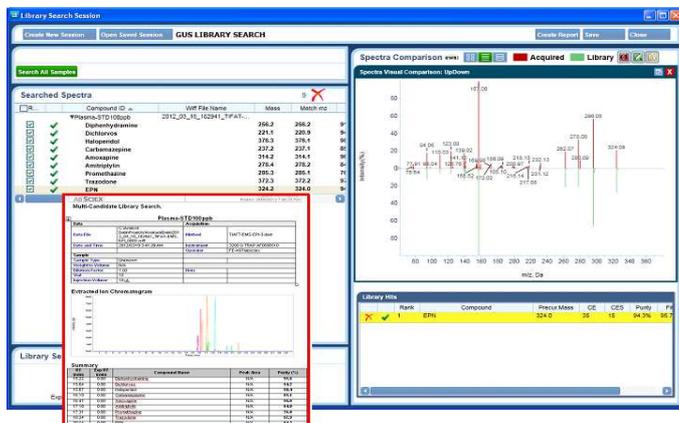
Compounds detected and identified by LibraryView™ Software MS/MS spectral library searching with Enhanced Product Ion spectra generated from Multi-Targeted, General Unknown Screening and MTS+GUS workflows. The % purity score for each compound match are shown

Figure 9. Interactive Library Search Results from Multi-Targeted Screening



Compounds identified with their respective purity scores are shown as well as an example of an acquired spectrum/library spectrum match

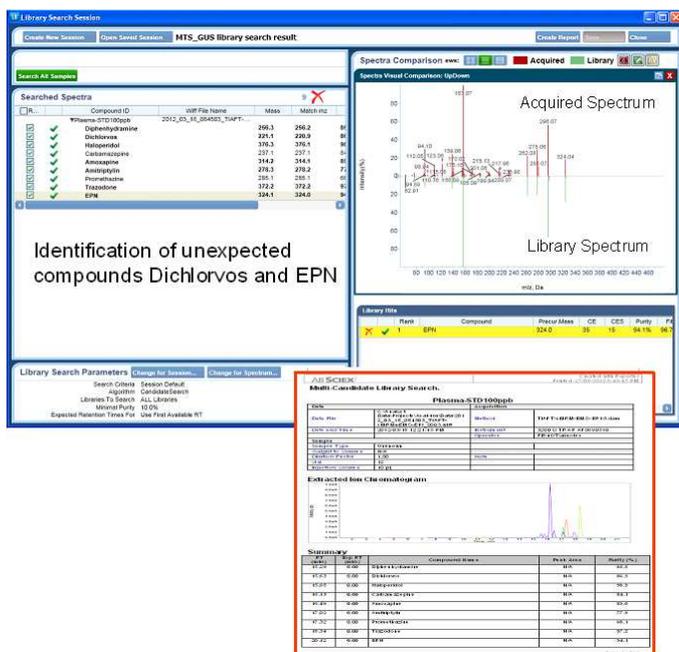
Figure 10. LibraryView™ Software General Unknown Screening Results with a Generated Report



A total of nine compounds were detected and confidently identified using the GUS workflow and LibraryView™ Software, including unexpected pesticides EPN and Dichlorvos that the MTS workflow missed.

The results of the LibraryView™ Software spectral library searching with IDA triggered EPIs generated from the MTS+GUS workflow are shown in Table 2 and Figure 11. This novel screening approach has allowed the identification of all nine compounds comprehensively from a single injection with greater than 70% library matching purity scores for all compounds, including the unexpected pesticide compounds Dichlorvos and EPN.

Figure 11. MTS+GUS Workflow LibraryView™ Software Results with a Generated Report.



LibraryView™ Software has several sample acquired and library spectra visualization comparison options. Shown here is the UpDown option.

Conclusions

LibraryView™ Software is a powerful tool, allowing for advanced automated spectral library searching for compound identification in forensic toxicology samples.

A number of LC/MS/MS drug screening approaches have been performed to identify “known” and “unknown” compounds from a plasma sample. The Q TRAP® technology allows the generation of highly sensitive full scan MS and MS/MS spectra which is amenable to the targeted and general unknown screening workflows. LibraryView™ Software has been successfully used to identify all the compounds in a plasma sample based on % purity spectral library matching scores of IDA triggered EPI spectra generated from each of the screening workflows tested. The novel approach, MTS+GUS, has been shown to be considered a feasible and efficient method for screening of forensic toxicological analysis. Both conceivable as well as unexpected compounds can be identified and confirmed with confidence based on MS/MS spectral library searches using the LibraryView™ Software, all from a single injection.

KEY POINTS OF THE LIBRARYVIEW™ SOFTWARE

- Ability to search across multiple libraries (and merge libraries)
- Interactive MS/MS library searching and improved MS/MS library search matches
- Automated report generation

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