K27 Advanced Automated Library Searching for Compound Identification in Forensic Toxicology Samples

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After attending this presentation, attendees will learn about the different complimentary screening techniques that can be performed on a hybrid quadrupole linear ion trap to confidently identify targeted and unknown compounds. Attendees will learn about using acquired Tandem Mass Spectrometry (MS/MS) data to search against MS/MS libraries and utilizing a new advanced automated library searching with the capability to dynamically review the collected MS/MS information. Attendees will see that the software substantially improves the "data mining" process and provides an elegant solution to automated processing of the forensic toxicology screening data to confidently identify compounds.

This presentation will impact the forensic science community by demonstrating the advantages of the new automated library-searching approach in improving typical forensic toxicology screening workflows.

Liquid Chromatography with Tandem Mass Spectrometry (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 the 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.

Forensic toxicology samples were analyzed using generic sample preparation procedures with two AB SCIEX LC/MS systems: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument. The tandem mass spectrometric measurements were performed using the Collision Energy Spread (CES) feature which ensures the detection of the fragment ions generated in low-, medium-, and high-collision energy regimes. All the collected MS/MS spectra were searched against an AB SCIEX Forensic Drug Spectral Library comprised of over 1,250 compounds. The data processing was performed with the new AB SCIEX prototype library searching tool equipped with two library search algorithms. The accuracy, flexibility, speed, and robustness of the new library searching approach was successfully demonstrated in the processing of the data specifically acquired in different experimental set-ups. The ion trap screening data were collected in three screening workflows that consisted of several looped experiments as follows:

1. Multitargeted Screening:
   a. MRM detection of 300 analytes with the *Scheduled* MRM™ algorithm.
   b. Enhanced Product Ion (EPI) dependent scans set to automatically collect MS/MS fragmentation spectra for the targets detected in experiment 1.
2. General Unknown Screening:
   a. Enhanced Mass Spectrum (EMS) monitoring for the detection of the unknown analytes.
   b. EPI dependent scans set to automatically collect MS/MS fragmentation spectra for the unknowns detected in experiment 1.
3. Combined multitargeted and unknown screening:
   a. MRM detection of 300 analytes with the *Scheduled* MRM™ algorithm.
   b. EMS monitoring for the detection of the unknown analytes.
   c. EPI dependent scans set to automatically collect MS/MS fragmentation spectra for the targets identified in experiment 1 and unknowns identified in experiment 2.

The hybrid quadrupole-time-of-flight data collected using a TOF/MS survey scan with IDA-triggering of up to 20 product ion scans was also processed. In all specified cases, both targeted and unknown drugs and metabolites were identified in selected samples with a high level of confidence (based on the values of purity, fit, and reverse fit). Utilization of the new advanced automated library searching with the capability to dynamically review the collected MS/MS information has been demonstrated to substantially improve the "data mining" process and provide an elegant solution to automated processing of the forensic toxicology screening data.

LC/MS/MS, Library Searching, Hybrid Linear Ion Trap