Simultaneous quantitative determination and screening of pesticides using Orbitrap MS Technology

Hans Mol, Paul Zomer, Marc Tienstra
Outline

Introduction pesticide residue analysis
Instrument and method (LC)
Quantitative analysis
Identification
Qualitative screening
What about GC?
Conclusions
Pesticide residue analysis in food

The analytical challenge: theory

Pesticide Manual: **1630 entries**
- World: ~700 in use, others obsolete
- EU: 462 approved

But: residues imported illegal pesticides but not gone

100s of different food matrices of varying complexity

MRLs: 0.01-10 mg/kg
Pesticide residue analysis in food

The analytical challenge: practice

Detection rate of pesticides amenable to LC-MS based multi-residue analysis

- 89 never found, 15 only once, in 10,000 samples
- Quantitative analysis with extensive AQC = waste of time
- Qualitative analysis with automated detection more appropriate

127 pesticides ≥2x found in 10,000 samples

**Quantitative** analysis with extensive AQC justified:
- Manual check XICs
- LOQ
- Linearity
- Recovery
- Repeatability
- Measurement uncertainty

Data 2011-2013 compiled from NVWA, https://www.vwa.nl/

Detection frequency in ~10,000 fruit/veg samples
The challenge and the solution

Analysis request:
a) are any pesticides present; b) if so: at what level?

New solution:

Solution 1: (majority of routine labs)
- LC-MS/MS (triple quad)
- Quantitative analysis
  - ~250 pesticides

Solution 2:
- LC-Q-HRMS
- Quantitative analysis for usual suspects
- Qualitative analysis for others

Solution 3:
- LC-MS/MS (triple quad)
- + LC-fs-HRMS (TOF, Orbitrap™ technology)
- Quantitative analysis
  - ~250 pesticides
- Qualitative analysis
  - >500 pesticides
Outline work flow

1. Sample
   - homogenisation

2. homogenised sample
   - extraction/cleanup

3. Extract

4. LC-Q-HRMS analysis
   - quantification + identification
   - detection (identification)

5. Raw data
   - < LOQ or xx mg/kg
   - positive or negative

6. Positive?
   - positive or negative
   - Re-run with calibrants
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Instrument used

Thermo Scientific™ Exactive™ Plus MS

**Thermo Scientific™ Q Exactive™ MS**
Thermo Scientific™ Q Exactive™ Focus MS
Thermo Scientific™ Q Exactive™ Plus MS
Thermo Scientific™ Exactive™ Plus EMR MS
Thermo Scientific™ Q Exactive™ HF MS

Resolution FWHM @ m/z 200 (scan speed)
17,500 (12 Hz); 35,000 (6 Hz); 70,000 (3 Hz); 140,000 (1.5 Hz) [Focus: up to 70,000]
m/z 50-6000 (2000)
Mass accuracy: internal < 1 ppm RMS; external < 3 ppm RMS
Polarity switching: one full cycle pos&neg in <1 sec (R=35,000)
Variable precursor ion isolation width selection from 0.4 Da to full mass range

vDIA is not available in the U.S.
Various acquisition options

**Non-target acquisition**

- without fragmentation (Full Scan)
- with fragmentation in HCD cell
  - AIF = all-ion-fragmentation
  - vDIA = variable Data Independent Acquisition

**Targeted acquisition**

- without fragmentation (SIM = Selected Ion Monitoring)
- with fragmentation
  - ddMS/MS = data-dependent MS/MS with inclusion list
  - t-MS/MS = targeted MS/MS
  - PRM = Parallel Reaction Monitoring

**Combinations of the above**

vDIA is not available in the U.S.
Full scan acquisition

FS: m/z 100-1000

Upper & lower m/z cut-off
Set up of acquisition method: full scan

General source parameters, AGC settings: TFS default recommendations

Full scan measurement:
m/z range: 135-1000
mass resolution: \( \geq 50,000 \) for reliable mass accuracy in complex samples to ensure for selectivity and quantification*
Here: 70,000 FWHM @ m/z 200

* Kellmann et al, JASMS, 2009, 20, 1464–1476
Extracting pesticides from the raw data

Extract signal of exact mass ± x Da (ppm), e.g. Dimethoate $[\text{M+H}]^+ 230.0069 \pm 5\text{ ppm} (\pm 0.0012 \text{ Da})$

- M/z 230.0069
  - C$_5$H$_{12}$NO$_3$PS$_2$
  - Dimethoate

- M/z 230.0536
  - C$_9$H$_6$F$_3$N$_3$O
  - Flonicamid

- M/z 365.1449
  - C$_{19}$H$_{25}$CIN$_2$OS
  - Pyridaben

- M/z 202.0854
  - C$_7$H$_{12}$CIN$_5$
  - Simazine

- M/z 343.5290
  - C$_{12}$H$_{14}$N$_4$O$_4$S$_2$
  - Thiophanate-methyl

Leek spiked @ 10 ppb,
Full scan m/z 135-1000; Res = 70,000
Set up of acquisition method: fragmentation

Generation of fragments:
1) needed for identification, 2) improve screening selectivity

For optimum detection and identification:
full scan acquisition without and with fragmentation in 1 run

Non-targeted fragmentation:
two options: AIF and vDIA

vDIA is not available in the U.S.
Combined Full scan + AIF acquisition

vDIA is not available in the U.S.
Combined Full scan + AIF acquisition

FS: m/z 100-1000
AIF 1 m/z 100-1000
FS: m/z 100-1000
AIF 1 m/z 100-1000

Upper & lower m/z cut-off

vDIA is not available in the U.S.
Combined Full scan + vDIA acquisition

- FS: m/z 100-1000
- v: 100-200
- v: 200-300
- v: 300-400
- v: 300-400
- v: 500-1000
FS: m/z 100-1000

Upper & lower m/z cut-off
m/z isolation window

vDIA is not available in the U.S.
Combined Full scan + vDIA acquisition

FS: m/z 100-1000

Upper & lower m/z cut-off
m/z isolation window

vDIA is not available in the U.S.
Combined Full scan + vDIA acquisition

FS: m/z 100-1000
 lied: 100-200  lied: 200-300  lied: 300-400  lied: 300-400  lied: 500-1000  FS: m/z 100-1000

Upper & lower m/z cut-off
m/z isolation window

vDIA is not available in the U.S.
Combined Full scan + vDIA acquisition

<table>
<thead>
<tr>
<th>FS: m/z 100-1000</th>
<th>500-1000</th>
<th>4: 100-200</th>
<th>4: 200-300</th>
<th>4: 300-400</th>
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</table>

Upper & lower m/z cut-off
m/z isolation window

vDIA is not available in the U.S.
AIF vs. vDIA

Dimethoate 10 ppb in wheat

- **FS**
  - m/z 135-1000
  - RP = 70,000

- **AIF**
  - m/z 67-1000
  - RP = 70,000

- **vDIA**
  - m/z 195-305
  - RP = 35,000

Carbaryl 10 ppb in wheat

- **AIF**
  - m/z 67-1000
  - RP = 70,000

- **vDIA**
  - m/z 195-305
  - RP = 35,000

⇒ vDIA preferred: improved selectivity & sensitivity + beneficial for identification

vDIA is not available in the U.S.
Dealing with exception 1: interfering analytes

Simazine $C_7H_{12}ClN_5$ and Carbaryl $C_{12}H_{11}NO_2$: difference $[M+H]^+ = 0.9$ mDa (4 ppm)

XIC $202.0854 \pm 5$ ppm:
- $202.0844-202.0864$

Simazine $[M+H]^+ 202.0854$

XIC $202.0863 \pm 5$ ppm:
- $202.0853-202.0873$

Carbaryl $[M+H]^+ 202.0863$

Simazine Fragment 124.0869

Carbaryl Fragment 145.0648

Spiked LeekFS 70K, vDIA 35K

vDIA is not available in the U.S.
Dealing with exception 2: interfering matrix

Thiophanate-methyl in leek spiked @10 ppb

\[
\begin{align*}
(M+H)^+ &= 343.0529 \\
(M+1)^+ &= 344.0563 \quad (^{13}C, \quad ^{15}N) \\
(M+2)^+ &= 345.0487 \quad (^{34}S) \\
\end{align*}
\]

\[
C_{12}H_{14}N_4O_4S_2
\]

Fragment 1: 151.0325 \( (C_7H_7N_2S)^+ \)

Fragment 2: 93.0573 \( (C_6H_7N)^+ \)

Spiked Leek FS 70K, vDIA 35K

vDIA is not available in the U.S.
Method used

Sample preparation: QuEChERS (AOAC version)
10 g homogenised sample + 10 mL Acetonitrile/1% HAc
Shake 30 min
4 g MgSO₄ + 1 g NaAc, centrifuge (no dSPE cleanup)
Dilute acetonitrile phase 1:1 with water

LC: Thermo Scientific™ Dionex™ UltiMate™ 3000 system:
Injection: 5 µL
Column: 100×3 mm ID, 3 µm Atlantis T3; T=35°C
Gradient: water/methanol, 2 mM NH₄HCOO
Flow: 0.30 mL/min

HRMS: Q Exactive MS with H-ESI-II source
Heated capillary: 320°C

FS+vDIA
Cycle time 978 ms
full scan: no fragmentation
m/z 135-1000@70K

HCD: 30 and 80 NCE, ACG: 10⁶

Data handling: Thermo Scientific™ TraceFinder™ 3.2 software

vDIA is not available in the U.S.
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## Quantitative validation

Frequently found + others to widen range of phys/chem properties

<table>
<thead>
<tr>
<th>Active Ingredient</th>
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<td>Mesosulfuron-methyl</td>
<td>Pinoxaden</td>
<td>Spiromesifen</td>
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</tbody>
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*in red = ESI−*
Quantitative data review

Review by pesticide (compound view):
XIC mass extraction window: ±5 ppm
For each quan pesticide: click through the samples and check assignment/integration of quantifier (main adduct) and qualifier (fragment), adjust when needed

Quantifier OK
Qualifier OK
Quantitative data review
Verification of linearity

**ng/mL Solv. Lett Oran**

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**ng/mL area Orangyl**

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**ng/mL area Spinosyn-A**

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**ng/mL area Thiophanate-methyl**

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Recoveries and RSDs

Recovery range

RSD range

Recovery range

RSD range
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### Identification

**Guidance document:** EU SANCO/12571/2013

**Chromatography:**
\[ t_r > 2t_0 \]; retention time deviation $< \pm 0.2 \text{ min}$

**Mass spectrometry**

<table>
<thead>
<tr>
<th>Table 4. Identification criteria for different MS techniques</th>
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<tbody>
<tr>
<td><strong>MS mode:</strong></td>
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<tr>
<td><strong>Typical systems (examples):</strong></td>
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<tr>
<td><strong>Acquisition mode:</strong></td>
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<tr>
<td><strong>Requirements for identification:</strong></td>
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<tr>
<td><strong>Ion ratio(s):</strong></td>
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</tbody>
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### Table 4. Identification requirements for different MS techniques

<table>
<thead>
<tr>
<th>MS detector / characteristics</th>
<th>Typical systems (examples)</th>
<th>Acquisition</th>
<th>Requirements for identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit mass resolution</strong></td>
<td>quadrupole, ion trap, TOF</td>
<td>full scan, limited m/z range, SIM</td>
<td>3 ions</td>
</tr>
<tr>
<td><strong>MS/MS</strong></td>
<td>triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap</td>
<td>selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>2 product ions</td>
</tr>
<tr>
<td><strong>Accurate mass measurement</strong></td>
<td>High resolution MS: (Q-)TOF (Q-)Orbitrap, FT-ICR-MS sector MS</td>
<td>full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof</td>
<td>2 ions with mass accuracy ≤ 5 ppm²,³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined single MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>2 ions: 1 molecular ion or adduct ion with mass acc. ≤ 5 ppm, 1 MS/MS product ion</td>
</tr>
</tbody>
</table>

1) For definition of terms relating to mass spectrometry see Murray et al. (2013) Pure Appl. Chem., 85:1515–1609
2) preferably including the molecular ion or adduct ion ([M-H], [M+H]⁺, [M+NH₄]⁺, M+Na⁺, etc)
3) including at least one fragment or product ion

EU SANCO/12571/2013 under revision......
Table below: under construction/discussion......
Ion ratio

Full scan acquisition with/without fragmentation:

⇒ Various options for ratio determination:

\[
\begin{align*}
\text{area F2} & \quad \text{area F1} \\
\text{area [M+H]^+} & \quad \text{area [M+Na]^+} \\
\text{area F2} & \quad \text{area [M+H]^+} \\
\text{area F1} & \quad \text{area [(M+2)+H]^+}
\end{align*}
\]

difenoconazole \( C_{19}H_{17}Cl_2N_3O_3 \) in Lettuce (10 ppb)

\[
\begin{align*}
[M+H]^+ &= 406.07190 \\
[M+Na]^+ &= 428.0539 \\
[(M+2)+H]^+ &= 408.0600 \quad (^{37}\text{Cl})
\end{align*}
\]

Fragment 1: 251.0025 \( [C_{13}H_9OCl_2]^+ \)

Fragment 2: 188.0387 \( [C_{12}H_9Cl]^+ \)

vdIA is not available in the U.S.
## Identity confirmation

### Examples: isopyrazam and clofentezine

<table>
<thead>
<tr>
<th>Solvent standards isopyrazam</th>
<th>Solvent standards clofentezine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mL</td>
<td>ion ratio (%)</td>
</tr>
<tr>
<td>5</td>
<td>5.08</td>
</tr>
<tr>
<td>10</td>
<td>5.40</td>
</tr>
<tr>
<td>50</td>
<td>5.10</td>
</tr>
<tr>
<td>100</td>
<td>4.68</td>
</tr>
<tr>
<td>250</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Reference ion ratio: 5.11

- tolerance -30%: 3.58
- tolerance +30%: 6.65

<table>
<thead>
<tr>
<th>µg/kg</th>
<th>Lettuce</th>
<th>Orange</th>
<th>µg/kg</th>
<th>Lettuce</th>
<th>Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4.62</td>
<td>5.53</td>
<td>10</td>
<td>53.28</td>
<td>50.05</td>
</tr>
<tr>
<td>50</td>
<td>4.95</td>
<td>4.88</td>
<td>50</td>
<td>52.16</td>
<td>50.08</td>
</tr>
<tr>
<td>200</td>
<td>4.97</td>
<td>3.87</td>
<td>200</td>
<td>49.77</td>
<td>51.10</td>
</tr>
</tbody>
</table>

vDIA is not available in the U.S.
Outcome Quantitative Method Validation

Selectivity: no significant response in blank lettuce and orange

Adequate linearity in most cases

Recovery and RSD\textsubscript{r} meet requirements for majority of pesticides

- exceptions included: acequinocyl, aminopyralid, clopyralid, quinmerac, fluroxypyr, triclopyr

Quantitative performance and identification capabilities similar to triple quadrupole MS/MS / fit-for-purpose

=> Q Exactive suitable to replace triple quad
Outline

Introduction pesticide residue analysis
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Qualitative screening
What about GC?
Conclusions
Qualitative screening: method set up

Same raw data, different data review

High number of target pesticides, low probability of detection
Manual verification of all XICs too time consuming
⇒ Automated pesticide detection by the software

Various options:
TraceFinder SW (screening module), Thermo Scientific™ ToxFinder™ ID software, ....
Here: quan module (but without any quan)

Default settings for pesticide detection:
Mass extraction window: exact m/z ±5 ppm
Time window: database RT 0.5 min
Requirement: signal found for pre-set adduct AND fragment ion
Output: report of samples showing only pesticides found
Review by sample (sample view):
For each sample, click through the pesticides found:
Check: 2 peaks present? Matching peak profile/RT?
Optional: isotope pattern, additional fragments

<table>
<thead>
<tr>
<th>Sample</th>
<th>Active</th>
<th>Flags</th>
<th>Status</th>
<th>Filename</th>
<th>Sample Type</th>
<th>Height</th>
<th>Area</th>
<th>Expected RT</th>
<th>Actual RT</th>
<th>Compound</th>
<th>Height</th>
<th>Area</th>
<th>Expected RT</th>
<th>Actual RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>QEx_140918_021</td>
<td>Unknown</td>
<td>std 250 mg/l solvent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>QEx_140918_029</td>
<td>Unknown</td>
<td>std 250 mg/l solvent</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>QEx_140918_030</td>
<td>Unknown</td>
<td>Blank Lettuce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>QEx_140918_034</td>
<td>Unknown</td>
<td>Blank Lettuce</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Prosulfocarb?

⇒ Reject
Screening: data review

Terbutylazine?

(upon quantification: << 1 ppb)
Screening method: validation

Guidance document: EU SANCO/12571/2013*

Initial validation:
Required for each individual pesticide, for each commodity group
Establish SDL: screening detection limit = lowest concentration for which it has been demonstrated that a pesticide can be detected in ≥95% of the samples

≥20 samples (m matrices in n-fold, with n≥2) reflecting scope of laboratory
Spike each sample at anticipated SDL
Include a blank for each matrix

Supplemented by on-going validation (QC sample added to routine analysis):
Cover additional matrices
Demonstrate performance over time/routine conditions

Criteria:
False negative rate ≤ 5%
False positive rate: no requirement
(any detect triggers identification/quantification/confirmatory analysis)

Validation parameters:
Count # pesticides found in each sample
⇒ detection rate / false negatives
⇒ blank samples: false positives
Screening method: validation

Overall detections in spiked samples (4026 pesticide/matrix combinations per level):
0.01 mg/kg: 91.9%
0.05 mg/kg: 97.2%
0.20 mg/kg: 98.3%

Detection rates in % of spiked pesticides / sample:
Screening method: validation

Screening detection limits:

![Graph showing the number of pesticides detected at different fortification levels (µg/kg).]
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GC-full scan MS

Required for further coverage + highly useful complementary technique

> 1990s: GC-EI-single quad / GC-ion trap / TOF
> mid 2000s: GCxGC-EI-hs-TOF-MS
> mid 2000s: GC-EI-hr-TOF-MS (RP 5-10K)

> 2010: GC-EI-hr-TOF-MS and GC-EI-Q-TOF-MS (RP 15-25K)
GC-APCI-Q-TOF-MS (RP > 20K)

APCI: con: can’t use EI-MS libraries
pro: molecular ion or adduct ion
generation of fragment ions, same approach as in LC-ESI-HRMS

EI: pro: simple, one acquisition event to get multiple accurate mass ions
use of existing EI-MS libraries 100thousands of compounds
con: molecular ion not always present

> 2015: GC-EI-Orbitrap MS (RP >60K @ m/z 200)
Mass accuracy in complex matrix RP 15,000

GC-EI-Orbitrap MS
RP = 15,000
FWHM @ m/z 200

XIC m/z 179.11789 ± 25 ppm
Diazinon fragment C\textsubscript{10}H\textsubscript{15}N\textsubscript{2}O\textsuperscript{+}

MS spectrum
profile m/z 179

+15 ppm
Mass accuracy in complex matrix RP 60,000

GC-EI-Orbitrap MS
RP = 60,000
FWHM @ m/z 200

XIC m/z 179.11789 ± 5 ppm
Diazinon fragment C_{10}H_{15}N_{2}O^+

MS spectrum profile m/z 179

+0.06 ppm

RIKILT
WAGENINGEN UR
GC-Orbitrap MS: example kresoxim-methyl

Exact mass most abundant fragment ions:

\[ C_{11}H_{12}NO_3^+ \quad 206.08117 \]
\[ C_9H_9N^+ \quad 131.07295 \]
\[ C_8H_6N^+ \quad 116.04948 \]
Simulated unit resolution MS: MEW: ± 500 mDa

1 µL inj. GC-Orbitrap MS
Leek spiked @ 10 ppb,
Full scan m/z 50-500; Res = 60,000
Narrowing down the MEW: ± 100 ppm

1 µL inj. GC-Orbitrap MS
Leek spiked @ 10 ppb,
Full scan m/z 50-500; Res = 60,000
Narrowing down the MEW: ± 25 ppm

1 µL inj. GC-Orbitrap MS
Leek spiked @ 10 ppb,
Full scan m/z 50-500; Res = 60,000
Narrowing down the MEW: ± 5 ppm

1 µL inj. GC-Orbitrap MS
Leek spiked @ 10 ppb,
Full scan m/z 50-500; Res = 60,000
Comparison with GC-MS/MS (triple quad)

Kresoxim-methyl in leek @ 10 ppb
1 µL inj. GC-MS/MS (TSQ 8000 Evo)
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Conclusions

**Acquisition:**
Full scan combined with vDIA: optimum way of non-targeted measurement; provides best sensitivity, selectivity, fragments without sacrificing scope

**Quantification [top 100-150 frequently found, with calibrants]:**
Performance comparable with triple quadrupole instruments, sensitivity fit-for-purpose for pesticide residue analysis

**Identification:**
Meets EU requirements (SANCO/12571/2013)

**Screening [for the other 100s, without calibrants]:**
Fully automated output, low # false positives, easy manual accept/reject of hits
Overall detection rate 92% @ 10 ppb
SDLs 10 ppb for majority of pesticides tested

GC-Orbitrap MS highly promising to complement LC-based quan/qual analysis

vdIA is not available in the U.S.
Acknowledgement

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Thank you for your attention!