# Approaches to drug screening using Triple Quadrupole, QTRAP® and QTOF technologies

With the emergence of novel psychoactive substances (NPS), forensic toxicology labs have undergone an evolutionary change in their analytical testing and technology usage as the demands in the detection and identification of these new compounds have required different testing regimes.

No longer is the modern forensic toxicology lab able to solely utilize targeted screening even with a panel of a few hundred drugs. Nowadays, comprehensive screenings often require targeting for more than 1000 drugs including monitoring of their metabolites. The increased potency of these new substances has demanded rapid and comprehensive analytical methods that can provide identification of these drugs with high confidence and quantify them at low concentrations with good accuracy and reproducibility in a broad range of biological matrices.

Liquid chromatography (LC) coupled to tandem Mass Spectrometry (MS/MS) is a powerful analytical tool used in many forensic testing laboratories to detect drugs and their metabolites from a variety of biological matrices. When identifying and quantifying hundreds of compounds in challenging samples, the increased sensitivity of the latest generation mass spectrometers enables simplified workflows by allowing extensively dilution of sample extracts or the ability to utilize less sample volume when sample limited. This is an effective way to eliminate ion suppression caused by matrix components and the extended linear dynamic range allows quantification of more compounds to meet the most challenging forensic toxicology workflows.

For targeted screening, triple quadrupole and QTRAP mass spectrometers are the gold standard for routine high sensitivity detection and quantification of drug analytes. Multiple Reaction Monitoring (MRM) is the most common mode of employing triple quadrupole MS/MS for quantitative analysis. MRM functionality of these systems provide selective and sensitive quantification with the lowest limits of detection, excellent reproducibility and linear range. Using MRM ratios is a way to identify compounds with high confidence, that includes the ratio of quantifier and qualifier MRM transition. Despite the high selectivity of MRM detection, there is however always a risk of false positive findings due to interfering matrix signals. Acquiring full scan MS/MS data in an Enhanced Product Ion (EPI) experiment, using QTRAP® functionality, allows for searching against mass spectral libraries and can significantly increase confidence in identification. The combination therefore of triple quadrupole and QTRAP system functions allows for quantification and identification with MS/MS spectra in a single LC run.

The SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready is the latest offering of nominal systems that builds on the SCIEX legacy of groundbreaking innovation for quantitative performance. The continuing advancements in mass spectrometric technology from the ionization source all the way through the ion guide enabled improvement in the efficiency of ion capture and transmission, resulting in more sensitivity through sampling more ions with no sacrifice in robustness and reliability. The improved ion generation and sampling results in higher sensitivity and up to 6 orders of linear dynamic ranges, allowing quantification of more compounds across a wider range of chemical properties without the requirement for extensive sample preparation.

Forensic scientists are also concerned about screening for and identifying non-targeted compounds, including metabolites. High resolution and accurate mass LC-MS/MS systems are capable of performing highly sensitive and fast MS scanning experiments to search for unknown molecular ions while also performing selective and characteristic MS/MS scanning for further compound structural elucidations and, therefore, is the instrument

of choice for this challenging task. General unknown screening workflows do not use a target analyte list and compound detection is not based on any prior knowledge, including retention times and information on possible molecular and fragment ions. Therefore, acquired chromatograms are information-rich and can easily contain thousands of ions from both any compounds present in the sample as well as from the sample matrix. Powerful software tools are required to allow the exploration of such data and aid in the efficient data reduction to the significant components and identification of the unexpected compounds. Data processing include a combination of automated sample-control-comparisons followed by MS/MS library searching, empirical formula finding, and structural database searching.

For these untargeted workflows, the combination of the SCIEX X500R QTOF System and SCIEX OS Software provide a comprehensive solution designed for routine testing to deliver reliable and sensitive results in the forensic toxicology laboratory. The X500R QTOF System was designed with performance in mind and engineered to simplify screening and quantification of unknowns in complex biological samples. The X500R QTOF System is a flexible system that can be used for both high specificity, targeted quantification as well as non-targeted screening using acquisition methods such as IDA or SWATH® Acquisition to collect high resolution spectra from single sample sets in a routine testing laboratory environment. These non-targeted data acquisition strategies enable generation of high quality TOF MS and TOF-MS/MS spectra, which provide comprehensive compound fragmentation on all the analytes present in the sample. Because these fragments are acquired in high resolution, the detected compounds can be accurately identified through extraction of specific accurate mass fragment ions. These fragment ions can in turn be matched for identification through spectral library matching using the spectral database searching functionality of the software.

In addition to providing the ability to optimize, acquire, process and review the data in a streamlined and integrated fashion, SCIEX OS Software also enables retrospective data analysis (or data mining) of additional analytes missed in initial screens, which is becoming extremely relevant with the constant flux of new synthetic substances on the drug market. Full quantitative and qualitative analysis can be performed in one centralized platform that provides quick, intuitive and streamlined data processing power to produce accurate and reliable results.

To conclude, forensic testing has seen the transition into the adoption of tandem MS workflows with the routine use of triple quadrupole and QTRAP instrumentation. Developments in this technology in combination with continual software improvements have allowed for more compound coverage in a single workflow and helped streamline the process of getting to the right result, every time. As focus turns to identifying the significant components in a forensic sample in an untargeted workflow, high resolution and accurate mass LC-MS/MS systems such as the SCIEX TripleTOF® and QTOF Systems are quickly developing as the tool of choice with the capability to capture all information about a sample. That data can be processed using a targeted approach to identify known compounds and still quantify them at low concentrations with good accuracy and reproducibility. Most significantly, the same data can be processed using non-targeted approaches to identify the new, unknown compounds – all from a single instrument.



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