QTRAP® Functionality: Is it useful to toxicology labs?

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Overview

• QTRAP® technology
  – Hardware overview and scan functions
  – QTRAP® vs. triple quadrupole

• Increasing confidence in compound identification
  – Unique QTRAP® MRM-EPI with library searching

• Increasing selectivity for quantitation
  – Unique QTRAP® MRM³ to remove interferences

• More information to identify unknown compounds
  – Combining the power of high sensitivity and fast MS, MS/MS, MS³ and enhanced resolution scanning
Why talking about QTRAP® Technology Almost 10 Years After First Launch?

• Technical improvements in hardware and software throughout the years, i.e. *Scheduled MRM™* algorithm, Linear Accelerator™ trap technology...

• Leading forensic laboratories increasingly employ QTRAP® based methods
  – Shift from pure target quantitation to multi-target screening and non-target screening
  – Availability of MS/MS libraries, increasing number of compounds in libraries, improving spectral quality, optimized library search algorithm
  – Need for increased selectivity for quantitation
Technology Overview
QTRAP® Technology
Hybrid Triple Quadrupole Linear Ion Trap (LIT)

Turbo V™ source
Curtain Gas™ interface

Q0 Q1 LINAC® collision cell Q2 Q3

Ion production
Ion filtering
Ion filtering

Ion transport
Fragmentation
Ion detection

Trapping / Scanning
QTRAP® Scan Functions
QTRAP® Features

• A QTRAP® system is two different mass spectrometers under one hood.
  – All triple quadrupole scan functions (i.e. MRM, Scheduled MRM™ algorithm, precursor scan, neutral loss scan)
  – No compromise in triple quadrupole performance, no loss in sensitivity and no loss in selectivity

• A QTRAP® system offers additional features.
  – Fast and sensitive MS scan
  – Fast and sensitive MS/MS scan
  – Fast and sensitive MS/MS/MS scan
  – Highly selective MRM³
  – Enhanced Resolution scan for high selectivity quantitation
  – All scan types can be combined in Information Dependent Acquisition (IDA) methods to maximize information per sample
Multiple Reaction Monitoring (MRM)
Multiple Reaction Monitoring (MRM)

- Highest selectivity and sensitivity for quantitation
  - Mass filtering in both Q1 and Q3: *fast, sensitive and selective*
  - LINAC® collision cell (Linear Accelerator): *shortest dwell times per MRM transition without cross talk*
  - Scheduled MRM™ algorithm: *maximum number of MRM transitions and optimal accuracy and reproducibility*
Identical MRM Performance (Signal and S/N) using Triple Quad and QTRAP® Systems
Enhanced Product Ion (EPI) Scan
Trapping and Scanning in a Linear Ion Trap

(1) Trapping

(2) Scanning
Enhanced Product Ion Scan (EPI)

- Fast and sensitive MS/MS scan
  - Precursor ion filtering in Q1
  - Fragmentation in the LINAC® collision cell
  - Trapping in Q3 (fixed or dynamic fill time)
  - Scanning of fragment ions at 4000 Da/s and up to 20,000 Da/s (QTRAP® 5500 systems)
Linear Accelerator™ Trap Technology

- **Addition of axial fields within the linear ion trap**
  - Move ions towards the extraction region just before the mass scan
  - Increased ion trap sensitivity
  - Faster scan speeds
  - Overall reduction of cycle time

- **Pulsed gas supply at the end of the linear ion trap**
  - More efficient in-trap fragmentation
  - Reduced cooling time
  - Shorter MRM³ and MS³ cycle time
  - More Efficient In-Trap Fragmentation
Example MS/MS QTRAP® vs. QqQ
Higher Sensitivity Maintaining the Characteristic Fragmentation Pattern

QTRAP® system - EPI
970000cps

Triple Quadrupole - PI
4010cps
Information Dependent Acquisition (IDA)

MRM $\rightarrow$ EPI
Information Dependent Acquisition (IDA)
Multi-Target Screening and Quantitation with MS/MS Identification

Scheduled MRM™ survey

EPI

Threshold

Library Search

intensity threshold

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Advantage of QTRAP® Technology for Multi-Target Screening with Identification

- **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
MRM-EPI to Quantify and Identify
Molecular Fingerprint for Highest Confidence in Compound Identification

MRM to quantify

EPI to confirm by library search
Example 1: Detection/identification

MS/MS Library Search at Low Levels

Good Library Match Although only 3400 cps in MRM Mode

Retention Time: 2.56 minutes
Q1/Q3: 267.05/241.000 0 e
Fit (%): 100.0  FRT (%): 83.2

Acquired Spectrum

Collision Energy = 35 ± 15 eV

Library Spectrum

MRM of Oxazepam 287.1/241.0

(Detection)

Acquired spectrum

(Identification)

Library spectrum of Oxazepam

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Peak Area</th>
<th>Purity (%)</th>
<th>Visual Check?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Oxazepam</td>
<td>1.40e+004</td>
<td>83.2</td>
<td></td>
</tr>
</tbody>
</table>
Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP® LC-MS/MS system and library searching

S. Dresen • N. Ferreirós • H. Gnann • R. Zimmermann • W. Weinmann

The EPI scans were performed at a scan range of 50 to 700 amu after a fixed fill time of 50 ms with a scan rate of 4,000 amu/s applying a CES of 35±15 eV. Q0 trapping was activated to accumulate ions in Q0 while concurrently scanning ions from the linear ion trap and the pause time after the EPI scans was set to 5 ms. The declustering potential, entrance potential, gas values, and source temperature were the same as used for the MRM mode. Analyst® version 1.5 and Cliquid® 2.0 (both Applied Biosystems/MDS Sciex) were used to operate the LC-MS/MS system.

The LC-MS/MS system consisted of a 3200 Q TRAP® triple-quadrupole linear ion trap mass spectrometer fitted with a TurboIonSpray interface (Applied Biosystems/MDS Sciex) in a survey scan and an IDA-triggered dependent scan. As a survey scan an MRM method with 700 transitions in positive ionization mode for 700 analytes was established by using our MS/MS library and the MRM catalogue of Cliquid® 2.0 to automatically select the precursor mass, the most intensive product ion, and its corresponding collision energy. The MRM transitions were only analyzed at a time window of ±60 s and the total cycle time of the MRM mode was 2.1 s including the pause time between the MRM transitions of 2 ms. The compounds with corresponding MRM transitions and retention times are shown in Table S1.
QTRAP® Acquisition and Data Processing Workflow with MasterView™

• Screening and identification followed by quantitation and confirmation
  1. MRM-EPI: MasterView™ (single MRM only?)
  2. Scheduled MRM™ Pro: MultiQuant™

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Method Verification: 40 Drugs at 10 ng/mL

MS/MS spectra collected for all drugs with corresponding matching library spectra
Unknown – Screening and Identification

Alprazolam positive identification; RT and Library Hit
Quick Single Point Calibration Quantification - 72ng/mL

Unknown 1

Control - standard 10 ng/mL

Methadone acquired MS/MS spectrum

Methadone Library MS/MS spectrum
Enhanced MS (EMS) Scan
Advantage of QTRAP® Technology for Unknown Screening and Identification

Triple Quad
- MRM Quantitation
- ID with MRM ratio
- Low sensitivity and slow MS and MS/MS

QTRAP® system
- MRM Quantitation
- ID with MRM ratio
- High sensitivity and fast MS, MS/MS, MS/MS/MS, and ER

information to detect and identify compounds
Enhanced MS Scan (EMS)

- Fast and sensitive MS scan
  - No Precursor ion filtering in Q1
  - No Fragmentation in the LINAC® collision cell
  - Trapping in Q3 (fixed or dynamic fill time)
  - Scanning of fragment ions at 4000 Da/s and up to 20,000 Da/s (QTRAP® 5500 systems)
Information Dependent Acquisition (IDA)
Non-Target Screening MS/MS Identification
General Unknown Screening on QTRAP®

Example: EMS-EPI

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Peak Area (counts)</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextromethorphan</td>
<td>1.37e+008</td>
<td>85.6</td>
</tr>
</tbody>
</table>

Acquired spectrum (Identification)

Library spectrum of Dextromethorphan

XIC of EMS peak (272.2-272.8) (Detection)
MRM³ Quantitation
Advantage of with QTRAP® Technology for Quantitation and Identification

Triple Quad

- MRM Quantitation
- ID with MRM ratio

QTRAP® system

- MRM Quantitation
- ID with MRM ratio
- MRM³ Quantitation

increasing selectivity for quantitation
**MRM³**

- Precursor selection in Q1.
- Collisional activation in the Q2 Qurved LINAC collision cell
- Trapping of first-generation fragment ions in the Linear Accelerator Trap
- Isolation of 2nd precursor in the LIT by RF/DC isolation.
- Collisional activation of 2nd precursor by Single Frequency Excitation
- 2nd generation fragments are scanned out of the Linear Accelerator Trap
- During steps 3-6, ions for the next cycle are accumulated in Q0 for enhanced sensitivity (Q0 Trapping).
Example 1: THC-COOH in hair

MRM vs. MRM$^3$ – THC-COOH in Human Hair
100 pg/mL Solvent vs. Matrix

XIC of -MRM (3 pairs): 343.1/299.2 Da from...
Max. 6706.3 cps.

MRM
343.1/299.2
100 pg/mL in solvent

S/N = 37.8
Peak Int.(Subt.)=6.3e+3
3xStd.Dev.(Noise)=1.7e+2

TIC of -MS3 (343.10),(299.20): from Sample...
Max. 3.4e6 cps.

MRM$^3$
343.1/299.2/245.1
100 pg/mL in solvent

S/N = 59.7
Peak Int.(Subt.)=3.4e+6
3xStd.Dev.(Noise)=5.7e+4

XIC of -MRM (3 pairs): 343.1/299.2 Da from...
Max. 2.6e4 cps.

MRM
343.1/299.2
100 pg/mL in matrix

S/N = 26.1
Peak Int.(Subt.)=2.6e+6
3xStd.Dev.(Noise)=9.9e+4

TIC of -MS3 (343.10),(299.20): from Sample...
Max. 2.6e6 cps.

MRM$^3$
343.1/299.2/245.1
100 pg/mL in matrix

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Example 2: THC-COOH in oral fluid

Top: 10 pg/mL; Bottom: 30 pg/mL

MRM

MRM³

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