Demonstrate new features and capabilities that enable confident identification and quantification of unknown impurities
• Present new GC-MS technology

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Overview

- Present new GC-MS technology
- Demonstrate new features and capabilities that enable confident identification and quantification of unknown impurities
- Demonstrate pharmaceutical intermediate impurity analysis with real-world samples analyzed in partnership with AstraZeneca
Analysis of pharmaceutical intermediate impurities

- Volatile Impurities
- Semi-Volatile Impurities
- Non-Volatile Impurities
- Elemental Impurities
Analysis of pharmaceutical intermediate impurities

Volatile Impurities

Semi-Volatile Impurities

Non-Volatile Impurities

Elemental Impurities

- GC-MS
- GC-HRMS
- EI & CI
- Library
- Substructure & Composition
Requirements for Identification of Unknowns

RT & m/z

Empirical Formulae

Structure

Substructure

Name & CAS

Amount

C_{15}H_{16}O_{2}

80-05-7

4,4'-(propane-2,2-diyl)diphenol

Bisphenol-A
AstraZeneca study objectives

“High mass accuracy is an advantage when it come to looking at identifying compounds. This increases the confidence in structural characterisation.”

- **Aim:** Analyze low molecular weight compounds, typical of starting materials
  - Analyze at relevant concentrations (1% to 0.0001% (v/v) for liquid samples)
  - Unambiguously confirm structure of intermediate and impurities through accurate mass measurement and sub-structural analysis.
  - Assess performance across concentration ranges.
Introducing Thermo Scientific™ Q Exactive™ GC

Quadrupole-Orbitrap GC-MS/MS system
Integration of 3 highly successful technologies

Thermo Scientific™ TRACE™ 1310 GC
rapid heat cycling
unique modular injector and detector design
Integration of 3 highly successful technologies

Thermo Scientific™ ExtractaBrite™ ion source technology
Routine grade robustness
Removable without breaking vacuum
Patented RF lens protects ion guide and quads
Integration of 3 highly successful technologies

Thermo Scientific™ Orbitrap™ mass analyzer technology

Incredible HR/AM performance

Proven Thermo Scientific™ Q Exactive™ platform
Chemical ionization for molecular ion

Remove entire ion source or change to CI source in under 2 minutes without venting...
• Thermo Scientific™ Mass Frontier™ software can be used for sub-structural interpretation
• Sub ppm accurate mass allows for higher confidence in fragment IDs
Breakthrough in GC-MS capability

The Power of Q Exactive GC

Resolution
- Up to 120,000 at m/z 200
- Highest available
- Maximum selectivity
- Fast enough for GC!

Mass Accuracy
- < 1ppm
- Every scan
- All concentrations
- In complex matrix
- Across the mass range from 30 – 3000 m/z
- Everyday!

Sensitivity
- ppt
- In full scan
- High selectivity
- High spectral fidelity

Dynamic Range
- >6 orders
- Excellent coverage in sample profiling
- “Triple quad grade” quantitation in full scan
Scan speed and accurate mass error across a peak

PPM Error Measured Mass

R > 60,000
Peak width = 3s
Scans = 18

Ethyl octanoate m/z 129.0910
Unprecedented quantitation power for HR/AM GC-MS

PPT Sensitivity

Huge linear range

- Low fg on-column detection limits (~best triple quads)
- > 6 Orders linear range*
- Excellent precision
- Highest selectivity
- “triple quad grade” quantitation

*application specific

Detection limit = 2 fg
Breakthrough in GC-MS Performance

Unprecedented quantitation power for HR/AM GC-MS

OFN 10 fg - 10 ng

\[ y = 295.06x^{0.9345} \]

\[ R^2 = 0.9999 \]
Breakthrough in GC-MS Performance

Unprecedented quantitation power for HR/AM GC-MS

>6 Orders Linearity

OFN 10 fg - 10 ng

\[ y = 295.06x^{0.9345} \]
\[ R^2 = 0.9999 \]

Low residual error

OFN 10 fg to 10 ng, m/z 50-300, 10.4% RSD
Breakthrough in GC-MS Performance

Unprecedented quantitation power for HR/AM GC-MS

>6 Orders Linearity

Low residual error

Always low mass error

OFN 10 fg to 10 ng, m/z 50-300, 10.4% RSD

y = 295.06x^{0.9545}

R^2 = 0.9999

Blank Subtracted Response Factor

Amount Injected (fg)

Mass Error (ppm)

Amount Injected (fg)
Breakthrough in GC-MS Performance

To get data this good, it’s really fast and simple...

Easy set-up

- Familiar Q Exactive environment
- Simple status
- Automated leak checking
- Automated tuning & calibration
- Source and lens tuning
- Mass calibration
- Ready to go < 5 minutes
Results
“Impurity detection is important within the pharmaceutical industry. **High mass accuracy is an advantage** when it come to looking at unknown sample impurities.”
• Aim: demonstrate mass accuracy across a range of test compounds:

  • “All five compounds were identified by their elemental formula.”

  • “Each was identified with a mass accuracy of less than 0.9 ppm”

<table>
<thead>
<tr>
<th>Compound Analysed</th>
<th>Mass Accuracy ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Compound 1]</td>
<td>0.6</td>
</tr>
<tr>
<td>![Compound 2]</td>
<td>0.0</td>
</tr>
<tr>
<td>![Compound 3]</td>
<td>0.9</td>
</tr>
<tr>
<td>![Compound 4]</td>
<td>0.7</td>
</tr>
<tr>
<td>![Compound 5]</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Scan Speed

“For quantitative analysis in GC/MS, a minimum of 10 scans is needed for an idealized peak shape.”

• 4-fluorobenzonitrile (2.5 µg/mL (w/v) in methanol) was analysed in CI mode.

• The main component peak has a peak width of 3.6 seconds.
Scan speeds & data points
Scan speeds & data points

$R \geq 120,000$

11 scans/peak
Scan speeds & data points

R≥ 60,000  16 scans/peak

R≥ 120,000  11 scans/peak
Scan speeds & data points

R≥ 30,000  
25 scans/peak

R≥ 60,000  
16 scans/peak

R≥ 120,000  
11 scans/peak
Scan speeds & data points

- $R \geq 15,000$: 33 scans/peak
- $R \geq 30,000$: 25 scans/peak
- $R \geq 60,000$: 16 scans/peak
- $R \geq 120,000$: 11 scans/peak
Scan speeds & data points

- B ≥ 15,000
  - 33 scans/peak
- B ≥ 30,000
  - 25 scans/peak
- B ≥ 60,000
  - 16 scans/peak
- B ≥ 120,000
  - 11 scans/peak

- Acquisition rate more than sufficient for narrow GC peaks
- Quantitative capabilities even at exceptionally high B ≥ 120,000
Mass Accuracy Across the Peak

RT: 4.45 - 4.52  SM: 3B

R = 91800

NL: 4.66E10
TIC MS AZ15june00 1

Mass Accuracy Across the Peak

NL: 4.66E10
TIC MS AZ15june00 1

Mass Accuracy Across the Peak

NL: 4.66E10
TIC MS AZ15june00 1

Mass Accuracy Across the Peak

NL: 4.66E10
TIC MS AZ15june00 1

Mass Accuracy Across the Peak

NL: 4.66E10
TIC MS AZ15june00 1
Mass Accuracy Across the Peak

- High mass accuracy on EVERY scan (<1ppm)
- 16 data points even on very narrow peak @ R ≥ 90,000
Linearity

- Aim: show linearity across relevant concentration range

- Linearity of **N,N,N’-trimethylethylenediamine** across a concentration range of 0.0001 to 0.1250 % (v/v in DMSO).

- Analysed at a resolution of **60,000 RP** (at m/z 200) using EI

- Extracted ion chromatogram of m/z 102.11515 (mass window 2 ± ppm) and corresponding peak area were used in calibration curve.
### Linearity Results

#### Compound Results

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Expected RT</th>
<th>RT</th>
<th>Level</th>
<th>Height</th>
<th>Area</th>
<th>m/z (Expected)</th>
<th>m/z (Apex)</th>
<th>m/z (Delta(ppm))</th>
<th>ISTD Amt</th>
<th>ISTD Response</th>
<th>IR</th>
<th>Fragment 1</th>
<th>Fragment 1 (mass delta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>diamine</td>
<td>3.19</td>
<td>3.14</td>
<td>0.125</td>
<td>1223778</td>
<td>3859374</td>
<td>3.14</td>
<td>102.11515</td>
<td>102.11530</td>
<td>1.42398</td>
<td>N/A</td>
<td>N/A</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>AZ16June_019</td>
<td>0.06</td>
<td>620650</td>
<td>1801138</td>
<td>3.14</td>
<td>102.11515</td>
<td>102.11528</td>
<td>1.27435</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>AZ16June_018</td>
<td>0.03</td>
<td>338565</td>
<td>945272</td>
<td>3.15</td>
<td>102.11515</td>
<td>102.11530</td>
<td>1.42398</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>AZ16June_017</td>
<td>0.015</td>
<td>149369</td>
<td>438038</td>
<td>3.14</td>
<td>102.11515</td>
<td>102.11522</td>
<td>0.47685</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>AZ16June_015</td>
<td>0.0001</td>
<td>3243</td>
<td>18670</td>
<td>3.19</td>
<td>102.11515</td>
<td>102.11526</td>
<td>1.05041</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>AZ16June_014</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/F</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
</tbody>
</table>

#### Calibration Curve

![Calibration Curve Image]

<table>
<thead>
<tr>
<th>Concentration % (v/v)</th>
<th>Theoretical mass</th>
<th>Measured mass</th>
<th>Mass difference [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>102.11515</td>
<td>102.11527</td>
<td>1.1</td>
</tr>
<tr>
<td>0.007</td>
<td>102.11515</td>
<td>102.11525</td>
<td>0.9</td>
</tr>
<tr>
<td>0.015</td>
<td>102.11515</td>
<td>102.11525</td>
<td>0.9</td>
</tr>
<tr>
<td>0.03</td>
<td>102.11515</td>
<td>102.11526</td>
<td>1.0</td>
</tr>
<tr>
<td>0.06</td>
<td>102.11515</td>
<td>102.11525</td>
<td>0.9</td>
</tr>
<tr>
<td>0.125</td>
<td>102.11515</td>
<td>102.11524</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Linearity Results

- Linearity demonstrated > 4 orders of magnitude
- Mass accuracy not affected by concentration
• Aim: analyse *(3S)-3-methylmorpholine* and to identify the impurities within the sample.

API Intermediate  

Impurities
<table>
<thead>
<tr>
<th>Retention (min)</th>
<th>Compound ID</th>
<th>Exact mass (EI)</th>
<th>Measured mass (EI)</th>
<th>Δppm</th>
<th>Exact mass (PCI)</th>
<th>Measured mass (PCI)</th>
<th>Δppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.55</td>
<td><img src="image1.png" alt="Image" /> (3S)-3-methylmorpholine Molecular Formula = C₅H₁₀NO</td>
<td>101.08352</td>
<td>101.08358</td>
<td>0.6</td>
<td>102.09134</td>
<td>102.09136</td>
<td>0.2</td>
</tr>
<tr>
<td>3.75</td>
<td><img src="image2.png" alt="Image" /> Molecular Formula = C₅H₁₀NO</td>
<td>115.09917</td>
<td>115.09925</td>
<td>0.7</td>
<td>116.10699</td>
<td>116.10703</td>
<td>0.4</td>
</tr>
<tr>
<td>4.32</td>
<td><img src="image3.png" alt="Image" /> Molecular Formula = C₅H₁₀NO</td>
<td>129.11482</td>
<td>129.11486</td>
<td>0.4</td>
<td>130.12264</td>
<td>130.12268</td>
<td>0.3</td>
</tr>
<tr>
<td>5.06</td>
<td><img src="image4.png" alt="Image" /> Molecular Formula = C₅H₁₀NO</td>
<td>155.13047</td>
<td>155.13048</td>
<td>0.1</td>
<td>156.13829</td>
<td>156.13825</td>
<td>0.3</td>
</tr>
<tr>
<td>5.87</td>
<td><img src="image5.png" alt="Image" /> Molecular Formula = C₆H₁₂NO₂</td>
<td>129.08624</td>
<td>129.08634</td>
<td>0.0</td>
<td>130.08626</td>
<td>130.08634</td>
<td>0.6</td>
</tr>
<tr>
<td>6.23</td>
<td><img src="image6.png" alt="Image" /> Molecular Formula = C₆H₁₂NO₂</td>
<td>143.09010</td>
<td>143.09141</td>
<td>0.4</td>
<td>144.10193</td>
<td>144.10193</td>
<td>0.2</td>
</tr>
<tr>
<td>Retention (min)</td>
<td>Compound ID</td>
<td>Exact mass (EI)</td>
<td>Measured mass (EI)</td>
<td>Δppm</td>
<td>Exact mass (PCI)</td>
<td>Measured mass (PCI)</td>
<td>Δppm</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>----------------</td>
<td>--------------------</td>
<td>------</td>
<td>----------------</td>
<td>---------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.55</td>
<td><img src="" alt="3S)-3-methylmorpholine" />-3-methylmorpholine</td>
<td>101.08352</td>
<td>101.08358</td>
<td>0.6</td>
<td>102.09134</td>
<td>102.09136</td>
<td>0.2</td>
</tr>
</tbody>
</table>

| 3.75 | ![3S)-3-methylmorpholine](attachment:3S)-3-methylmorpholine | 115.09917       | 115.09925          | 0.7  | 116.10699       | 116.10703           | 0.4  |

- **Change from EI to CI in minutes**
- **Mass Accuracy maintained in CI – all <1ppm**
- **“Delivers unambiguous identification”**

| 4.32 | ![3S)-3-methylmorpholine](attachment:3S)-3-methylmorpholine | 130.12264       | 130.12268          | 0.3  | 156.13829       | 156.13825           | 0.3  |

| 5.06 | ![3S)-3-methylmorpholine](attachment:3S)-3-methylmorpholine | 156.13047       | 156.13048          | 0.1  | 156.13829       | 156.13825           | 0.3  |

| 5.87 | ![3S)-3-methylmorpholine](attachment:3S)-3-methylmorpholine | 129.07843       | 129.07843          | 0.0  | 130.08626       | 130.08634           | 0.6  |

| 6.23 | ![3S)-3-methylmorpholine](attachment:3S)-3-methylmorpholine | 143.09408       | 143.09414          | 0.4  | 144.10193       | 144.10193           | 0.2  |
PCI-MS/MS allows full unambiguous structural elucidation

- MS/MS can be performed on any ion
- Full sub-structural composition can be performed
- Thermo Scientific™ Mass Frontier™ software can be used for comprehensive sub structural peak identification
More confidence in search

- Spectra can be identified through proprietary **nominal mass** library search:
  - NIST
  - WILEY
  - Pfleger / Maurer / Weber
  - Existing in-house libraries
More confidence in search

- Spectra can be identified through proprietary **nominal mass** library search:
  - NIST
  - WILEY
  - Pfleger / Maurer / Weber
  - Existing in-house libraries

- Or **high resolution accurate mass** MS/MS libraries
  - Thermo Scientific™ High-Resolution Accurate-Mass MS/MS Spectral Libraries
  - mzCloud.org freely available to search online
mzCloud™

- Free
- Advanced high resolution mass spectral database
- Search spectrum, name, structure, substructure, and m/z
- Identify compounds even when they are not present in the library through substructure search

www.mzcloud.org
AstraZeneca Conclusions
The Q Exactive GC MS has been evaluated for both qualitative and quantitative analysis in support of pharmaceutical uses.
AstraZeneca Conclusions

The Q Exactive GC MS has been evaluated for both qualitative and quantitative analysis in support of pharmaceutical uses.

“The accurate mass measurement across a dynamic range, linearity and the ability to identify impurities in both EI & CI have shown that this instrument is a powerful tool.”
AstraZeneca Conclusions

The Q Exactive GC MS has been evaluated for both qualitative and quantitative analysis in support of pharmaceutical uses.

“The accurate mass measurement across a dynamic range, linearity and the ability to identify impurities in both EI & CI have shown that this instrument is a powerful tool.”

“The speed and efficiency of the Q Exactive GC gives confidence in impurity identification with only one elemental formula being identified.”
Requirements for Identification of Unknowns

**RT & m/z**

**Empirical Formulae**

C\(_{15}\)H\(_{16}\)O\(_2\)

**Structure**

![Structure of Bisphenol-A](image)

**Substructure**

![Substructure](image)

**Name & CAS**

4,4'-(propane-2,2-diyl)diphenol

Bisphenol-A

80-05-7

**Amount**

[Scale icon]
Requirements for Identification of Unknowns

- **RT & m/z**
- **Empirical Formulae**: C_{15}H_{16}O_{2}
- **Structure**
- **Substructure**
- **Name & CAS**: 4,4’-(propane-2,2-diyl)diphenol (Bisphenol-A)
- **Amount**
www.thermoscientific.com/QExactiveGC

Data in press:
Rapid Communications in Mass Spectrometry, circa April 2016