Accelerate your Quantitative LC/MS Workflows with a Fully Integrated UHPLC-QQQ Platform

Remco Swart

Pittcon
March 8-12, 2015
Outline

- Thermo Scientific™ Vanquish™ UHPLC System
- Thermo Scientific™ TSQ Endura™ Triple Quadrupole Mass Spectrometer (MS) and Thermo Scientific™ Quantiva™ Triple Quadrupole Mass Spectrometer
- Thermo Scientific™ Dionex™ Chromelone™ Chromatography Data System (CDS) Software 7.2 for LCMS
Why a New UHPLC?

Customers told us they need:
- More resolution
- More sensitivity
- Easier and less method development
- More productivity
- Easier interaction and operation
- More robust and more reliable
- More sample capacity
- More access to experts
- More and fast service
- New, fresh & exciting instruments
- Better results in shorter time
The New Thermo Scientific Vanquish UHPLC System

We heard you! And we:

- Started with a complete new design in line with MS portfolio
- Put in 30 years of experience and know-how
- Developed a new and unique technology
- Closed gaps and pushed beyond previous limitations
- Boosted robustness

- Developed a new column technology
- Built the best UPHLC around the column/user
- Further leveraged our gold standard CDS software
- Boosted serviceability beyond established concepts
Vanquish System…Provides More Solutions!

- Vanquish UHPLC is not only a system – it is a problem-solving solution philosophy

Better integration with the world’s best mass spectrometers

Maximum speed and resolution with new Thermo Scientific™ Accucore™ Vanquish™ columns

Revolutionary ease-of-use experience by new Chromeleon CDS features and workflows

More throughput capacity with the new Thermo Scientific Vanquish Charger module
Vanquish System is…

**Confidence**

Controlling the separation with more confidence
- 2 thermostatting modes
- 5 °C to 120 °C temperature range
- Active preheating

**Power**

Driving the separation with more power
- 1500 bar (22,000 psi) of pump pressure
- Flow rate up to 5 mL/min
- 2 × 3 solvent channels

**Sensitivity**

Detecting the analyte with more sensitivity
- Linear up to 3000 mAU
- Noise levels down to ±3 µAU
- Lowest dispersion with Thermo Scientific™ LightPipe™ technology

**Accuracy**

Handling the sample with more accuracy
- Up to 23 well plates for up to 8832 samples
- Unsurpassed sample dosage
- Automation of workflows with barcode reading
# Vanquish Binary Pump

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500 bar / 22,000 psi Up to 5000 µL/min</td>
<td>Flexibility for all working conditions</td>
</tr>
<tr>
<td>New parallel dual piston principle</td>
<td>very small pressure ripple =&gt; column and sensitivity</td>
</tr>
<tr>
<td>Adaptive Thermal Effect Compensation (ATEC™)</td>
<td>best flow and gradient performance =&gt; RT stable</td>
</tr>
<tr>
<td>2 × 3 solvent channels</td>
<td>9 different solvent combinations</td>
</tr>
<tr>
<td>Fingertight check valve design</td>
<td>Easy to service</td>
</tr>
</tbody>
</table>
# Vanquish Split Sampler

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>New switching valve</td>
<td>no maintenance</td>
</tr>
<tr>
<td>Adjustable GDV</td>
<td>easy method transfer</td>
</tr>
<tr>
<td>Automatic sample pre-compression</td>
<td>• reduced physical stress on column = longer lifetime</td>
</tr>
<tr>
<td></td>
<td>• better retention time reproducibility</td>
</tr>
<tr>
<td>New air-to-air cooling concept</td>
<td>no condensation</td>
</tr>
<tr>
<td>Increased sample capacity</td>
<td>4 racks or well plates</td>
</tr>
<tr>
<td>Automated barcode reader</td>
<td>Rack tracking (esp. with Charger)</td>
</tr>
</tbody>
</table>

![Image of Vanquish Split Sampler](image-url)
Vanquish Charger Module

- Fully integrated robotic unit with less than 1 min cycle time
- Hosts up to 9 additional racks/deep well plates or 20 shallow well plates
- Up to 9000 samples – temperature controlled
- Precise temperature control using new designed air stream cooling concept
  - 4 – 40 °C
- Integrated barcode reader

One weekend full of results

23 × 96 wells
100 s cycle time
2208 samples
# The Vanquish Column Compartment

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Pre-Heater</td>
<td>Perfect temperature control (QC)</td>
</tr>
<tr>
<td>Two thermostatting modes</td>
<td>Easy method transfer</td>
</tr>
<tr>
<td>Three independent column compartments</td>
<td>Method scouting, different temperature zones</td>
</tr>
</tbody>
</table>

![ThermoFisher Scientific Logo]
Vanquish Column Compartment for MS integration

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column compartment is vertical</td>
<td>The column outlet is closer to the MS ion source</td>
</tr>
<tr>
<td>Position of the column compartment can be to the left or to the right</td>
<td>Better integration with all Thermo Scientific Mass Spectrometers</td>
</tr>
</tbody>
</table>
Vanquish Diode Array Detector

- LightPipe technology for an unmatched detection experience
  - 10 mm standard flow cell
  - 60 mm high sensitivity flow cell
- Best signal-to-noise performance
- Lowest baseline noise
- Ultra-wide dynamic range
  - Up to 3000 mAU
  - For simultaneous detection of highly concentrated compounds and trace impurities
- Variable slit width for optimum resolution
  - 1 – 8 nm
- Low peak dispersion
- Reduced RI and thermal effects
Vanquish System Features & Benefits for LC-MS Users

• Improved separation performance

<table>
<thead>
<tr>
<th>FEATURE</th>
<th>BENEFIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Better resolution</td>
<td>• Less matrix effects</td>
</tr>
<tr>
<td>• Minimal peak dispersion</td>
<td>• More consistent quantification</td>
</tr>
<tr>
<td>• More consistent sensitivity</td>
<td></td>
</tr>
<tr>
<td>• Faster analysis</td>
<td>• Improved LCMS productivity</td>
</tr>
<tr>
<td>• Rock solid retention time stability</td>
<td>• More reliable identification</td>
</tr>
<tr>
<td>• More reliable quantification</td>
<td>• More reliable quantification</td>
</tr>
<tr>
<td>• New core enhanced 1.5 µm columns</td>
<td>• Less method development</td>
</tr>
<tr>
<td></td>
<td>• Less peaks of interest in the void volume</td>
</tr>
</tbody>
</table>
Challenges in Quantitative Mass Spectrometry

- Large Sample Numbers
- Low Concentration

Triple Quads

Thermo Fisher Scientific
TSQ Endura MS and TSQ Quantiva MS

**TSQ Endura MS**

**Extreme Quantitative Value**
- Best-in-class sensitivity
- Unprecedented usability
- Ultimate robustness

**TSQ Quantiva MS**

**Extreme Quantitative Performance**
- Attogram sensitivity
- Unprecedented usability
- Exceptional robustness
Sensitivity, Speed & Robustness for High-Throughput Laboratories

TSQ Quantiva MS

Extreme quantitative performance
- Designed for the most challenging assays.
- For scientists needing to stay at the forefront of analytical technology

<table>
<thead>
<tr>
<th></th>
<th>TSQ Quantiva MS</th>
<th>TSQ Endura MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Range</td>
<td>10-1850</td>
<td>10-3400</td>
</tr>
<tr>
<td>Max SRM Number</td>
<td>30,000 SRMs</td>
<td>30,000 SRMs</td>
</tr>
<tr>
<td>SRM/Sec</td>
<td>500 SRMs/sec</td>
<td>500 SRMs/sec</td>
</tr>
</tbody>
</table>
| Ion Optics          | Active Ion Management (AIM)
                      • Ion Max NG source
                      • Electrodynamc ion funnel
                      • Ion beam guide with neutral blocker
                      • 6 mm HyperQuad quadrupoles with asymmetric RF drive
                      S-LENS with Beam Blocker Technology
| Quadrupole Design   | 4mm Quadrupoles with Asymmetric RF |
| Reserpine Specification | 100,000 :1 S/N for 1 pg Reserpine | 10,000 :1 S/N for 1 pg Reserpine |

TSQ Endura MS

Extreme quantitative value
- Designed for non-stop operation.
- For scientist who need to run routine samples day in and day out.
Low Sample Concentration

Challenge

Demanding assays which require the absolute lowest limit of detection

TSQ Quantiva MS

Powered by AIM (Active Ion Management) technology, the TSQ Quantiva MS is the world’s most sensitive triple quadrupole MS, detecting compounds at the ppt level.
Large Sample Numbers

Challenge

Need to analyze multi-residue analytes in very short run times, using UHPLC with robust performance 24/7

Vanquish System with Charger and TSQ Quantiva MS

The AIM technology includes an ultra fast collision cell designed for fast SRMS with no loss in sensitivity. Neutral clusters eliminated for robustness.
Routine Quantitation

**Challenge**

Robust and reliable quantitation

**TSQ Endura MS**

LODs and LOQs unrivaled in its class. It delivers this best-in-class quantitation run-after-run and day-after-day regardless of sample type or matrix.
Challenge

Analysts don’t have time to waste learning new software or optimizing methods.

Vanquish System and TSQ MS with Chromeleon CDS Control

The entire software workflow has been redesigned to remove un-needed parameters.
Introduction

• How can you include MS in your laboratory CDS?
  • Currently it’s on a workstation and there is no way to work remotely

• Chromeleon™ 7.2 Chromatography Data System is the first CDS to support MS instrument control and data processing with all main front-end separation techniques (GC, LC, IC) in an enterprise environment
Quantiva & Endura - Fully Integrated within Chromeleon CDS

• Chromeleon 7.2 CDS supported workflows
  • Compliance, scalability, suite of quantification features
  • MS-specific data organization at component level
  • Context menu to open data in MS applications (e.g. Thermo Scientific™ Xcalibur™ Software)

• Key benefits
  • Only need to install, validate and learn one software package
  • Can use all compliance and processing features of Chromeleon CDS
  • Enhanced data security
  • Easily start analyses with eWorkflows™
  • Additional MS-specific detection algorithms (Genesis, ICIS)
MS Data Visualization

Seeing is believing...
Component and Channel Centric Data Viewing

All channels and components listed and multi-selectable

Filter box to quickly narrow list of components or channels

Components are tagged with tool tips, indicating analysis results
MS Data Views – Total Ion Chromatogram (TIC)
MS Data Views – MS Quantitation Channel

Overlay of all Quantitation XICs

Results for selected component
MS Data Views – Mass Spectrum Plot

Peak apex spectrum

Click on component name or peak and select mass spectra

Also show leading/tailing spectra & library hits – stack or overlay
MS Data Views – Component Traces

Quantitation and confirmation ions displayed...

Click on component name or peak
Data Visualization – Multiple Components / Injections

- Using SmartLink and Component Traces...

...in rows...

...or overlaid

...components in columns

...and pin injections / components

Quantitation and confirmation ions

Select SmartLink...
MS Data Processing
MS Data Processing

• With multiple traces and possibly hundreds of components, how can you quickly and easily process your MS data?
• How can you rapidly and accurately setup your component table?
• Existing data processing tools in Chromeleon CDS are now complimented by a suite of MS-specific tools for fast and efficient MS data processing, including:
  • Easy extracted ion chromatogram (XIC) creation
  • Additional MS-specific detection algorithms
  • Full processing control on a component-by-component basis
  • MS Library searching
MS Data Processing

- Apply all existing calibration modes and options to MS
- Easy extracted ion chromatogram creation
- Additional MS-specific detection algorithms
  - Genesis, ICIS
- Full processing control on a component-by-component basis
  - Additional MS-specific parameters in component table – quantitation and confirming ions, CAS numbers, molecular formulas, etc.

[Image: Extract MS Channel window with options for Trace, Filter, Mass range, and Channel name]
MS Data Processing – Extracted Ion Chromatograms

• Manual extraction of XIC

Right-click on Channel or MS Plot
MS Data Processing – Extracted Ion Chromatograms

- Graphical extraction

Click below the axis...
MS Data Processing – Extracted Ion Chromatograms

- Graphical extraction

New channel added to list and displayed

... and drag out a range
Integrated NIST Library Search

- Use NIST libraries for spectral library screening and ad-hoc library search
Import from Compound Databases and NIST Libraries

- Quickly populate processing method component tables
- Use library searches
  - NIST
  - User libraries
- Use predefined compound data bases
  - Thermo Scientific™ TraceFinder™ software
- Use Xcalibur software raw files with component info
MS Data Reporting
MS Data Reporting

• Can be a very time-consuming activity!
• Multiple components and traces make generic reporting tricky, if not impossible
• How do you effectively report such large quantities of data?
• Several new additions to Chromeleon CDS reporting tools
  • New MS based reporting objects
    • Mass Spectral Plot
    • Component Traces Plot
    • MS related report variables and tables
  • New tools for reporting of large amounts of multi-channel data
    • Consolidated Report Tables
    • Autorepeat Area Enhancements
MS Data Reporting

MS peaks often only appear in certain detection channels (filters) – how do you create a report for your peaks of interest?
MS Data Reporting – Consolidated Report Tables

Chromeleon CDS Consolidated Report Tables

- Automatically report for the Channel with the highest response

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak Name</th>
<th>Area counts*min Highest Response</th>
<th>Channel Highest Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>PCB#18</td>
<td>15392</td>
<td>TIC_F01</td>
</tr>
<tr>
<td>10</td>
<td>PCB#28</td>
<td>19480</td>
<td>TIC_F01</td>
</tr>
<tr>
<td>11</td>
<td>PCB#33</td>
<td>17945</td>
<td>TIC_F01</td>
</tr>
<tr>
<td>25</td>
<td>PCB#52</td>
<td>5455</td>
<td>TIC_F03</td>
</tr>
<tr>
<td>34</td>
<td>PCB#95</td>
<td>3640</td>
<td>TIC_F06</td>
</tr>
<tr>
<td>50</td>
<td>PCB#70</td>
<td>2364</td>
<td>TIC</td>
</tr>
<tr>
<td>79</td>
<td>PCB#156</td>
<td>3650</td>
<td>TIC_F06</td>
</tr>
<tr>
<td>145</td>
<td>PCB#126</td>
<td>2757</td>
<td>TIC_F06</td>
</tr>
<tr>
<td>179</td>
<td>PCB#153</td>
<td>4781</td>
<td>TIC_F07</td>
</tr>
<tr>
<td>219</td>
<td>PCB#188</td>
<td>1100</td>
<td>TIC_F10</td>
</tr>
<tr>
<td>225</td>
<td>PCB#170</td>
<td>889</td>
<td>TIC_F10</td>
</tr>
</tbody>
</table>

- Useful for reporting large quantities of MS data in a generic, compact table
Antibiotics 1 ppb
36 Antibiotics Targeted Screening

Accucore Vanquish C18 column, 1.5µm x 2.1mm x 100mm

Overlaid selected reaction monitoring chromatograms showing detection of 36 antibiotics within a 5 minute detection window, binary Vanquish system and TSQ Vantage MS

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>10</td>
</tr>
<tr>
<td>4.375</td>
<td>90</td>
</tr>
<tr>
<td>5.000</td>
<td>90</td>
</tr>
<tr>
<td>5.125</td>
<td>10</td>
</tr>
<tr>
<td>8.750</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1. LC gradient conditions

Mobile phase A: 0.1% formic acid / Water
Mobile phase B: 0.1% formic acid / MeOH
Flow rate: 400 µL/min
Column temperature: 40 °C, active preheating
Injection volume 2 µL
47 Drugs in 4 Minutes

Overlaid selected reaction monitoring chromatograms showing detection of 47 drugs within a 4 minute detection window, binary Vanquish system and TSQ Vantage MS

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>10</td>
</tr>
<tr>
<td>0.16</td>
<td>10</td>
</tr>
<tr>
<td>2.88</td>
<td>90</td>
</tr>
<tr>
<td>3.20</td>
<td>90</td>
</tr>
<tr>
<td>3.28</td>
<td>10</td>
</tr>
<tr>
<td>5.60</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1. LC gradient conditions

Mobile phase A: 10 mM ammonium acetate in water
Mobile phase B: 0.1% formic acid in methanol
Flow rate: 500 µL/min
Column temperature: 50 °C, with active preheating
Injection volume: 2 µL
Summary – Bringing It All Together

Chromeleon CDS has all the tools to help you process and report your MS data in the shortest time!
Thank You!

Any Questions?