Using High Resolution, Accurate Mass Instruments for the Simultaneous Quantitative and Qualitative Analysis of Banned and Hazardous Compounds in Food and Environmental Analysis

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Agilent Technologies
EMEAI & Nordic Team
”Accurate and sensitive qualitative and quantitative analysis of any compound in food and environmental samples is probably one of the biggest challenges for an analytical chemist”
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Trace amounts (low ppt)

Difficult matrices
Accurate and sensitive qualitative and quantitative analysis of any compound in food and environmental samples is probably one of the biggest challenges for an analytical chemist.

Trace amounts (low ppt)

Difficult matrices

Identification/Confirmation issues

Multimethods
BACKGROUND

Triple quadrupole type of instruments dominates

• Why QQQ?
  • ”MS/MS is required to get sufficient selectivity”
  • ”MS/MS is required to get sufficient sensitivity”
  • ”The only instrument type robust enough to work in routine
  • ”Minimum user dependant results”
  • ”Superior sensitivity”
  • ”Superior linearity”
..but why not high resolution instruments like TOF?

• High **resolving power** and **accurate mass** for selective and sensitive quantification
• No need to setup **MRM** transitions
• No need to decide upfront **what to detect**
• ”All the ions all the time”. Speed and sensitivity (Ferrer et.al.)
• Possibility to build **accurate mass MS/MS libraries**
• Modern Tof/Q-Tof’s are **easy to operate**
• Modern Tof/Q-Tof’s are **stable and reliable**
Ultra High Definition
Optimizing all Analytical Dimensions

Signal Response
• Sensitivity
• Dynamic Range
• Linearity

Chromatogram
• Separation Speed
• Peak Capacity

Mass Spectrum
• Mass Accuracy
• Resolving Power
• Acquisition Rate
e.g. Caffeine, C8H10N4O2

**Tof/Q-Tof:**
Resolution: 25.000

**Quadrupole:**
Resolution: 280
Mass accuracy:
e.g. Caffeine

Tof/Q-Tof:
195.0877±1ppm
195.0875 - 195.0879m/z

Quadrupole:
195.1±0.1Da
195.0 - 195.2m/z
or ~ ±500ppm
**Mass accuracy:**
e.g. Caffeine

Tof/Q-Tof:
195.0877±1ppm
195.0875 - 195.0879m/z

Quadrupole:
195.1±0.1Da
195.0 - 195.2m/z
or ~ ±500ppm

Number of hits searching the METLIN DB.....~45,000 cpd´s

1 (one)

24 (twenty four)
Effect of resolution and mass accuracy
EIC of m/z 144.1627

Isolation window ±10ppm
s/n ~39.000

Isolation window ±500ppm
s/n ~650
Statement 1 and 2:

• ”MS/MS is required to get sufficient selectivity”
• ”MS/MS is required to get sufficient sensitivity”
Statement 1 and 2:

• “MS/MS is required to get sufficient selectivity”
• “MS/MS is required to get sufficient sensitivity”

• Low resolving power instruments e.g. Quadrupole based, requires MS/MS to achieve sufficient selectivity and sensitivity
6540 UHD Accurate Mass Q-TOF LC/MS
The Highest Performing Benchtop Q-TOF

Exceptional Ultra High Definition Performance…
With No Trade-Offs

• 40,000 resolution
• Excellent isotopic fidelity
• Mass accuracy < 1 ppm
• 5 orders of linear dynamic range
• Femtogram-level sensitivity with Agilent Jet Stream
• Fast acquisition for UHPLC – up to 20 spectra/second

Made Possible by Continuing Technology Breakthroughs

• Ion Beam Compression (IBC) cools & focuses ion beam
• Extended Flight Tube with Enhanced Mirror Technology (EMT)
• New Photonis Fast Bipolar Detector

The Ultimate Qualitative Analysis System

• Proteomics/Metabolomics
• Non-targeted food/environmental screening
• Impurity analysis
• Metabolite ID
Technology Innovation

Ion Beam Compression Technology (resolution + mass accuracy)

Dual-stage ion mirror (resolution)

INVAR flight tube (mass accuracy)

ADC (dynamic range)

4 GHz electronics (resolution, mass accuracy, sensitivity, dynamic range)

Ion acceleration in hexapole collision cell (faster MS/MS spectra)
Ultra High Speed Acquisition
From Agilent’s Leadership in GHz Speed Electronics

- 4 GHz Acquisition for Maximum Resolving Power and <1ppm Mass Accuracy
- 5 Decades of in-Spectrum Dynamic Range from 2-Channel x 2 GHz Dual Gain Mode
- 4 GHz (8 bit) Analog-Digital-Converter Adapted from Agilent’s High Speed Oscilloscope Systems
- Ultra High Speed FPGAs process and store transients in real time

Making Research Grade Performance possible in a Benchtop Format
"All the ions all the time"
"All the ions all the time"

Full sensitivity all the time
"All the ions all the time"

Full sensitivity all the time

No need to setup MRM
"All the ions all the time"

Full sensitivity all the time

No need to setup MRM

No need to decide upfront what to detect
**6540 Ultra High Definition Q-TOF**

**Mass Accuracy – Repetitive Injections**

**40 pg reserpine on-column, 10 injections**

<table>
<thead>
<tr>
<th>Run</th>
<th>Error (ppm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

**Mean** 0.25  
**Std Dev** 0.32

**250 ppb mass accuracy calibration and very accurate isotopic ratios**
Food Safety Screening for Triazines in Tomato Matrix

**TIC**

6230 TOF, MS Mode, Extended Dynamic Range

**EICs**

<table>
<thead>
<tr>
<th>Compound</th>
<th>% RSD (3 inj.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simazine</td>
<td>0.3</td>
</tr>
<tr>
<td>Prometon</td>
<td>1.6</td>
</tr>
<tr>
<td>Atrazine</td>
<td>0.3</td>
</tr>
<tr>
<td>Ametryn</td>
<td>2.2</td>
</tr>
<tr>
<td>Propazine</td>
<td>2.6</td>
</tr>
<tr>
<td>Prometryn</td>
<td>2.6</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Note: on-column amount is 5 pg (10 µL x 500 ppt).
Identification done by database searching
Rapid Analysis of Pharmaceutical Compounds

TOF - MS with 40 Hz acquisition rate over 39-second run time

H2O/ACN
Flow = 1.8 ml/min
5-90% B in 0.5 min
Stop time = 0.65 min
80°C, ACR
MS 40 Hz
100-1000 Da

Atenolol
Metoprolol 0.36s
Primidone 0.36s
Verapamil 0.42s
Beclomethasone-dipropionate 0.36s

Flow = 1.8 ml/min
5-90% B in 0.5 min
Stop time = 0.65 min
80°C, ACR
MS 40 Hz
100-1000 Da
Analysis of Sudan I: Reproducibility, Sensitivity, Quantitation

- Sudan I - 249.1023 m/z

![Chemical structure of Sudan I]

Reproducibility N=20
2.95% RSD

Reproducible
3% RSD

Sensitive (2 pg)

Calculated LOD (S/N=3) = 1.81 pg

Linear
Analysis of Sudan I: Mass Accuracy, Resolution

Sudan I - 249.1023 m/z

Resolution ~ 30,000

Mass accuracy 0.29 ppm
# Sudan Dyes: Outstanding Mass Accuracy and Resolution Enable Quantitation

<table>
<thead>
<tr>
<th>Name</th>
<th>m/z</th>
<th>Mass error (ppm)</th>
<th>Resolution</th>
<th>Signal/noise (2 pg)</th>
<th>Calc. LOD at 3x RMS noise (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl (Butter) Yellow</td>
<td>226.1339</td>
<td>-0.11</td>
<td>28884</td>
<td>43.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Sudan I</td>
<td>249.1023</td>
<td>0.29</td>
<td>29851</td>
<td>3.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Sudan II</td>
<td>277.1336</td>
<td>-0.59</td>
<td>31267</td>
<td>6.9</td>
<td>0.87</td>
</tr>
<tr>
<td>Para Red</td>
<td>294.0873</td>
<td>0.64</td>
<td>31413</td>
<td>13.5 (20 pg)</td>
<td>4.4</td>
</tr>
<tr>
<td>Sudan III</td>
<td>353.1397</td>
<td>-0.34</td>
<td>32653</td>
<td>3.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Sudan Red 7B</td>
<td>380.1870</td>
<td>0.31</td>
<td>32526</td>
<td>11.3</td>
<td>0.50</td>
</tr>
<tr>
<td>Sudan IV</td>
<td>381.1710</td>
<td>0.32</td>
<td>33515</td>
<td>8.9 (20 pg)</td>
<td>6.7</td>
</tr>
<tr>
<td>Sudan Red B</td>
<td>381.1710</td>
<td>0.56</td>
<td>34131</td>
<td>18.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>443.2330</td>
<td>-0.14</td>
<td>35607</td>
<td>58.4</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Average: Mass error = 0.37 ppm, Standard deviation = 0.19 ppm

< 0.5 ppm mass error
Vitamin B Analysis in Food

By courtesy to Q&Q Laboratories, Sweden
R Wahlström, E. Hermansson, G. Hägglund
TOF Pesticide Screening
250 pesticides, 50 pg on column

Counts vs. Acquisition Time (sec)

-1.8 ppm

Peak Width=0.8 sec
23 data points

Spectral Resolution & Isotopic Fidelity

Thiabendazole
1290 UHPLC with 6540 UHD Q-TOF
250 pesticides in 90 seconds

1-naphthol
Mass [M+H]^+ = 145.064891
Resolution = 24909
0.06 ppm

Cpd 151: Spinosyn A
Mass [M+H]^+ = 145.064819
Resolution = 24909
0.06 ppm

Cpd 201: Naphthol, 1-

Fenpyroximate E
Mass [M+H]^+ = 422.208059
Resolution = 38636
-0.25ppm

Cpd 265: Fenpyroximate(E)
Mass [M+H]^+ = 732.468714
Resolution = 47094
-0.77ppm

Spinosad A
Mass [M+H]^+ = 732.468714
Resolution = 47094
-0.77ppm
Identification of 29 Pesticides in Pepper Matrix at 10 ppb

**Methomyl**
- Formula: 163.05348 (M+H)+
- Concentration: -0.90 ppm
- Retention Time: R =24,200

**Imidacloprid**
- Formula: 268.15425 (M+H)+
- Concentration: -0.06 ppm
- Retention Time: R =28,900

**Diethofencarb**
- Formula: 270.13975 (M+H)+
- Concentration: -0.33 ppm
- Retention Time: R =29,100

**Triflumuron**
- Formula: 360.04026 (M+H)+
- Concentration: -0.60 ppm
- Retention Time: R =31,700

**Flufenoxuron**
- Formula: 492.04627 (M+H)+
- Concentration: -0.34 ppm
- Retention Time: R =34,500

**Total Ion Chromatogram (TIC)**
# Successful Identification of Isobaric Co-eluting Species by 1290/ 6540 UHD QTOF System

<table>
<thead>
<tr>
<th>Formula</th>
<th>m/z</th>
<th>Compound</th>
<th>ppm difference</th>
<th>Identify by</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9H7N3S</td>
<td>190.0433</td>
<td>1a tricyclazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C9H16ClN5</td>
<td>190.0667</td>
<td>1b propazine F1 Cl-37</td>
<td>123</td>
<td>RT difference</td>
</tr>
<tr>
<td>C14H21NO4</td>
<td>226.1074</td>
<td>2a diethofencarb F1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14H15N3</td>
<td>226.1339</td>
<td>2b cyprodinil</td>
<td></td>
<td>MS difference</td>
</tr>
<tr>
<td>C15H18ClN3O</td>
<td>294.1181</td>
<td>3a cyproconazole Cl-37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15H20ClN3O</td>
<td>294.1368</td>
<td>3b paclobutrazol</td>
<td></td>
<td>MS difference</td>
</tr>
<tr>
<td>C13H11Cl2F4N3O</td>
<td>372.0288</td>
<td>4a tetraconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C21H13N3O4</td>
<td>372.0979</td>
<td>4b Azoxystrobin-F1</td>
<td></td>
<td>MS difference</td>
</tr>
</tbody>
</table>

**Complex sample challenge:**

*Insufficient chromatographic or mass spectrometry resolution for these isobaric coeluting species (ICS)*
SUMMARY

Modern Tof and Q-Tof instruments offers...

- High **resolving power** and **accurate mass** for selective and sensitive quantification
- No need to setup **MRM** transitions
- No need to decide upfront **what to detect**
- **Excellent linearity** over 3-3.5 orders of magnitude
- Possibility to build **accurate mass MS/MS libraries**
- Modern Tof/Q-Tof’s are **easy to operate**
- Modern Tof/Q-Tof’s are **stable and reliable**
- Still sensitivity gap to QQQ systems (10-20 times)
MassHunter Software
MassHunter Workstation

Future proof with support of Windows 7 (64-bit)

- **New and updated components (Feb-2011)**
  - MassHunter Acquisition for QQQ B.04.01
  - MassHunter Acquisition for TOF/Q-TOF B.04.00
  - MassHunter Qualitative Analysis B.04.00
  - MassHunter BioConfirm B.04.00
  - MassHunter PCDL Manager B.04.00
  - MassHunter Forensics/Tox PCD B.04.00
  - MassHunter Forensics/Tox PCDL B.04.00 (Broecker, Herre & Pragst)
  - MassHunter METLIN AMRT PCD B.04.00 - **NEW**
  - MassHunter METLIN PCDL B.04.00 - **NEW**
  - MassHunter Pesticide PCD B.04.00

- **All run on Win 7 64-bit (in 32-bit mode), supports Excel 2010**
  - Future proof your analytical instrument software NOW
  - Prepare for future native 64-bit versions of MassHunter Qual and Quant
MassHunter Workstation

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  - MassHunter Acquisition for QQQ B.04.01
  - MassHunter Acquisition for TOF/Q-TOF B.04.00
  - MassHunter Qualitative Analysis B.04.00
  - MassHunter BioConfirm B.04.00
  - MassHunter PCDL Manager B.04.00
  - MassHunter Forensics/Tox PCD B.04.00
  - MassHunter Forensics/Tox PCDL B.04.00 (Broecker, Herre & Pragst)
  - MassHunter METLIN AMRT PCD B.04.00 - **NEW**
  - MassHunter METLIN PCDL B.04.00 - **NEW**
  - MassHunter Pesticide PCD B.04.00

- **All run on Win 7 64-bit (in 32-bit mode), supports Excel 2010**
  - Future proof your analytical instrument software NOW
  - Prepare for future native 64-bit versions of MassHunter Qual and Quant
Agilent Personal Compound Database
Food Safety Screening, Metabolomics, Forensics

- Endogenous metabolite database (METLIN) ~45,000 compounds including lipids
- Pesticide database (1600 pesticides)
- Forensic, toxicology, pharmaceutical database (7000 compounds)
- Create your own compound database

Public Metlin: www.metlin.scripps.edu

Scoring based on
- monoisotopic mass
- isotope distribution
- isotope spacing

Accurate mass and optional RT databases (AMRT)
MassHunter PCDL Manager B.04.00
Create/edit customized local PCDs and PCDLs

• Personal Compound Database and Libraries (PCDLs) for accurate mass database and MS/MS library search with optional retention time
• Add compounds, AM MS info, RT info and AM MS/MS spectra at multiple collision energies using the new PCDL Manager software
• Use create and edit PCDs and PCDLs on your personal PC (keep proprietary data in house!)

SUPPORTS:
METLIN AMRT PCD B.04.00
METLIN AMRT PCDL B.04.00
For/Tox AM PCD B.04.00
For/Tox AM PCDL B.04.00
Pesticide AM PCD B.04.00

=> The most comprehensive offering of content PCDs and PCDLs
The Forensics/Tox accurate mass PCD contains 7500 compounds.
New Forensics/Tox accurate mass PCDL contains **7900 MS/MS spectra** for over 2600 compounds from 3 collision energies mostly in positive mode.
The first commercially available **accurate mass MS/MS library**!
New METLIN AMRT PCD and PCDL B.04.00

MS/MS spectra for > 2300 endogenous metabolites!

- Now contains ~ 45,000 endogenous metabolites
- New METLIN AMRT PCD added retention times for 700 compounds on a RP column. Additional RTs on a hydrophilic column in progress.
- New METLIN AMRT PCDL contains MS/MS spectra from over 4,600 compounds from up to 4 collision energies in positive and/or negative mode (~ 27,000 spectra total)
**Targeted Workflow**

- Highest productivity via automatic Finding, identifying and confirming targeted compounds via **Find by Formula** using accurate mass MS info.
- Easily create a **Personal Compound Database and Library (PCDL)** from targeted compounds via **new PCDL Manager**.
- Confirm identity via accurate mass MS/MS spectra library search in a PCDL via **new Directed MS/MS** in the first run.

**Untargeted Workflow**

- Highest productivity via automatic extraction of accurate mass MS and MS/MS info via **new Find by MFE with MS/MS spectral extraction** from Auto MS/MS data files.
- Automatically conduct a PCDL search and Molecular Formula Generation using MS and (!) MS/MS information.
- Highest confidence via **new combined scoring and viewing of results from AM DB search, AM library search and MFG**.
New 6550 iFunnel QTOF

10X Sensitivity Gain Enables Applications

Sensitivity

• Dramatically improved quantitative capabilities
• New Qual/Quan Workflows
• Non-targeted compound screening

Comprehensive Performance Enhancements

• Mass Resolution >40,000
• 50 spectra/sec MS and 33 spectra/sec MS/MS
• 5 orders of linear dynamic range
• <1 ppm MS mass accuracy; <2 ppm MS/MS
• Unrivalled sensitivity
iFunnel Technology Captures 6x More Ions

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

**Hexabore Capillary**
- Samples 6 times more ion rich gas from the source with 6 capillaries
- Captures the majority of the gas from the source region

**Dual Ion Funnel**
- Removes the gas but captures the ions
- Removes neutral noise
- Extends turbo pump life
Agilent Jet Stream
Thermal Gradient Focusing Technology

Dramatic **Sensitivity Gains** for Premium TOF, Q-TOF, and Triple Quad Ions Focused in a Collimated Thermal Confinement Zone

Improved Ionization Efficiency and Sampling

**Effective Across a Broad Range of Analyte Classes, including many APCI compounds**
Two Stage Ion Funnel Manages the Gas Load

Offset ion funnels to prevent neutrals from going straight through to MS
**Ion Funnel Operation**

- **RF Voltage** focuses the ions to the center.
- **Unfocused Ions and Gas Enter**
- **DC Voltage accelerates the ions to the exit.**
- **RF Drive**
- **Focused Ions Out**

March 28, 2012
Ion Funnel Construction

Previously, many metal plates made cleaning a priority because of the large, active surface area… The high capacitance also required larger power supplies to provide RF power.

The 6490 design uses printed circuit board technology giving a greatly reduces surface area. This low capacitance enables the use of small power supplies, and enables fast polarity switching.
Simple and Easy Ion Funnel Cleaning Procedure

• As with all LC/MS systems routine cleaning is necessary periodically.

• The high pressure ion funnel should be cleaned periodically, although this could vary from 3 months to 1 year depending on the quantity and type of samples

• The high pressure ion funnel is easily removed

• Clean by sonicating the ion funnel assembly in 100% isopropanol for 15 minutes.
Dramatically Improving Detection Levels for Non-Targeted Screening

10 ppb Detection of Low Response Compounds

European Reference Lab for Pesticides

- 15% of tested pesticides have detection limits between 20 ppb to 100 ppb

- International actionable level is 10 ppb

- Challenge: Detect low responding compounds at <10 ppb using 6550 iFunnel QTOF

Chromatograms of 300 pesticides spiked in pepper extract at conc. 10 ug/kg (10 ppb)
6550 Linearity and Precision for Fluazifop-butyl

- Pepper matrix
- Linearity = 0.9999
- Limit of detection < 1 ppb
- 3.5 orders dynamic range

\[ y = 89278.335723 \times x - 10213.428054 \]

\[ R^2 = 0.99997214 \]

Type: Linear, Origin: Ignore, Weight: None

Successfully Detected in Pepper at 1 ppb

0.01 ng/mL to 55 ng/mL
Dramatic Increase in Pesticide Detection
Over 10 Fold Gain in Detection for Fluazifop-butyl

Extracted Ion Chromatogram for fluazifop-butyl at 10 ppb

MS spectrum of fluazifop-butyl
Mass error ~0.5 ppm
Score 98
## Compound Detection Below 10 ppb

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pepper</th>
<th>Pepper</th>
<th>Orange</th>
<th>Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorfluazuron</td>
<td>6530</td>
<td>6550</td>
<td>6530</td>
<td>6550</td>
</tr>
<tr>
<td>Cymoxanil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuron</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluazifop-butyl</td>
<td>6530</td>
<td></td>
<td>6530</td>
<td></td>
</tr>
<tr>
<td>Fluroxypr</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Propaquizafop</td>
<td>6530</td>
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</tr>
<tr>
<td>Quizalofop-ethyl</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rotenone</td>
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</tr>
<tr>
<td>Bromacil</td>
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<tr>
<td>Fenproximate</td>
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</tr>
<tr>
<td>Tribenuron</td>
<td>6530</td>
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<td>6530</td>
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</tr>
<tr>
<td>Aldoxicarb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

85% of all pesticides successfully detected by 6530 QTOF in tomato, pepper, leek, orange matrices
THANK YOU!