Introducing the Agilent G6410A Triple Quadrupole Mass Spectrometer

Michael Zumwalt
Overview of QQQ Hardware

QQQ Mechanical Layout

Common w/ QTOF

Leveraged w/ Q & QTOF
MRM (Multiple Reaction Monitoring)

Spectrum with background ions (from ESI)

Quad Mass Filter (Q1)
- Target ion 210 passes through Q1
- Collision cell breaks ion 210 apart

Quad Mass Filter (Q3)
- Monitors only characteristic fragments 158 and 191 from ion 210 for quant and qual.

No chemical background
Autotune and Calibration System
Built-in calibrant delivery system is unique to Agilent

Built-in calibrant delivery system used for fast auto-calibration

Calibrant solutions
Calibrant Delivery System

Calibrant delivered to MS via the API Interface

Valve can also be utilized to divert flow from the HPLC via software
The analyzer story - minimizing noise

1. Agilent’s outstanding Patented orthogonal spray sources maximize ionization while minimizing solvent and matrix noise.
2. Counter-Current Drying gas **Cold Dielectric capillary**...enhances desolvation while minimizing chemical noise.
3. RF Octopole guide......High efficiency ion capture while optimizing wide mass bandwidth ion transmission.
4. L2 RF.......greatly enhances high mass ion transmission. Patented!
5. Quad 1....hyperbolic quads optimize ion transmission and spectral resolution.
6. RF quadrupole segment.....enhances ion transmission into and out of the collision cell.
7. Collision cell.....high pressure collision cell with linear acceleration optimizes MS-MS fragmentation while eliminating cross-talk even at very low dwell times. Small diameter high frequency hexapole assembly assists with capturing and focusing fragmented ions.
8. Quad 2 ....hyperbolic quads optimize ion transmission and spectral resolution.
10. Multiplier has extremely long life since ions never touch the surface, only electrons.
Liquid enters the grounded nebulizer

A charged aerosol is made in the ESI Zone

The aerosol is dried by IR lamps

Neutral analytes and ESI charged analytes pass through the APCI Zone

ESI and APCI ions enter the capillary
## Agilent Multimode Source - Compound Detection

<table>
<thead>
<tr>
<th>Compound Ion Polarity</th>
<th>Dedicated APCI (+)</th>
<th>Dedicated APCI (-)</th>
<th>Dedicated ESI (+)</th>
<th>Dedicated ESI (-)</th>
<th>Simultaneous (+)</th>
<th>Simultaneous (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetazolamide</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
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<tr>
<td>Butyl 4-aminobenzoate</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
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<tr>
<td>Cortisone</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Gemfibrozil</td>
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<td>0</td>
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<tr>
<td>Hexahydro-...dione</td>
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<td>Morin</td>
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<td>Paclitaxel</td>
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<td>Phenylbutazone</td>
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<tr>
<td>Progesterone</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Sulfamethoxazole</td>
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<tr>
<td>Tolazamide</td>
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<tr>
<td>Uracil</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
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<tr>
<td><strong>Detected by Polarity</strong></td>
<td><strong>67%</strong></td>
<td><strong>56%</strong></td>
<td><strong>67%</strong></td>
<td><strong>50%</strong></td>
<td><strong>78%</strong></td>
<td><strong>61%</strong></td>
</tr>
<tr>
<td><strong>Detected by Source</strong></td>
<td><strong>89%</strong></td>
<td><strong>89%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
Environmental Compounds, Alternating Positive/Negative Mixed Mode Ionization

Positive mixed mode TIC, primarily \([M+H]^+\) and \([M+Na]^+\):

- Diquat
- Paraquat (respond in ESI only)
- Oxamyl
- Methomyl
- Fenuron
- Monuron
- Simazine
- Propoxur

Negative mixed mode TIC, primarily \([M-H]^−\) and \([M+acetate]^-\):

- 4-Nitrophenol (responds best in APCI)
- Dicamba
- 2,4-D
- Monuron
- 2,4,5-T

5 ng each analyte; 2.1 x 150 mm Zorbax XDB-C18, 3.5 µ, 60 °C, water/methanol gradient (3-90% methanol) with 1mM ammonium acetate, scan mode \(m/z\) 130-330; sample dissolved in 80:20 water/methanol containing 1% acetic acid. * denotes impurity
Broad Mass Range Transmission and Transmission Efficiency – Why a Hexapole?

Variables include:

• Number of poles (i.e. quad, hex, octo)
• Inscribed diameter \( (R_0) \)
• Drive Frequency

Evaluation included:

• Theoretical modeling
  – Calculation, Simulations
• Experimental results

Ion focusing: quad > hex > octo
Collision Cell Design and Electrical Drive Specifics

- Hexapole construction
- $2R_0 = 4.5$ mm
- Length = 150 mm
- Experimental press. range = $0.1 - 20$ mTorr
- In coming ion energy range = $0 - 250$ eV
- Accelerating potential range = $0 - 10$ V
- RF Drive voltage range $10 - 550$ V

RF Voltage = 10 - 550 V
Collision Cell Clearing Profile

Collision gas = 7mTorr  0 V collision energy  5 V Applied Axial Potential

Beam Turn-off Characteristics

Collision Cell Clearing Profile

Collision gas = 7mTorr  0 V collision energy  5 V Applied Axial Potential

Beam Turn-off Characteristics

Collision Cell Clearing Profile

Collision gas = 7mTorr  0 V collision energy  5 V Applied Axial Potential

Beam Turn-off Characteristics
Fast MRM Selection

**Transition 1**
- Q1 = 622m/z
- CE = 30V
- AAP = 7V
- Q3 = 540m/z
- 5msec settling
- 5msec sampling

**Transition 2**
- Q1 = 600m/z (no signal)
- CE = 30V
- AAP = 7V
- Q3 = 540m/z
- 5msec settling
- 5msec sampling

Sample:
Tuning Soln, Infused
Dwell Time Evaluation

200 pg Alprazolam 100, 20, 5 ms dwell times

<table>
<thead>
<tr>
<th>Dwell</th>
<th>Area</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ms</td>
<td>14860</td>
<td>0.61</td>
</tr>
<tr>
<td>20 ms</td>
<td>13605</td>
<td>0.74</td>
</tr>
<tr>
<td>5 ms</td>
<td>13202</td>
<td>2.25</td>
</tr>
</tbody>
</table>

5 ms dwell

10. Multiplier has extremely long life since ions never touch the surface, only electrons.
The Analyzer Story - Pump aspects:

Newly designed manifold with a new single 3-stage turbomolecular pump, backed by a single mechanical (rough) pump.

3-staged partitioning removes the need for two turbomolecular pumps.
Reserpine Sensitivity
Specification: 500 fg with S/N 20:1, %RSD < 5
Reserpine Dilution Series (in mobile phase)  
Demonstration of Sensitivity via a Standard  
Reserpine (609>195) EIC, acquiring 3 transitions

$r^2 = 0.987$
Alprazolam Calibration Results
1 pg – 10 ng, Positive ESI Mode

Alprazolam Linearity

\[ Y = 77.2x + 1593.4 \]
\[ R^2 = 0.9974 \]
New Agilent MassHunter Workstation Software

**Instrument Control**
- Real-time monitoring
- Method set-up
- Autotune

**Qualitative Analysis**
- Chromatographic results
- Spectral results
- Find compounds

**Quantitative Analysis**
- User filters
- Compound results
- Calibration curve
Full Integration: The Lab, Business Applications, OpenLAB, the Company Network

The Lab

- GC/MS
- GC
- LC
- CE
- UV-vis
- ICP-MS
- Lab-on-a Chip

Business Applications

- MS Office
- Legacy Data
- Images
- PDF
- ELN
- Drawings
- LIMS
- Research
- Data

Agilent OpenLAB (Laboratory Informatics)

Intranet

Internet
Data Acquisition

- Instrument status
- Data display
- Acquisition settings
- Sequence list
Auto or Manual Tune
## Triple (QQQ) Reporting (Excel) - Tune

### QQQ Tune Report

**DataPath**: C:\Agilent\QQQTuneData\Reports\AutoTune\Tune.xml

**ReportType**: AutoTune

**DateTime**: 20:10:05 17:41

**ReportDate**: 20:10:05 17:41

### Profiles

<table>
<thead>
<tr>
<th>Profile Mass 1</th>
<th>Profile Mass 2</th>
<th>Profile Mass 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass: 322.1</td>
<td>Mass: 622.5</td>
<td>Mass: 922.0</td>
</tr>
<tr>
<td>FWHM: 0</td>
<td>FWHM: 0</td>
<td>FWHM: 0</td>
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<tr>
<td>Height: 3725</td>
<td>Height: 3945</td>
<td>Height: 4603</td>
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</table>

<table>
<thead>
<tr>
<th>Profile Mass 4</th>
<th>Profile Mass 5</th>
<th>Profile Mass 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass: 652.9</td>
<td>Mass: 652.9</td>
<td>Mass: 322.1</td>
</tr>
<tr>
<td>FWHM: 0</td>
<td>FWHM: 0</td>
<td>FWHM: 0</td>
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<tr>
<td>Height: 3725</td>
<td>Height: 3725</td>
<td>Height: 3725</td>
</tr>
</tbody>
</table>

### Graphic MS1 Full Range Scan

**Mass: 322.1**

**Mass: 622.5**

**Mass: 922.0**

**Mass: 652.9**

**Mass: 652.9**

**Mass: 322.1**

**Mass: 322.1**

**Mass: 622.5**

**Mass: 922.0**

**Mass: 652.9**

**Mass: 652.9**

**Mass: 322.1**

**Mass: 322.1**

**Mass: 622.5**

**Mass: 922.0**

**Mass: 652.9**
Reporting (Excel)

- Macros in Excel Adding
- Add tables and graphics via tool buttons
- Drag and drop additional columns from XML results
- Formatting of Font and numbers in Excel
- Print Preview in Excel
### Agilent Quantitative Analysis

**Batch processing**

<table>
<thead>
<tr>
<th>Sample</th>
<th>THC(d) Method</th>
<th>THC(d) Results</th>
<th>THC(d) ([STD]) Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>OF_THC_01.d</td>
<td>Dill</td>
<td>6</td>
<td>5.032</td>
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<td>OF_THC_02.d</td>
<td>Dill</td>
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<td>9.430</td>
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</table>

**Calibration Curve**

- **Response** = 24.8555
- **Response** = 22.655
- **Response** = 20.15
- **Response** = 17.55
- **Response** = 15.05
- **Response** = 12.55
- **Response** = 10.05
- **Response** = 7.55
- **Response** = 5.05
- **Response** = 2.55

**Concentration (ppm)**

- **C** = 0.032 * 10
- **C** = 0.1991
- **C** = 1.991
- **C** = 19.91
- **C** = 199.1
- **C** = 1991
- **C** = 19910
- **C** = 199100
- **C** = 1991000
- **C** = 19910000

**Acquisition Time (Min)**

- **Acquisition Time** (Min) = 3.0
- **Acquisition Time** (Min) = 4.0
- **Acquisition Time** (Min) = 5.0
- **Acquisition Time** (Min) = 6.0
- **Acquisition Time** (Min) = 7.0
- **Acquisition Time** (Min) = 8.0
- **Acquisition Time** (Min) = 9.0
- **Acquisition Time** (Min) = 10.0
- **Acquisition Time** (Min) = 11.0
- **Acquisition Time** (Min) = 12.0

**Response Ratio**

- **Response Ratio** = 24.8555
- **Response Ratio** = 22.655
- **Response Ratio** = 20.15
- **Response Ratio** = 17.55
- **Response Ratio** = 15.05
- **Response Ratio** = 12.55
- **Response Ratio** = 10.05
- **Response Ratio** = 7.55
- **Response Ratio** = 5.05
- **Response Ratio** = 2.55

**Relative Response**

- **Relative Response** = 24.8555
- **Relative Response** = 22.655
- **Relative Response** = 20.15
- **Relative Response** = 17.55
- **Relative Response** = 15.05
- **Relative Response** = 12.55
- **Relative Response** = 10.05
- **Relative Response** = 7.55
- **Relative Response** = 5.05
- **Relative Response** = 2.55
Quantitative View - Method Editor
Parameter-Free Integrator – Optimal for QQQ data

- All peak list level and peak level info available
- Visualization of baseline regression
- Handling of merged peaks
- Peak validation – Quality Factor and ‘Traffic Light Coloring’
New “Parameterless” Integrator

- Handles low level spiky data (statistically sets peak start and end)
- Auto adjustment of threshold
  - Eliminates need for manual re-integration of peaks for low-level MRM signals
THC in QuantAnalysis

THC retention time < 4.5 min.

Overlap of quant and qual ion areas

D0 quant ion

D3 quant ion

THC Qualifier ion normalized

THC Qualifier ion un-normalized
Triple (QQQ) CurveFit Assistant
Calibration curve data point information

- Calibration Curve:
  - Type: Linear
  - Origin: Ignore
  - Weight: None
  - THCD0 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 2 QCs
  - Equation: \( y = 0.0250 \times x - 0.0073 \)
  - \( R^2 = 0.99838706 \)

- Level 5:
  - Type: Calibration
  - Conc.: 50
  - Relative Response: 1.193
  - Percent Residual: -4.2167
  - Call Path: OF_THC_22.d OF_THC_22.d

- Warning: 4 overlapping points found. Please zoom in to select an individual point.
  - Level: 2, low

- Agilent Technologies
  - WW Seminar Tour
  - Spring 2006
Calibration curve data point zooming

QC (blue triangle) at 2 ng/mL

QC (blue triangle) at 5 ng/mL
Analytical Conditions – MS

MRM transitions

Assay #2, Cocaine, etc
Coc, quant = 304.1 > 182.0
Coc, qual = 304.1 > 82.0
D3 - Coc (ISTD), quant = 307.1 > 185.1
D3 - Coc (ISTD), qual = 307.1 > 85.1
MDMA, quant = 194.1 > 163.0
MDMA, qual = 194.1 > 135.0
D5 - MDMA (ISTD), quant = 199.1 > 165.0
D5 - MDMA (ISTD), qual = 199.1 > 135.0
Meth, quant = 150.1 > 91.0
Meth, qual = 150.1 > 119.0
D5 - Meth (ISTD), quant = 155.1 > 92.0
D5 - Meth (ISTD), qual = 155.1 > 121.0
Amp, quant = 136.1 > 93.0
Amp, qual = 136.1 > 119.0
D5 - Amp (ISTD), quant = 141.1 > 93.0
D5 - Amp (ISTD), qual = 141.1 > 124.0

16 MRM Total!
Using Agilent Qualitative Analysis for Coc, Meth, Amp, & MDMA

Qualitative Analysis Method Editor

EICs for all 16 MRMs defined and specified for peak integration
Using Agilent Qualitative Analysis for Coc, Meth, Amp, & MDMA

All 16 MRM transition EICs generated and integrated automatically

All peaks elute < 1.5 min!
G6410A Agilent QQQ

• Outstanding sensitivity for its price class
  – 0.5 pg RESERPINE on column at 20:1 S/N and 5% RSD with 3 simultaneous MRMs running concurrently – utilizing Autotune parameters for mass analyzer calibration.

• 1635 amu mass range (m/z 15 – 1650)
• Powerful and reliable AutoTune analyzer calibration software
• New, Powerful and flexible Quantitation Software
• New “Parameterless” integrator
• Intelligent Qualitative Data Analysis Software
• Flexible reporting based on Excel
• Single point LC and MS instrument control
• ESI, APCI, and MultiMode sources available