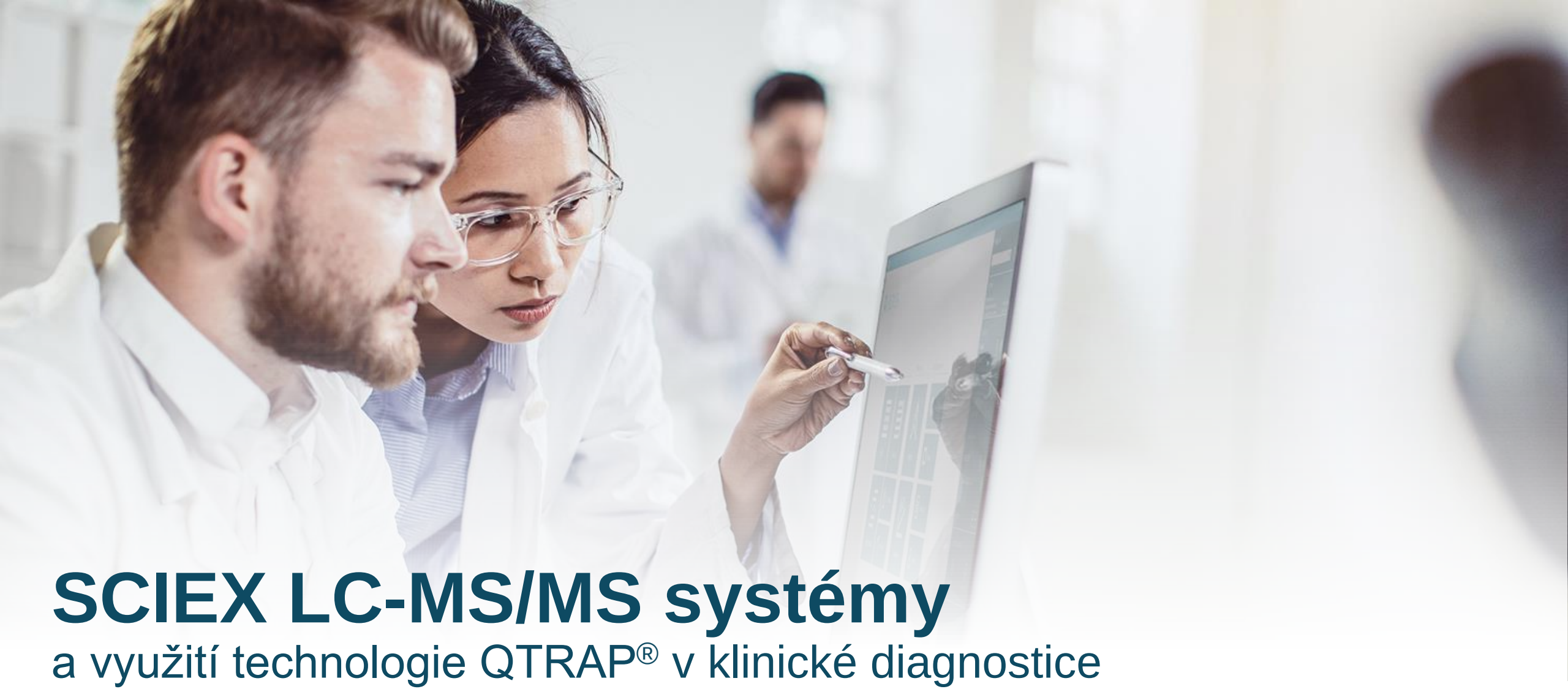


SCIEX LC-MS/MS systémy

a využití technologie QTRAP® v klinické diagnostice

František Laštovička - AMEDIS, 29.5.2023 VitaTox 2023

SCIEX
The Power of Precision

SCIEX mass spectrometry portfolio: 2023

SCIEX Triple Quad and QTRAP systems

Triple Quad 3500 system

QTRAP/TQ 4500 system

SCIEX 5500+ system

QTRAP/TQ 6500+ system

SCIEX 7500 system

Pushing the limits

X500R QTOF system

X500B QTOF system

ZenoTOF 7600 system

SCIEX HRMS products

SCIEX The Power of Precision

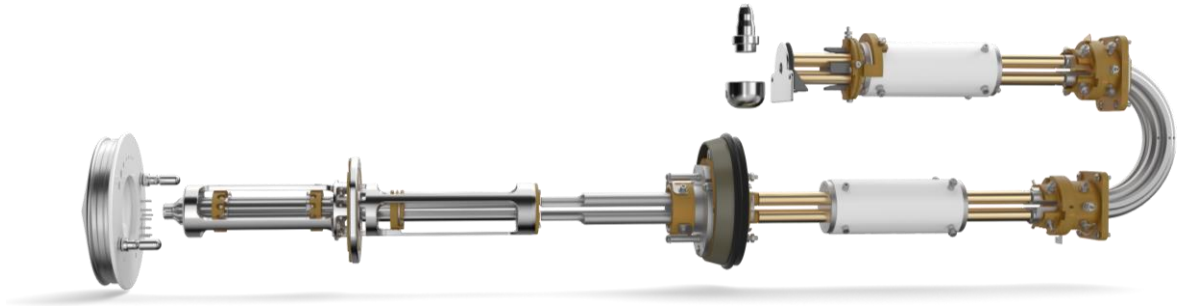
QTRAP – two instruments in one, System technology

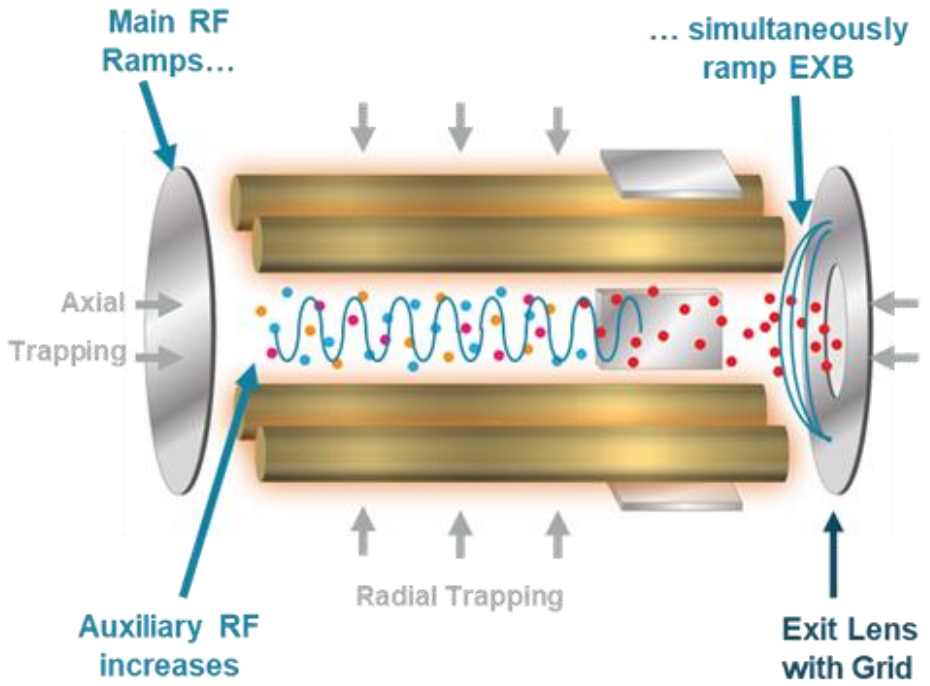
František Laštovička, AMEDIS, 27.4.2023 AMEDIS Day, Praha

SCIEX The Power of Precision

QTRAP - Built on a legacy of performance

SCIEX QTRAP SYSTEM



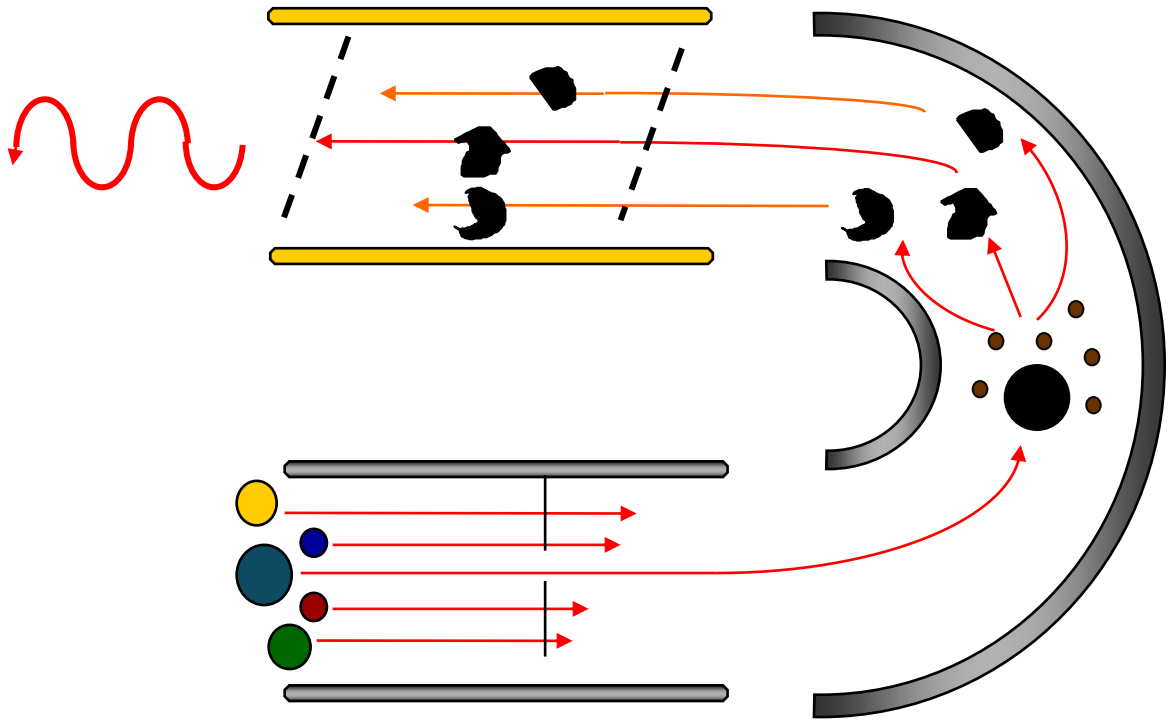


QTRAP - Triple Quadrupole / Linear Ion Trap MS

- All QqQ modes of operation
  - Identical sensitivity in MRM
  - Wide dynamic range for quantitation
  - Neutral Loss (NL)
  - Precursor Ion Scan (PI)
  - MRM
- Additional simultaneous functions of linear iont trap
  - **Enhanced MS (EMS)**
  - Enhanced Resolution (ER)
  - **Enhanced Product Ion Scan (EPI)**
  - **MS^3**
  - Enhanced Multiply Charged Scan (EMC)

Enhanced Product Ion Scan (EPI)

Fragmentation pattern with good sensitivity



Q1: **SIM**

Q2: **Fragmentation**

LIT (Q3): **Trap scan**

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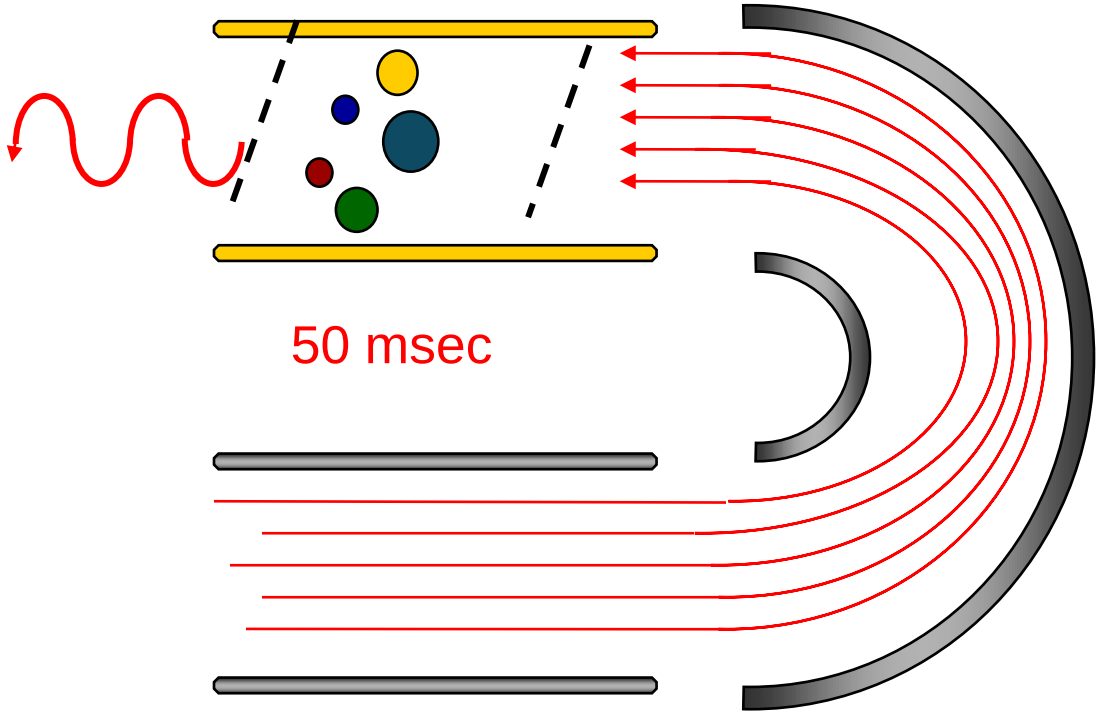
Enhanced MS Scan (EMS) - Search for Present Ions

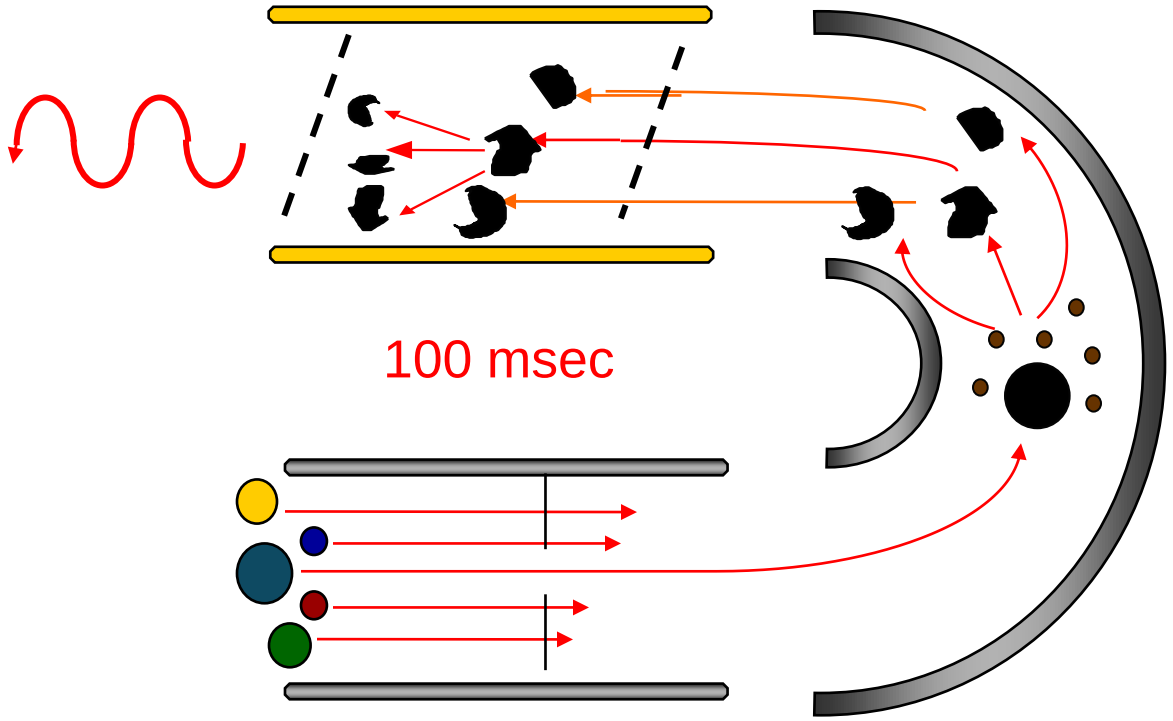


Q1: **RF only**

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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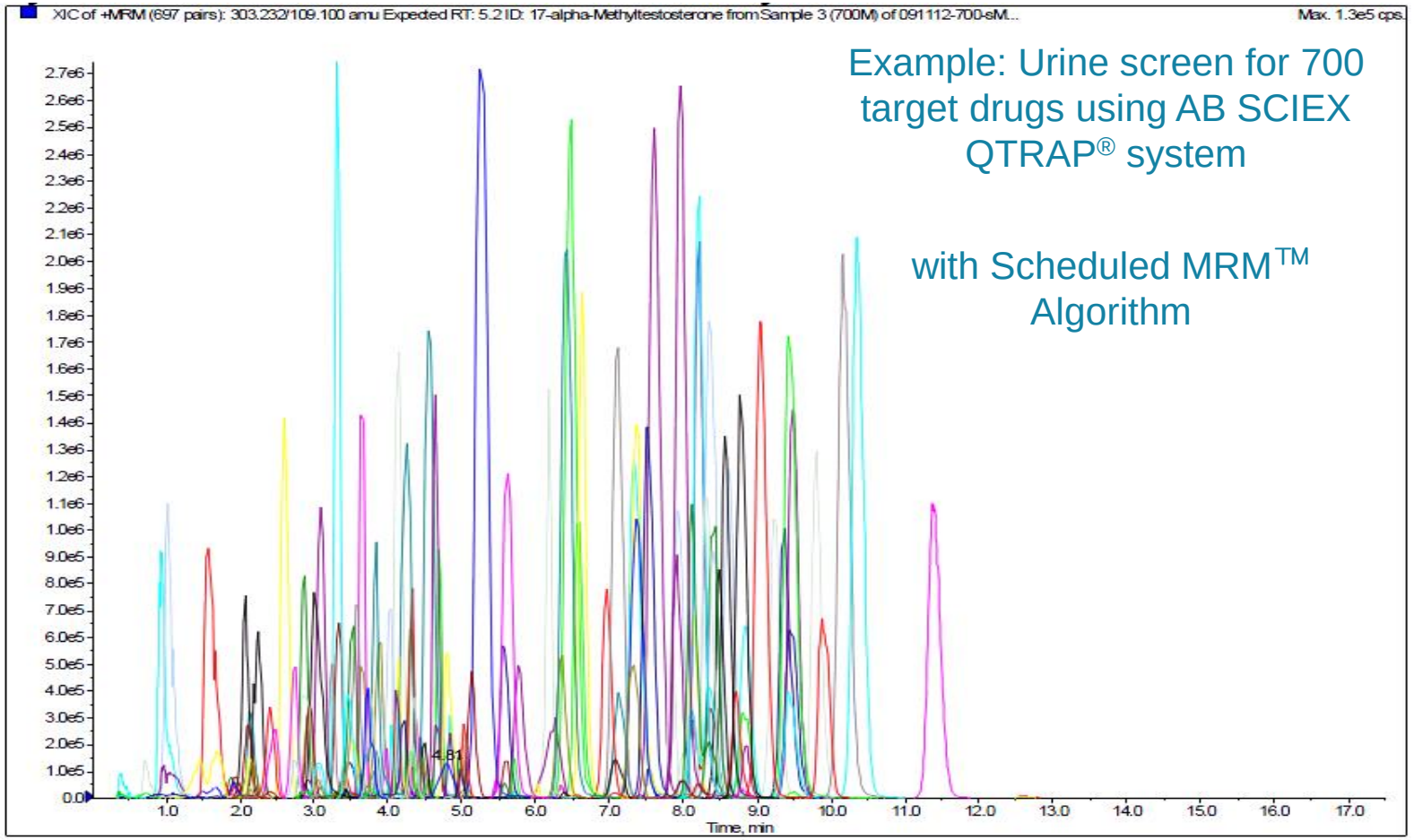
Multi-Target Screening (MTS) Approach with MRM

- MRM detection provides ultimate *sensitivity* and *selectivity*
- Two MRMs may be monitored, for additional confidence/confirmation



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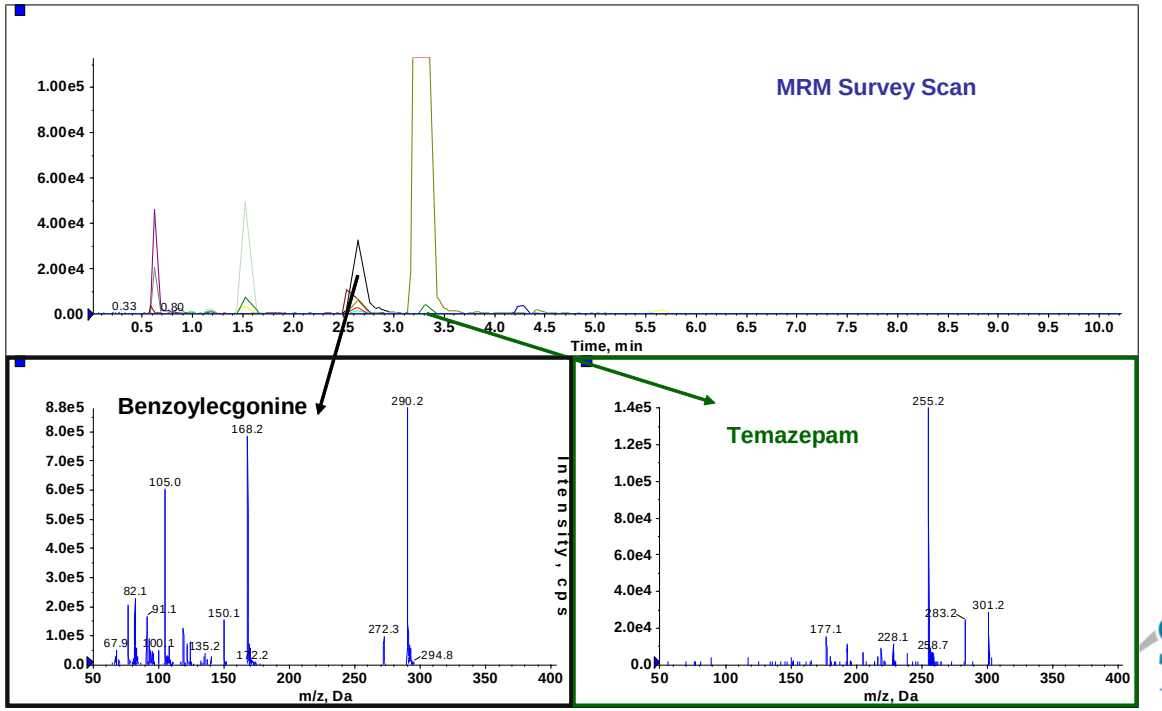
Multi-Target Screening + Confirmation

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

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



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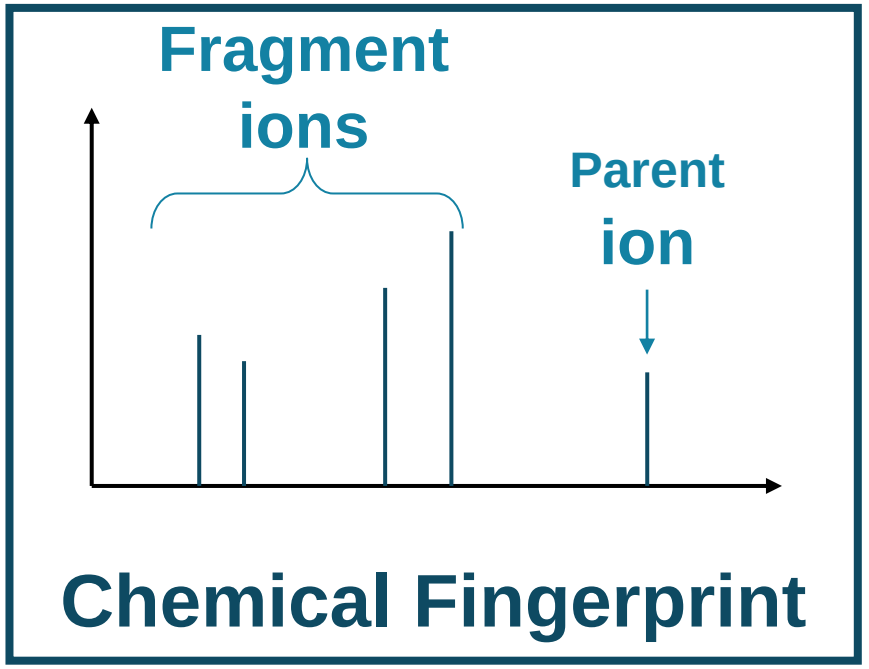
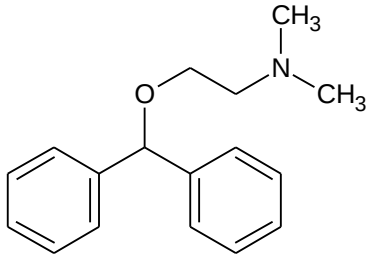
MS/MS Library Searching



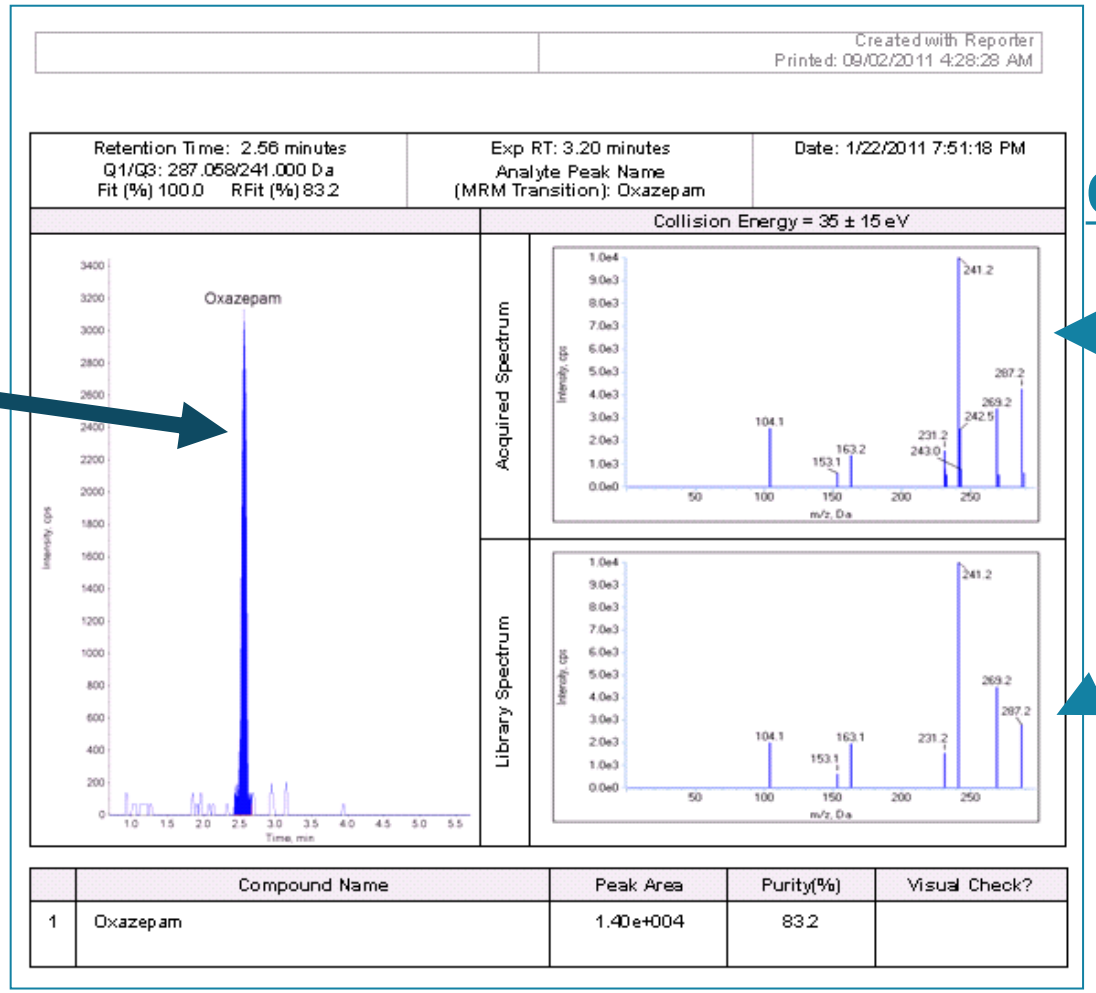


Multi-Target Screening + Confirmation

- Survey Scan: MRM, Dependent Scan: EPI

Detection

MRM of Oxazepam 287.1/241.0



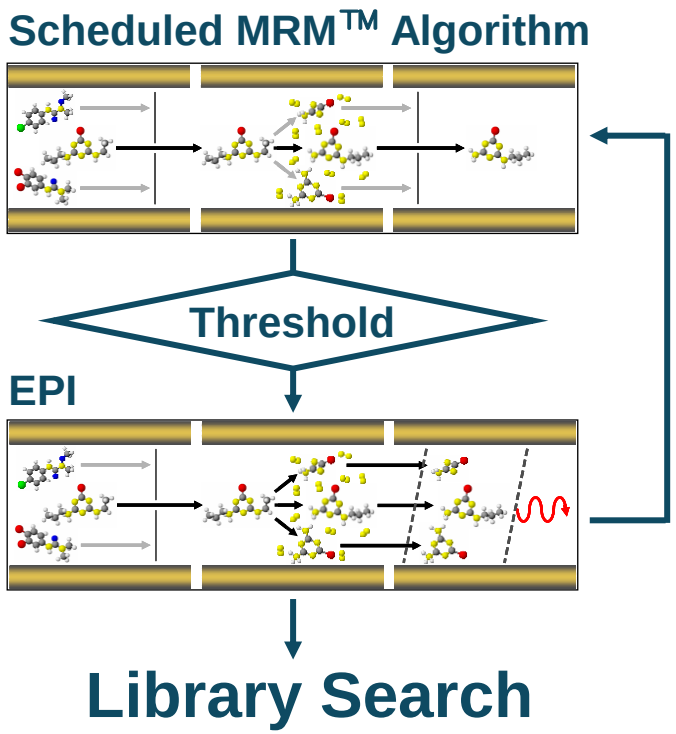
Confirmation

Acquired spectrum

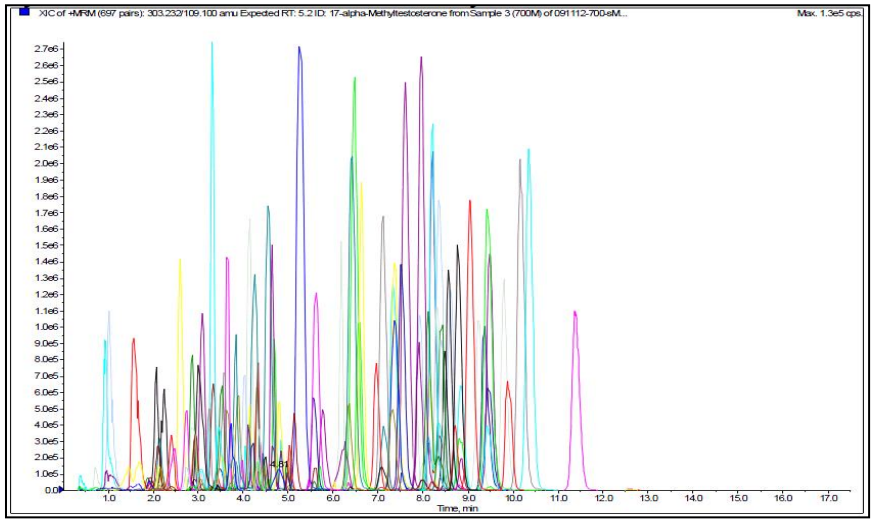
Library spectrum of Oxazepam

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



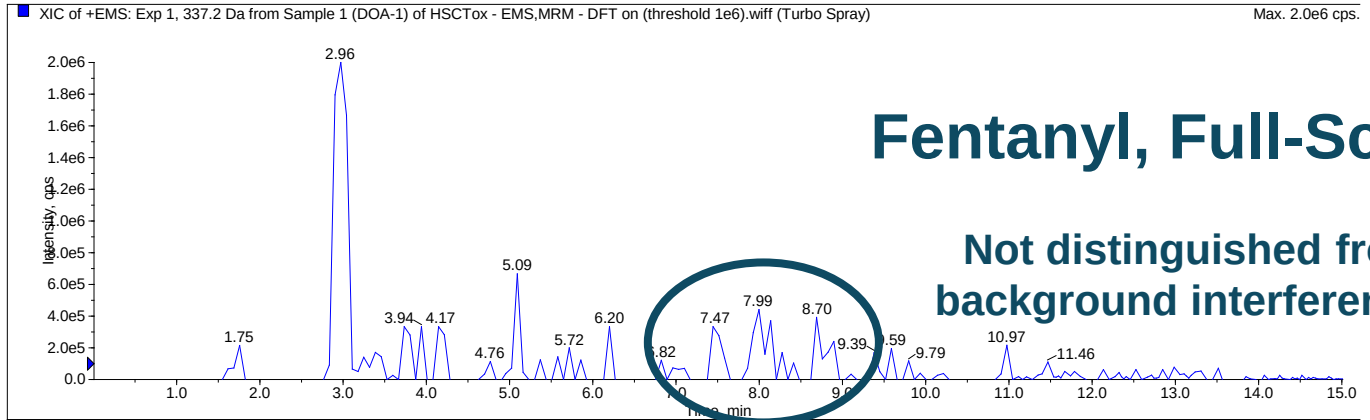
Example: Urine screen for 700 target drugs using AB SCIEX 3200 QTRAP® system

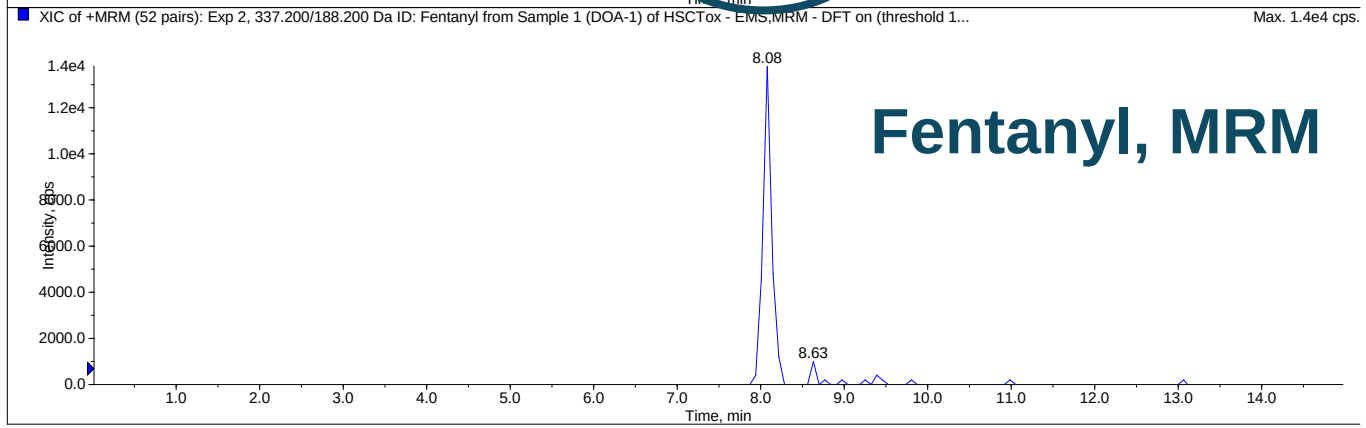


SCIEX The Power of Precision

Reminder: *Tandem* MS Provides Greater Selectivity

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





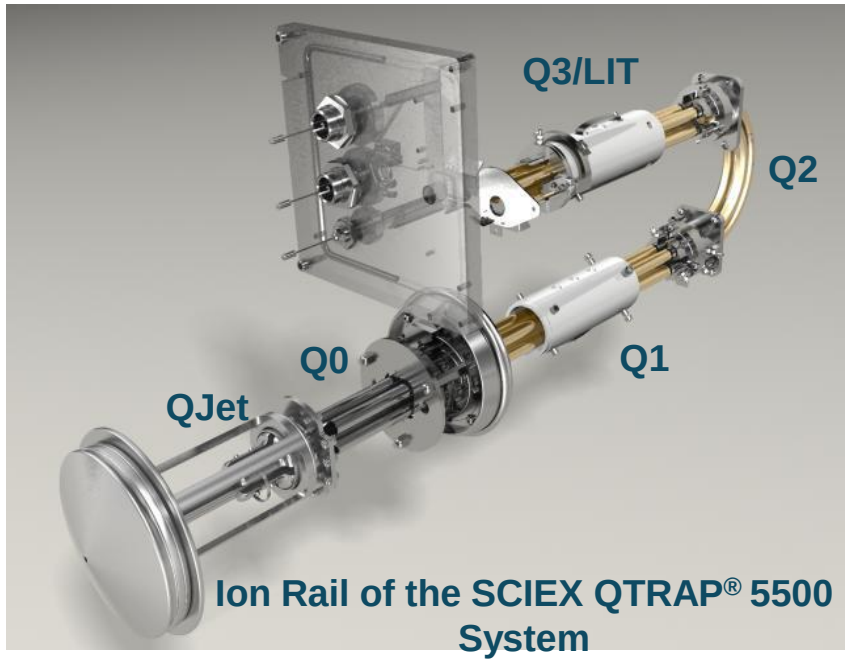
General Unknown Screening Approach

SCIEX The Power of Precision

General Unknown Screening + Confirmation on QTRAP®

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

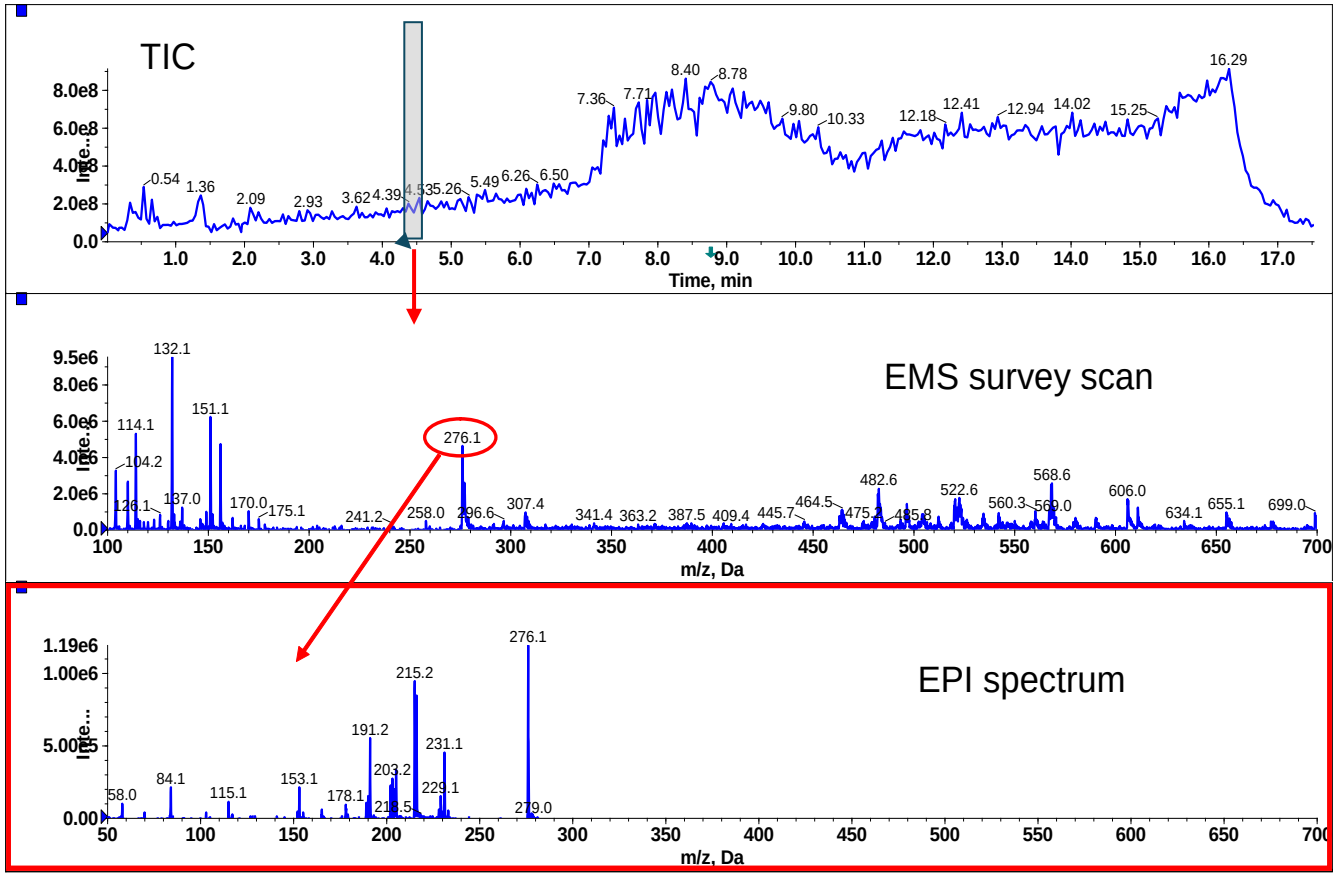
1. **EMS** survey scan rapidly screens for all compounds
2. IDA criteria is set to trigger dependent scans
3. **EPI** scans rapidly collect high-quality MS/MS data
4. Search MS/MS Library



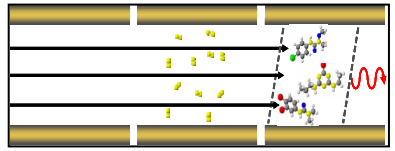
Ion Rail of the SCIEX QTRAP® 5500 System

General Unknown Screening + Confirmation on QTRAP®

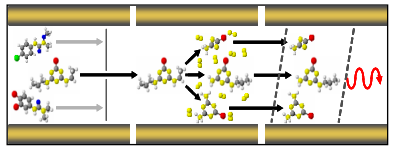
- EMS detection identifies all compounds and metabolites (whether they were anticipated or not)



Enhanced Mass Spectrum (EMS)



Enhanced Product Ion (EPI) scan



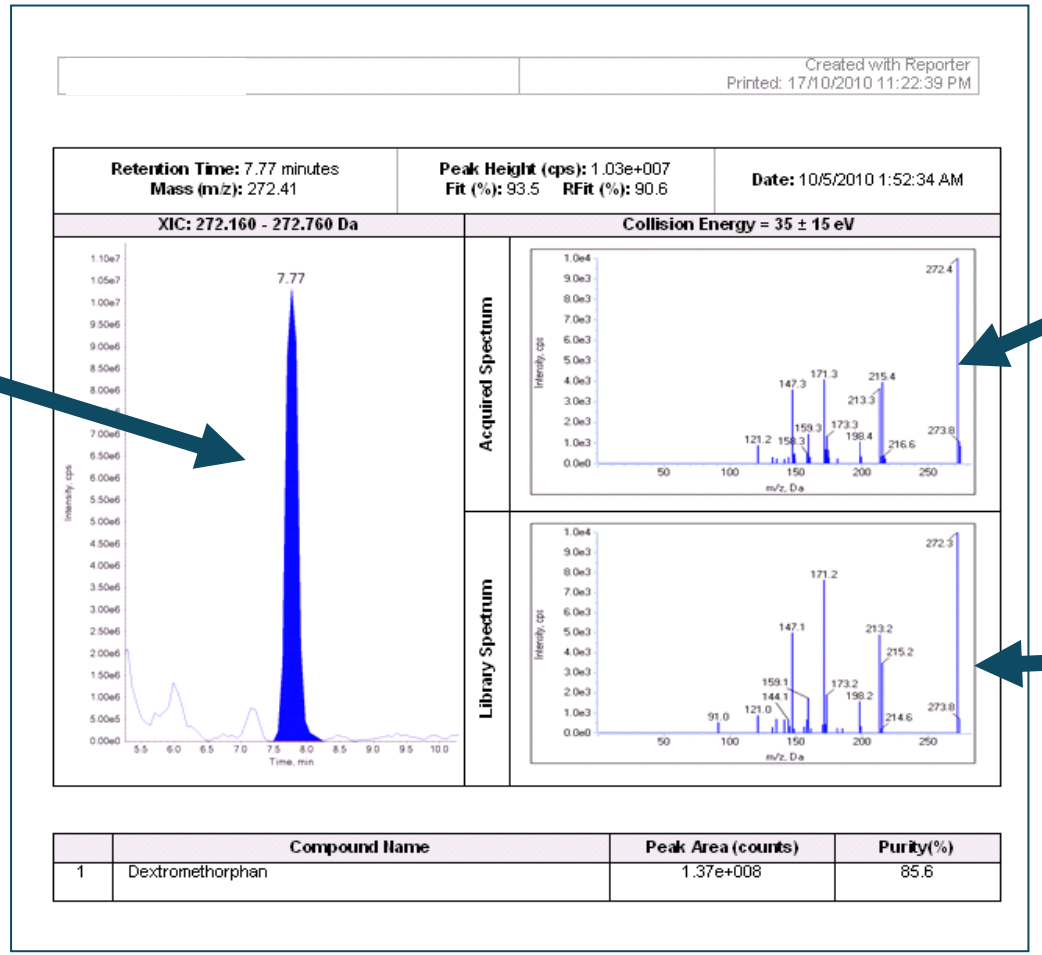
RUO-MKT-11-11650-C

General Unknown Screening + Confirmation on QTRAP®

- Survey Scan: EMS, Dependent Scan: EPI

Detection

XIC of EMS peak (272.2-272.8)



Confirmation

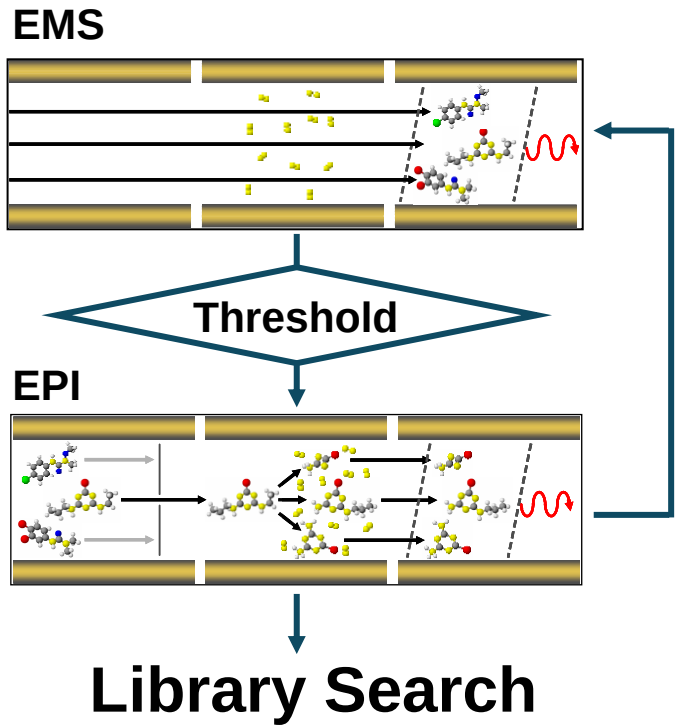
Acquired spectrum

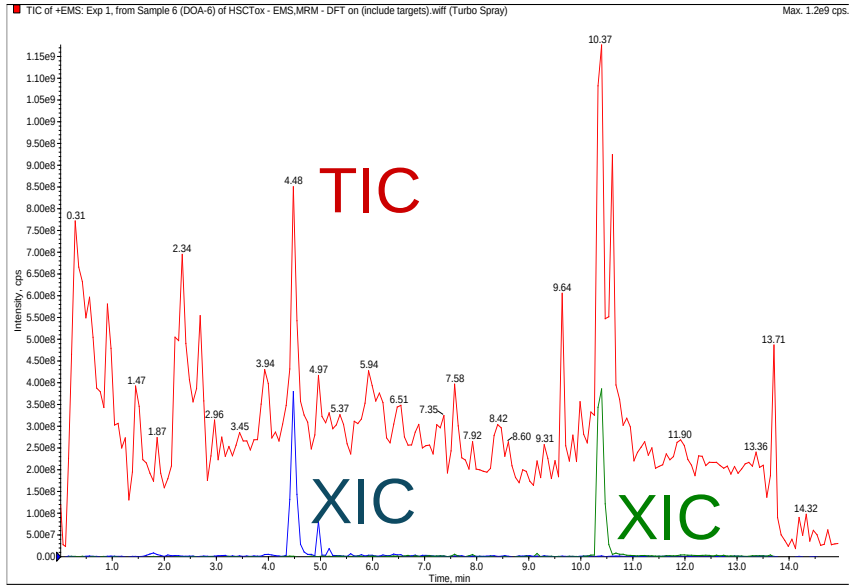
Library spectrum: Dextromethorphan

SCIEX The Power of Precision

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

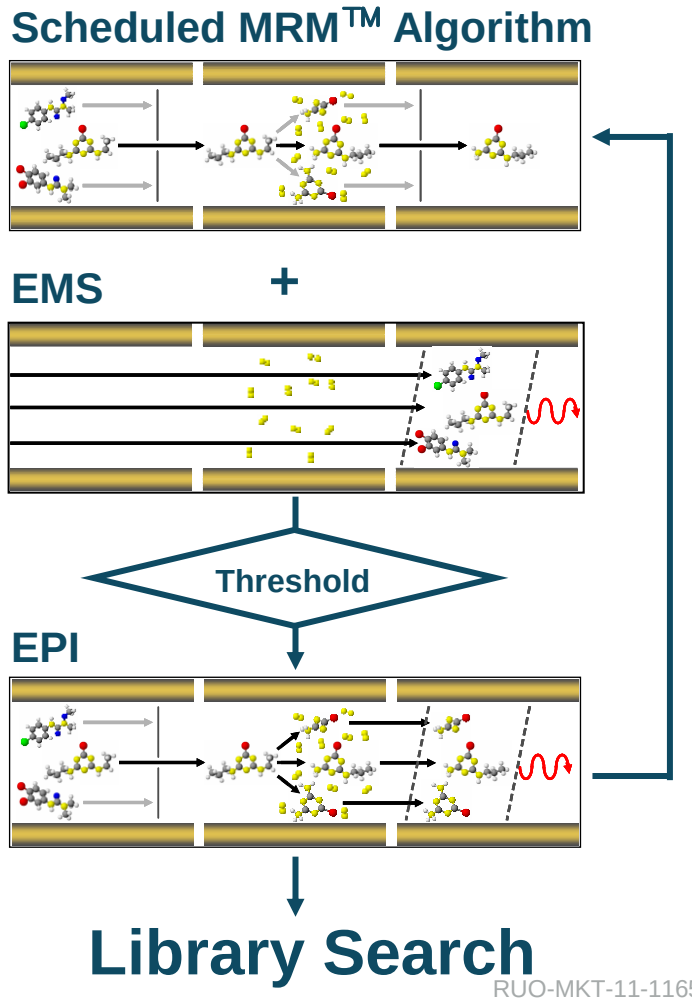
MTS and GUS are truly complementary screening techniques.

Combined Approach

SCIEX The Power of Precision

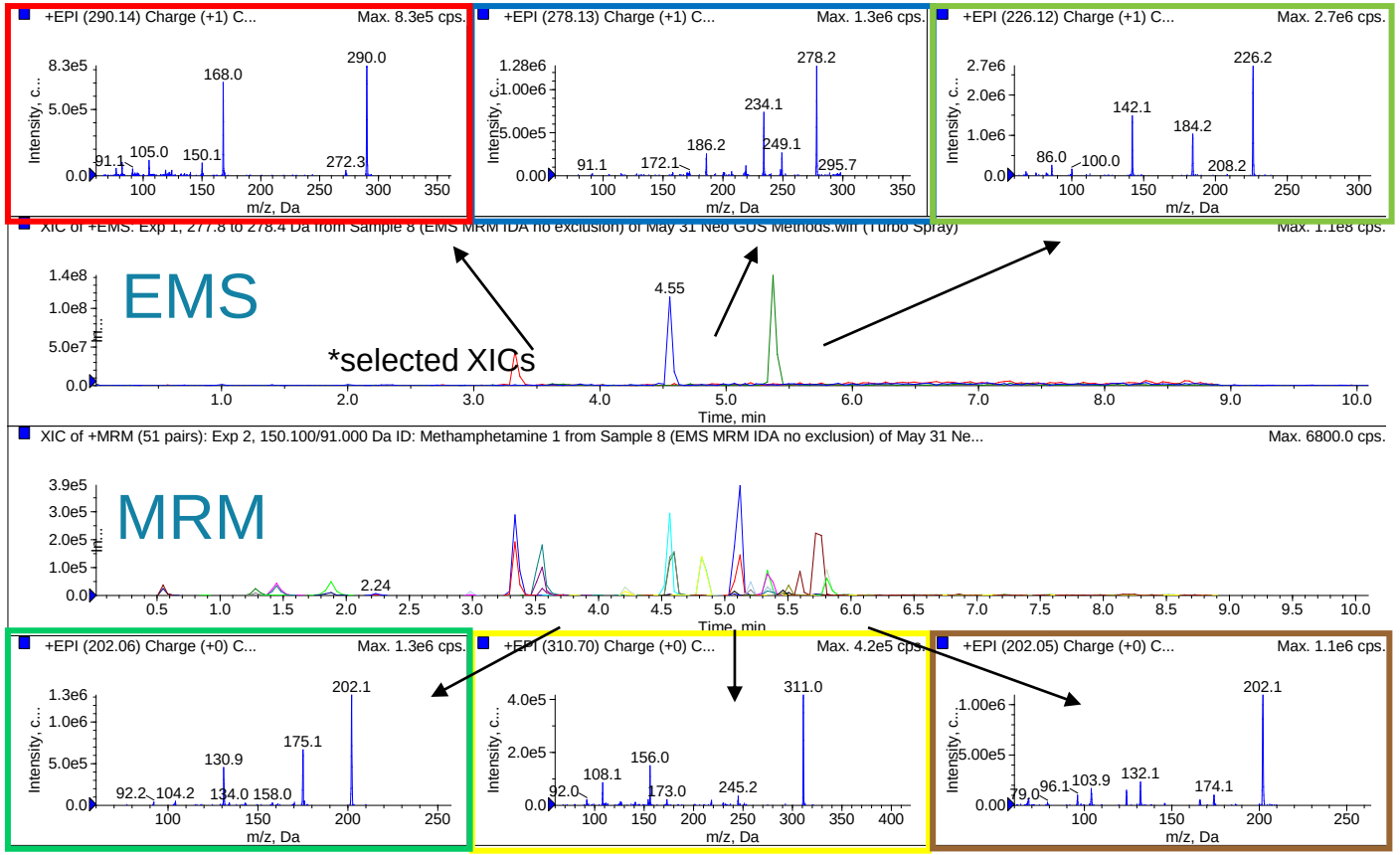
A New Approach: Combining Methodology

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



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



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

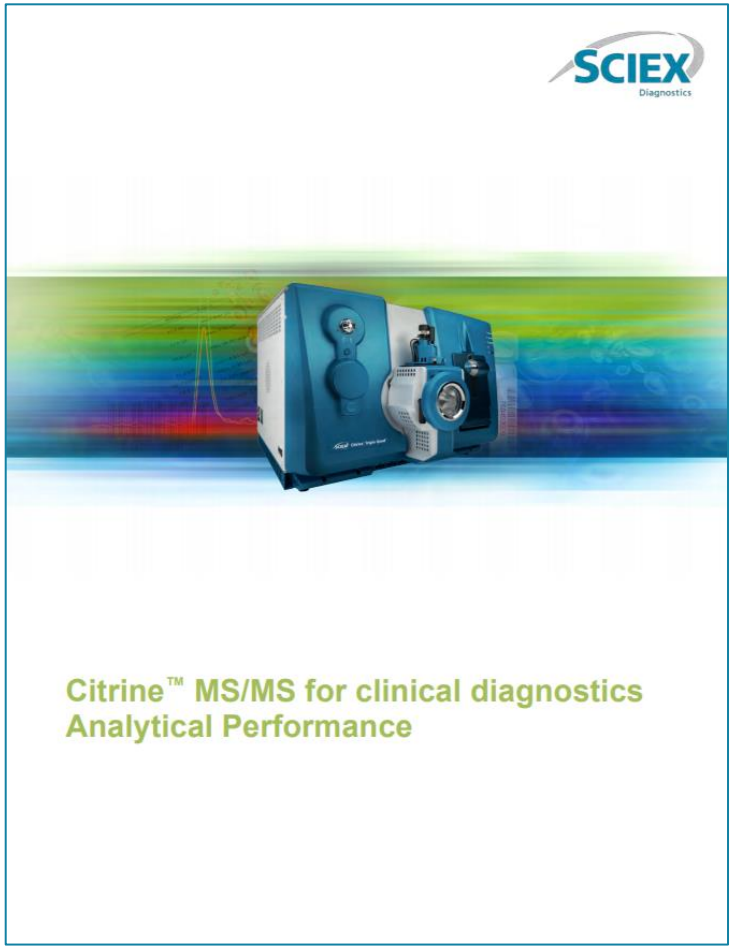
- The **QTRAP®** system allows rapid acquisition of full-scan MS/MS during MRM and EMS analysis, for additional confidence in IDs.

- 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.

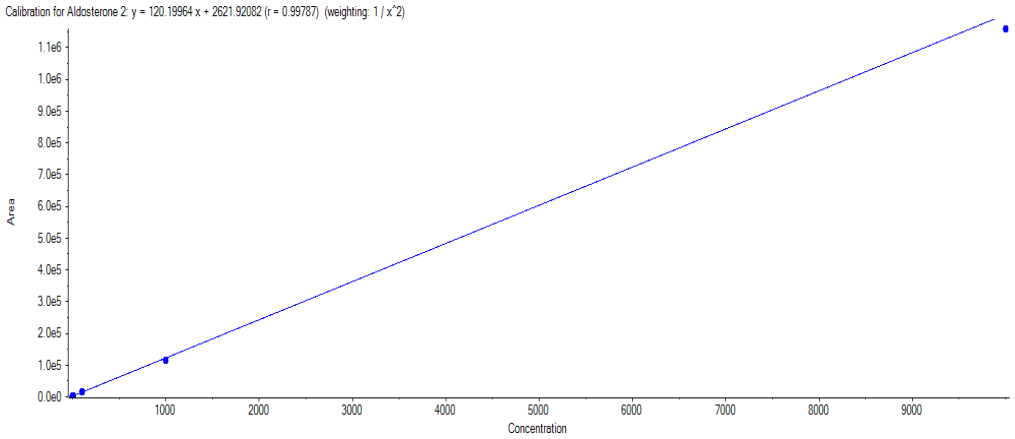
Performance Specification Booklet

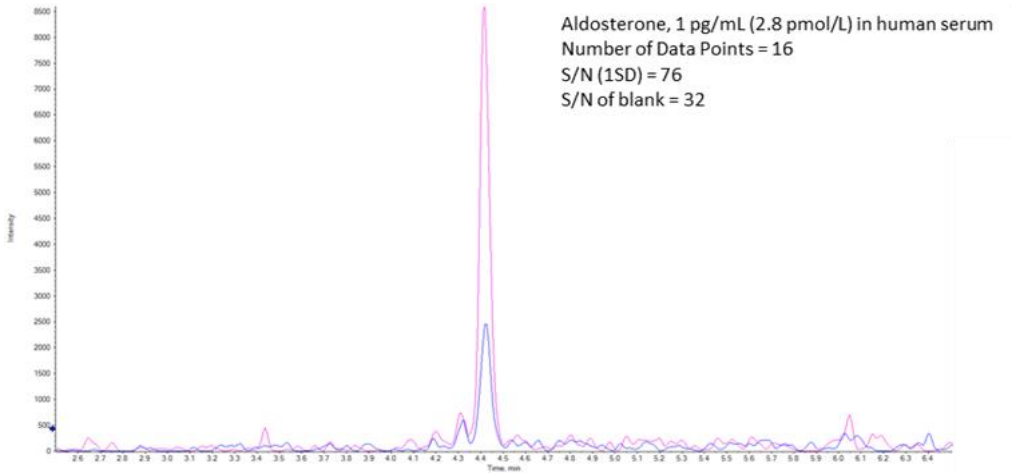
- Steroids, water-soluble vitamins and fat-soluble vitamins
- 1,25 Dihydroxyvitamin D2 & D3
- Estrone, Estradiol, Estriol
- FT3/FT4
- Aldosterone
- Low Level Testosterone
- Multi-Compound Drugs panel
- THC and metabolites in Hair (MRM^3)



Analytical Performance Specification Sheets

ALDOSTERONE





Compound	Range (units)	%CV (at LLOQ)	S/N
Aldosterone	1-10000 pg/ml	8.7%	44*

Aldosterone

- 5 orders LDR
- LLOQ 1pg/ml (2.8pmol/L)
- %CV at LLOQ 8.7%

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Analytical Performance Specification Sheets

STEROIDS & VITAMINS (“WELLNESS PANEL”)

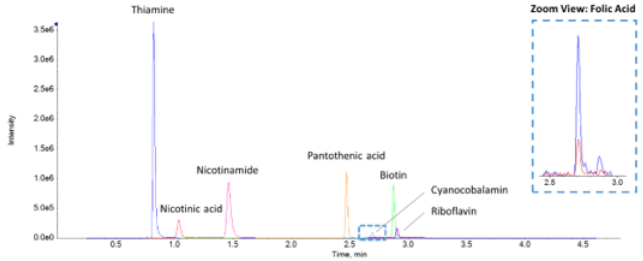


Figure 1. Chromatogram of 8 water-soluble vitamins in serum matrix using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

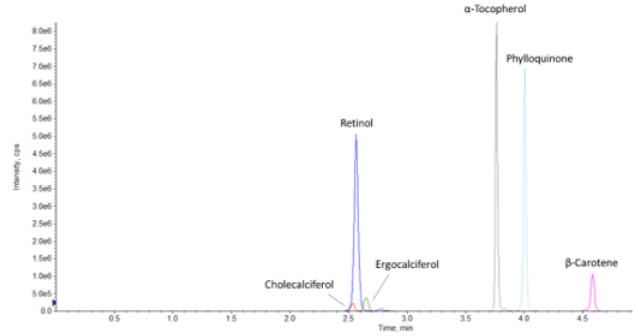
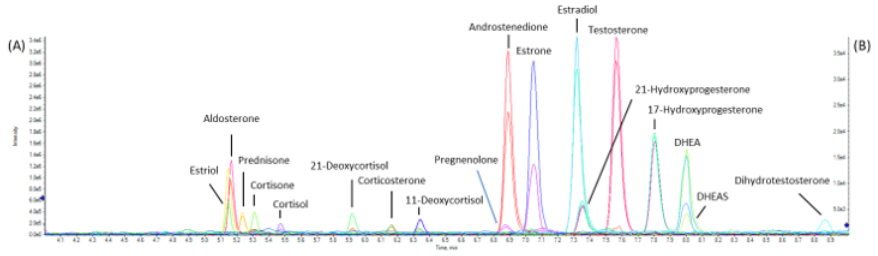


Figure 2. Chromatogram of 6 fat-soluble vitamins in serum matrix using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.



Compound	Range (ng/mL)	%CV (at LLOQ)	S/N
Thiamine	0.25-100	0.4%	207
Riboflavin	0.41-100	1.6%	87
Nicotinamide	0.07-102	2.7%	69
Nicotinic acid	0.1-100	11.3%	28
Pantothenic acid	0.5-1000	0.4%	118
Biotin	0.05-100	6.2%	9
Folic acid	10-1000	5.0%	11
Cyanocobalamin	0.2-100	1.7%	16
Retinol	10-2000	2.0%	15
β-Carotene	12-1200	2.7%	34
Cholecalciferol	1-100	2.3%	55
Ergocalciferol	4-100	3.1%	260
α-Tocopherol	200-20000	1.0%	1040
Phylloquinone	0.064-20	1.3%	41
Aldosterone	1-10,000	8.7%	44*
Estradiol	1-10,000	9.2%	31*
Estriol	1-10,000	18.4%	143
Estrone	1-10,000	4.6%	394
Androstenedione	1-10,000	8.7%	14*
Corticosterone	1-10,000	10.8%	6
Cortisol	1-10,000	13.5%	11
Cortisone	1-10,000	5.6%	30*
11-Deoxycortisol	1-10,000	4.0%	30*
21-Dexoycortisol	1-10,000	3.2%	4
DHEA	1-10,000	19.4%	15
17-Hydroxyprogesterone	1-10,000	12.0%	45*
21-Hydroxyprogesterone	1-10,000	5.4%	12*
Prednisone	1-10,000	9.0%	26
Testosterone	1-10,000	3.7%	22

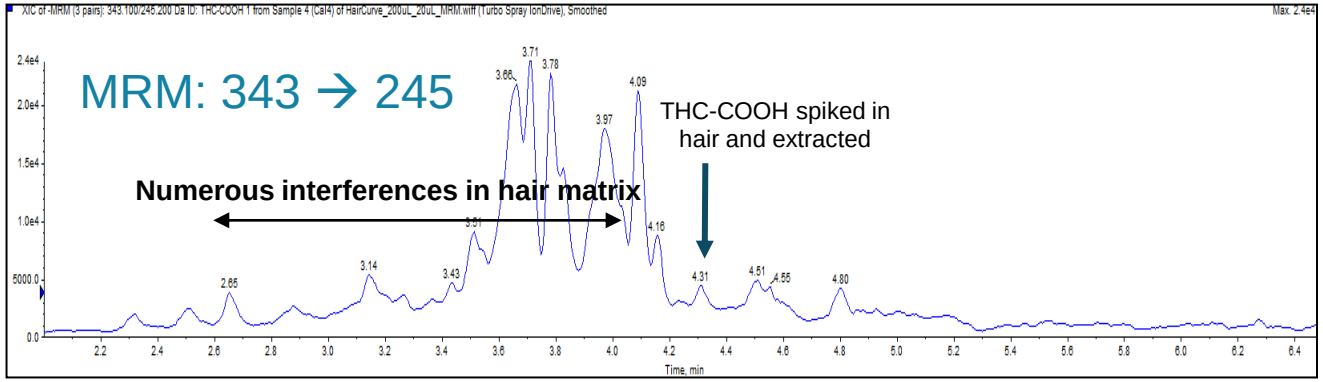
RUO-MKT-11-11650-C

QTRAP® MS/MS – Two Instruments in One

Improve your Limit of Detection using MRM^3 Scanning

In many cases isobaric interferences can limit the ability to accurately measure low levels of target analytes.

In these cases, the ability to use second-order fragmentation (MS/MS/MS) – using the QTRAP system – provides highly specific measurements, and can remove chromatographic interferences caused by isomers and background ions.



For the measurement of THC-COOH in hair samples, the sample matrix produces numerous interferences that cannot be resolved using traditional MRM (top pane).

QTRAP® MS/MS – Two Instruments in One

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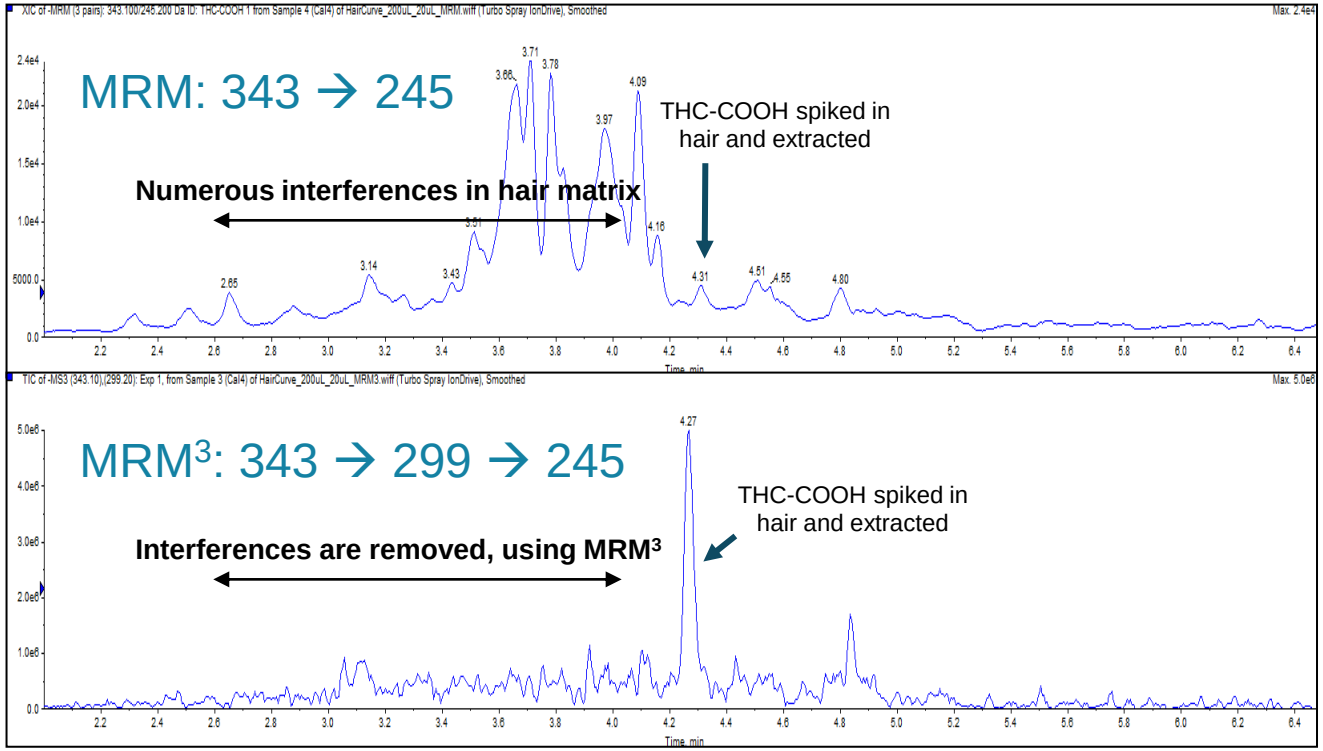
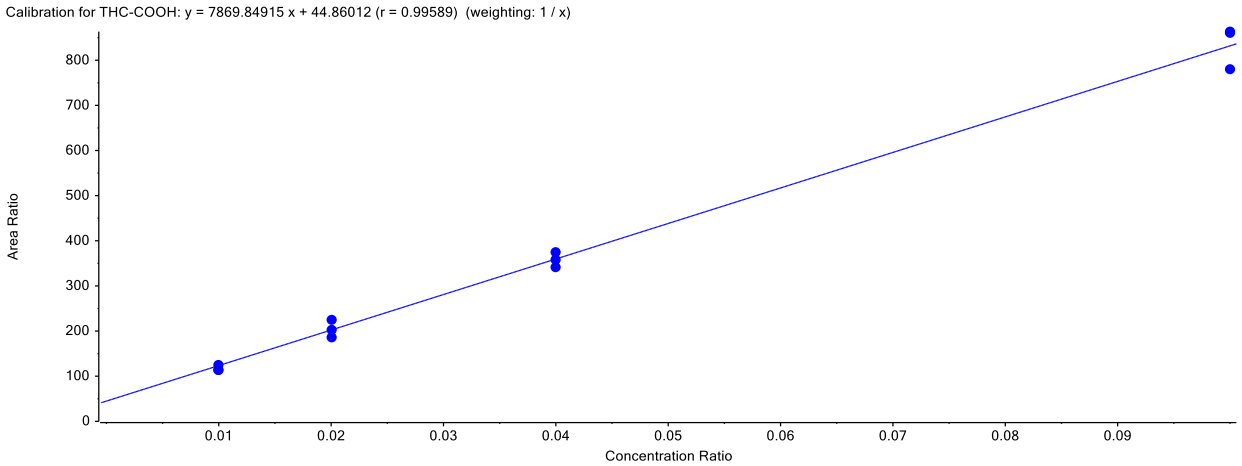
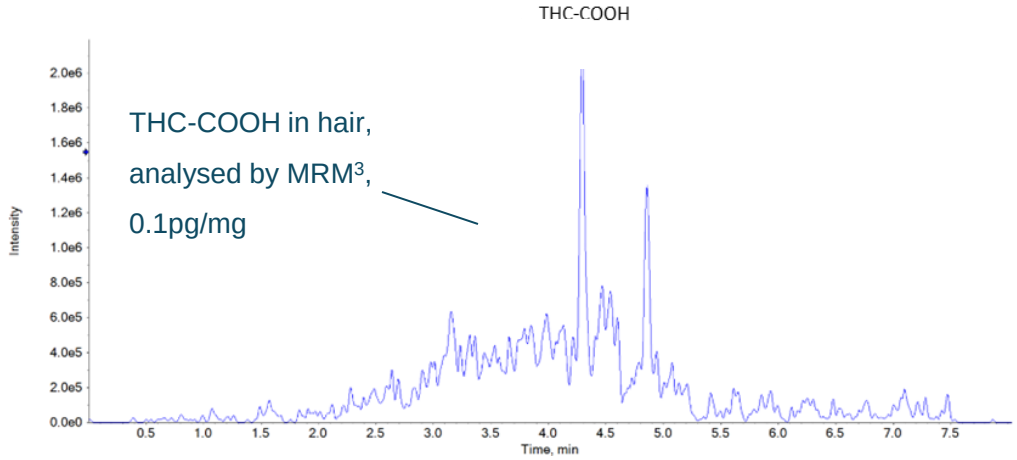


For the measurement of THC-COOH in hair samples, the sample matrix produces numerous interferences that cannot be resolved using traditional MRM (top pane). Using MRM^3 (bottom pane), the interferences are removed and the THC-COOH metabolite is clearly differentiated from background ions.

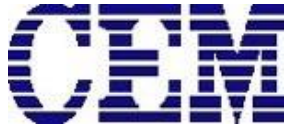
Analytical Performance Specification Sheets

THC METABOLITES IN HAIR (QUANTITATION WITH MRM^3)





Compound	Range (units)	%CV (at LLOQ)	S/N
THC-COOH	0.1-1 pg/mg	8.45%	26



All you need is ...

AMEDIS 30let s Vámi...