Triple quadrupole Vs (Q)TOF technology with RapidFire: - When to use what and how to get the optimum performance out of your instrument

Mass spectrometry

RapidFire user meeting
Waldbronn
November 14th, 2012
Moritz Wagner
Content

Overview of and comparison of technologies

Optimisation

1. Source
2. iFunnel
3. MS
4. RF

Pitfalls

Future developments
Agilent’s LC/MS Systems

Performance / sensitivity

Upgrade/ JetStream

Upgrade 2

iFunnel Technology

6420

6430

6460

6490

6520

6530

6540

6550

RapidFire User meeting Nov 14th
Agilent’s LC/MS Systems

Over 10 Years in LC/MS Atmospheric Sampling and Patented Orthogonal Geometry - Result in Industry Leading Sensitivity and Robustness
Operational Modes – Scan

• The quad is in Total Transmission Ion (TTI) mode. All ions are passed through the quad.
• There is no collision energy applied in the collision cell

Pass all ions

No collision energy applied.

Analyze mass peaks
Operational Modes: Product Ion Scan

Product Ion Scan - A mass selected precursor ion is chosen with Q1 and the collision cell generates fragment ions. The fragment ions are then analyzed.

- Specific mass passes through quad
- Collision energy applied
- Fragments analyzed
Operational Modes: MRM

MRM - A mass selected precursor ion is chosen with Q1 and the collision cell generates fragment ions. The fragment ions are then analyzed using selection of product ions with Q3.

Specific mass passes through quad

Collision energy applied

One fragment analyzed
Comparison QQQ/QTOF

QQQ MS2 scan
EIC 357.1
S/N 2

QTOF scan
EIC 357.119
S/N 500
Comparison QQQ/QTOF

QQQ Product ion scan
TIC 357.1
S/N 500

QTOF targeted MS/MS
TIC 357.119
S/N 250
Comparison QQQ/QTOF

+ESI MRM Frag=100.0V CID@15.0 (357.1000 -> 149.0000) GPD-10ng-MRM-001.d
Noise (PeakToPeak) = 10.06; SNR (3.414min) = 12784.9

QQQ MRM 357.1 → 149

S/N 12000
Comparison of instrument types, models and scan modes
Comparison of S/N across different MS

Comparison of fragmentor vs iFunnel QQQ

6460 QQQ

MRM

6490 QQQ with iFunnel

→ 20x signal intensity, same noise
Comparison of S/N across different MS

Comparison of fragmentor vs iFunnel QTOF in MS1

6530 QTOF

+ESI EIC(609.2810), Scan Frag=200.0V Reserpine_6530_MS.d
Noise (PeakToPeak) = 326.00; SNR (110.4 sec) = 26072.1

EIC 609.281 (MS1)
Scan/“SIM“

100 pg/µl

6550 QTOF with iFunnel

→ 8x signal intensity, 6x noise

+ESI EIC(609.2810), Scan Frag=365.0V Reserpine-6550-MS.d
Noise (PeakToPeak) = 2010.00; SNR (130.1 sec) = 7297.5

10 pg/µl

100 pg/µl
Comparison of S/N across different MS

Comparison of fragmentor vs iFunnel QTOF in MS/MS

6530 QTOF  Extracted product ion
609.28 → 195.06
„Hi-res MRM“

6550 QTOF with iFunnel
→ 10x signal intensity, no noise
Comparison of sensitivity in MS and MS/MS

EIC 711.724
TP53 peptide
Approx 100,000 counts

EIC 711.724 → 84.0808
TP53 peptide
Approx 30,000 counts
Comparison of S/N across different MS

Comparison of sensitivity in MS and MS/MS

- Noise in MS1: Approx 300 counts (amplitude)
- Noise in MS/MS: < 1 count (amplitude)
Comparison of S/N across different MS

Comparison of sensitivity in MS and MS/MS

Annotations show peak height and S/N (peak to peak)

Quantification in MS/MS approximately 100-fold more sensitive (1/3 signal but 1/300 noise)
Comparison of S/N across different MS

- 6530 MS1 S/N 83
- 6450 MRM S/N 299
- 6550 MS/MS S/N 33000
- 6550 MS1 S/N 128
- 6490 MRM S/N 7000
With Increases in Sensitivity comes “Opportunity”

Optimization - an act, process, or methodology of making something (as a design, system, or decision) as fully perfect, functional, or effective as possible

Simple View – 1 Compound

“Real” View – Multi Compound
Optimizing Agilent Jet Stream and iFunnel for highest sensitivity

AJS / ESI Optimization
The super-heated sheath gas collimates the nebulizer spray and creates a dramatically “brighter source”
Skimmer vs. iFunnel technology

6460 LC/MS  (Skimmer Configuration)

Cap | RF Ion Guide | Q1 | Coll Cell | Q3

Ion Detector

6490 LC/MS  (iFunnel Configuration)

HB Cap | Dual Ion Funnels | RF ion guide | Q1

Curved Collision cell
iFunnel technology components

**Agilent Jet Stream**
- Thermal confinement of ESI ion plume
- Efficient desolvation to create gas phase ions

**Hexabore Capillary**
- 6 capillary inlets
- Samples 12X more ion rich gas from the source

**Dual Ion Funnel**
- Removes the gas but captures the ions
- Helps to remove source generated noise

[Images of the technology components]
1) Drying Gas Temp Analysis for 7 Bad Actors – Chose 150° C

- **Aldicarb 2**
- **Chlorpyrifos-methyl**
- **Metosulam**
- **Fenitrothion**
- **Parathion-Methyl**
- **Terbufos**
- **Disulfoton**
Compound Types proposed

- Broad characterization of Compound Types based on iFunnel tuning profile in negative
- Type 1: Compound an optimum at the high end of the parameter setting
- Type 2: Compound with optimum at a plateau
- Type 3. Compound with optimum at the low end of the parameter set
Pesticide are generally more labile and the optimum is at a lower Funnel setting.
Drugs combined (6490/6550) Summary

Drugs are more stable hence the optimum is at a higher funnel setting

<table>
<thead>
<tr>
<th>LPRF/HPR</th>
<th>70</th>
<th>90</th>
<th>110</th>
<th>130</th>
<th>150</th>
<th>170</th>
<th>190</th>
<th>210</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0</td>
<td>2.564103</td>
<td>17.94872</td>
<td>10.25641</td>
<td>23.07692</td>
<td>15.38462</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>2.564103</td>
<td>25.64103</td>
<td>51.28205</td>
<td>71.79487</td>
<td>97.4359</td>
<td>69.23077</td>
<td>82.05128</td>
<td>33.33333</td>
</tr>
<tr>
<td>80</td>
<td>2.564103</td>
<td>20.51282</td>
<td>46.15385</td>
<td>79.48718</td>
<td>97.4359</td>
<td>100</td>
<td>69.23077</td>
<td>23.07692</td>
</tr>
<tr>
<td>100</td>
<td>2.564103</td>
<td>10.25641</td>
<td>35.89744</td>
<td>64.10256</td>
<td>84.61538</td>
<td>92.30769</td>
<td>48.71795</td>
<td>20.51282</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>2.564103</td>
<td>35.89744</td>
<td>79.48718</td>
<td>82.05128</td>
<td>79.48718</td>
<td>46.15385</td>
<td>17.94872</td>
</tr>
<tr>
<td>140</td>
<td>0</td>
<td>0</td>
<td>30.76923</td>
<td>41.02564</td>
<td>53.84615</td>
<td>74.35897</td>
<td>43.58974</td>
<td>41.02564</td>
</tr>
<tr>
<td>160</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.564103</td>
<td>10.25641</td>
<td>38.46154</td>
<td>41.02564</td>
<td>38.46154</td>
</tr>
</tbody>
</table>

Drugs Positive Ion Histogramm (Values above 90%)
Peptides Summary

<table>
<thead>
<tr>
<th>LPRF/HPR</th>
<th>70</th>
<th>90</th>
<th>110</th>
<th>130</th>
<th>150</th>
<th>170</th>
<th>190</th>
<th>210</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>54.05405</td>
<td>78.37838</td>
<td>67.56757</td>
<td>24.32432</td>
</tr>
<tr>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>59.45946</td>
<td>100</td>
<td>94.59459</td>
<td>83.78378</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>56.75676</td>
<td>75.67568</td>
<td>89.18919</td>
<td>94.59459</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>48.64865</td>
<td>91.89189</td>
<td>94.59459</td>
<td>83.78378</td>
</tr>
<tr>
<td>140</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>43.24324</td>
<td>83.78378</td>
<td>89.18919</td>
<td>70.27027</td>
</tr>
<tr>
<td>160</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16.21622</td>
<td>67.56757</td>
<td>64.86486</td>
<td>67.56757</td>
</tr>
</tbody>
</table>

Peptides will show a great sensitivity at the default as well as modified funnel setting.
• The iFunnel has different optimization characteristics for different compound classes.
  – These characteristics may be diverse for mixtures of labile and non-labile analytes
• It is necessary to optimize both AJS and iFunnel tuning in order to get the highest levels of sensitivity.
  – Optimization requires background knowledge and time!
• When optimizing a large number of compounds, e.g. multi-analyte pesticide methods, the tuning needs to be set for the best overall sensitivity of the entire compound set.
  – “One size may not fit all”
Quad resolution
Quad resolution

- Parent Quad Filter Q1
- Collision Cell
- Product Quad Filter Q3
- Detector
Quad resolution

<table>
<thead>
<tr>
<th></th>
<th>enhanced</th>
<th>unit</th>
<th>wide</th>
<th></th>
<th>enhanced</th>
<th>unit</th>
<th>wide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noise</td>
<td></td>
<td></td>
<td></td>
<td>Height</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>enhanced</td>
<td>1.3</td>
<td>2.9</td>
<td>2.3</td>
<td>19%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unit</td>
<td>1.4</td>
<td>2.6</td>
<td>3.1</td>
<td>31%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wide</td>
<td>2.1</td>
<td>3.1</td>
<td>4.3</td>
<td>40%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enhanced/Unit
Wide/Unit

ESI MRM Frag=380.0V CID@15.0 (517.3000 -> 791.4000) GH1-100nIU.d Noise (PeakToPeak) = 2.28; SNR (17.293min) = 293.4

ESI MRM Frag=380.0V CID@15.0 (517.3000 -> 791.4000) GH1-100nIU.d Noise (PeakToPeak) = 3.06; SNR (17.234min) = 367.3

ESI MRM Frag=380.0V CID@15.0 (517.3000 -> 791.4000) GH1-100nIU.d Noise (PeakToPeak) = 4.37; SNR (17.266min) = 364.6

ESI MRM Frag=380.0V CID@15.0 (517.3000 -> 791.4000) GH1-100nIU.d Noise (PeakToPeak) = 2.11; SNR (17.265min) = 300.7

ESI MRM Frag=380.0V CID@15.0 (517.3000 -> 791.4000) GH1-100nIU.d Noise (PeakToPeak) = 3.13; SNR (17.232min) = 380.6

Agilent Technologies
RapidFire User meeting Nov 14th
CitZ_03 peptide IEDIVTSEK, 280 amol on column
MRM 517.3 → 791.4 (z = 2 → y7)

In this case, the interferences were separated chromatographically.

Coeluting interferences will change Quantifier/qualifier ratios or could lead to false positives or overestimated quantitative results.
Goal: Automated ramping of RF parameters for method optimization
→ No optimization of solvent/buffer system

In this case, triplicate injections of 100 ng/ml QC PepMix were used
Pump 1: H2O + 0.1% FA
Pump 2+3: ACN/H2O 95/5 + 0.1% FA

1. Creation of plate map
96 well format
12 sequences x 3 injection

Use of matrix stations may be preferrable?
„Screening“ of RF methods

12 sequences using identical MS method

a) First subset of 7 sequences with identical cartridge chemistry (C4) but different RF methods, in this case the time for state 2 + 3 was ramped

b) Second subset of 5 sequences with identical RF method but different column chemistries
„Screening“ of RF methods

Extracted ion chromatograms for leucine enkephaline

3000 ms state 2
3000 ms state 3

4000 ms state 2
4000 ms state 3

5000 ms state 2
5000 ms state 3
„Screening“ of RF methods

Extracted ion chromatograms for leucine enkephaline
Pitfall 1: Unsufficient selectivity, isomers or close masses

MS optimization
Pitfall 1: selectivity/resolution

ISTD Omeprazol
Extracted ion chromatogram
m/z 346.1

Where does this come from?
Pitfall 1: selectivity/resolution

Accurate m/z of target: 346.136

Accurate m/z of ISTD: 346.122
Pitfall 1: selectivity/resolution

In this case, the high excess of the target (up to 80-fold more than ISTD) masked the ISTD → more selectivity by doing MS/MS on QTOF could be useful.

EIC m/z 346.136 ± 20 ppm

EIC m/z 346.122 ± 20 ppm
Pitfall 1: selectivity/resolution

Re-analysis in targeted MS/MS mode
Target: 346.1, quite generic collision energy of 20eV
Pitfall 1: selectivity/resolution

MS1: still the same mass shift issue/unresolvable masses
Pitfall 1: selectivity/resolution

Since Q1 operates at unit resolution, both 346.136 and 346.122 will be coisolated.
Pitfall 1: selectivity/resolution

The mixed spectrum has common fragments (coisolation) but also unique fragments.
Pitfall 1: selectivity/resolution

In MS/MS, quantification is possible using any product ion with excellent selectivity due to high resolution → „pseudo MRM“ or more precisely using product ion scanning.
Pitfall 2: Nonlinearity – detector saturation
Pitfall 2: detector saturation

Non-linear correlation between concentration and peak area due to ESI or detector saturation?
Corresponding spectrum of high concentration: Both C12 and C13 isotope are in detector saturation!

Detector saturation is automatically recognized and marked with an asterisk

Second isotope is unsaturated
Pitfall 2: detector saturation

Better linearity by selecting a less saturated mass → not ESI but detector saturation
Pitfall 2: detector saturation

Extracted ion chromatogram m/z 470.16 (second isotope)

High concentration

Low concentration

Even better linearity by selecting a nonsaturated mass
Pitfall 3: Nonlinearity – ion source saturation/ion suppression
Superimposed extracted ion chromatograms for Bumetanide

m/z 365.1166

10 ppm in matrix
10 ppm standard
1 ppm in matrix
0.1 ppm in matrix
Blind

Pitfall 3: ion suppression
Pitfall 3: ion suppression

Calibration curve Bumetanide 0.1 – 10 ppm

Quadratic fit due to saturation of the ESI source

Weighting 1/x, $R^2 = 1$

$y = -1.23774 \times 10^5 \times x^2 + 2.828778.919894 \times x + 25415.486346$

$R^2 = 1.00000000$
Pitfall 3: ion suppression

Superimposed EICs for 10 ppm samples in ACN and in 1000 ppm Arlamol E respectively

→ Only approximately 10% ion suppression/sensitivity loss compared to 95% in FIA!
Calibration curve
Bumetanide
0.1 – 10 ppm
Excellent linearity even using FIA analysis

However, using the matrix (Arlamol E) calibration the average standard concentration was approx. 189 ppm (10 ppm test compounds injected)

This means an observed ion suppression of approx 95% → 20x lower signal using FIA vs. LC (RF should be in-between)
Extracting the maximum from your RapidFire data or „Taking FIA to the next level“
MassHunter Workstation
*Increase your productivity significantly*

**High Throughput Quantitation**
- Study Manager to queue up quantitative assays incl. MRM optimization
- Optimizer quickly and easily optimizes MS/MS signal
- Dynamic MRM methods deliver robust assays faster
- WATSON LIMS Integration and Compliance support for BioAnalysis

**High Throughput Screening**
- Personal Compound Databases (PCDs) and Libraries (PCDLs) for accurate mass DB and MS/MS library search with optional RT
- Fully automated acquisition, data processing and reporting
- Supports targeted *and* untargeted screening
- New PCDL Manager SW to view and edit PCDs and PCDLs

**Proteomics / Metabolomics & Non-targeted Screening**
- Mass Profiler Professional (MPP)
  - Integrated workflow for feature finding, alignment, differential analysis, identification and pathway analysis
  - Powerful and easy-to-use statistical tools
- Pathway Analysis for direct biochemical pathway interrogation
MassCode Pathogen Detection

- Influenza A
- Influenza B
- RSV
- Adenovirus
- Parainfluenza
- Salmonella enteritidis
- Mycoplasma pneumoniae
- Bordetella pertussis

RapidFire User meeting Nov 14th
1. RT, PCR amplification of target sequences with mass-tagged primers

2. Purification of amplicons to remove excess tagged primers & primer dimers

3. Automated sample injection w/ in-line photo-release of MassCode tags and MS detection

4. Identification of MassCode tags - detection of target
Metabololomics as a data-driven approach to unravel mechanisms governing cellular metabolism

Metabolites are at the junction of environmental and genetic interactions, and they reflect the integrated response.
Enzymatic assay and genome-wide phenotyping by FIA-TOF/MS

1. Overexpress and purify protein (ASKA library, His-tag)

2. From profiles/ions/similarity SPECULATE on putative substrates.

3. Perform assay with on-line FIA-MS

4. Identify reaction products from accurate mass
   - **48 assays in parallel** (6 hours MS time)

KEIO Knock-out collection

Cultivation in **minimal medium** with glucose + amino acids

prep

FIA-TOF neg

FIA-TOF pos

processing

4’500 genes
2 clones/gene

8’600 samples

>17’000 analyses
>17’000 analyses

500 GB of data

prep

Cultivation in minimal medium with glucose + amino acids

FIA-TOF neg

FIA-TOF pos

processing

4’500 genes
2 clones/gene

8’600 samples

>17’000 analyses
>17’000 analyses

500 GB of data
High throughput, accurate mass metabolomics

Flow injection – Time of Flight
- 2 microL full loop injection
- Scanning 50-1000 m/z
- Inter-day CV 20%
- ca. 2000 injections/day
- Linear over >3 decades

Sample prep
- 5 minutes/plate
- NO drying

IT
- Storage & retrieval
- Automatization

Client software
- Data analysis
- Annotation
- Visualization

Cultivation
- 96 WP
Resolution & Sensitivity

50x sensitive, 10x in MS²

Coverage

5-8x more ions/compounds
RapidFire Profiling workflow

Total ion chromatogram after data splitting
RapidFire Profiling workflow – Peak picking using Molecular Feature extractor
RapidFire Profiling workflow

Profile spectrum of peak at 6 seconds
RapidFire Profiling workflow

Superimposed compound spectra

Counts vs. Mass-to-Charge (m/z)
RapidFire Profiling workflow

Approximately 700 compounds extracted

Total compound chromatogram after data splitting
RapidFire Profiling workflow – Export into statistical software

Worklist automation, export compound information into .CEF (compound exchange format) and import into GeneSpring/MPP
RapidFire Profiling workflow - Data import wizard

3 blanks @ well B1
3 medium concentration @ well E1
3 high concentration @ well H1
Filter on frequency, compounds must be present in each replicate → Getting rid of „one-hit-wonders“

Approximately **414 compounds** in 100% of each condition
RapidFire Profiling workflow

QC on samples
Sample quality can be assessed by examining the values in the PCA plot and other experiment specific quality plots.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>In00004-Reserpine QC-B1.1</td>
<td>B1</td>
</tr>
<tr>
<td>In00005-Reserpine QC-B1.1</td>
<td>B1</td>
</tr>
<tr>
<td>In00006-Reserpine QC-B1.1</td>
<td>B1</td>
</tr>
<tr>
<td>In00013-Reserpine QC-E1.1</td>
<td>E1</td>
</tr>
<tr>
<td>In00014-Reserpine QC-E1.1</td>
<td>E1</td>
</tr>
<tr>
<td>In00015-Reserpine QC-E1.1</td>
<td>E1</td>
</tr>
<tr>
<td>In00022-Reserpine QC-H1.1</td>
<td>H1</td>
</tr>
<tr>
<td>In00023-Reserpine QC-H1.1</td>
<td>H1</td>
</tr>
<tr>
<td>In00024-Reserpine QC-H1.1</td>
<td>H1</td>
</tr>
</tbody>
</table>

Legend - 3D PCA Scores
Color by Well:
- B1
- E1
- H1

Description
Algorithm: Principal Components Analysis
Parameters:
- Column indices = [1-9]
- Pruning option = [true,PrincipalComponents, 4]
- Mean centered = true
- Scale = true
- 3D scores = true
- PCA on = Columns
13 compounds are significantly different (p<0.01) and have changed in abundance more than 2-fold.
RapidFire Profiling workflow
- ID Browser for identification

Optional ID of compounds using a database search and/or Molecular formula generation
RapidFire Profiling workflow - ID Browser for identification

ID Browser results details - fluphenazine and ist metabolite hydroxyfluphenazin
RapidFire Profiling workflow
- Entity inspector for validation

Fluphenazine „levels“ across different injections/wells
Summary

It is simple to analyze 1000’s of samples per day

It is simple to find «markers» for everything

• A lot of the changes are irrelevant & trivial

Plenty of **new things can be discovered by HT-mass spec**

• Distal associations
• Novel enzymes
• Novel regulators
• Novel functions for «known» enzymes
• Predict response to drugs (toxicity)

**Pathway-based analysis!**

• Integrates prior information
• Improves interpretation
• Improves denoising

**Coverage is essential**

• Annotate everything!
• Fragment everything!