

Efficient Forensic Toxicological Screening and Quantitation Workflow Using QTOF LC-MS/MS System



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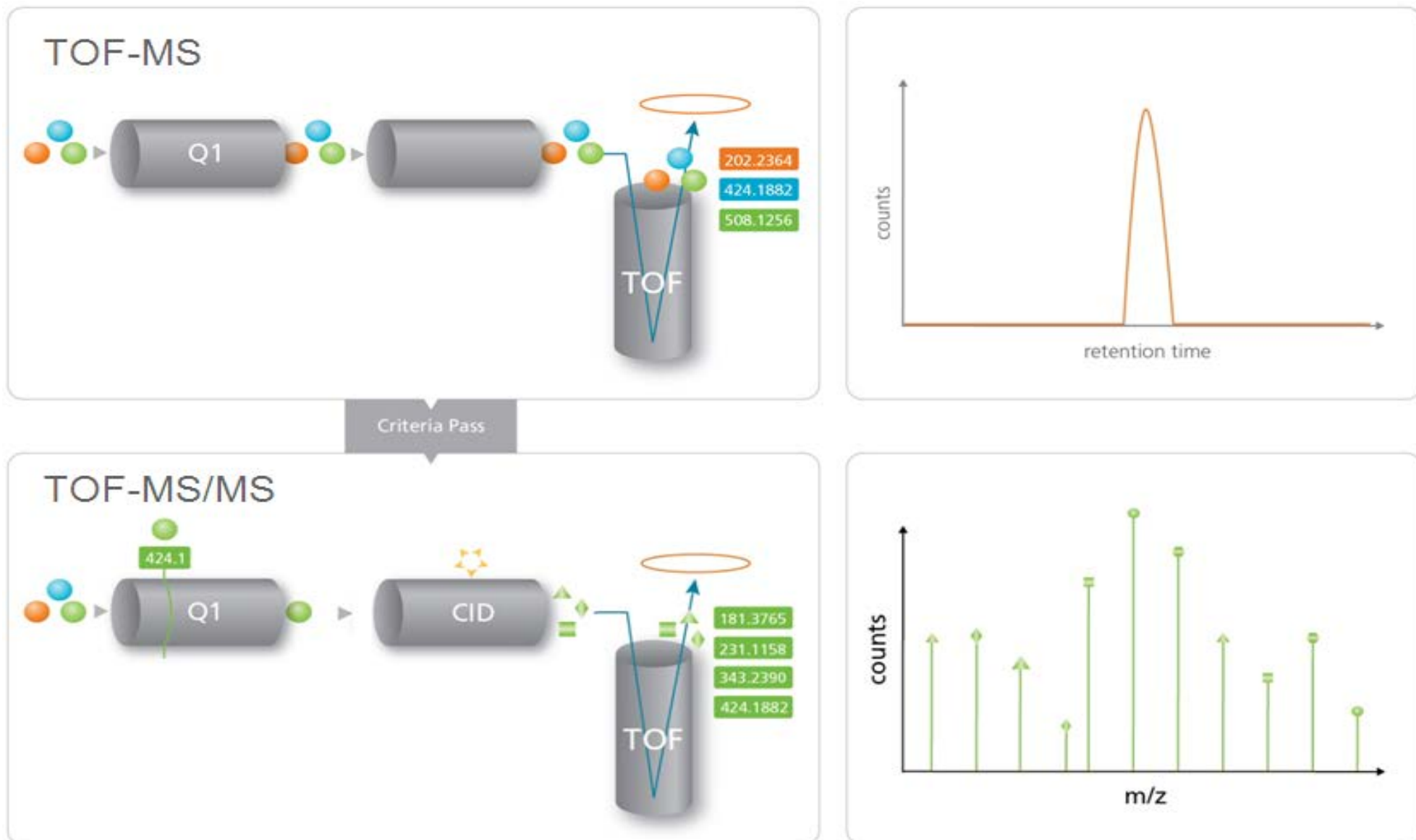
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INTRODUCTION

- Quadrupole Time-of-Flight (QTOF) mass spectrometry is becoming the desired technology for sensitive and selective screening workflows in a forensic toxicological setting.
- Using the accurate mass and mass resolution information from both TOF-MS and TOF-MS/MS acquired data allows for simultaneous highly specific targeted quantitation and non-targeted screening.
- Here we describe a new benchtop QTOF system with revolutionary N-optic designed flight path and new, intuitive software for easy adoption of accurate mass technology to forensic testing.
- We demonstrate that the new hardware and software combined allow a high level of confidence for compound identification and quantification from blood or urine samples in one seamless workflow.

MODES OF SCIEX X500R QTOF ACQUISITION

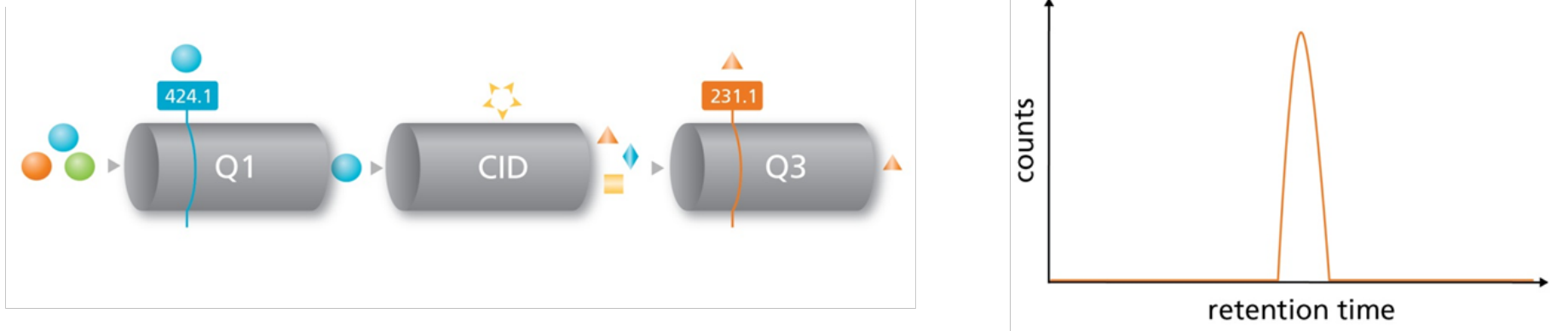
(1) Information Dependent Acquisition (IDA) of MS/MS



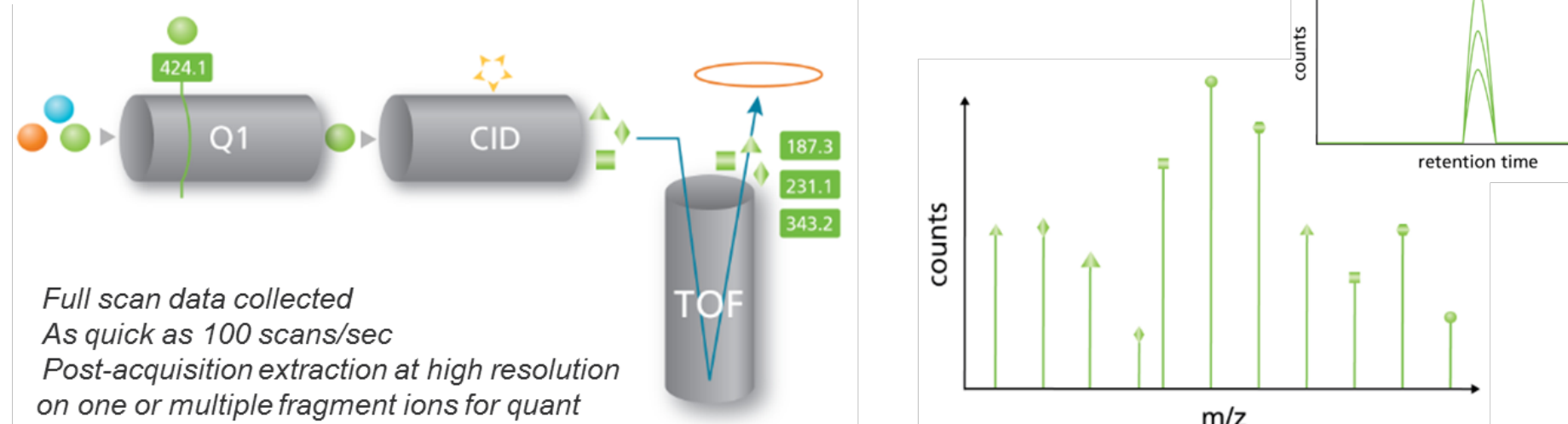
IDA-MS/MS provides the most interference-free fragmentation information

(2) Comparison of MRM^{HR} with traditional (unit resolution) MRM

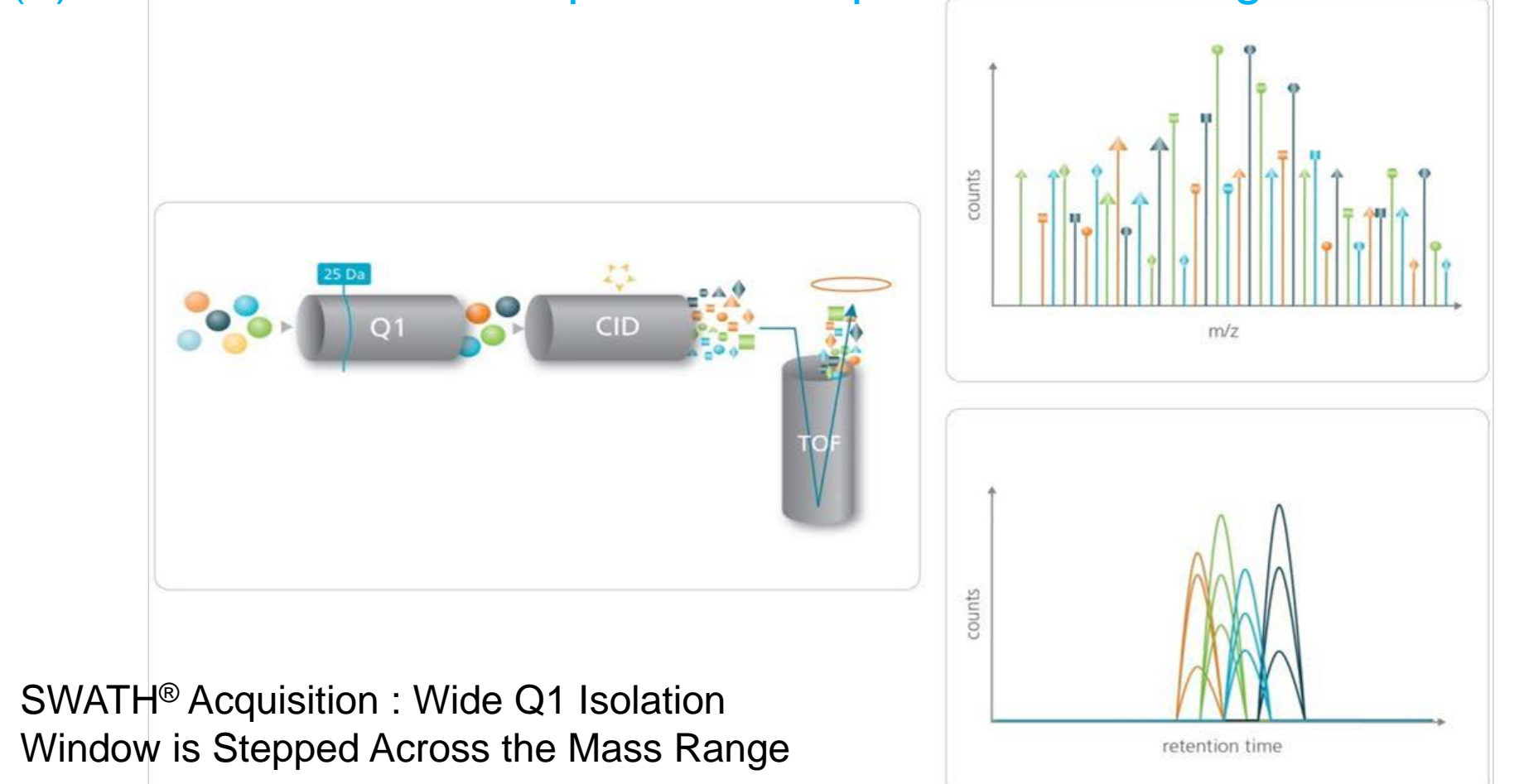
MRM (SCIEX Triple Quad™ or QTRAP®)



MRM^{HR} (QTOF: TripleTOF® or X500R QTOF)



(3) A Mode of Data Independent Acquisition Providing MS/MS^{ALL}



SWATH[®] Acquisition : Wide Q1 Isolation Window is Stepped Across the Mass Range

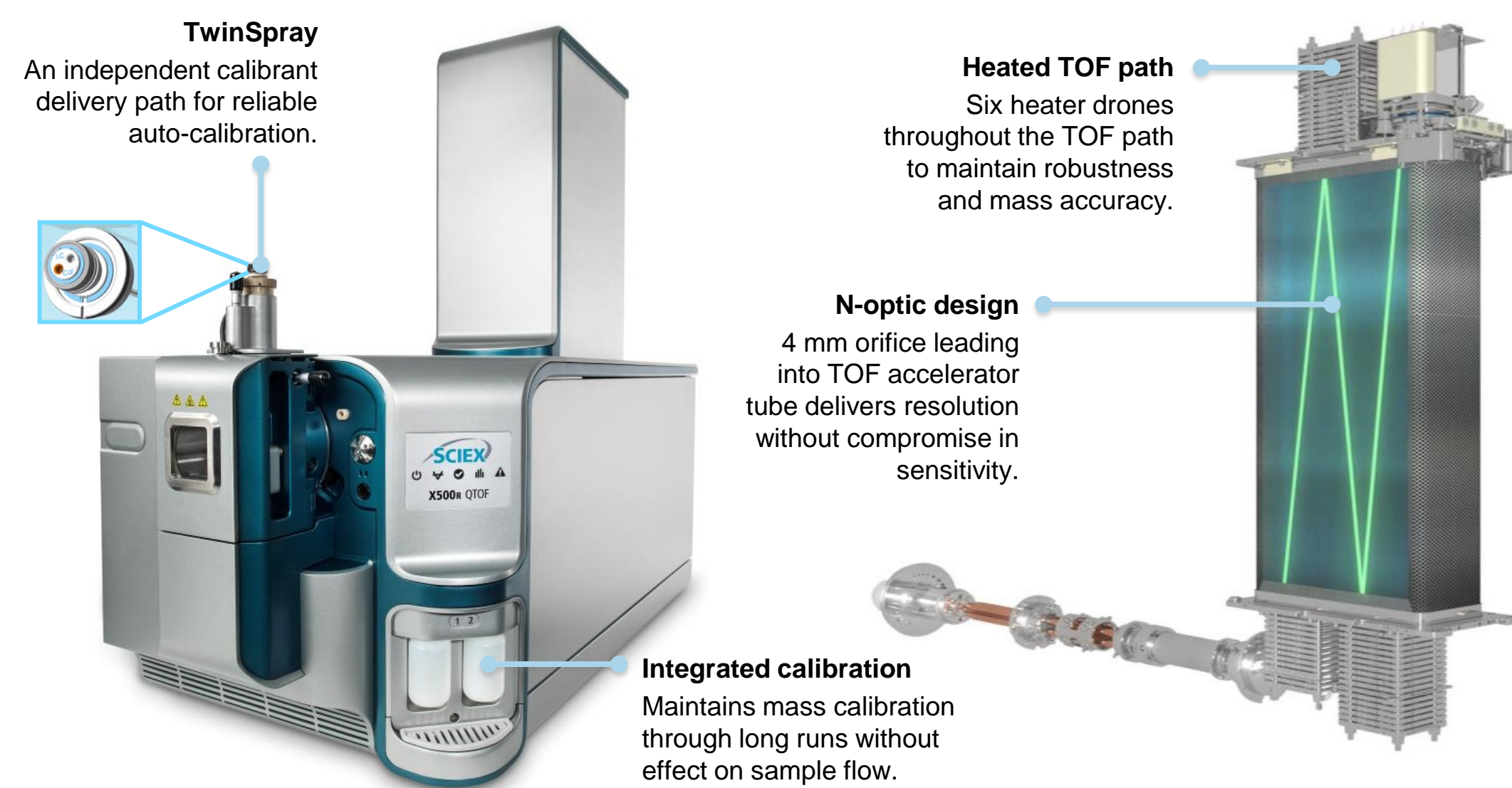
METHODS

Sample Preparation

Urine samples were diluted 10-fold in mobile phase A, centrifuged and 10 µL sample was injected.

HPLC Conditions

HPLC separation was performed using the SCIEX ExionLC™ AC system on a reverse-phase column (dimension: 50 × 2.1) at 30 °C with a 8.0 minute LC gradient. Mobile Phase A: H₂O with 5 mM NH₄FA; Mobile Phase B : MeOH with 0.05% formic acid



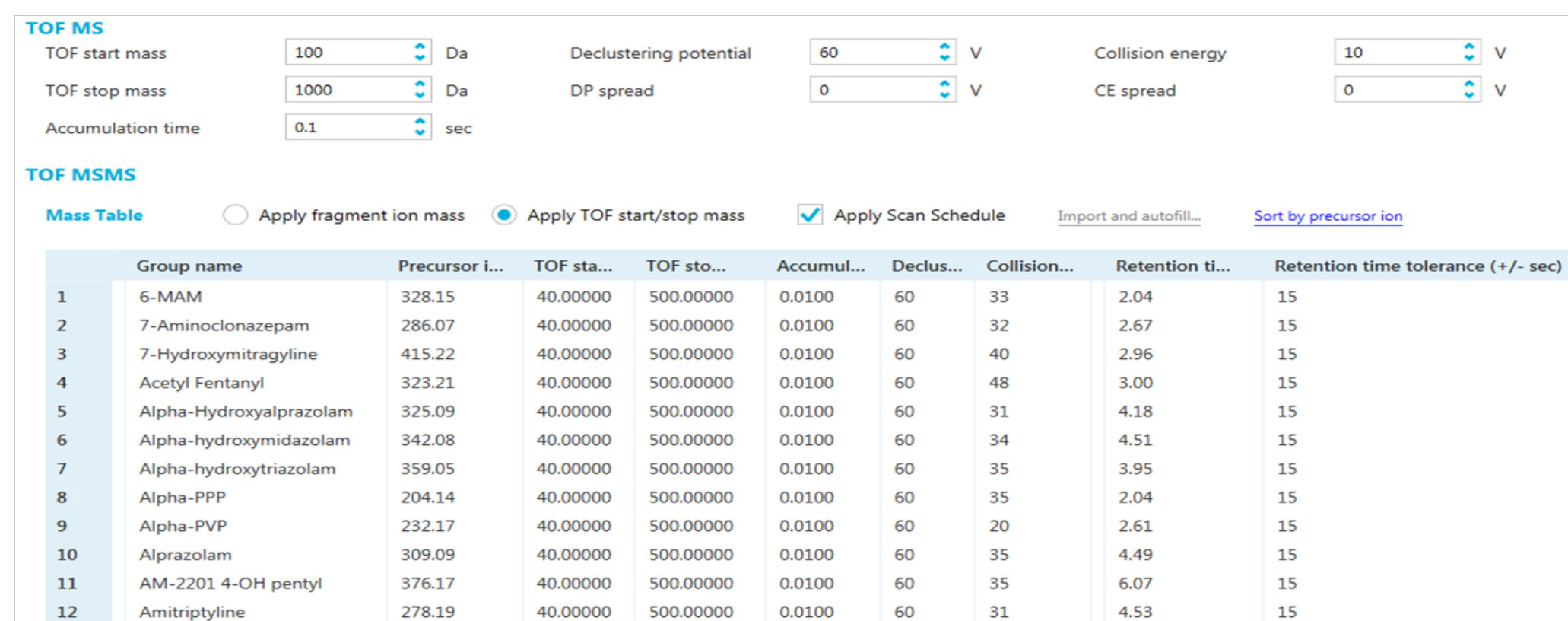
SCIEX X500R QTOF BENCHTOP SYSTEM AND DESIGN FEATURES

LC-30AD LC Gradient

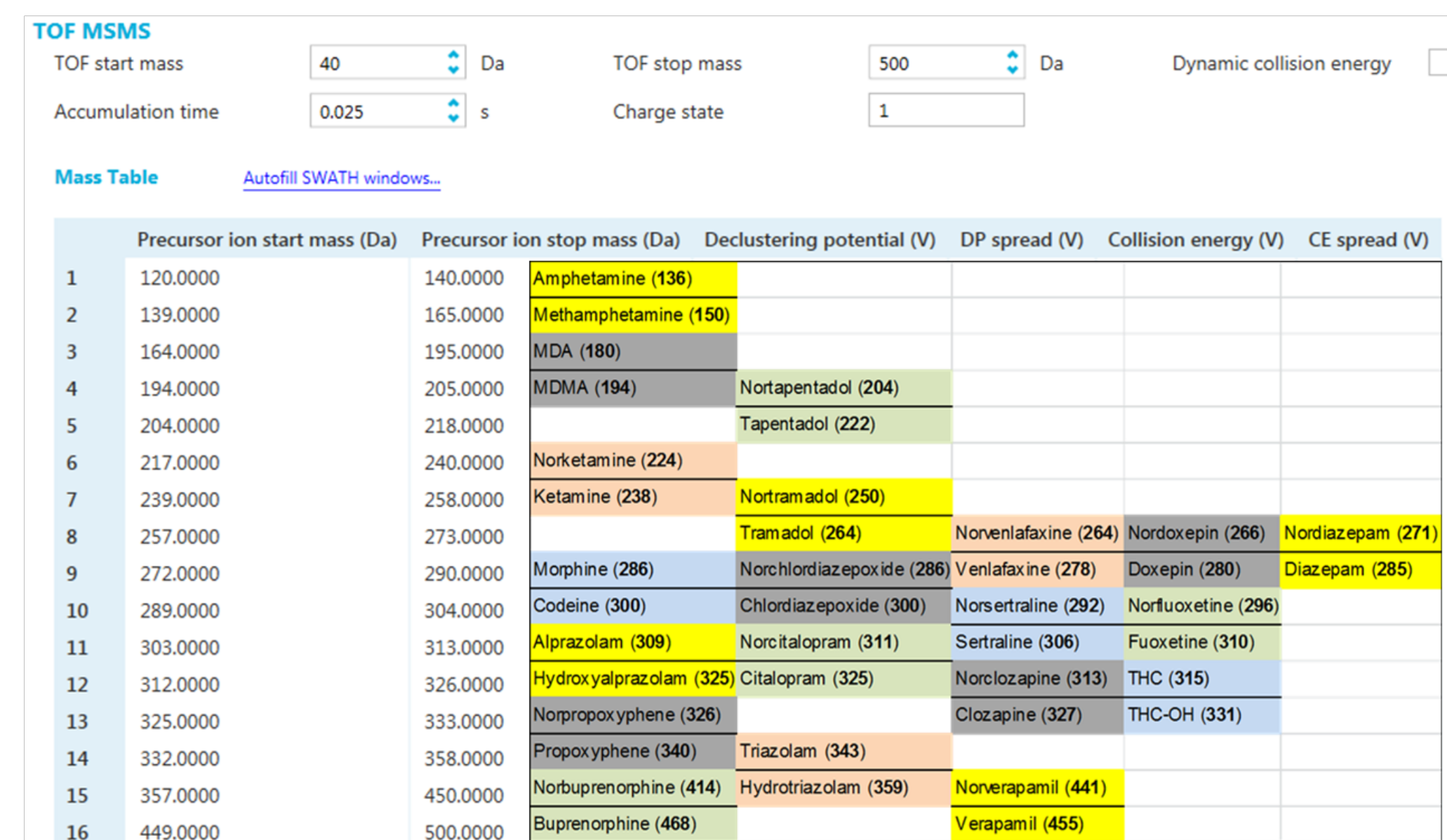
MS Acquisition

- 1) TOF-MS survey scan with Information Dependent Acquisition (IDA)-triggering up to 16 product ion scans
- 2) Scheduled MRM^{HR} or 3) SWATH[®] acquisition; the precursor isolation window width was varied for each of the 16 MS/MS experiments

Scheduled MRM^{HR}, 8 min, MS/MS full-scan



SWATH[®] Acquisition with variable windows



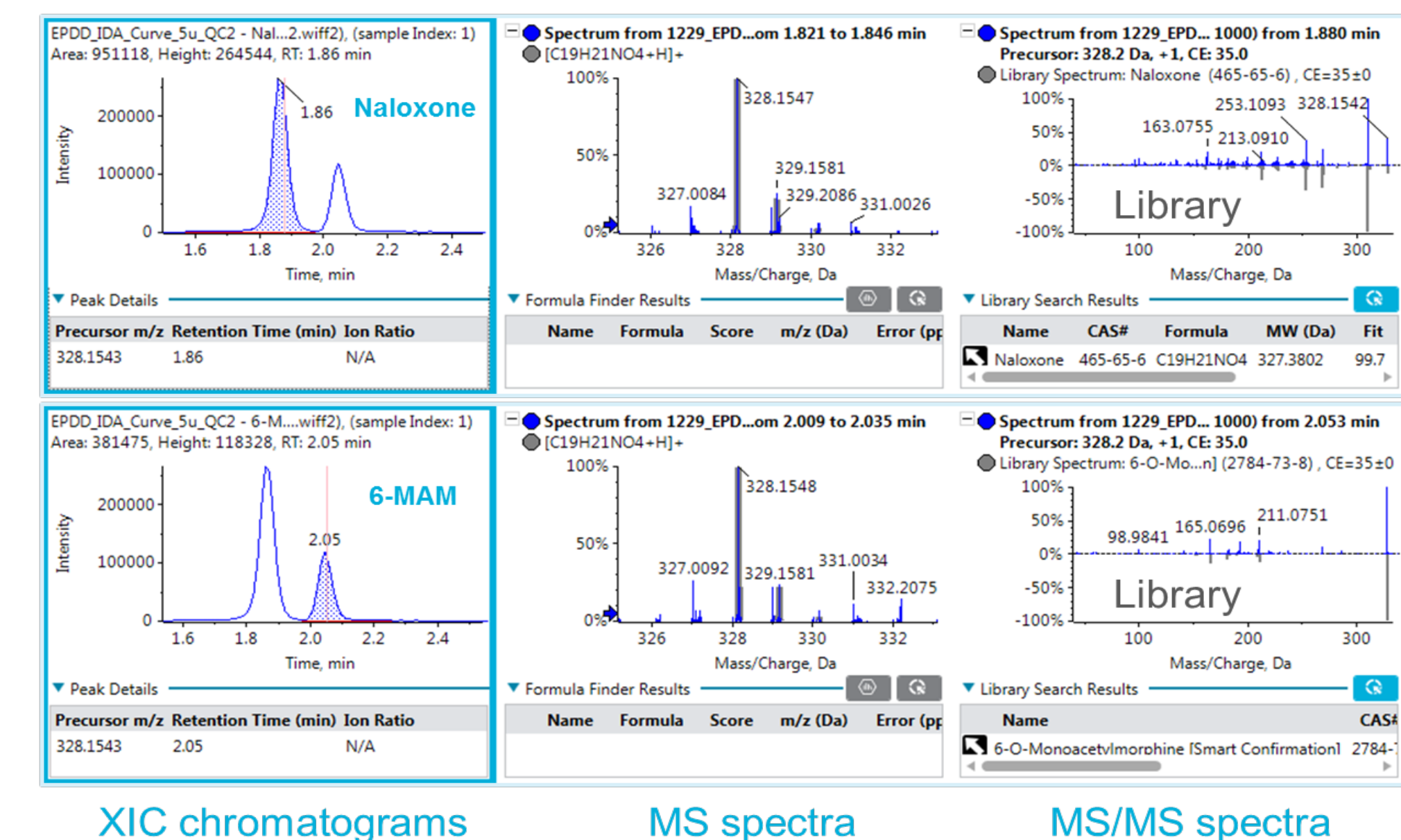
RESULTS

Review Quantitative and Qualitative Results

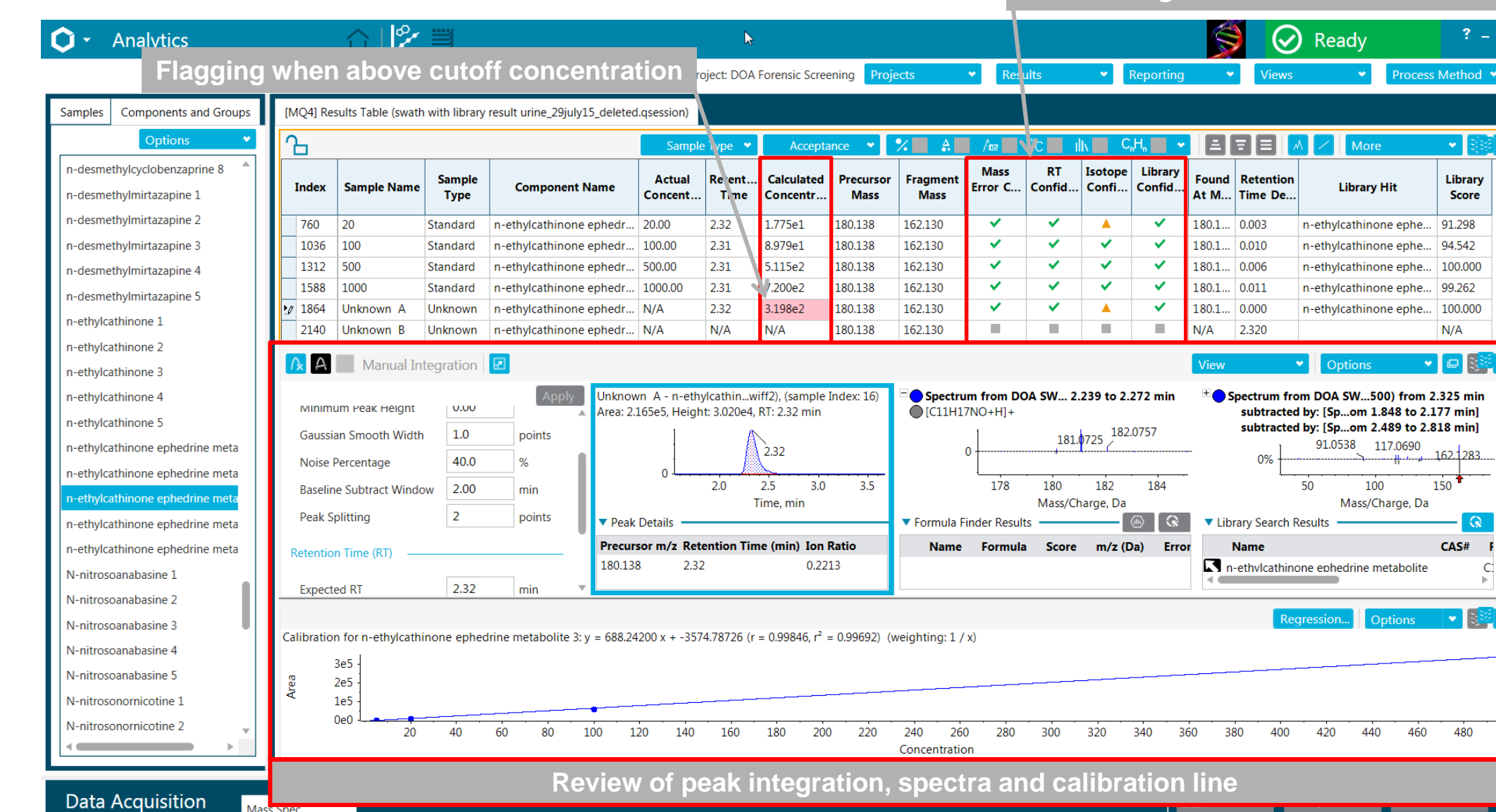


Targeted Data Processing of TOF-MS - Information Dependent Acquisition - TOF-MS/MS acquired data

Highest confidence gained through library MS/MS comparison

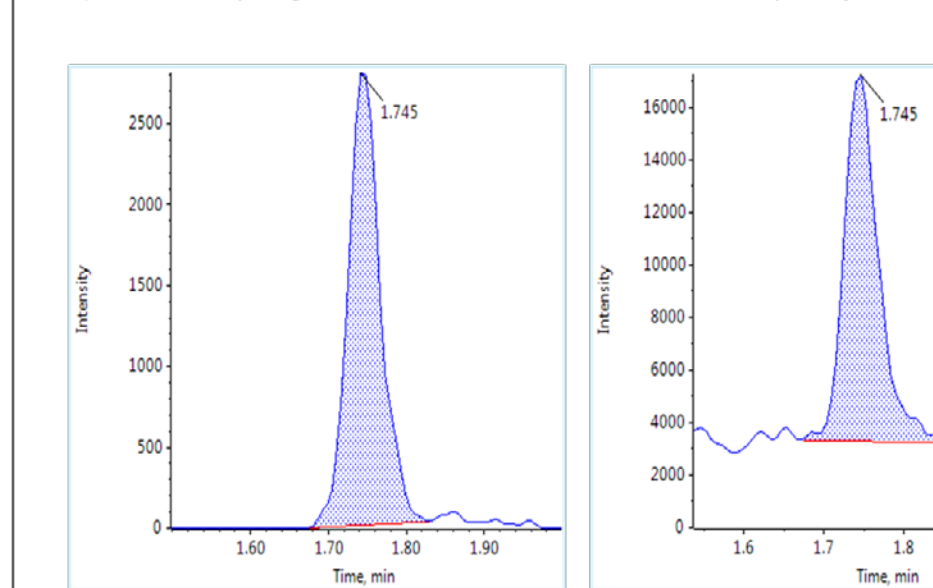


Review Quantitative and Qualitative Results



Scheduled MRM^{HR} (vs TOF scan)

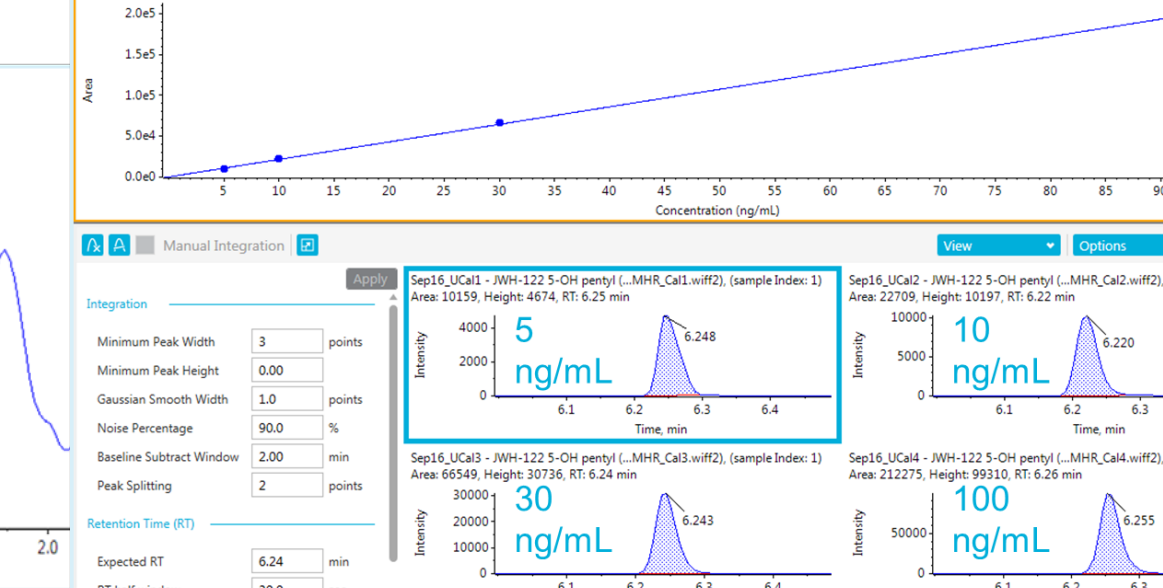
Buphedrone (5 ng/mL in urine, 10-fold dilution, 10 µL injection)



MRM^{HR}: 178.1→131.070±0.01 m/z TOF-MS: 178.1226±0.005 m/z

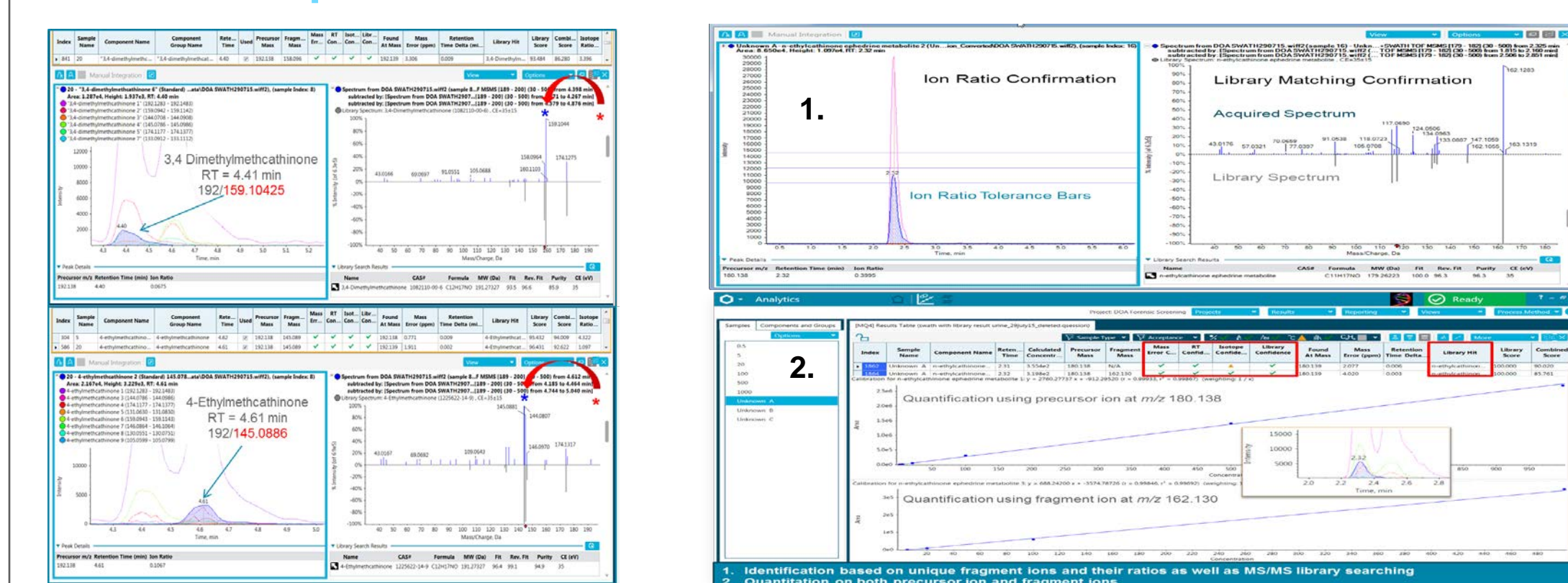
Scheduled MRM^{HR}, 372.2→169.0644±0.0100 m/z

JWH-122 5-OH pentyl in urine (Urine was diluted 10-fold, 10 µL injection)



MRM^{HR}: 372.2→169.0644±0.0100 m/z

SWATH[®] Acquisition with MS/MS^{ALL}



Confident Identification of Isomers using SWATH[®] Acquisition Based on Unique Fragment Ions and their Ratios

CONCLUSIONS

- A sensitive and selective workflow was developed for toxicological compound screening in a forensic setting using the new X500R QTOF system.
- Both the IDA-MS/MS and the novel MS/MS^{ALL} with SWATH[®] acquisition were used for the screening task.
- SWATH[®] acquisition provides consistent number of data points across the LC peak, and the resulted MS/MS data can not only be used for screening but also for quantitation when lower detection limit with complex matrix content in the samples.
- The newly designed software that accompanies the new benchtop QTOF instrument allows for simultaneous compound identification and quantitation in one step, all in one place.
- SCIEX OS software aims to allow the easy adoption of accurate mass technology to routine forensic workflows.

TRADEMARKS/LICENSING

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