



Answers for Science.
Knowledge for Life.™



It's Time to
See the
Real Evidence in
Your Samples

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

Approaches to Drug Screening using QTRAP® Systems

QTRAP® 4500 System



QTRAP® 5500+ System



QTRAP® 6500+ System

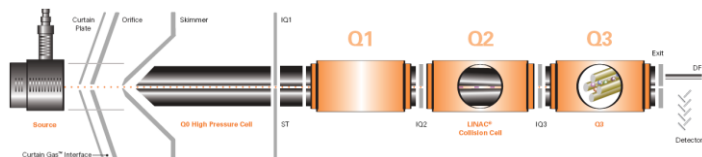


QTRAP® 7500 System



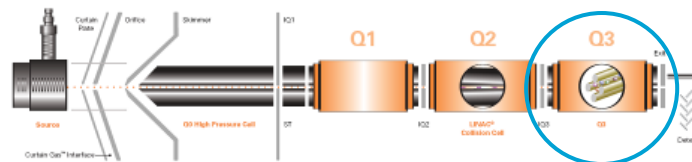
Advantage of QTRAP[®] System Technology

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

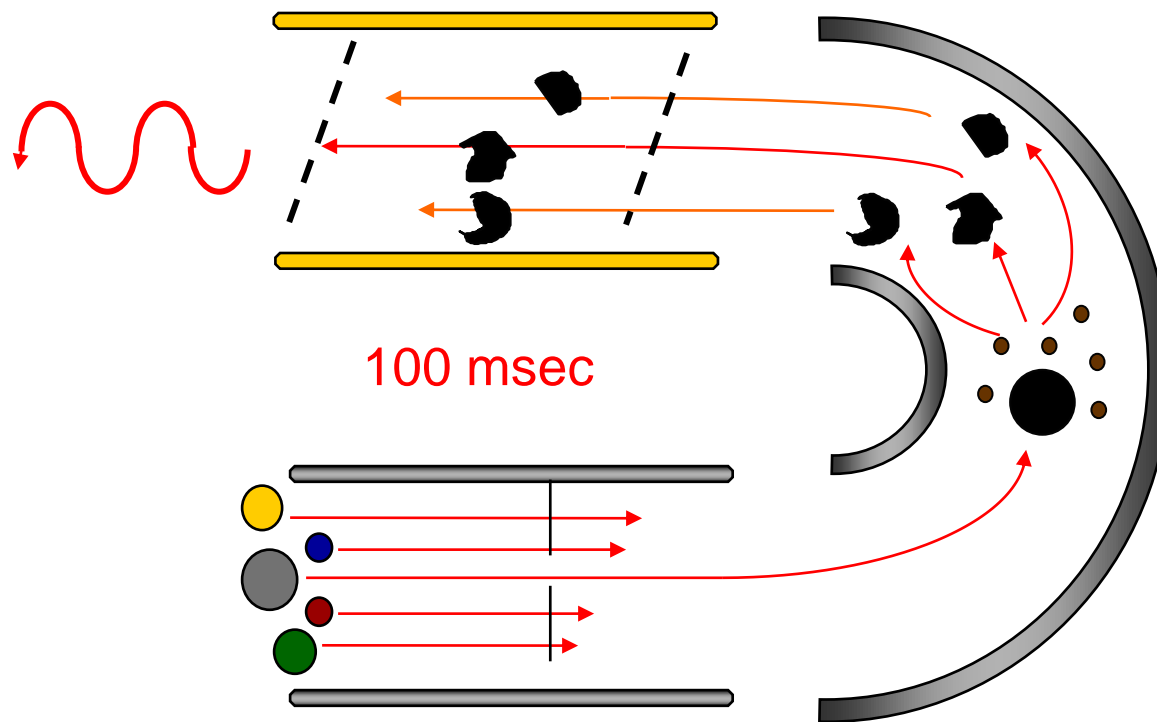


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Enhanced Product Ion Scan (EPI)

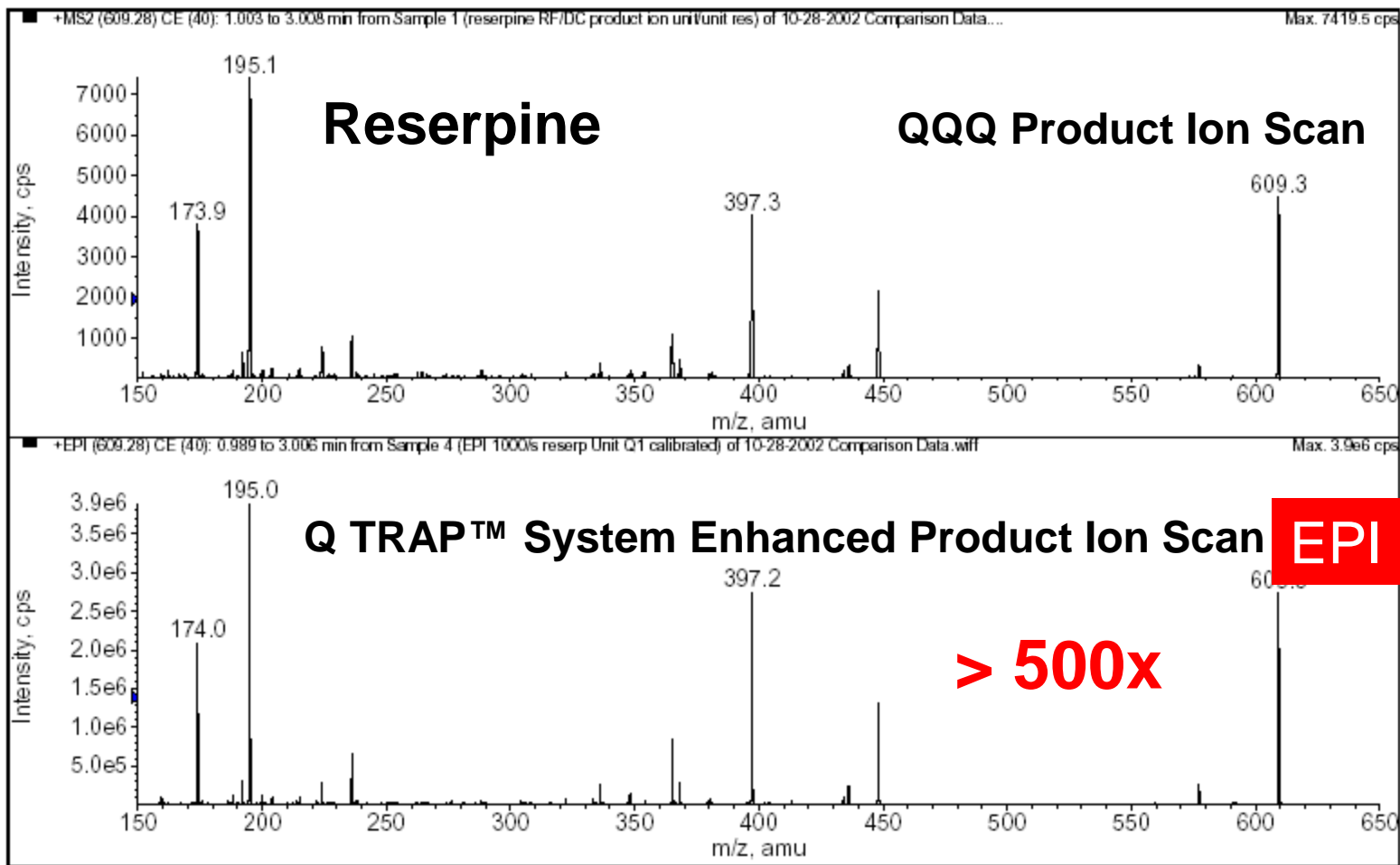


Q1: SIM

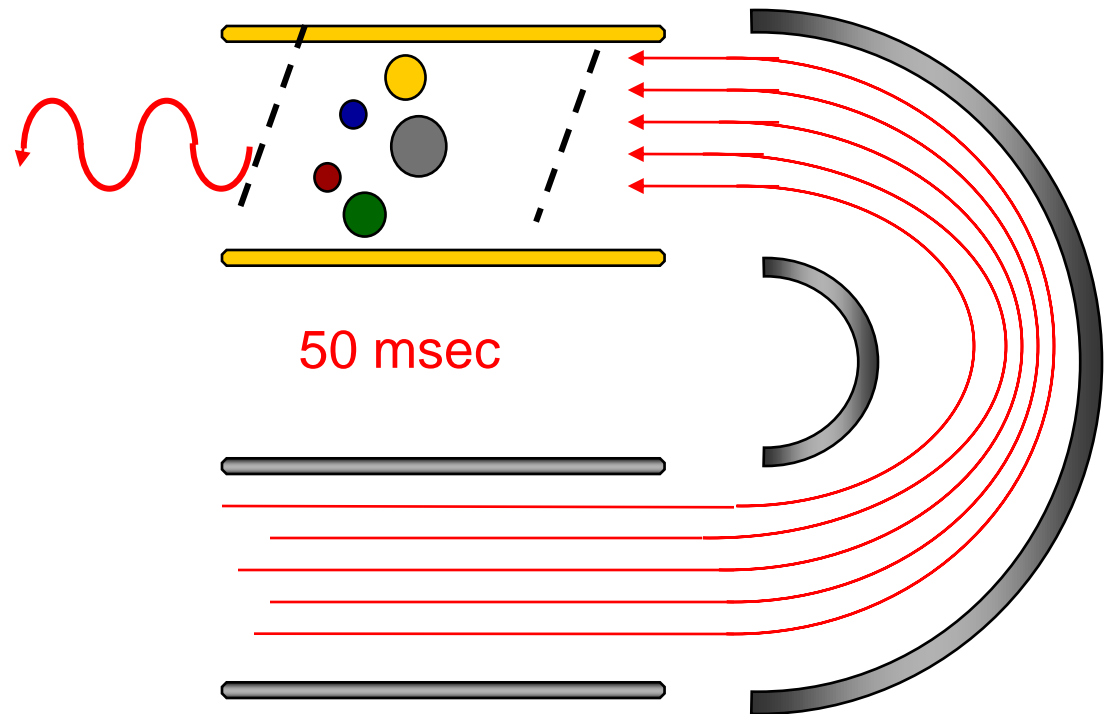
Q2: Fragmentation

LIT (Q3): Trap scan

Increased sensitivity - EPI



Enhanced MS Scan (EMS) - Search for Present Ions

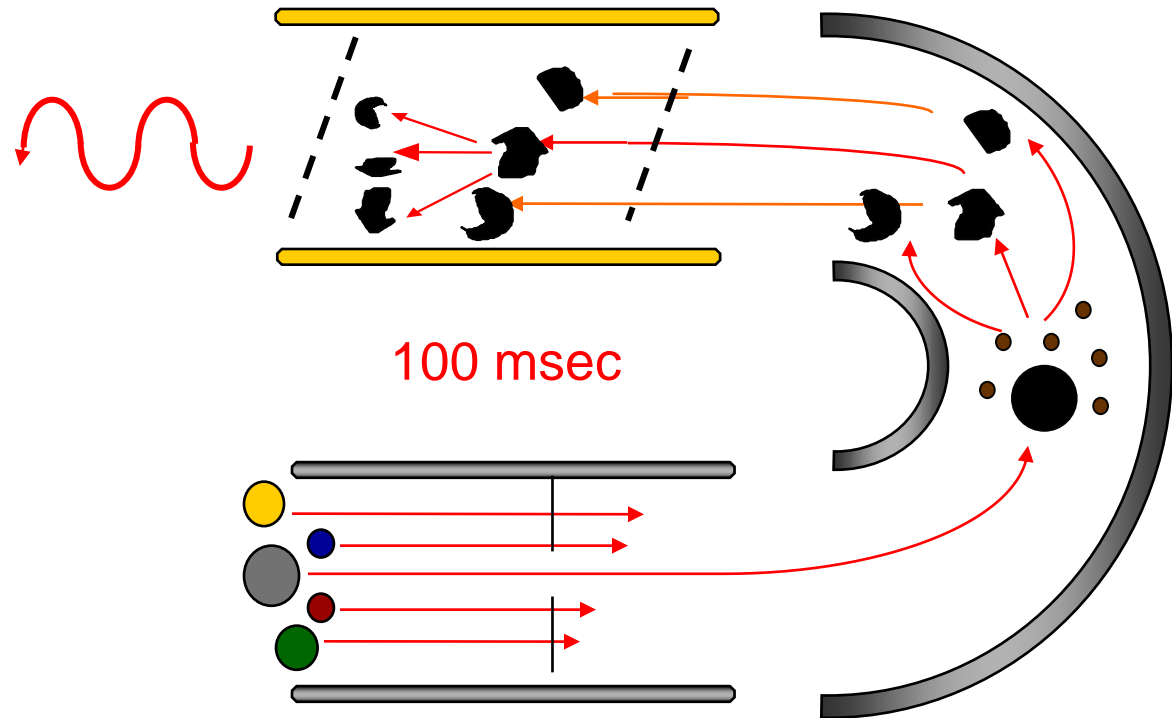


Q1: RF only

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



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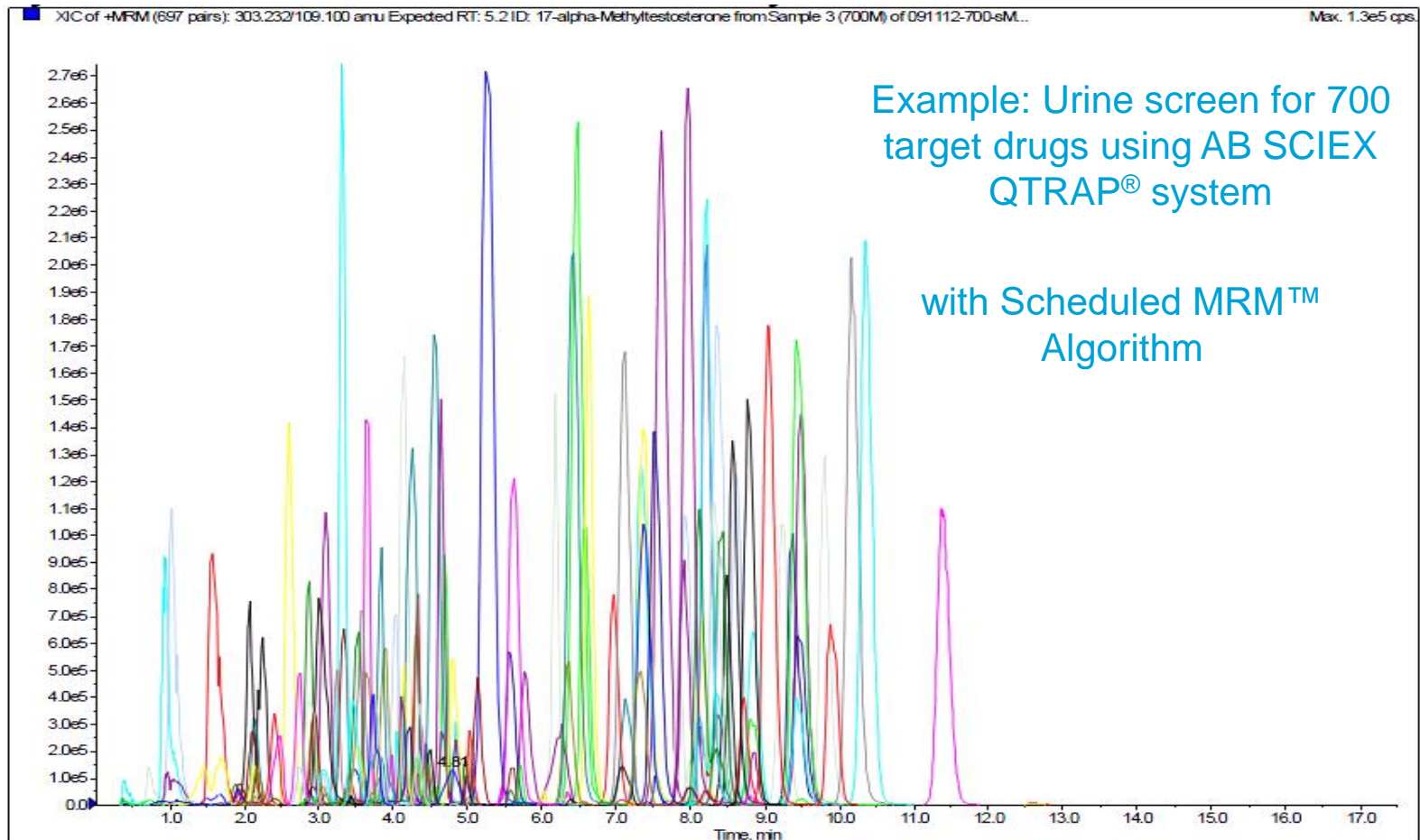
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Multi-Target Screening (MTS) Approach with MRM

MRM detection provides ultimate *sensitivity* and *selectivity*

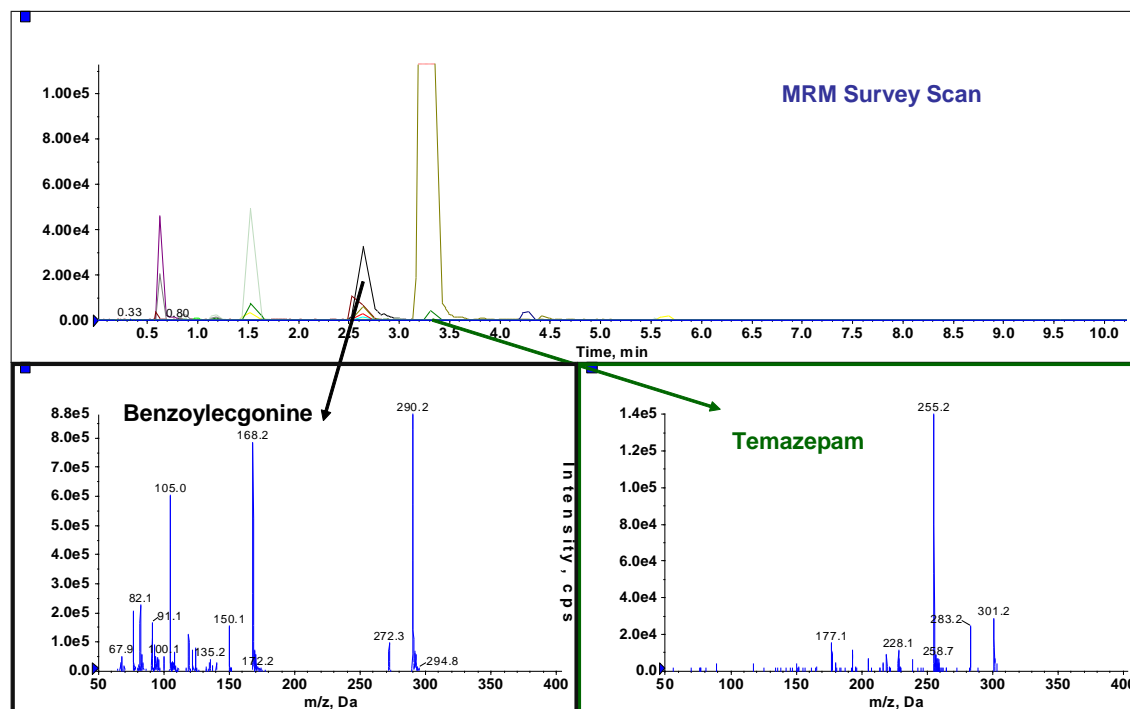
Two MRMs may be monitored, for additional confidence/confirmation



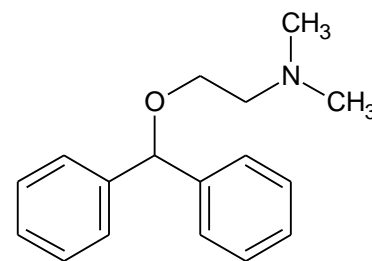
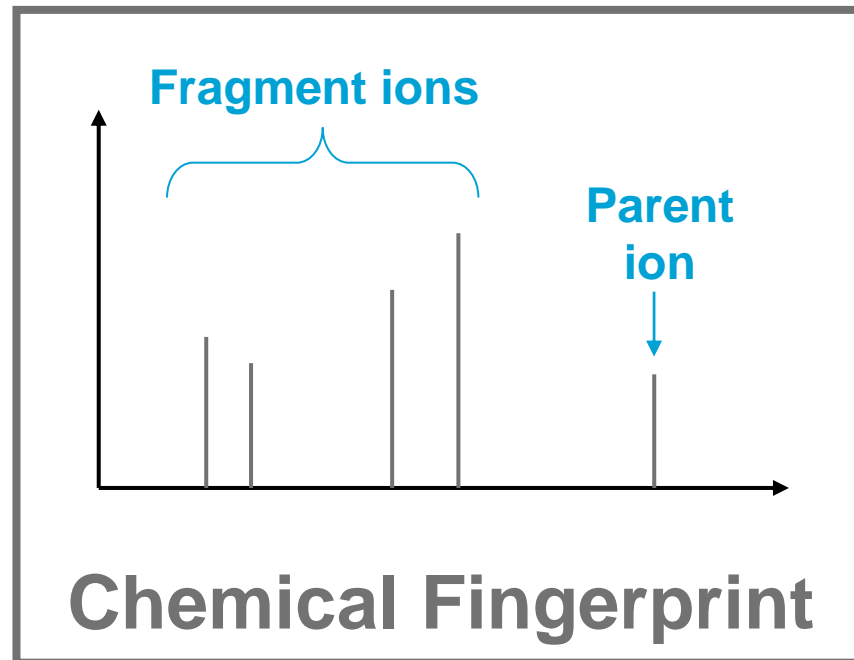
Multi-Target Screening + Confirmation

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

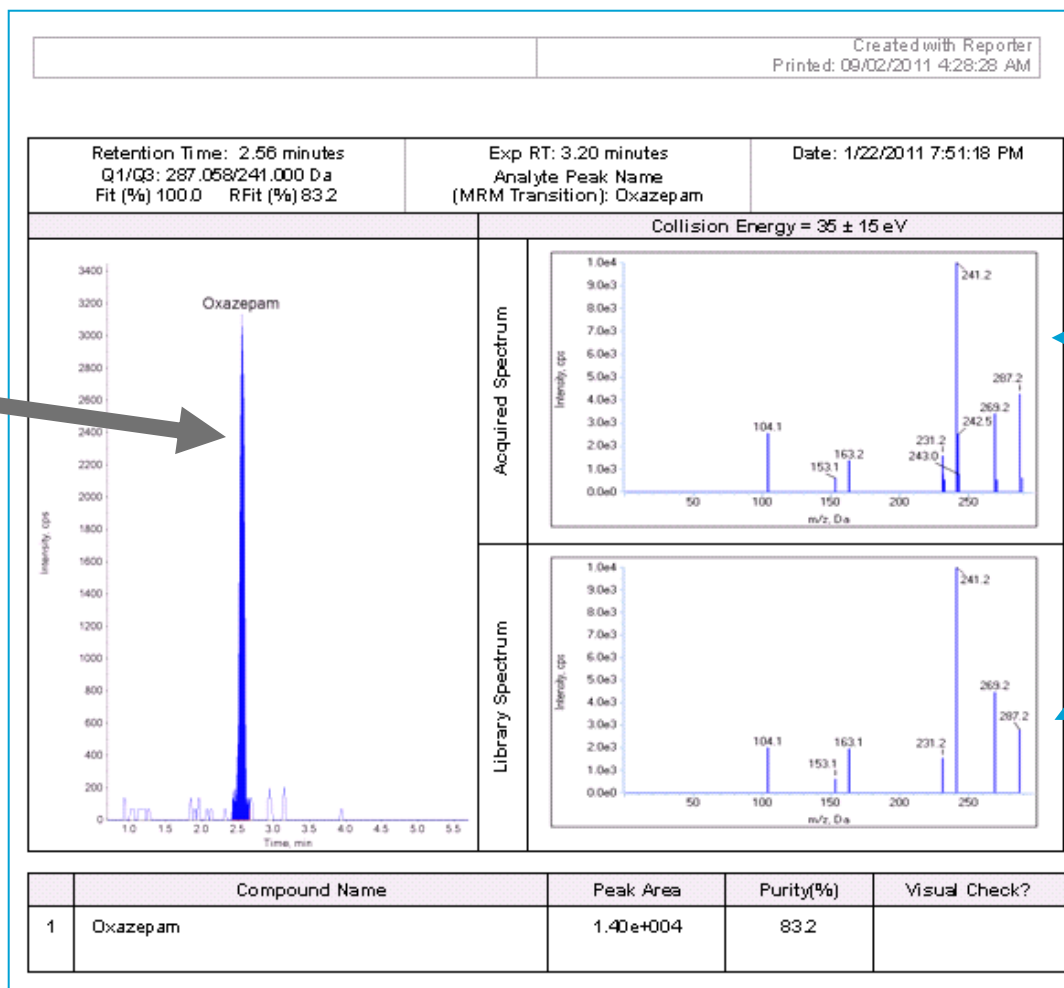
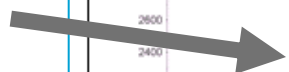


Multi-Target Screening + Confirmation

Survey Scan: MRM, Dependent Scan: EPI

Detection

MRM of Oxazepam
287.1/241.0



Confirmation

Acquired spectrum



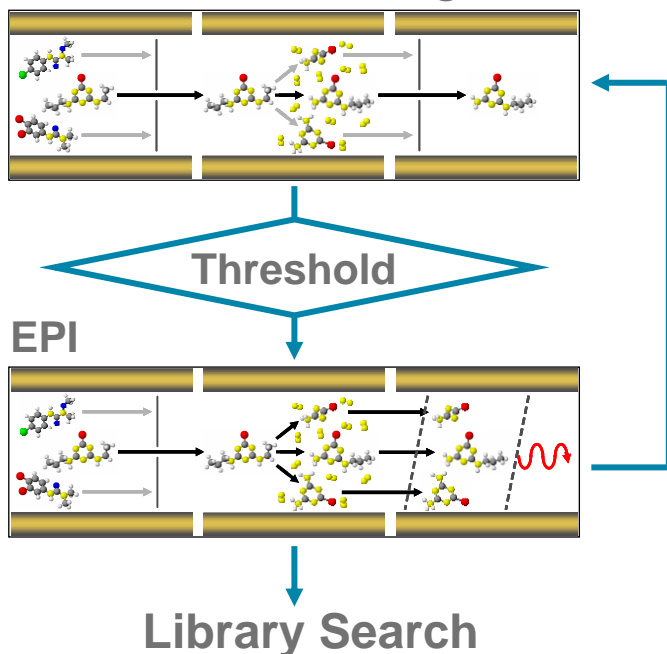
Library spectrum of Oxazepam



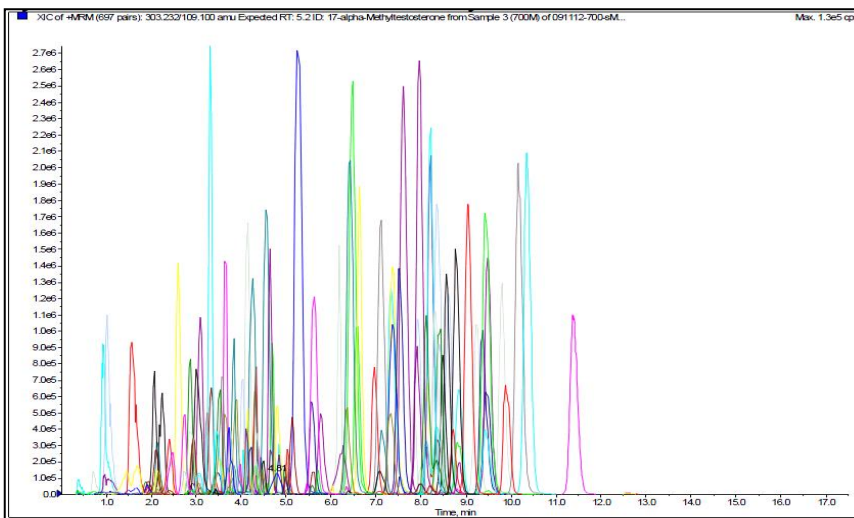
Summary: Multi-Target Screening Approach with MRM

- 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

Scheduled MRM™ Algorithm

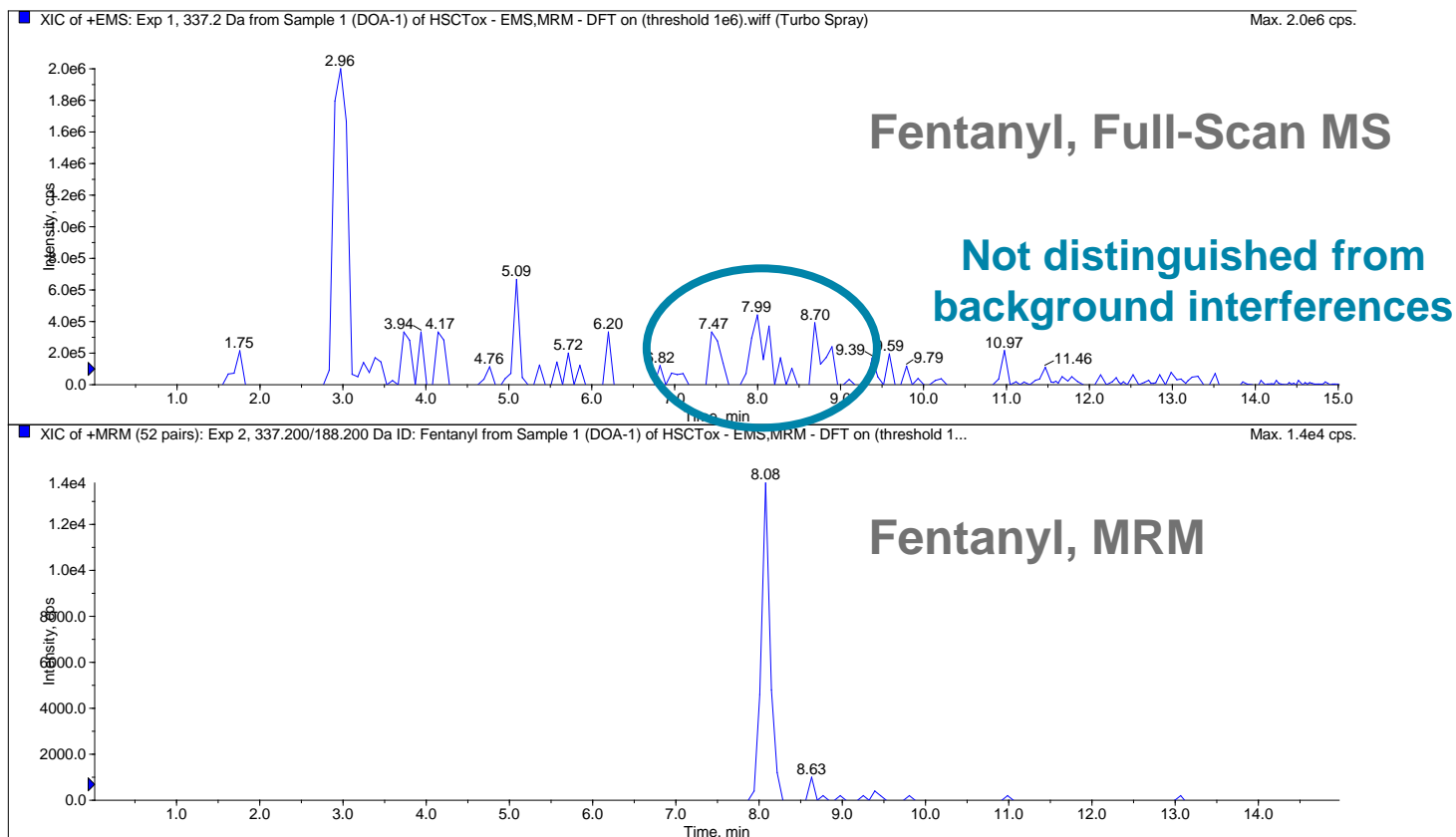


Example: Urine screen for 700 target drugs using AB SCIEX 3200 QTRAP® system



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



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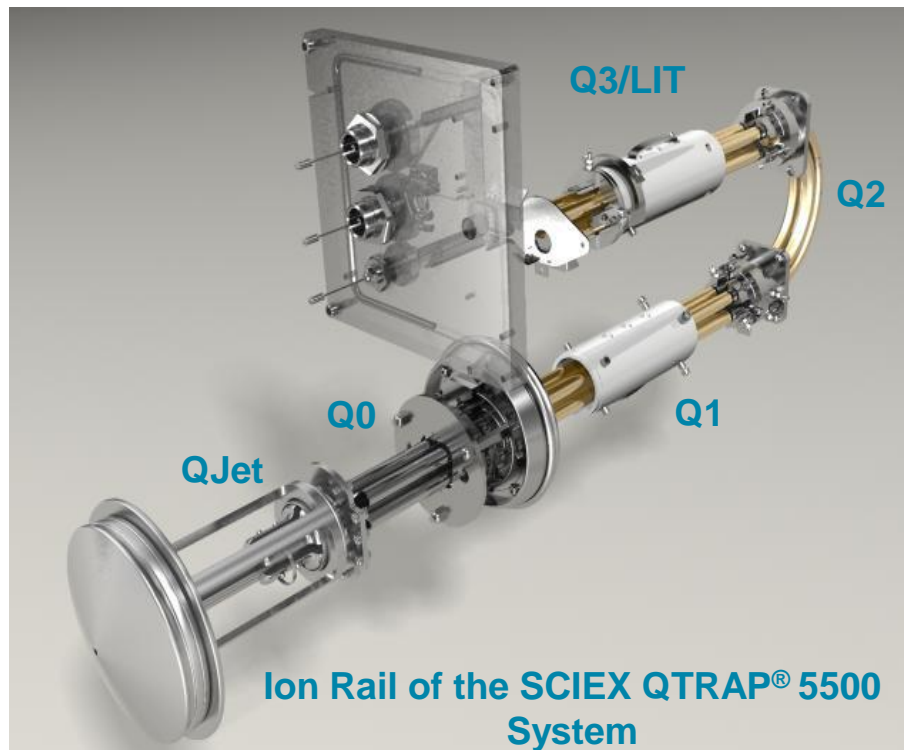
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General Unknown Screening + Confirmation on QTRAP®

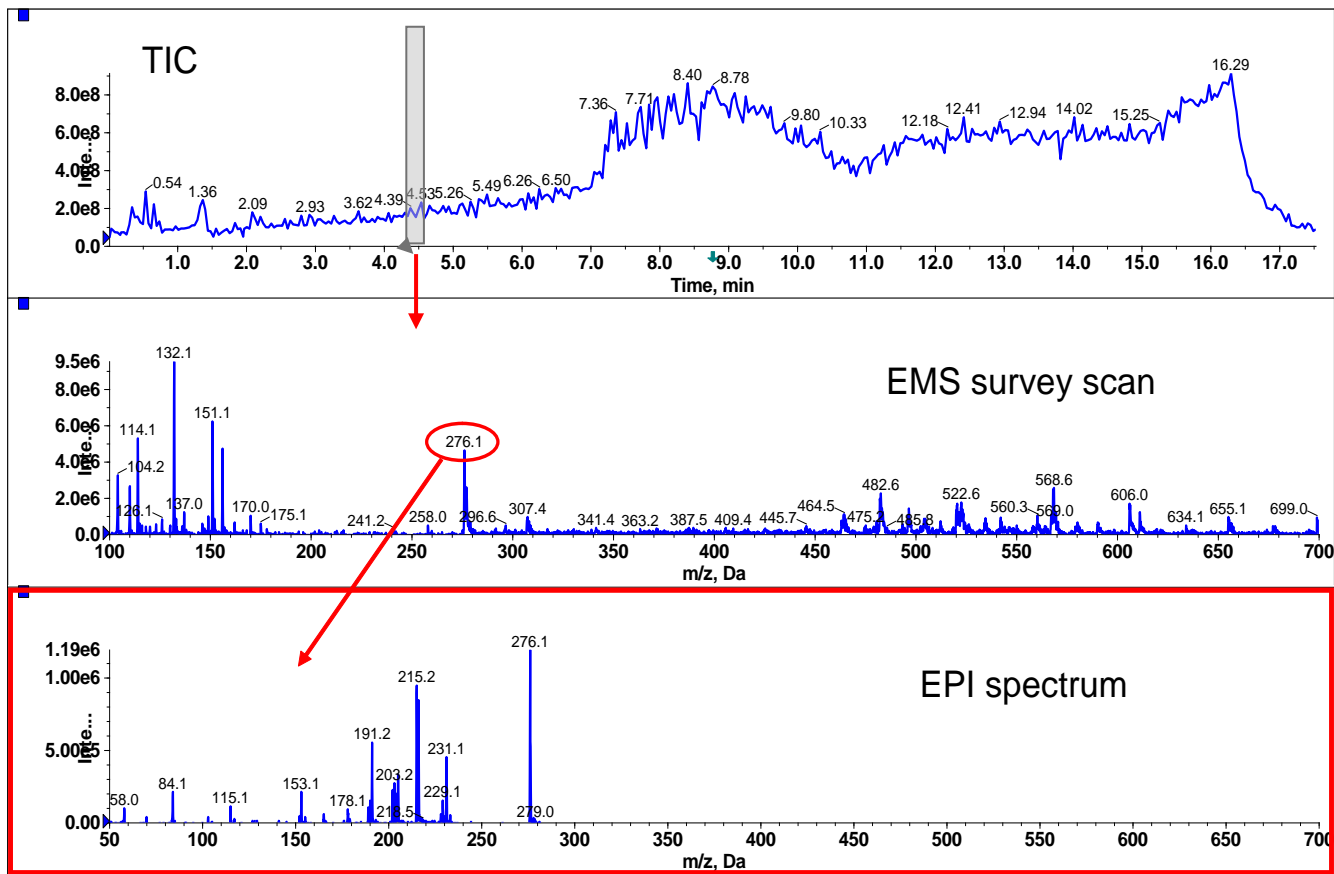
EMS-IDA-EPI workflow on QTRAP® LC-MS/MS systems

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

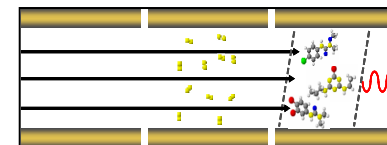


General Unknown Screening + Confirmation on QTRAP®

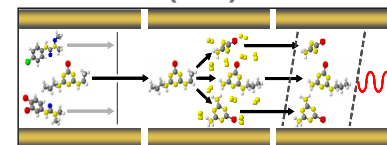
EMS detection identifies all compounds and metabolites (whether they were anticipated or not)



Enhanced Mass Spectrum (EMS)



Enhanced Product Ion (EPI) scan

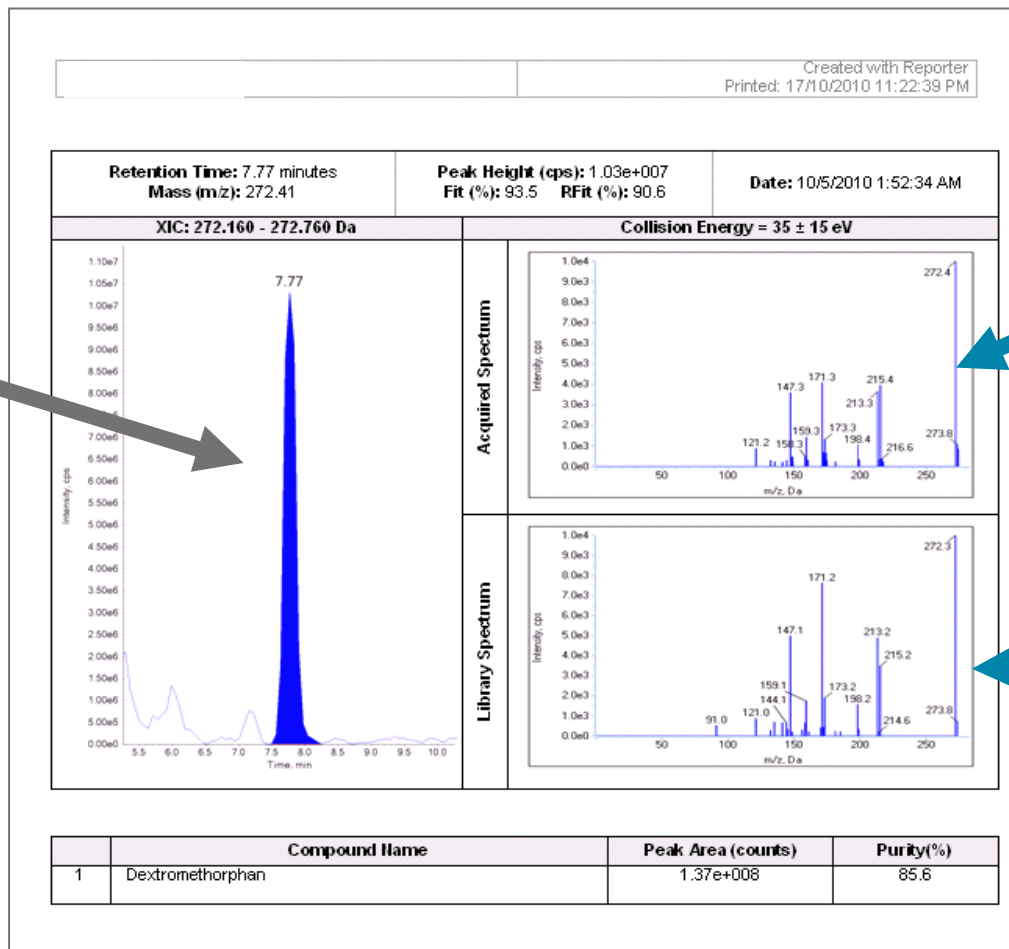


General Unknown Screening + Confirmation on QTRAP®

Survey Scan: EMS, Dependent Scan: EPI

Detection

XIC of EMS
peak
(272.2-272.8)



Confirmation

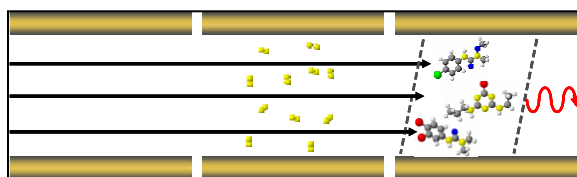
Acquired spectrum

Library spectrum:
Dextromethorphan

Summary: General Unknown Screening with EMS

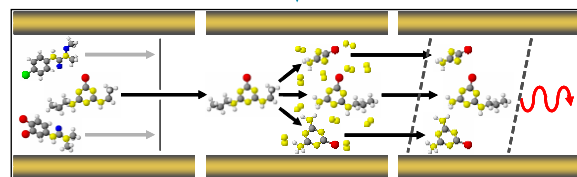
- Full-scan EMS detection identifies all compounds and metabolites
- MS/MS library searching provides confirmation
- Data “mining” is laborious; automated software is very helpful
- Sensitivity and selectivity is worse than MRM

EMS

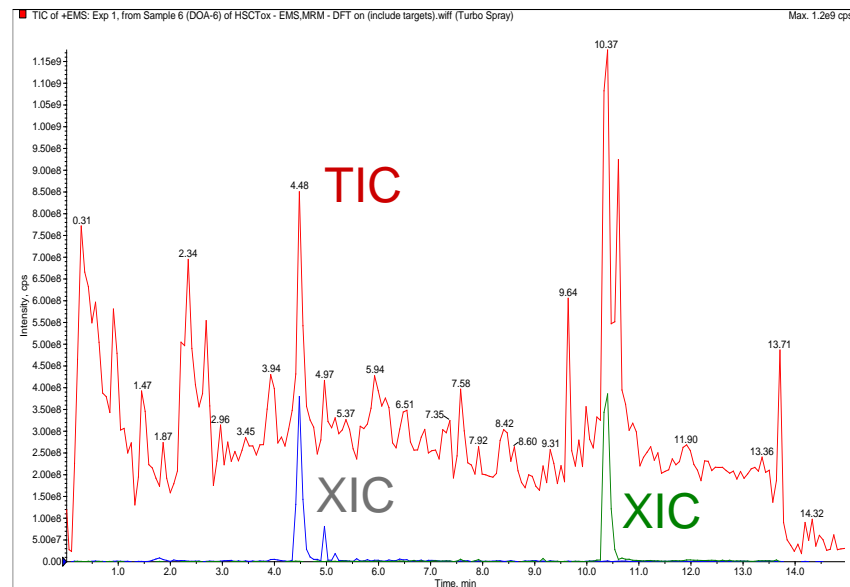


Threshold

EPI



Library Search



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



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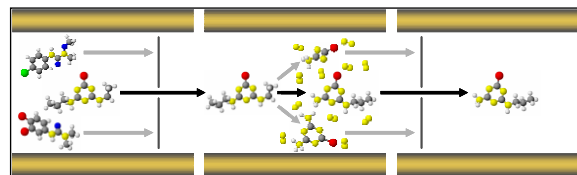
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A New Approach: Combining Methodology

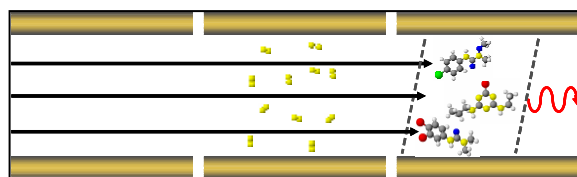
Why not combine “targeted” and “unknown” screening in a single method?

Scheduled MRM™ Algorithm



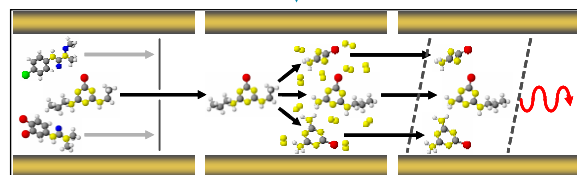
EMS

+



Threshold

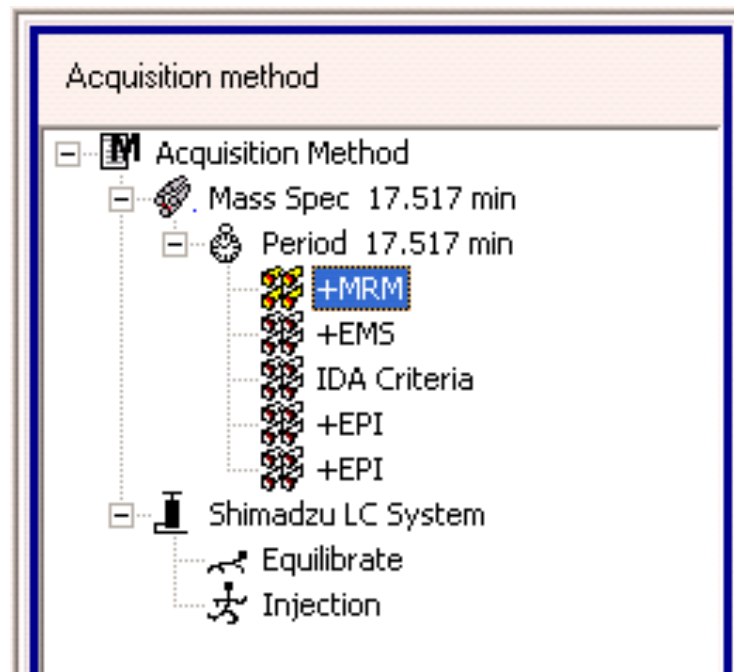
EPI



Library Search

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.

Acquisition method

- Acquisition Method
 - Mass Spec 17.517 min
 - Period 17.517 min
 - +MRM (1)**
 - LEMS
 - IDA Criteria
 - +EPI
 - +EPI
- Shimadzu LC System
 - Equilibrate
 - Injection

MS | Advanced MS

Experiment: 1 Scheduled MRM

Scan type: MRM (MRM)

Polarity: Positive Negative

MRM detection window: 90 (sec)

Target Scan Time: 1 (sec)

	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	370.238	200.200	6.5	Doxycycline	35.000

Period Summary

Duration: 17.517 (min) Delay Time: 0 (sec)

Cycles: 540 Cycle: 1.9464 (sec)

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).

The screenshot displays the software interface for configuring an acquisition method. On the left, a tree view shows the hierarchy: Acquisition Method > Mass Spec 17.517 min > Period 17.517 min > +EMS (2). The main configuration panel is titled 'MS | Advanced MS' and includes the following settings:

- Experiment: 2
- Scan type: Enhanced MS (EMS)
- Scan rate: 4000 (Da/s)
- Polarity: Positive
- MCA:
- Number of scans to sum: 1
- Total Scan Time (includes pauses): 0.2794 (sec)
- Buttons: Edit Parameters..., Import List, Optimize Masses
- Checkboxes: Center / Width, Parameter Range

A table of scan parameters is also visible, with the following data:

	Start (Da)	Stop (Da)	Time (sec)
1	100.000	280.000	0.0450
2	275.000	500.000	0.0563
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

At the bottom, a 'Period Summary' box shows:

- Duration: 17.517 (min)
- Delay Time: 0 (sec)
- Cycles: 540
- Cycle: 1.9464 (sec)

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.

Acquisition method

Acquisition Method

- Mass Spec 17.517 min
- Period 17.517 min
- +MRM
- +EMC
- IDA Criteria (3)**
- +EPI
- +EPI

Shimadzu LC System

- Equilibrate
- Injection

IDA - First Level Criteria | Include/Exclude | Isotope Pattern

Select 1 to 2 most intense peaks After Dynamic Background Subtraction of Survey scan ← DBS "on"

Survey -> IDA Experiment

- For ions greater than: 100 (m/z)
- For ions smaller than: 1700 (m/z)
- With charge state: 2 to 3
- Include unknowns
- Rolling Collision Energy Settings...

Which exceeds: 5000 (cps)

Exclude former target ions

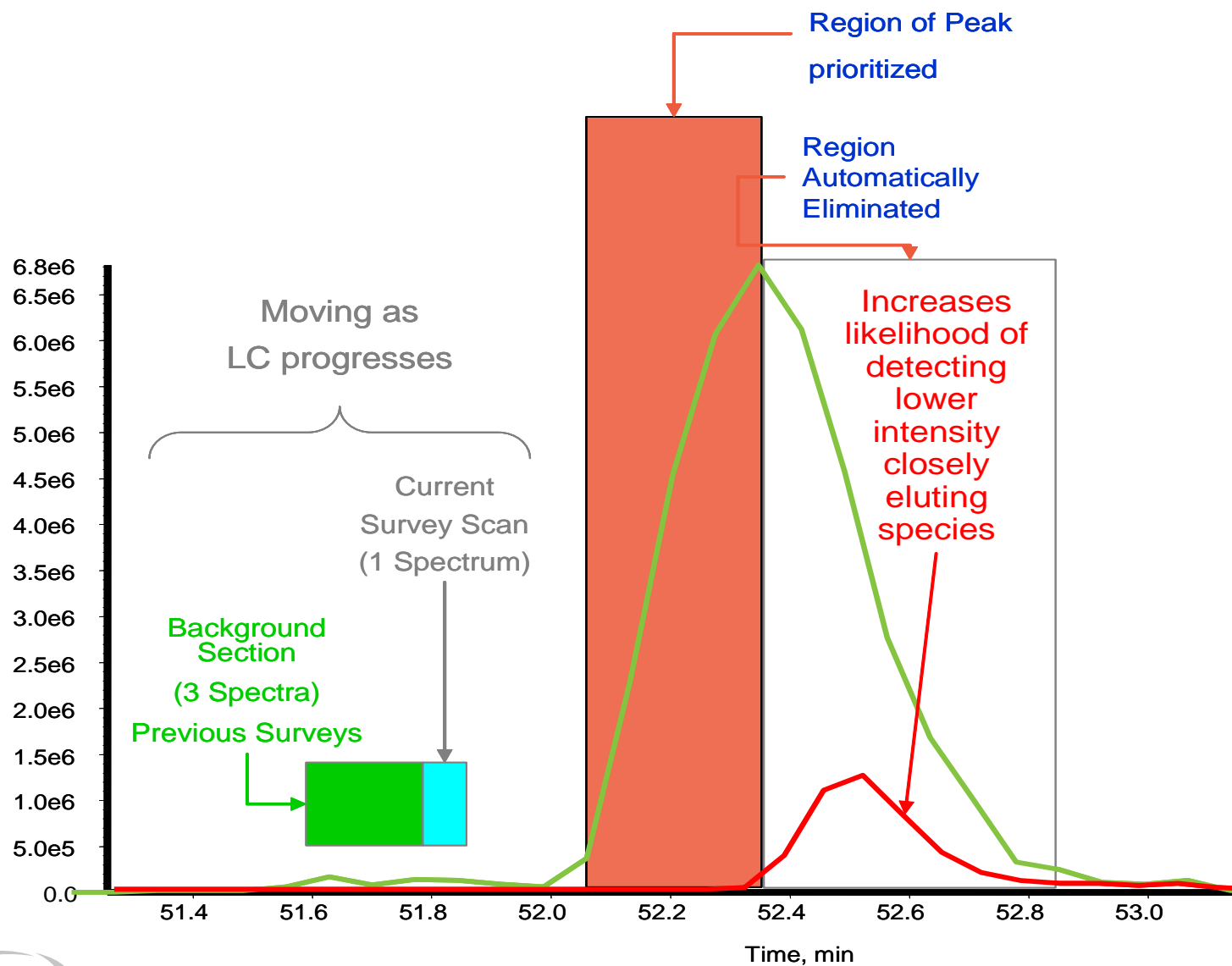
- Always
- Never
- After: 2 occurrence(s)
- For 10 (sec)

Mass Tolerance: 250 mDa ppm

- Exclude isotopes within: 4 (Da)

← Dynamic exclusion criteria

Dynamic Background Subtraction



Targeted + Unknown Screening on a QTRAP® System

(3) Information Dependent Acquisition (IDA)

The screenshot displays the QTRAP software interface for configuring Information Dependent Acquisition (IDA). The left sidebar shows the acquisition method tree, with 'IDA Criteria' highlighted and labeled with a blue circle and the number (3). The main window shows the 'IDA - First Level Criteria' configuration, with the 'Include/Exclude' tab selected. A blue arrow points from the 'Include/Exclude' tab to the 'Include List' table in the foreground window.

Include List

	Mass (Da)	Always	RT (min)	Width (sec)
1	303.200	<input type="checkbox"/>	5.200	60
2	232.000	<input type="checkbox"/>	6.200	60
3	243.100	<input type="checkbox"/>	5.600	60
4	333.100	<input type="checkbox"/>	4.300	60
5	180.100	<input type="checkbox"/>	3.900	60
6	208.100	<input type="checkbox"/>	4.900	60
7	194.100	<input type="checkbox"/>	4.400	60
8	433.900	<input type="checkbox"/>	2.900	60

Intensity: 5000 (cps) Default Width: 60 (sec)

Buttons: Import... Charge State... Clear Grid

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.

Acquisition method

- Acquisition Method
 - Mass Spec 17.517 min
 - Period 17.517 min
 - +MRM
 - +EMS
 - IDA Criteria
 - +EPI
 - +EPI
- Shimadzu LC System
 - Equilibrate
 - Injection

MS | Advanced MS

Experiment: 3

Scan type: Enhanced Product Ion (EF)

Scan rate: 4000 (Da/s)

Polarity: Positive Negative

MCA

Number of scans to sum: 1

Product Of: 30.000 (Da)

Total Scan Time (includes pauses): 0.3335 (sec)

Center / Width

Parameter Range

Import List

Optimize Masses

	Start (Da)	Stop (Da)	Time (sec)
1	50.000	70.000	0.0050
2	65.000	137.200	0.0181
3	132.000	500.000	0.0920
4			

Period Summary

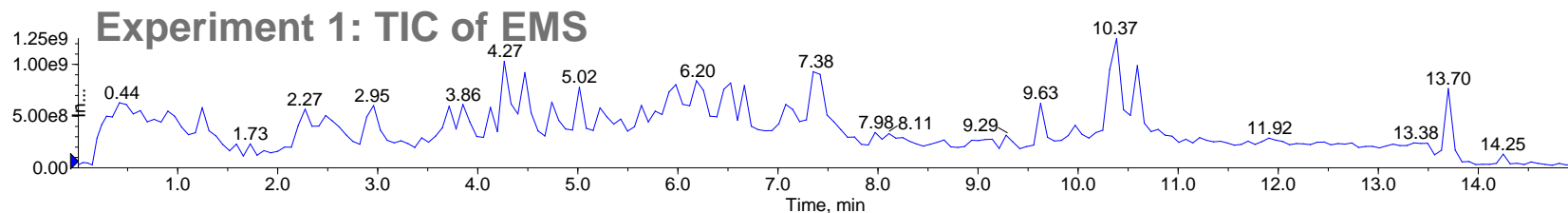
Duration: 17.517 (min) Delay Time: 0 (sec)

Cycles: 540 Cycle: 1.9464 (sec)

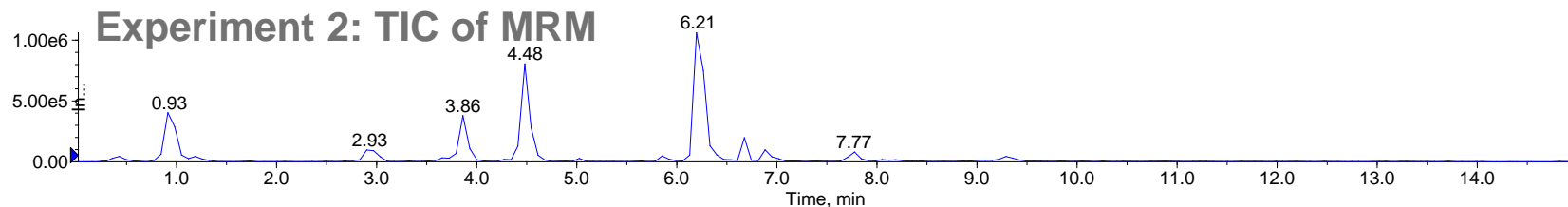
Targeted + Unknown Screening on a QTRAP® System



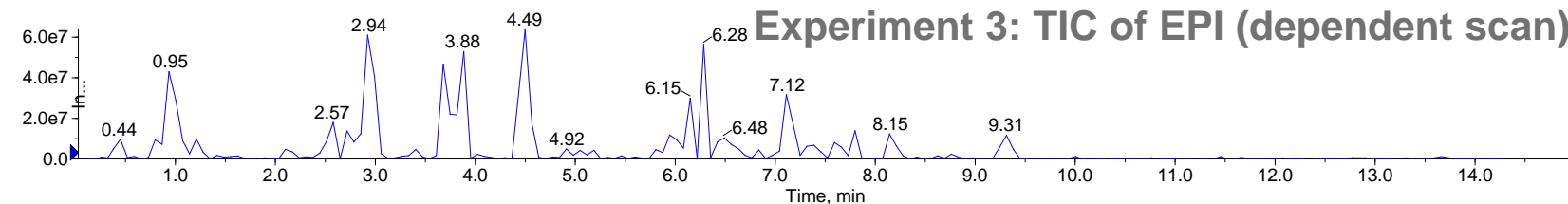
■ TIC of +EMS: Exp 1, from Sample 1 (DOA-1) of HSCTox - EMS,MRM - DFT on (include targets).wiff (Turbo Spray) Max. 1.2e9 cps.



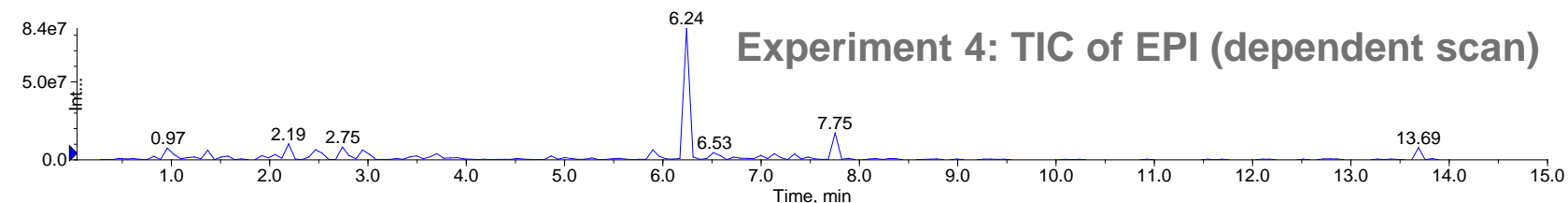
■ TIC of +MRM (52 pairs): Exp 2, from Sample 1 (DOA-1) of HSCTox - EMS,MRM - DFT on (include targets).wiff (Turbo Spray) Max. 1.1e6 cps.



■ TIC of +EPI: Exp 3, from Sample 1 (DOA-1) of HSCTox - EMS,MRM - DFT on (include targets).wiff (Turbo Spray) Max. 6.4e7 cps.

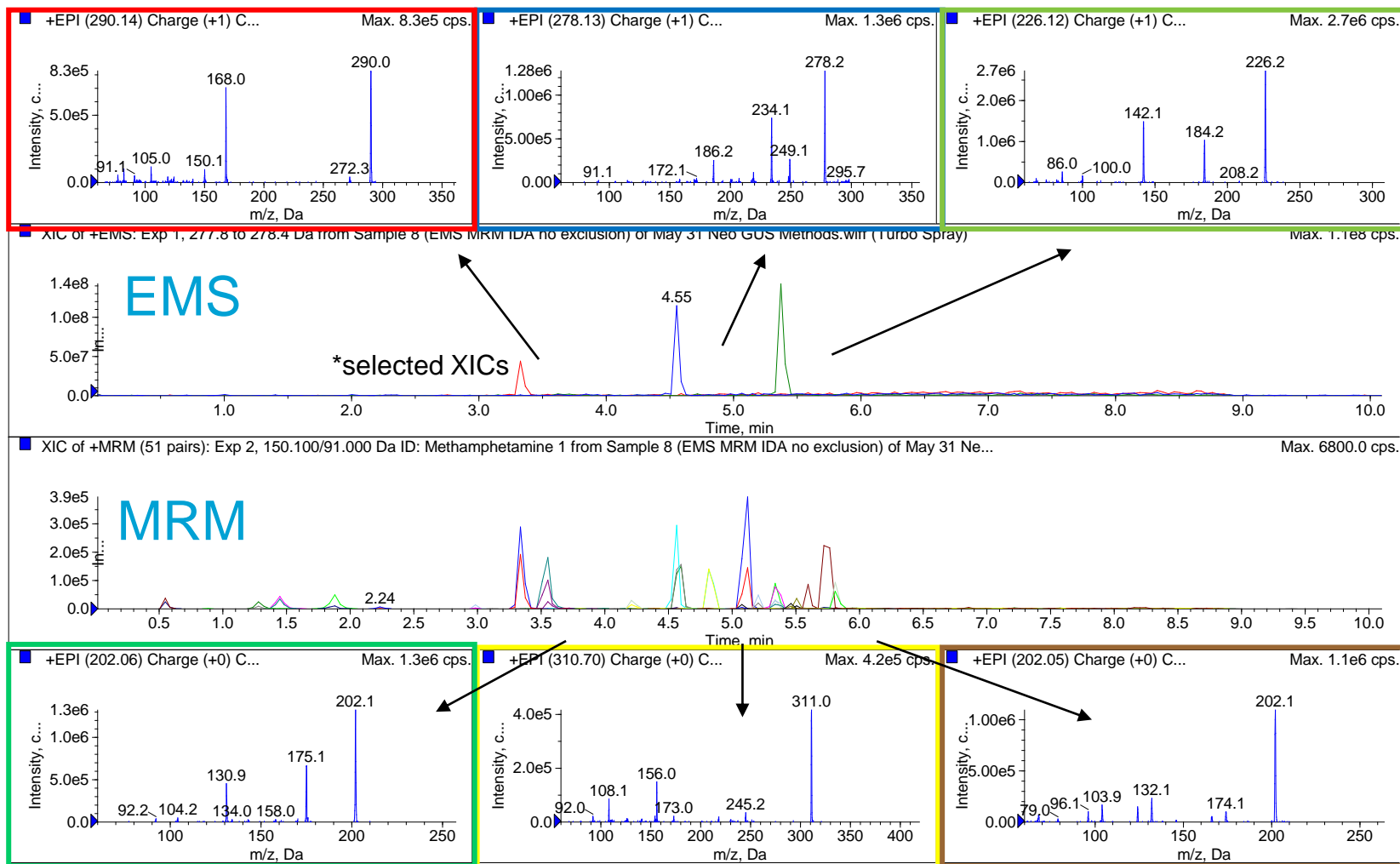


■ TIC of +EPI: Exp 4, from Sample 1 (DOA-1) of HSCTox - EMS,MRM - DFT on (include targets).wiff (Turbo Spray) Max. 8.4e7 cps.



Targeted + Unknown Screening on a QTRAP® System

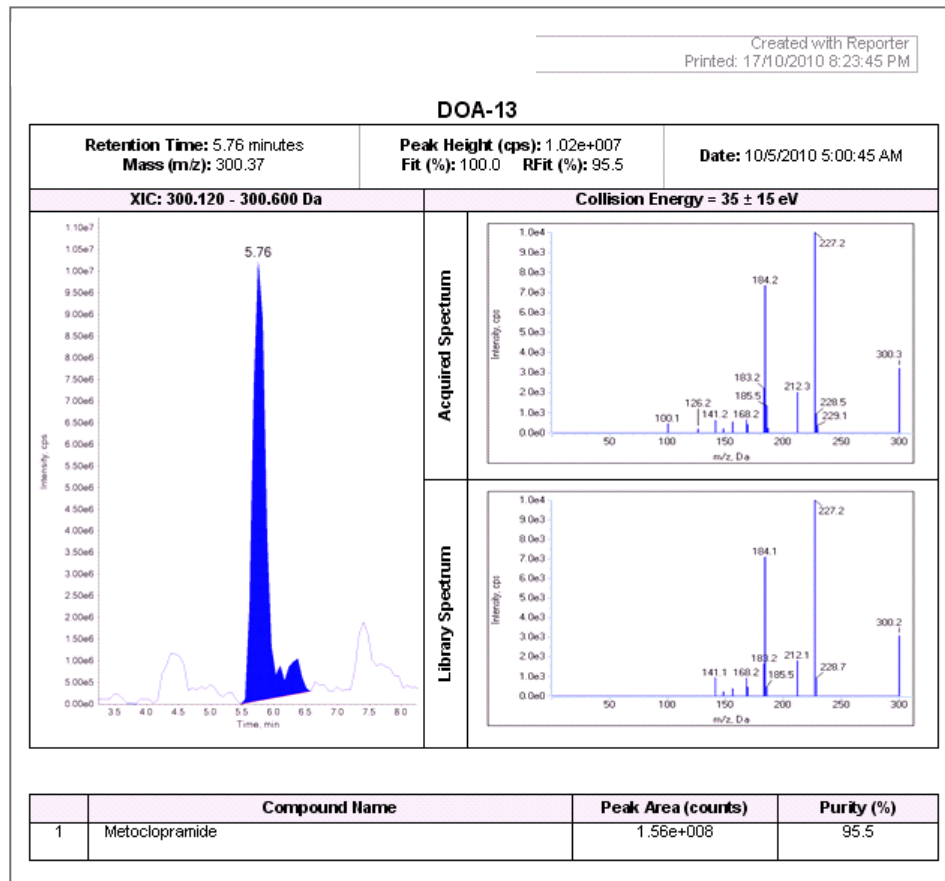
EPI spectra are triggered from EMS and MRM survey scans
EPI spectra are library-searched for confirmation of compound IDs



“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



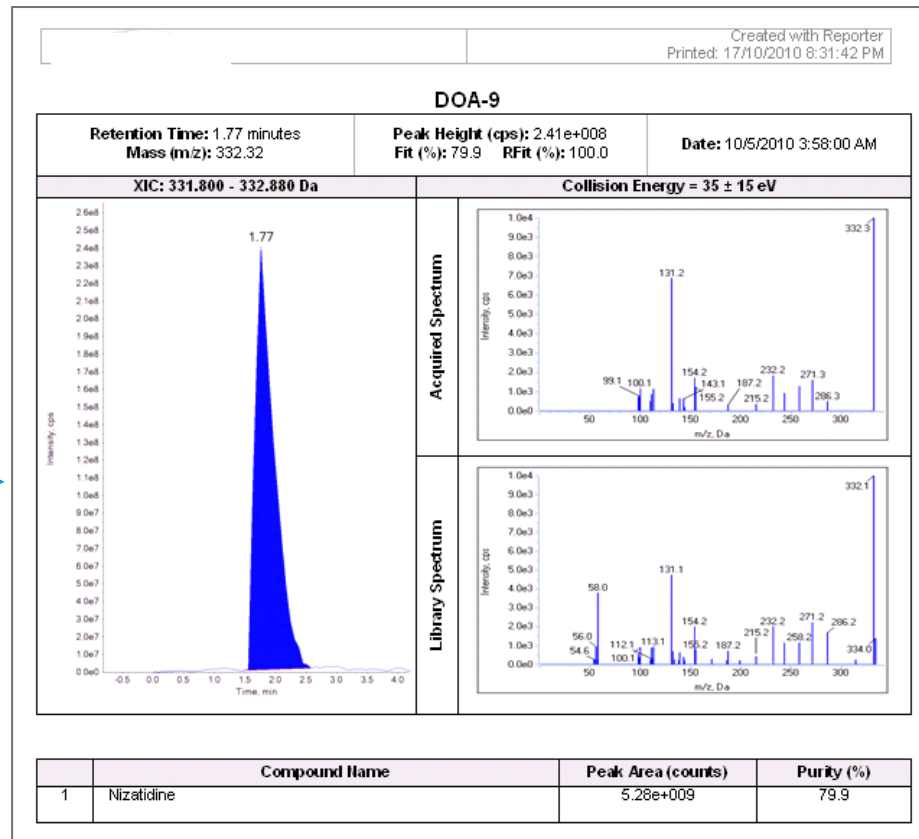
Acquired
spectrum

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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