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General Unknown Screening & Drug Screening Workflows using SCIEX QTRAP® LC-MS/MS Systems

RUO-MKT-11-7435-A

Approaches to Drug Screening using QTRAP® Systems

QTRAP® 4500 System

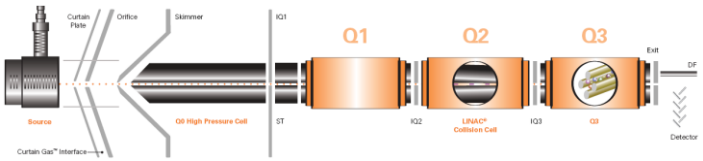
QTRAP® 5500+ System

QTRAP® 6500+ System

QTRAP® 7500 System

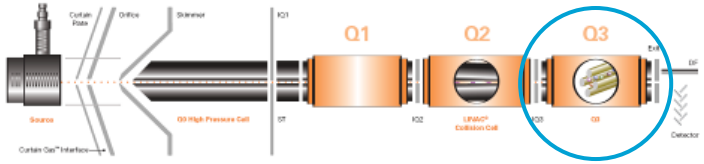
Advantage of QTRAP® System Technology

Triple Quad



- **Quantitation**
- ID with MRM ratio

QTRAP® system



- Quantitation
- ID with MRM ratio
- **ID with MS/MS library searching**

increasing confidence in compound ID

EPI – Enhanced Product Ion
EMS – Enhanced MS full scan
MS3 (MRM3) – MS/MS/MS

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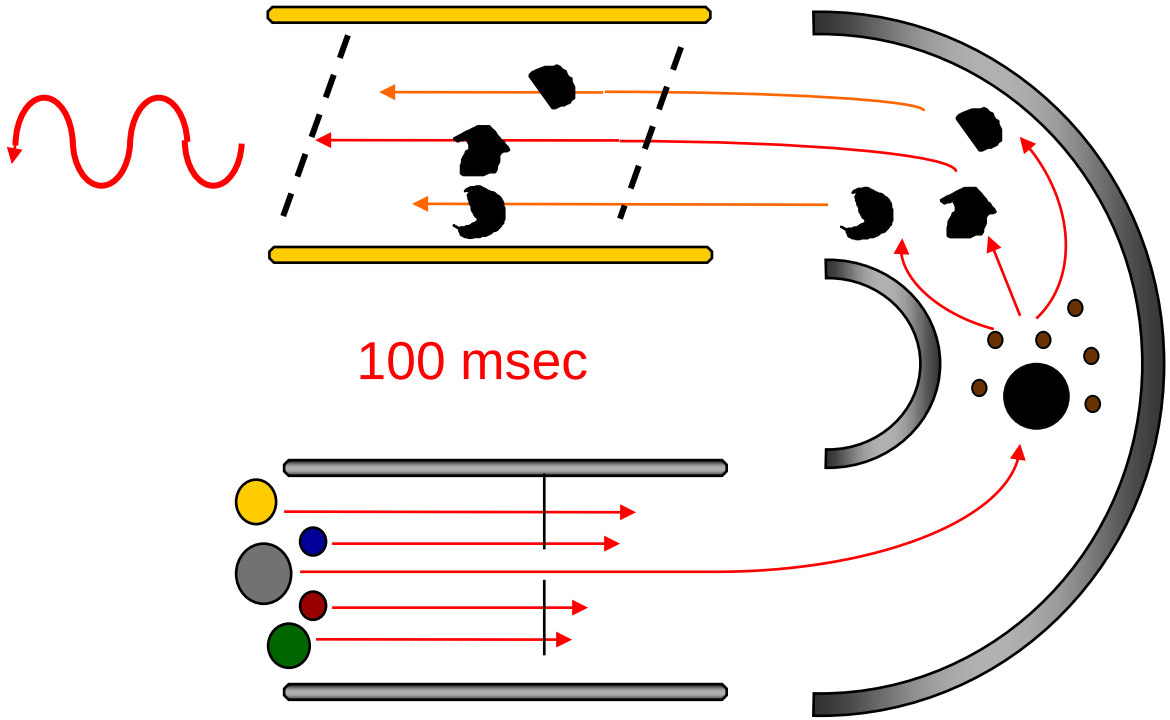
Enhanced Product Ion Scan (EPI)

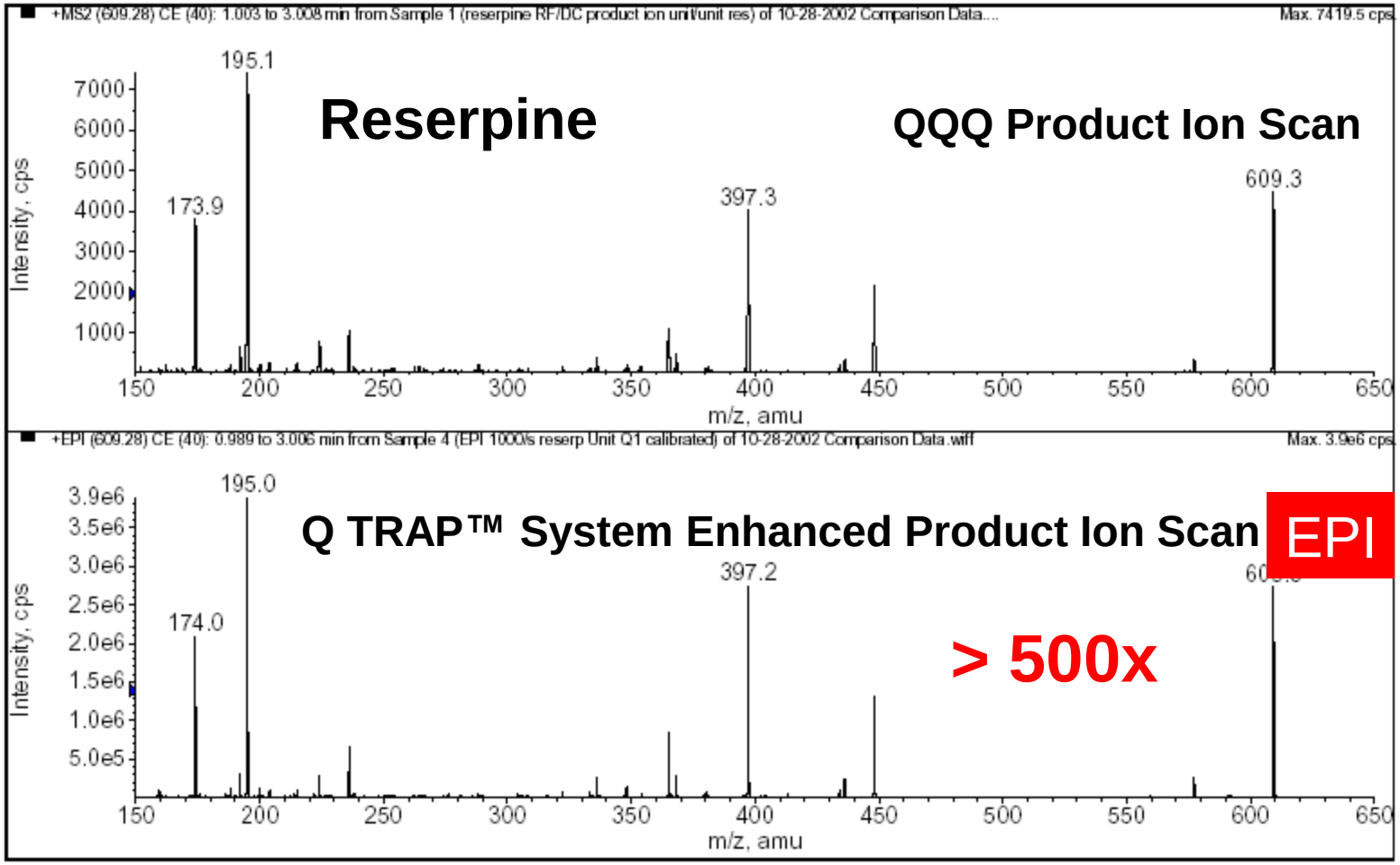


Q1: **SIM**

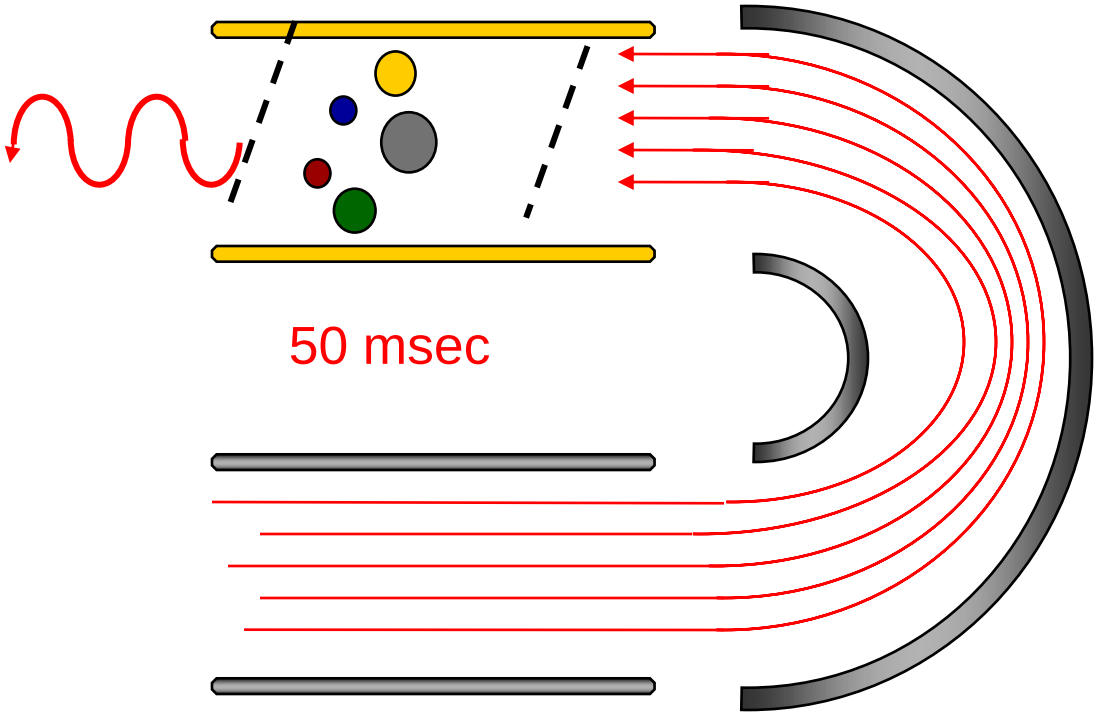
Q2: **Fragmentation**

LIT (Q3): **Trap scan**

Increased sensitivity - EPI



Enhanced MS Scan (EMS) - Search for Present Ions

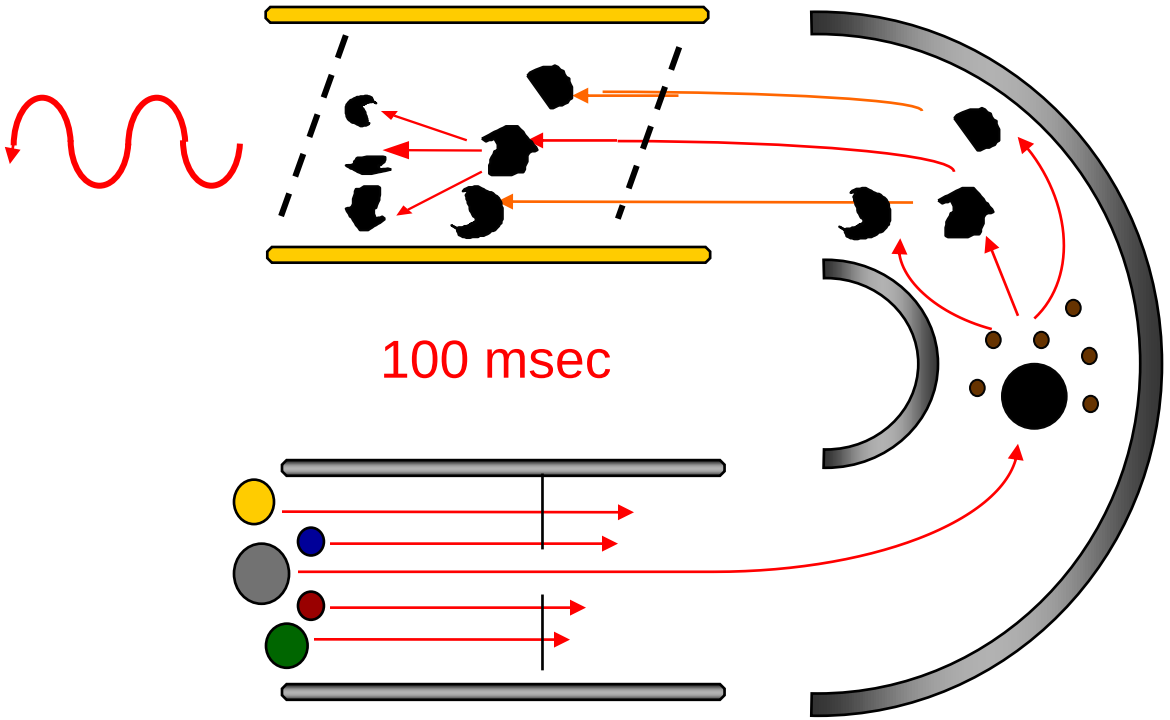


Q1: RF only

Q2: RF only

LIT (Q3): Trap scan

MS3 - additional structural information



Q1: **SIM** (selection of 1st precursor ion)

Q2: **Fragmentation**

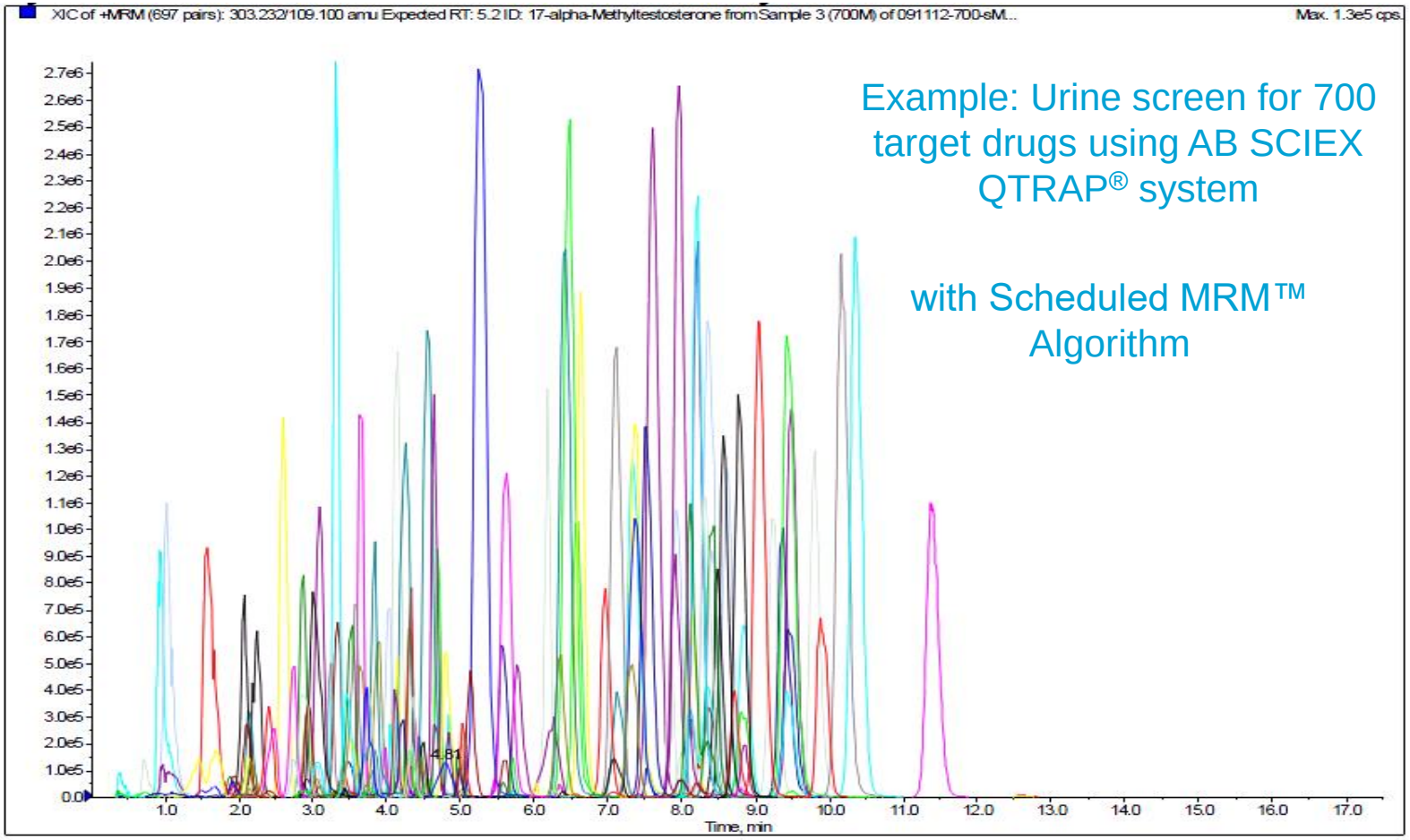
LIT (Q3): Trapping, isolation and fragmentation of 2nd precursor ion by single frequency excitation

Multi-Target Screening Approach

Multi-Target Screening (MTS) Approach with MRM

MRM detection provides ultimate *sensitivity* and *selectivity*

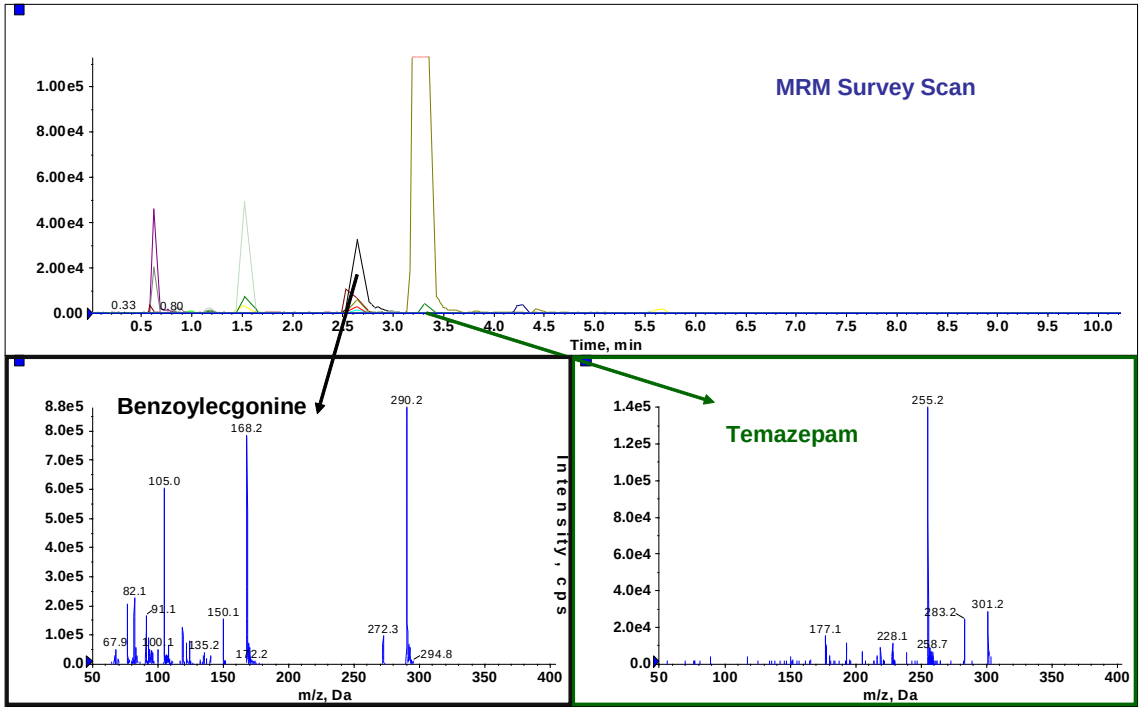
Two MRMs may be monitored, for additional confidence/confirmation



Multi-Target Screening + Confirmation

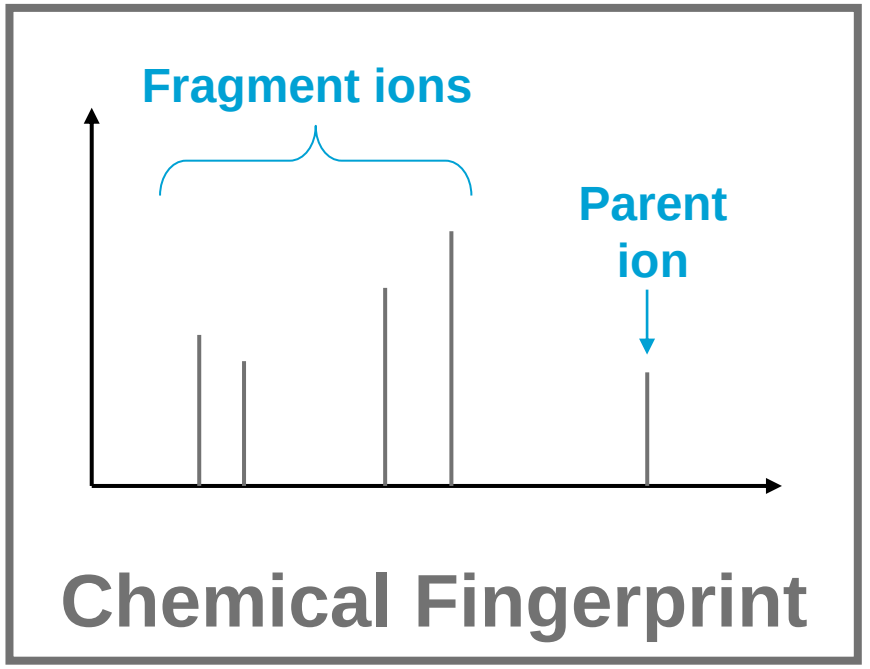
MRM-IDA-EPI workflow on QTRAP® LC-MS/MS systems

1. **MRM** survey scan screens for target compounds
2. IDA (Information Dependent Acquisition) criteria is set to trigger dependent scans
3. **EPI** (Enhanced Product Ion) scans rapidly collect high-quality MS/MS data
4. Search MS/MS Library

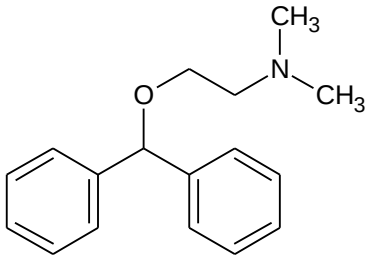


MS/MS Library Searching

Fingerprint



Chemical Fingerprint



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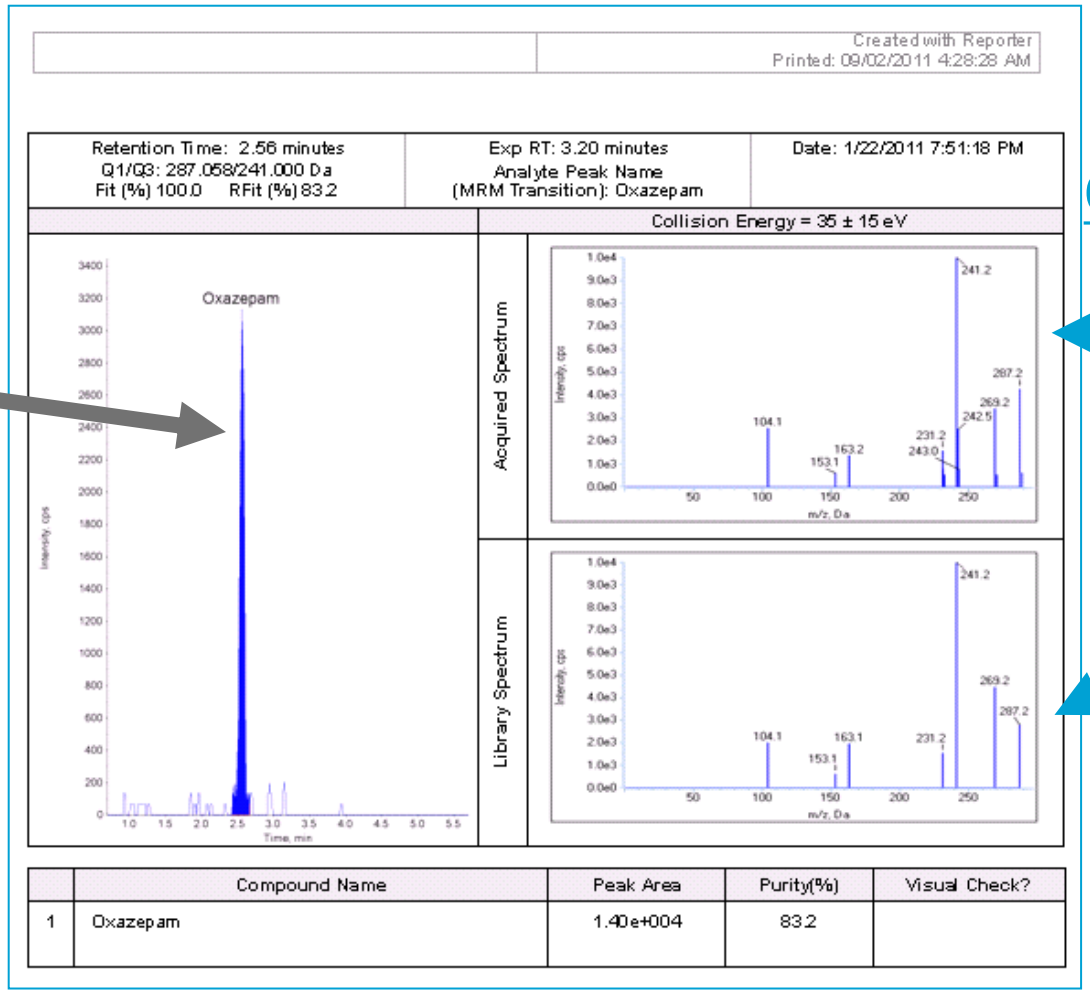
Multi-Target Screening + Confirmation

Survey Scan: MRM, Dependent Scan: EPI

Detection

MRM of Oxazepam 287.1/241.0



Confirmation

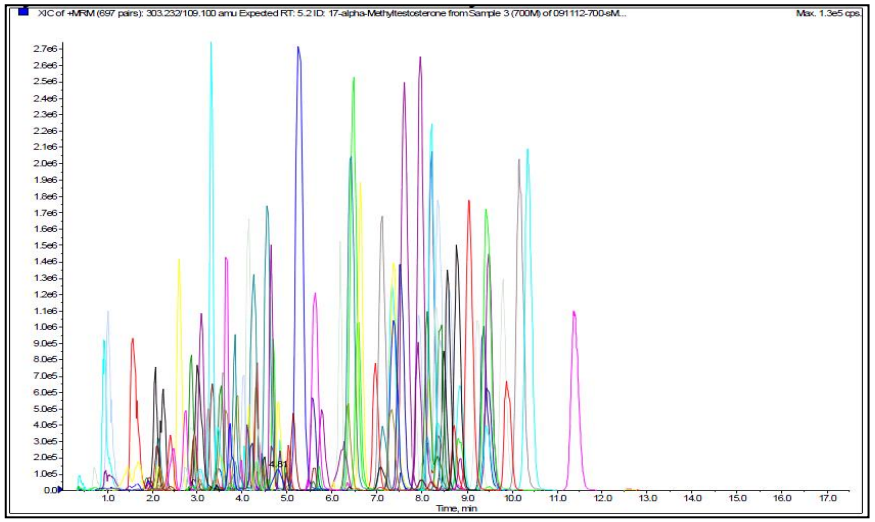
Acquired spectrum

Library spectrum of Oxazepam

Summary: Multi-Target Screening Approach with MRM

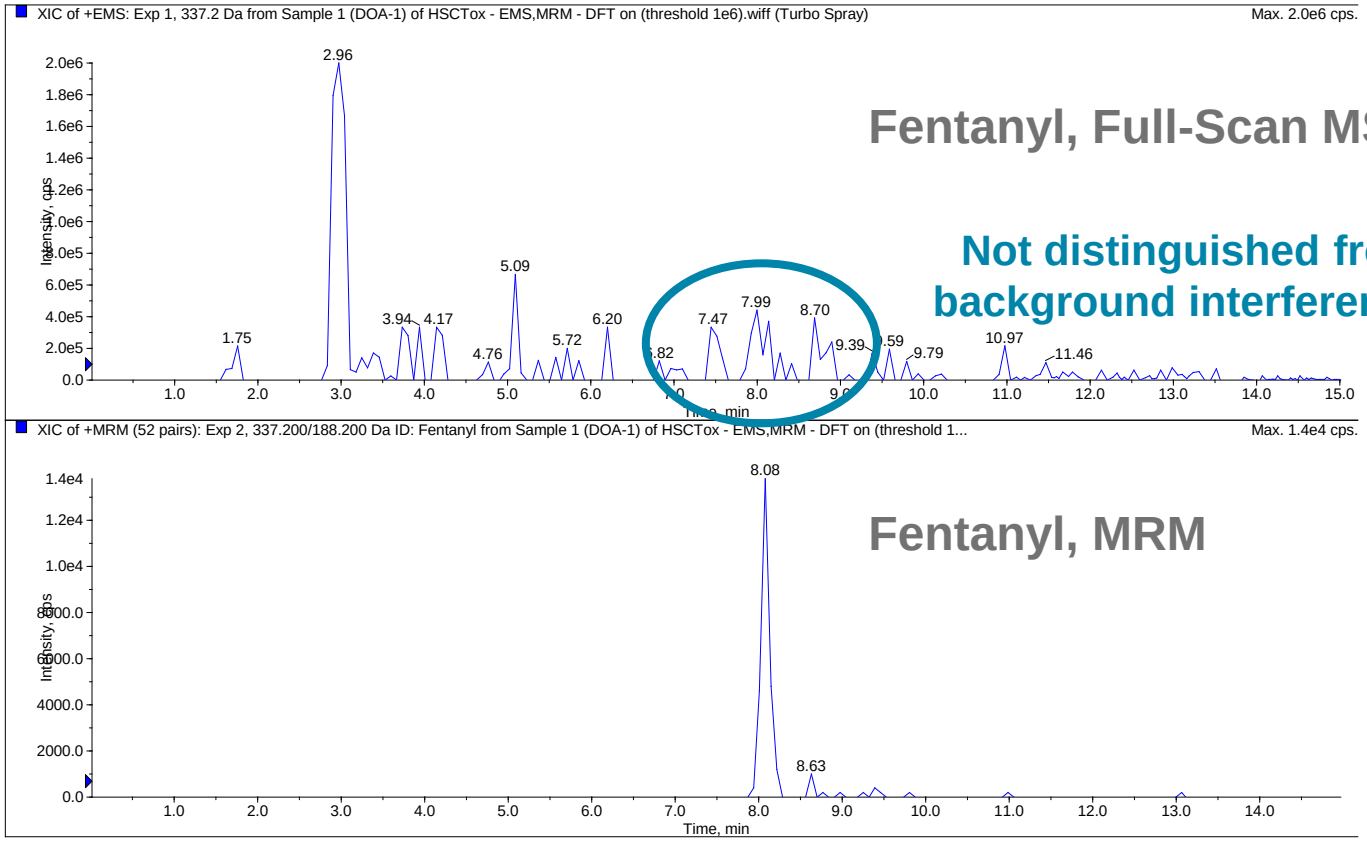
- MRM detection provides ultimate sensitivity and selectivity
- MS/MS library searching provides unambiguous confirmation
- Screening for hundreds of compounds is possible
- Only compounds on the “target list” are detected

Example: Urine screen for 700 target drugs using AB SCIEX 3200 QTRAP® system



Reminder: *Tandem* MS Provides Greater Selectivity

Single-stage mass spectrometry cannot provide the *selectivity* and *specificity* required to distinguish analytes at low levels

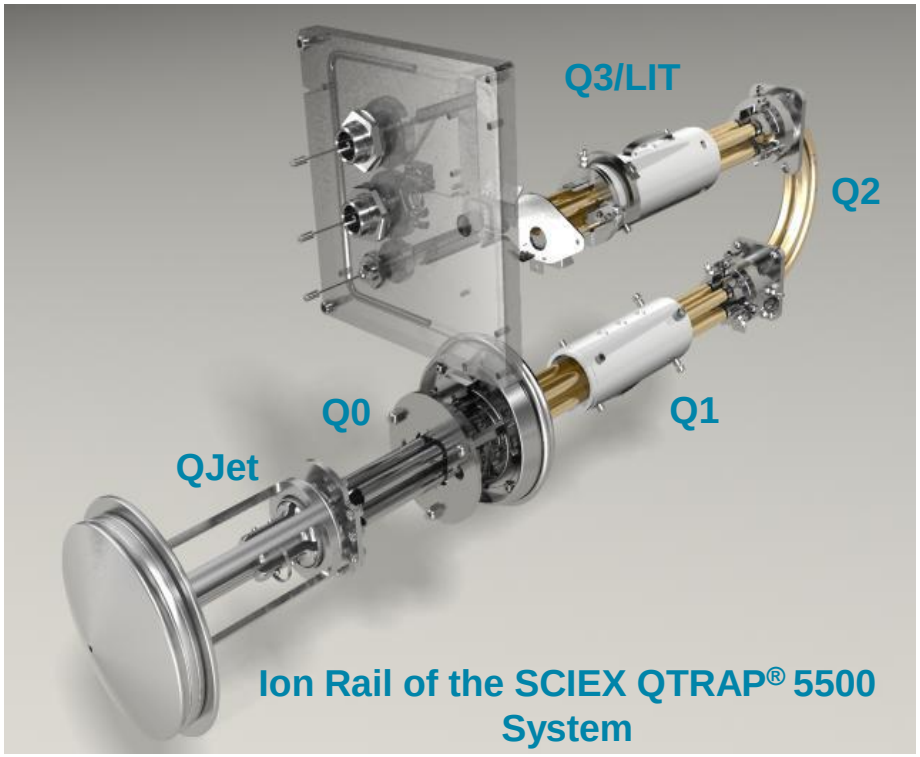


General Unknown Screening Approach

General Unknown Screening + Confirmation on QTRAP®

EMS-IDA-EPI workflow on QTRAP® LC-MS/MS systems

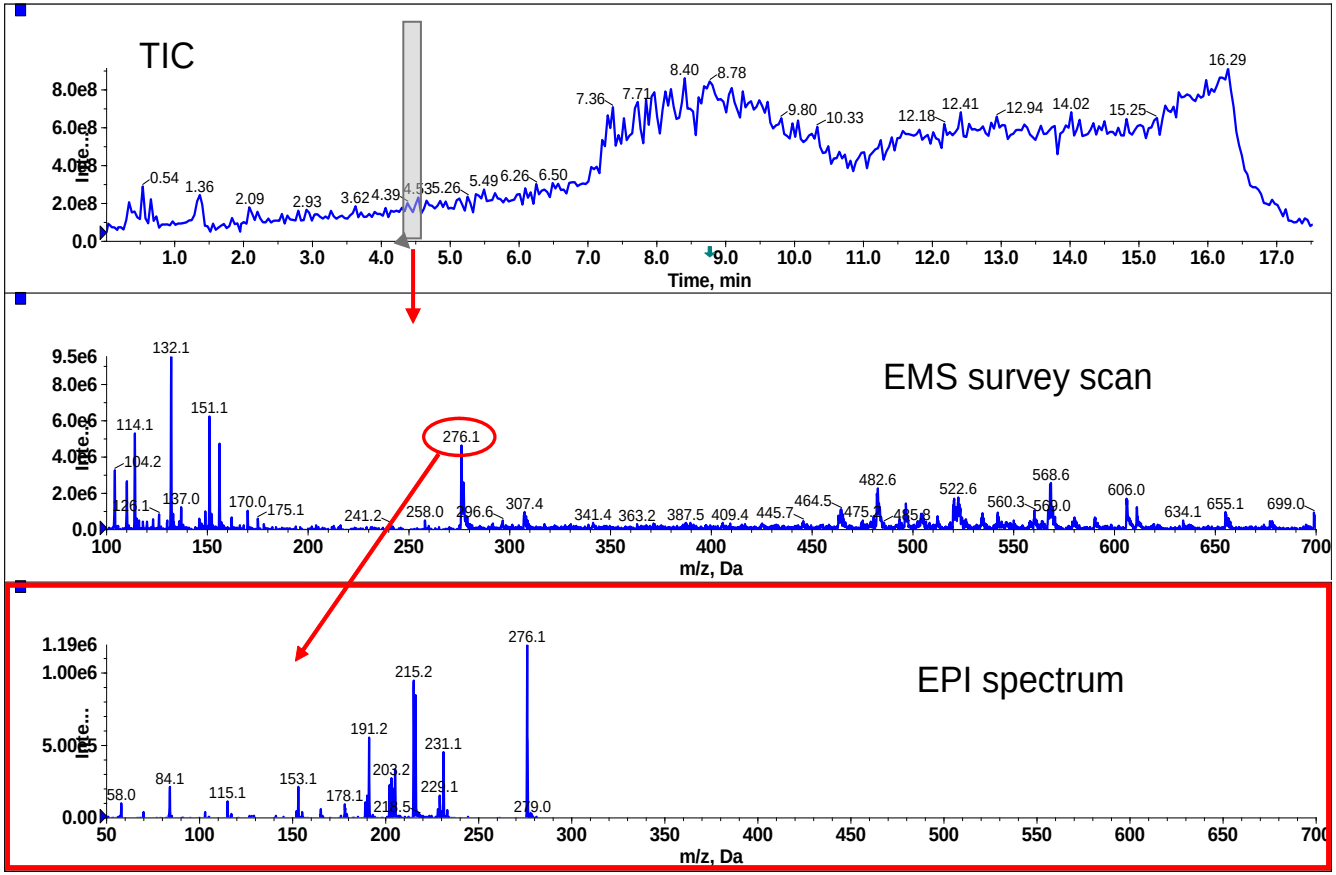
1. **EMS** survey scan rapidly screens for all compounds
2. IDA criteria is set to trigger dependent scans
3. **EPI** scans rapidly collect high-quality MS/MS data
4. Search MS/MS Library



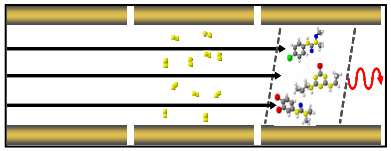
Ion Rail of the SCIEX QTRAP® 5500 System

General Unknown Screening + Confirmation on QTRAP®

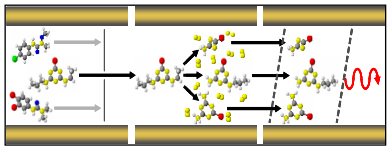
EMS detection identifies all compounds and metabolites (whether they were anticipated or not)



Enhanced Mass Spectrum (EMS)



Enhanced Product Ion (EPI) scan

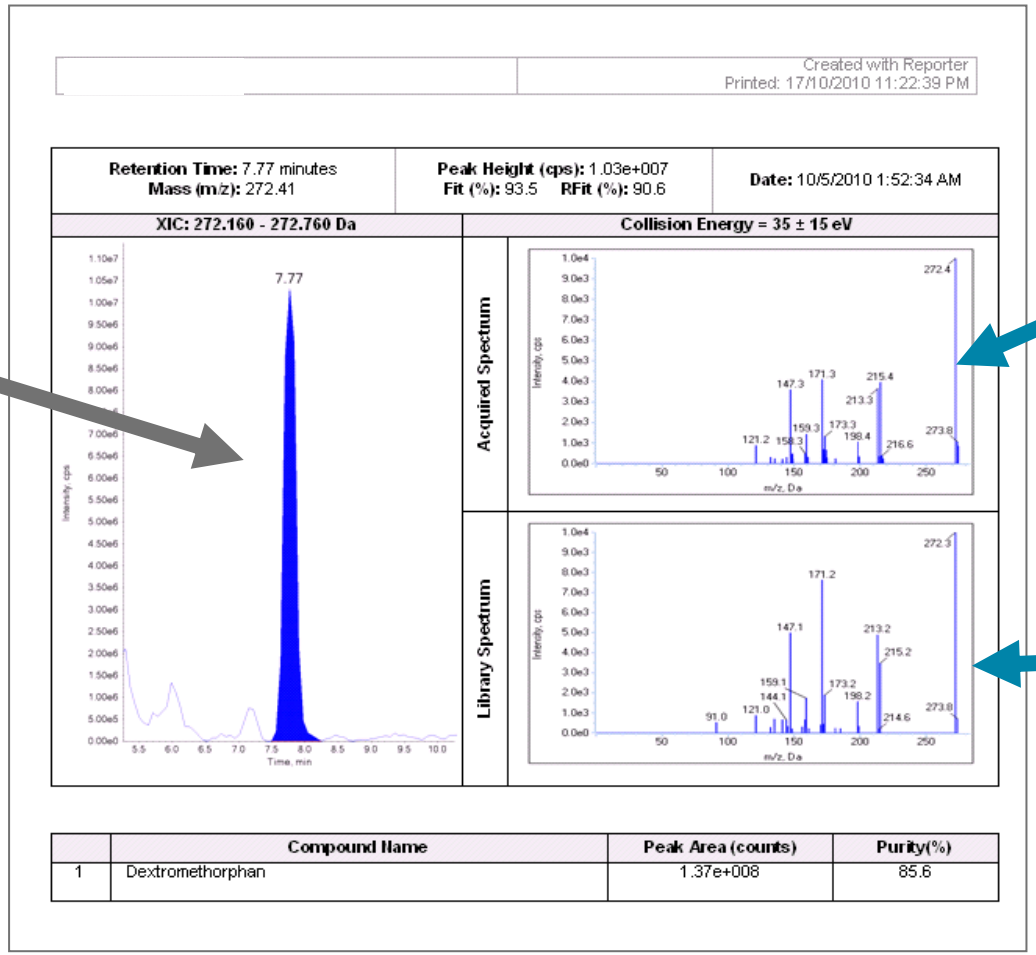


General Unknown Screening + Confirmation on QTRAP®

Survey Scan: EMS, Dependent Scan: EPI

Detection

XIC of EMS peak (272.2-272.8)



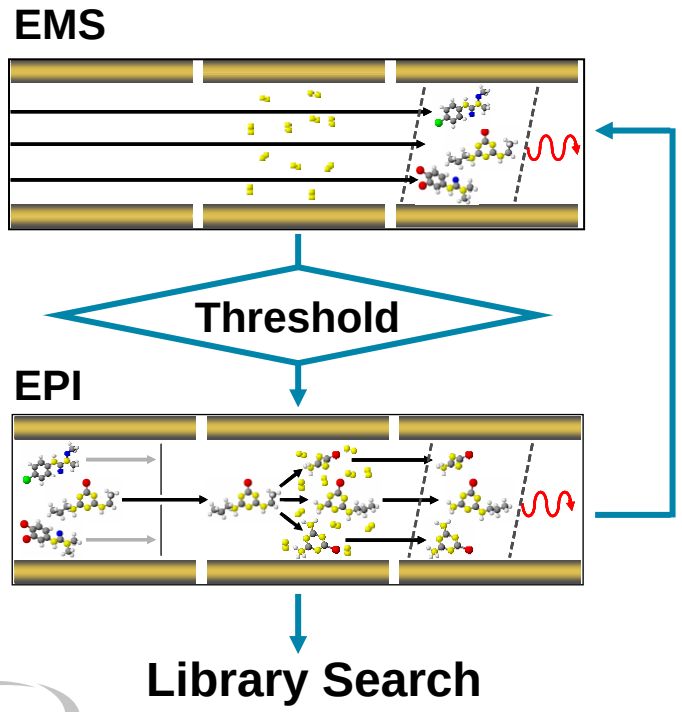
Confirmation

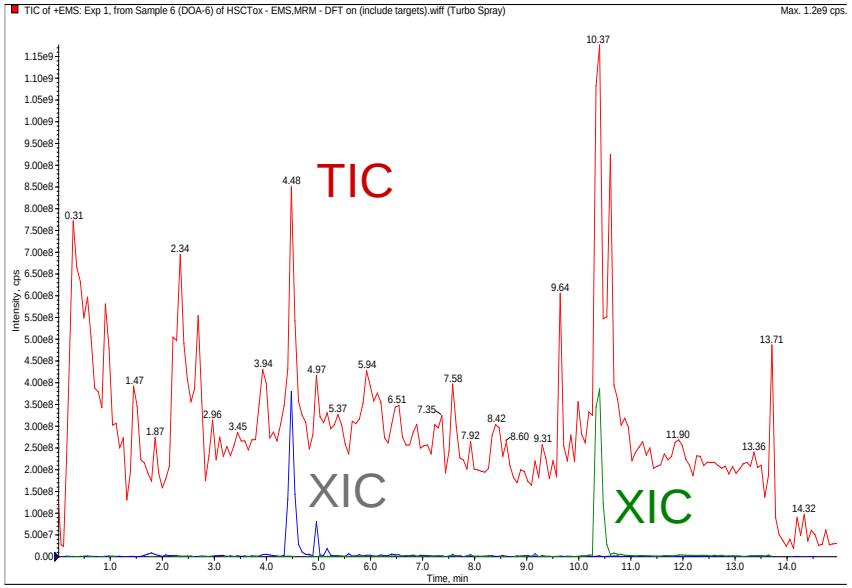
Acquired spectrum

Library spectrum: Dextromethorphan

Summary: General Unknown Screening with EMS

- Full-scan EMS detection identifies all compounds and metabolites
- MS/MS library searching provides confirmation
- Data “mining” is laborious; automated software is very helpful
- Sensitivity and selectivity is worse than MRM





Multi-target Screening vs. General Unknown Screening

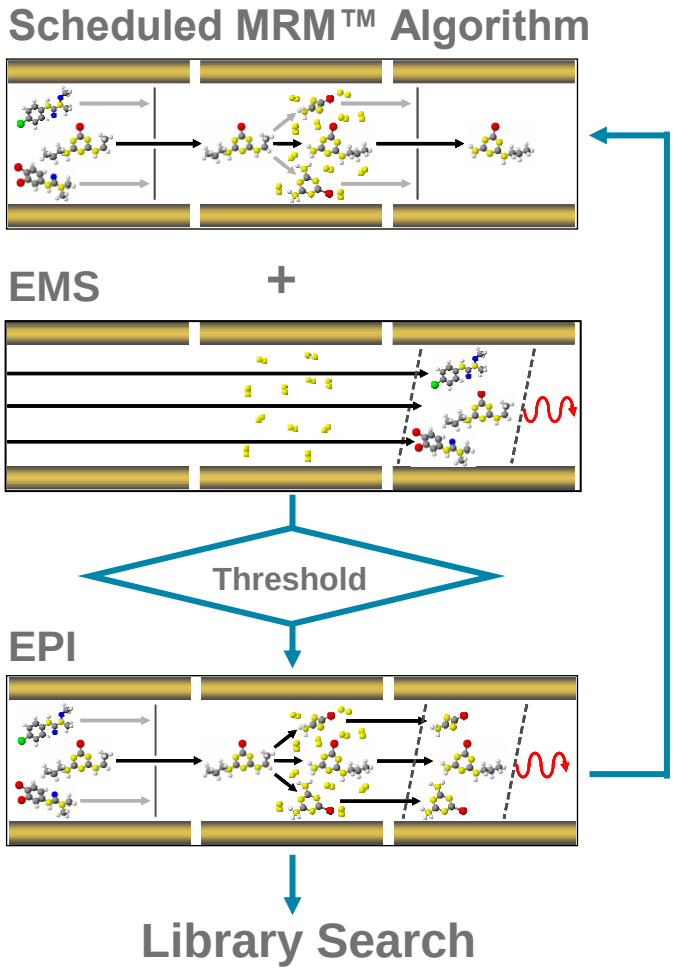
- Multi-target screening (with MRM) cannot detect unknowns
- Multi-target screening (with MRM) offers the best sensitivity, and will detect low-abundance *target* compounds
- General Unknown Screening (with EMS) can detect *all* compounds and metabolites
- General Unknown Screening (with EMS) will not detect all of the low-abundance compounds

MTS and GUS are truly complementary screening techniques.

Combined Approach

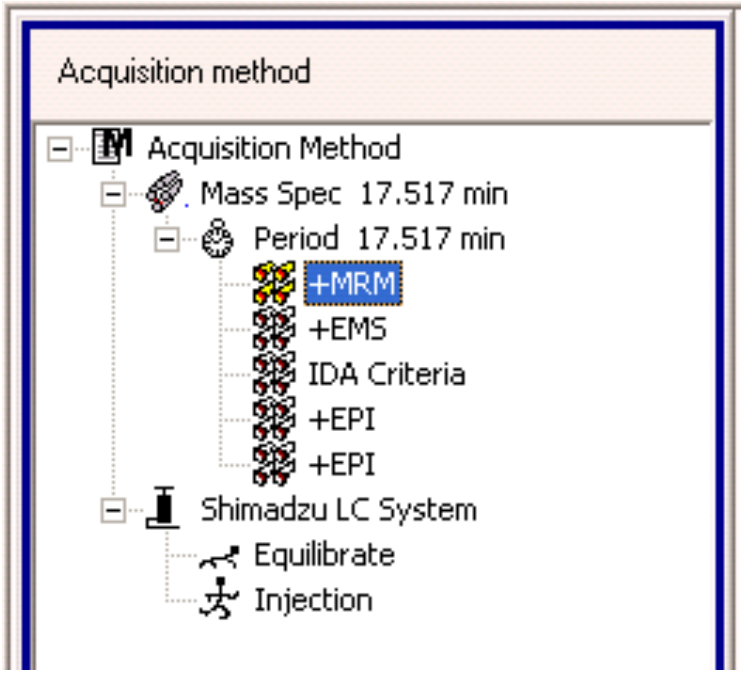
A New Approach: Combining Methodology

Why not combine “targeted” and “unknown” screening in a single method?



A New Approach: Combining Methodology

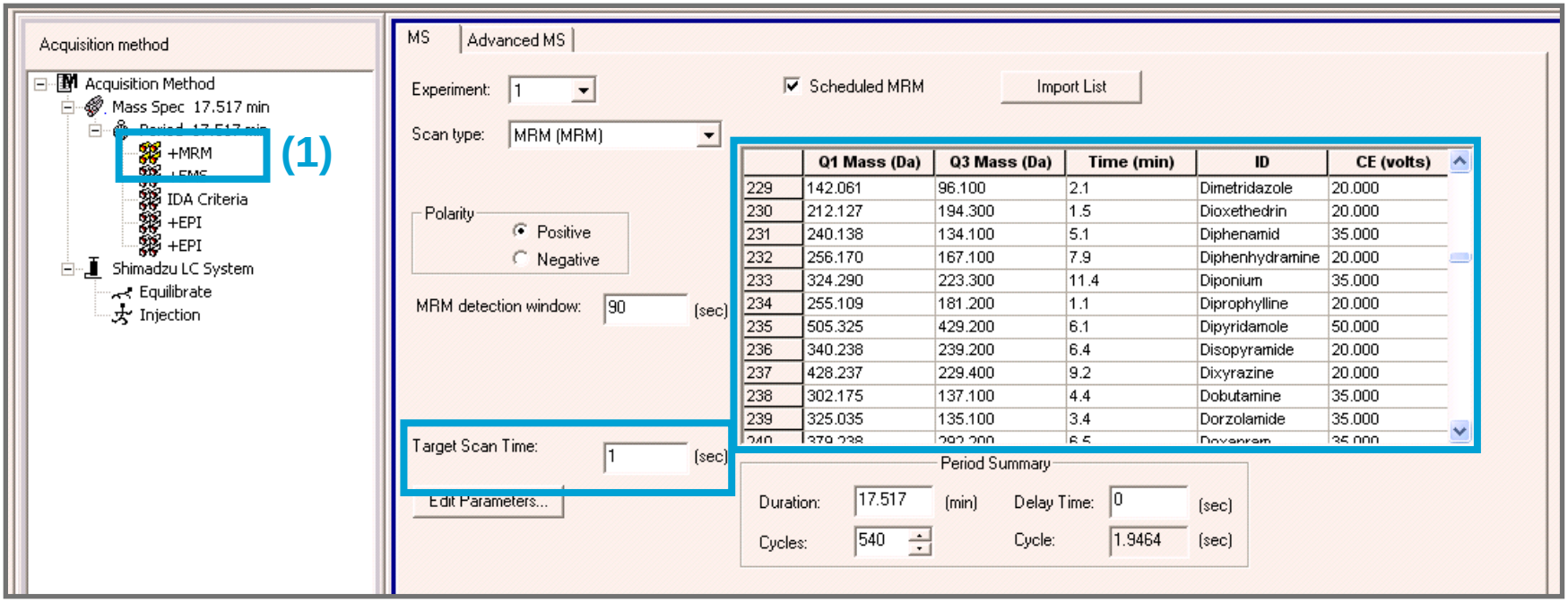
- MRM survey scan
  - Highly sensitive & selective MS/MS detection of target compounds
- EMS survey scan
  - “Full-scan” MS in linear ion trap
- IDA Criteria
  - Trigger dependent scan if ion signal (cps) exceeds a specified threshold
- EPI dependent scans
  - High-resolution, high-sensitivity “full-scan” MS/MS in linear ion trap



Targeted + Unknown Screening on a QTRAP® System

(1) MRM: Targeted screen

- The *Scheduled* MRM™ algorithm provides high-sensitivity detection of hundreds of target compounds in a single scan.
- In this example, the total scan time is 1 second.

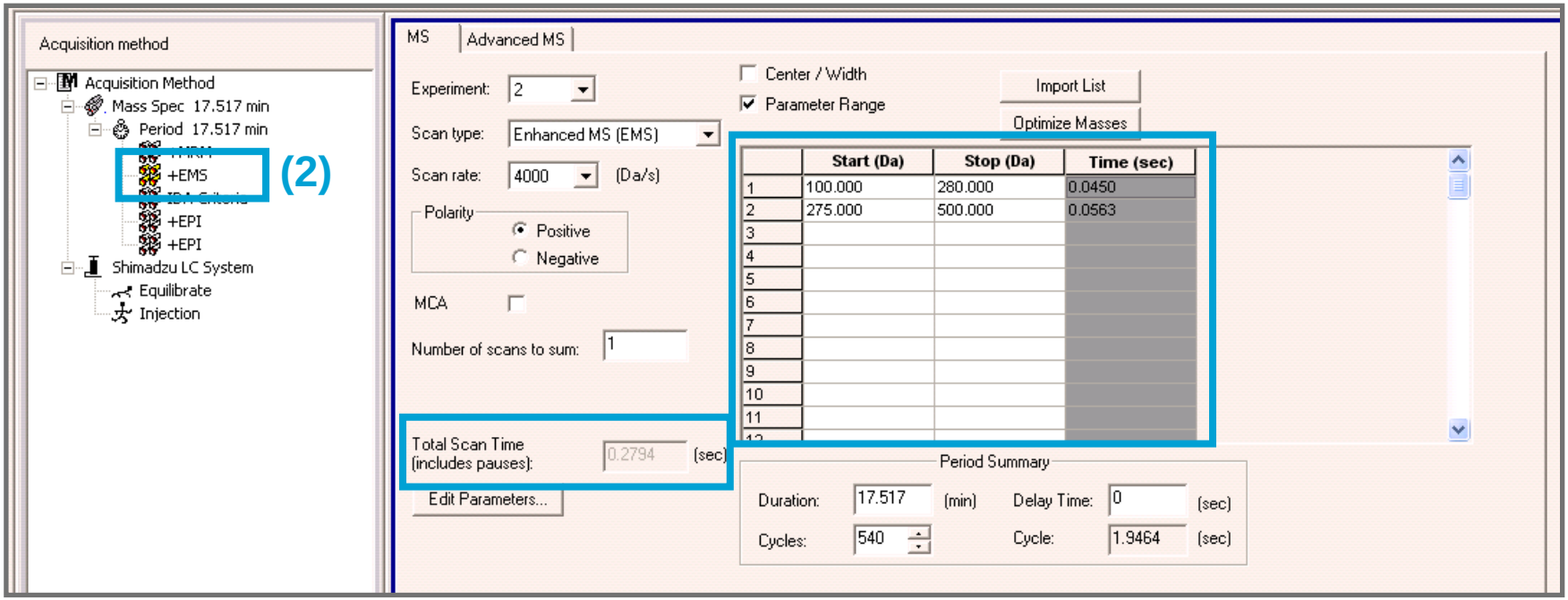


	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID	CE (volts)
229	142.061	96.100	2.1	Dimetridazole	20.000
230	212.127	194.300	1.5	Dioxethedrin	20.000
231	240.138	134.100	5.1	Diphenamid	35.000
232	256.170	167.100	7.9	Diphenhydramine	20.000
233	324.290	223.300	11.4	Diponium	35.000
234	255.109	181.200	1.1	Diprophylline	20.000
235	505.325	429.200	6.1	Dipyridamole	50.000
236	340.238	239.200	6.4	Disopyramide	20.000
237	428.237	229.400	9.2	Dixyrazine	20.000
238	302.175	137.100	4.4	Dobutamine	35.000
239	325.035	135.100	3.4	Dorzolamide	35.000
240	379.238	292.200	6.5	Doxepram	35.000

Targeted + Unknown Screening on a QTRAP® System

(2) EMS: Unknown screen

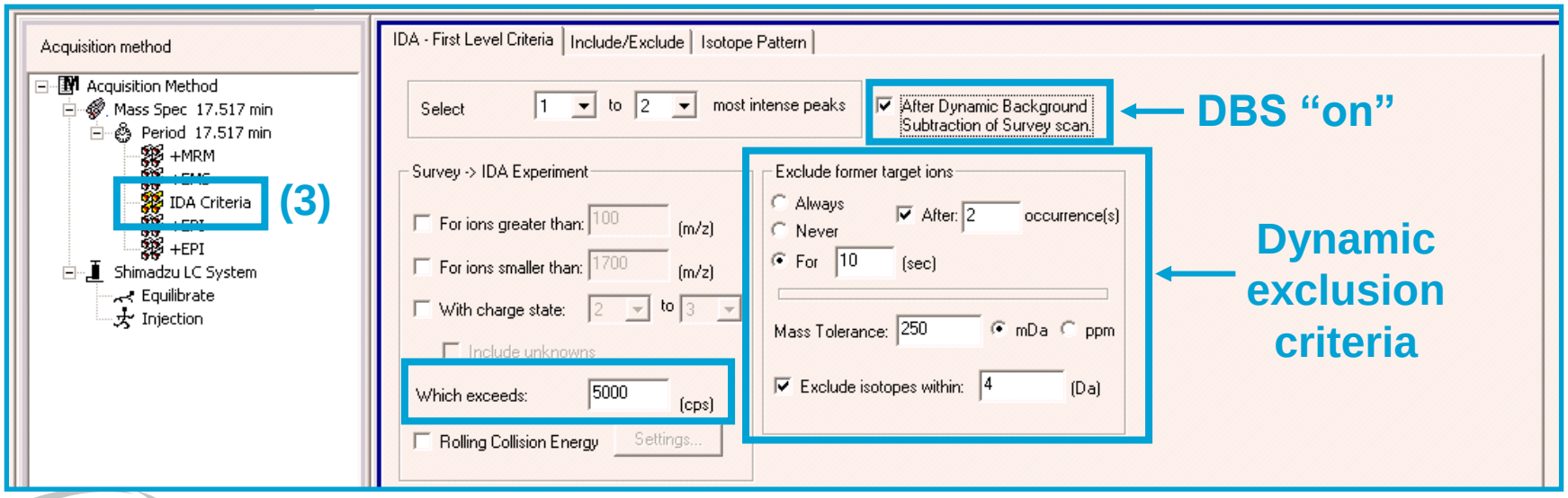
- Rapid and sensitive Enhanced Mass Spectrum (EMS) scan identifies any compounds not included in the MRM “target list”
- Acquisition of EMS data permits retrospective data processing (which is not possible with MRM-only data).



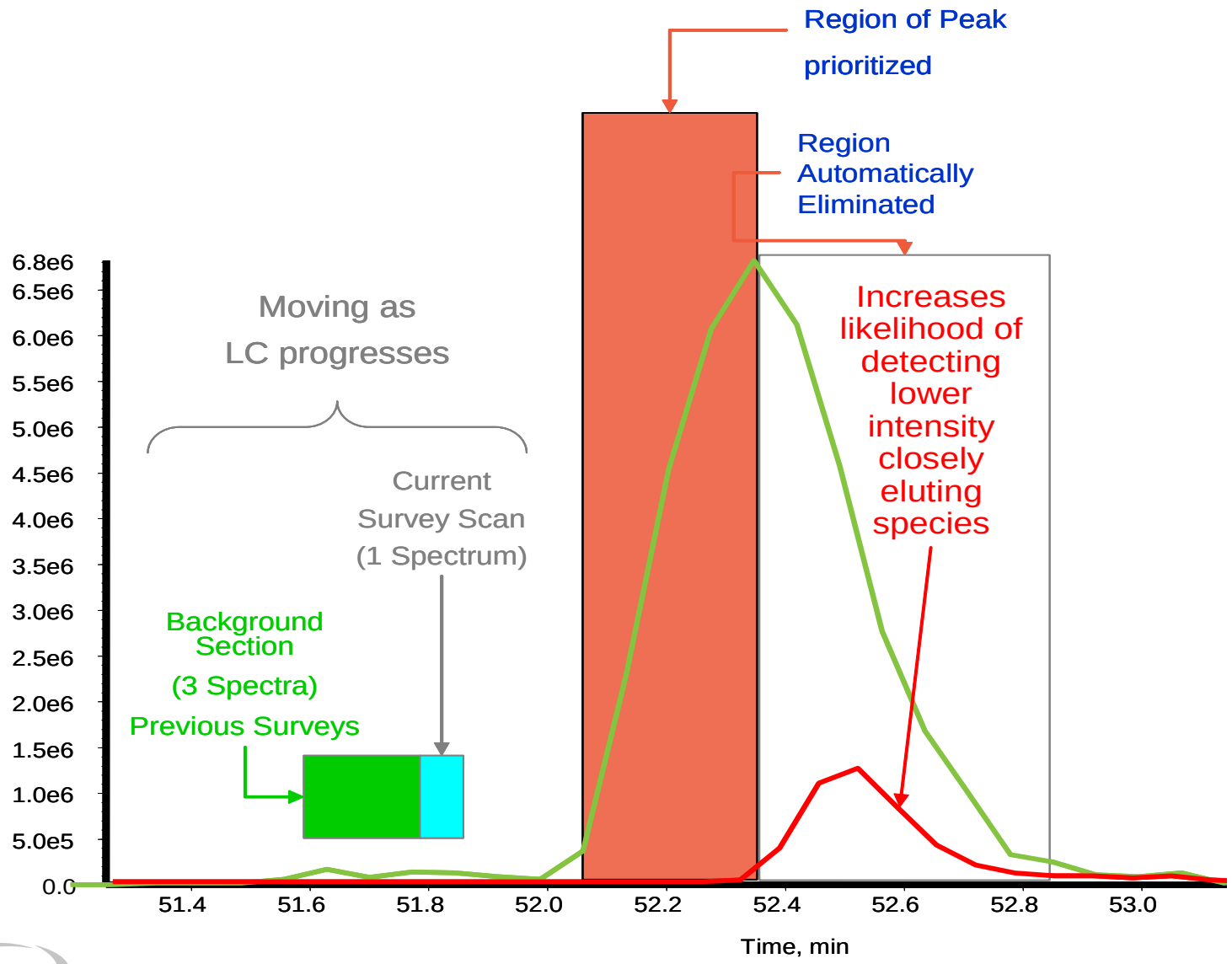
Targeted + Unknown Screening on a QTRAP® System

(3) Information Dependent Acquisition (IDA)

- Information Dependent Acquisition (IDA) criteria ensure that dependent scans are triggered when ion intensities exceed a specified threshold.
- Dynamic Background Subtraction (DBS) corrects for the presence of background ions, and increases likelihood of detecting co-eluting peaks.
- Dynamic exclusion criteria ensure that dependent scans are triggered for all co-eluting peaks.

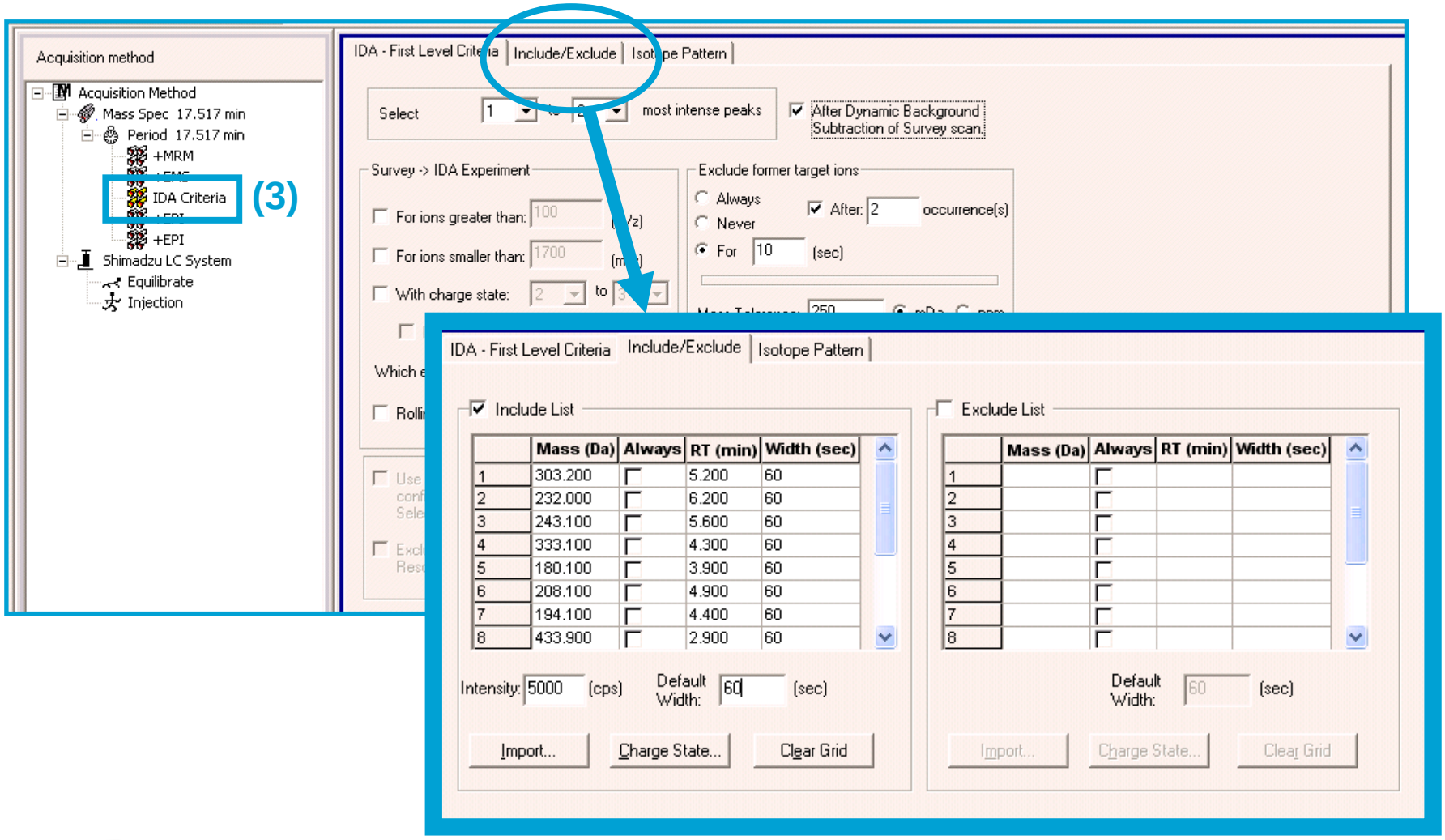


Dynamic Background Subtraction



Targeted + Unknown Screening on a QTRAP® System

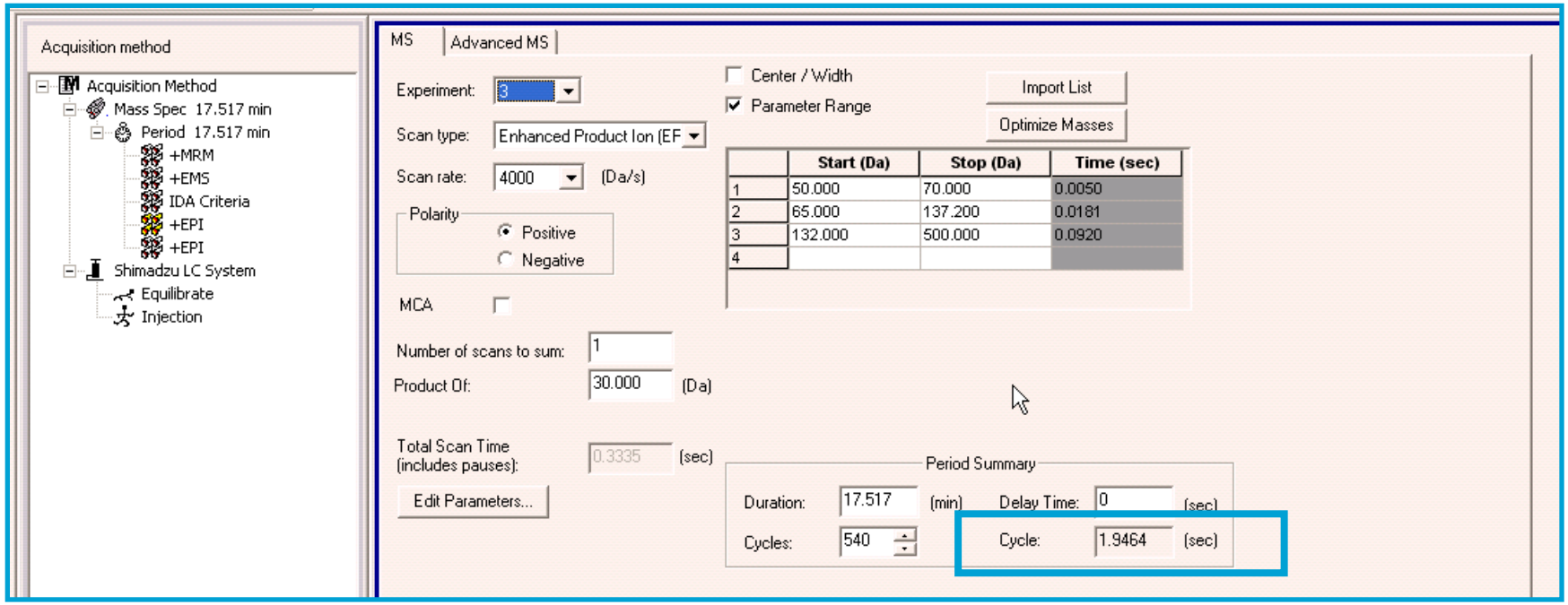
(3) Information Dependent Acquisition (IDA)



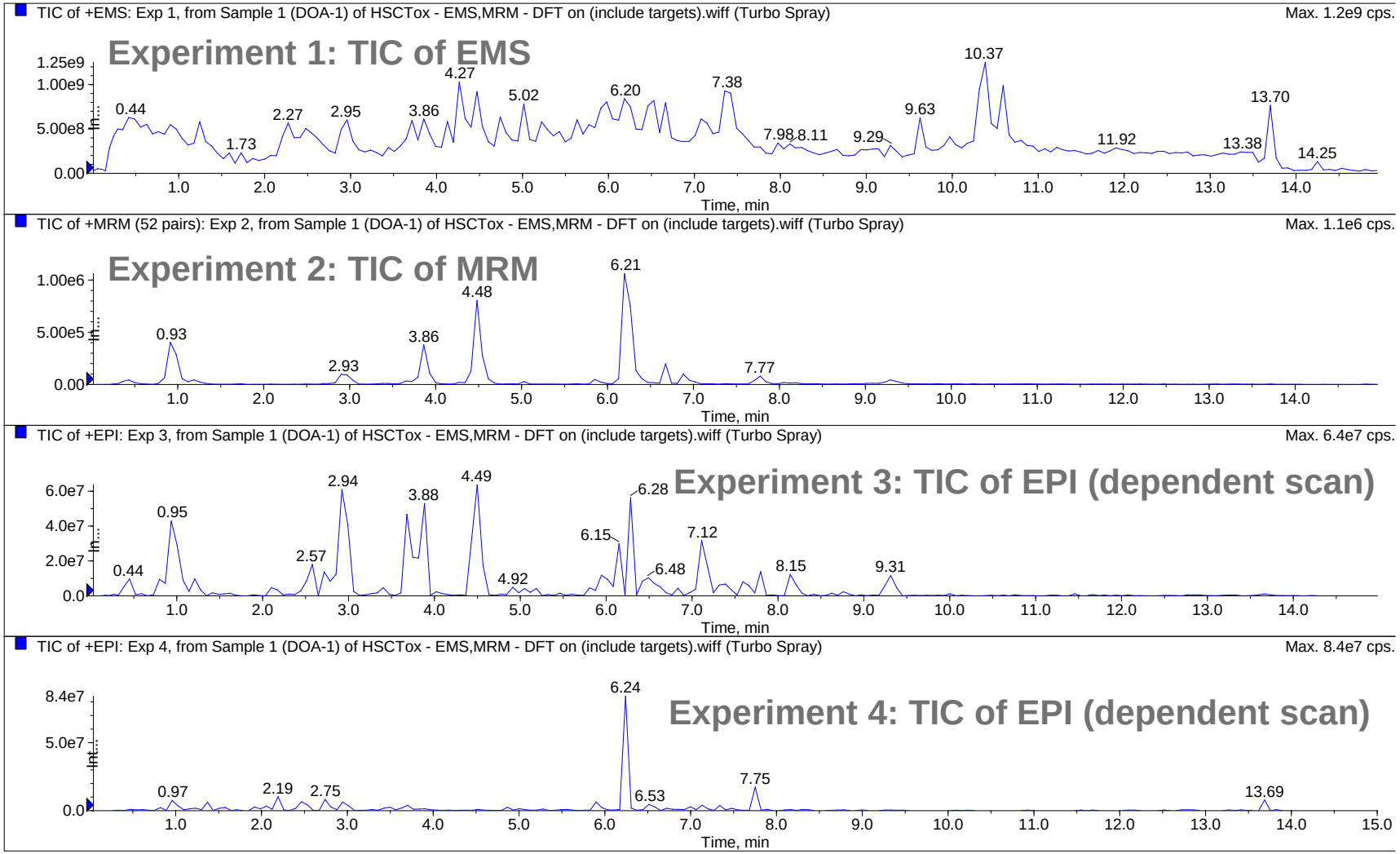
Targeted + Unknown Screening on a QTRAP® System

(4) EPI: MS/MS for Library Search Confirmation

- The information-dependent acquisition of EPI scans in the linear ion trap permits MS/MS spectral library searching, for confirmation of (i) “target” compounds, and (ii) “unknown” compounds.
- The total cycle time for this entire method (sMRM + EMS + EPI + EPI), on a QTRAP® system, is less than 2 sec.

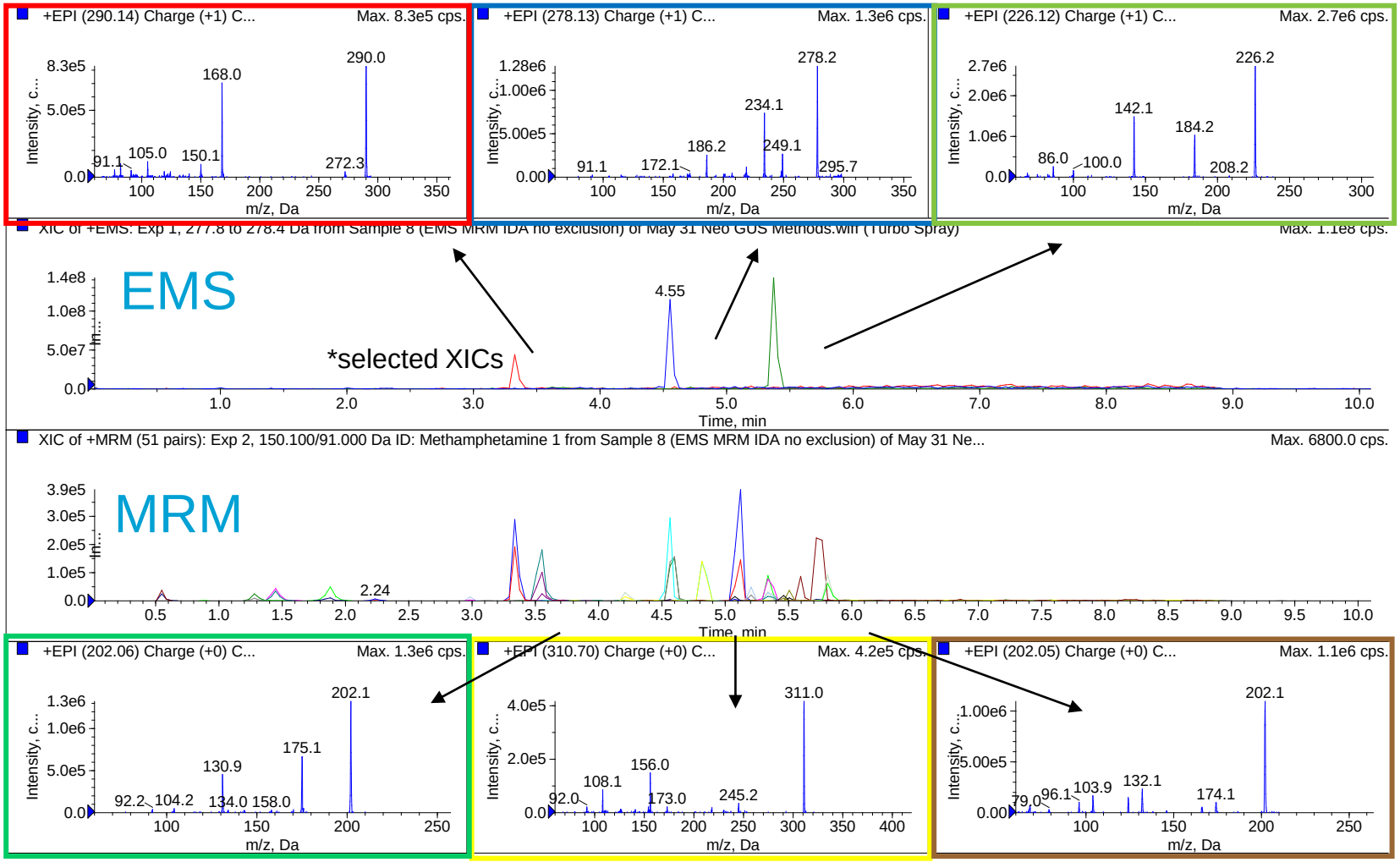


Targeted + Unknown Screening on a QTRAP® System



Targeted + Unknown Screening on a QTRAP® System

EPI spectra are triggered from EMS and MRM survey scans
EPI spectra are library-searched for confirmation of compound IDs

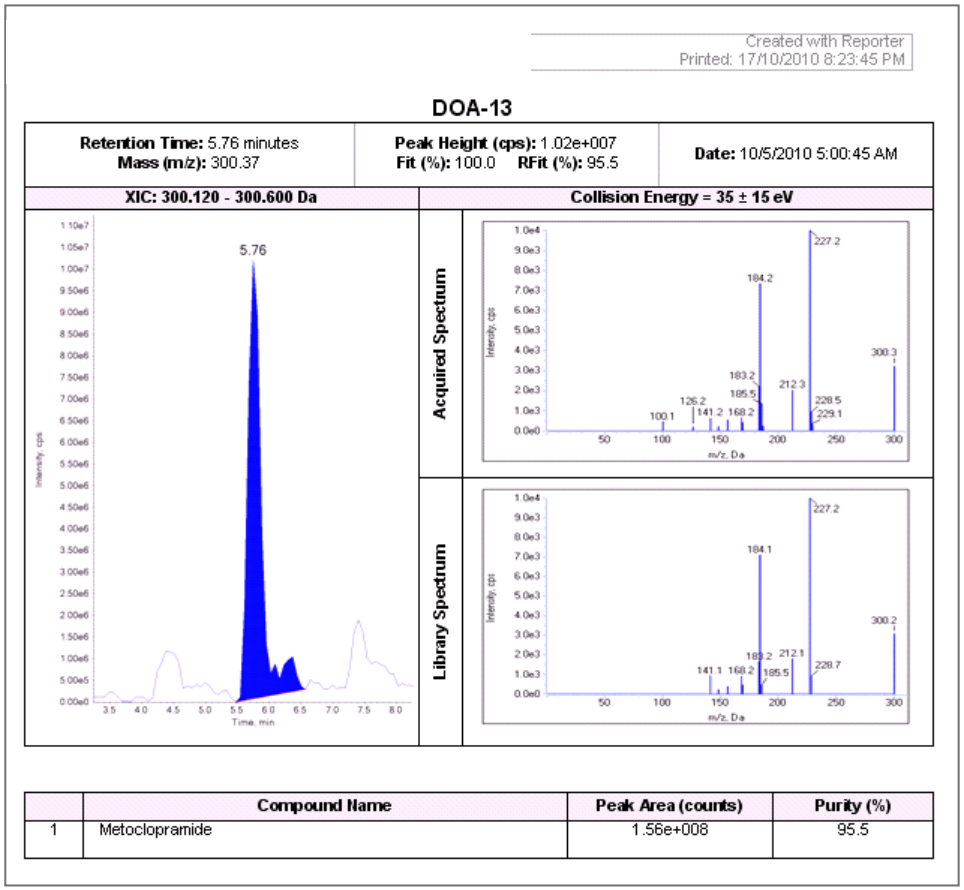


“Unknown” Detected and Confirmed by EMS-MRM-IDA-EPI

Example 1 (For Research Use Only. Not for use in diagnostic procedures)

- Metoclopramide: Antiemetic and gastroprokinetic agent. Used to treat nausea and vomiting, and to facilitate gastric emptying. Also used to treat migraines.

XIC of m/z 300.3 →



← Acquired spectrum

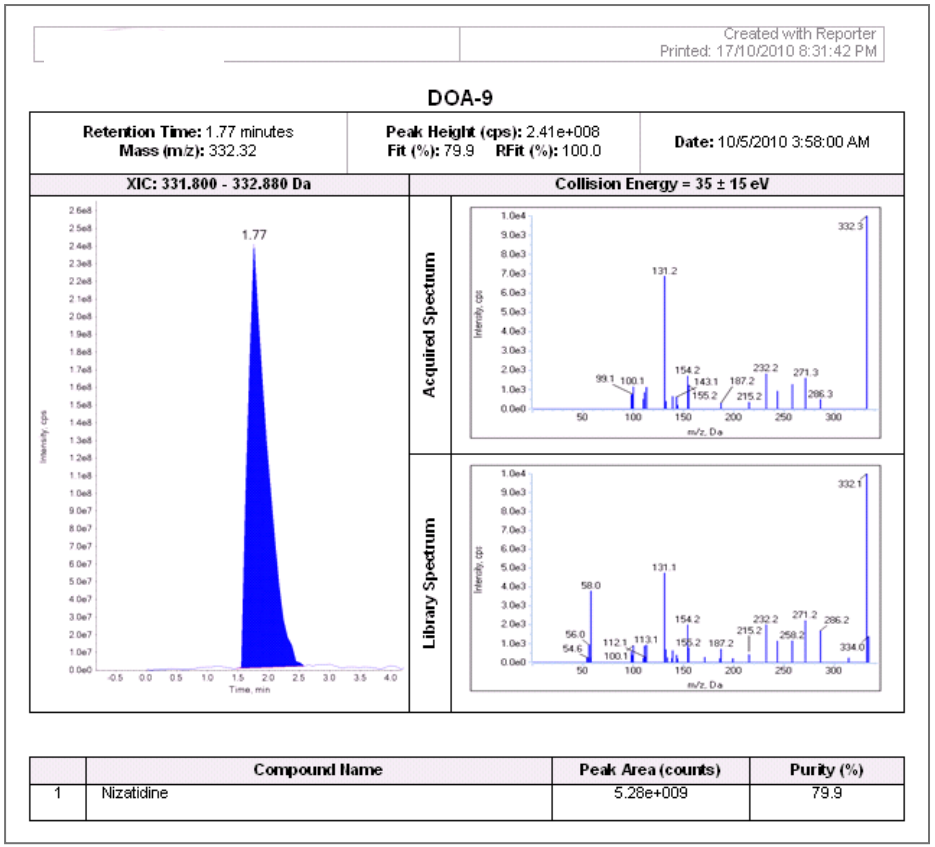
← Library spectrum of Metoclopramide

“Unknown” Detected and Confirmed by EMS-MRM-IDA-EPI

Example 2 (For Research Use Only. Not for use in diagnostic procedures)

- Nizatidine: Over-the-counter drug that inhibits stomach acid production. Used to treat peptic ulcer disease and gastroesophageal reflux disease. Also used to prevent weight gain associated with some antipsychotic drugs. Considered to be equipotent with ranitidine.

XIC of m/z 332.3



Acquired spectrum

Library spectrum of Nizatidine

Conclusions

- Multi-target Screening (MRM-based) and General Unknown Screening (EMS-based) are truly complementary techniques:
  - MTS provides the ultimate sensitivity for detection of low-abundance compounds
  - GUS provides detection of unknowns

- The QTRAP® system allows rapid acquisition of full-scan MS/MS during MRM and EMS analysis, for additional confidence in IDs.

- The speed and versatility of the QTRAP® system allows users to *simultaneously detect* "target" and "unknown" compounds, by combining two survey scan-types in a single experiment.

- The collection of EMS "full-scan" spectra throughout acquisition allows the user to re-interrogate the data for additional "unknowns" at a later date.

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