Analytical methods for determination of pesticides:

An Overview

Michele Mazzetti,
What is a Pesticide?

A 'pesticide' is something that prevents, destroys, or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport. The term includes, amongst others: herbicides, fungicides, insecticides, acaricides, nematicides, molluscicides, rodenticides, growth regulators, repellents, rodenticides, and biocides.

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.), including insecticide, herbicide, fungicide, and various other substances used to control pests.

http://ec.europa.eu/food/plant/pesticides_en

(EPA, 2009)
Definition of pesticide varied with times and countries. However, the essence of pesticide remains basically constant, i.e., it is a (mixed) substance that is poisonous and efficient to target organisms and is safe to non-target organisms and environments (hopefully n.d.r.)
History of pesticides

in the first phase (the period before 1870s) natural pesticides, for instance sulfur in ancient Greece, were used to control pests;

the second phase was the era of inorganic synthetic pesticides (the period 1870s-1945). Natural materials and inorganic compounds were mainly used during this period

the third phase (since 1945) is the era of organic synthetic pesticides. Since 1945, the man-made organic pesticides, e.g., DDT, 2,4-D, and later HCH, dieldrin, have terminated the era of inorganic and natural pesticides.

History of pesticides

In the earlier period of organic synthesized pesticides, there were mainly three kinds of insecticides,

- Carbamated insecticides:

- Organophosphorus insecticides

- Organochlorined insecticides.

Sooner after that herbicides and fungicides achieved a considerable development as well

Consumption of pesticides

The consumption of insecticides is estimated to decline gradually and the use of herbicides would be popular in the future. This trend may be found from the changes of the structure of pesticide consumption worldwide.

Worldwide consumption of pesticides (2013)

...for example Glyphosate

Consumption of pesticides

Note that pesticide sales in North America haven't grown very much — and usage actually seems to be declining in the United States (more on that below). The growth in Europe, meanwhile, is largely driven by a big uptick in sales in Eastern Europe. Meanwhile, sales are more or less stagnant in the Middle East and Africa.

The circulation of pesticides in nature (including crops)
The effects of using pesticides

Positive

- improvement in personal hygiene following the destruction of domestic insects (fleas, lice, ants)
- increased production of milk, eggs, meat and leather
- edible crop yields much increased
- food losses reduced during storage and transport
- limitation or elimination of many infectious diseases and epidemics transmitted by insects among farm animals and birds
- enhanced durability of industrial products like paper and textiles, and the prolonged usage of roads, railway lines and airports as a result of weed destruction

Negative

- contamination of water bodies and soils by pesticides carried by the wind or leached by torrential rains
- resistance of pathogens and pests to poisons
- destruction of all useful organisms inhabiting a given area
- direct threat to human health and life; accumulated in the body, they may be carcinogenic, neurotoxic, and may disrupt hormonal and enzymatic regulation

Trend in Analytical Chemistry, Vol. 30, No. 6, 2011
Article 3
Definitions
(d) ‘maximum residue level’ (MRL) means the upper legal level of a concentration for a pesticide residue in or on food or feed set in accordance with this Regulation, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers;
• 97% of samples analysed were within legal limits.
• Of these, 53.6% were free of quantifiable residues and 43.4% contained residues that were within permitted concentrations.
• Of the samples originating from EU/EEA countries, 1.6% contained residues exceeding legal limits; the corresponding figure for samples from third countries was 6.5%.
• No quantifiable residues were found in 91.8% of baby food samples.
• 98.8% of organic products were either free of residues or contained residues within legal limits.

EFSA concluded that exposure is unlikely to pose a threat to human health.
The 2014 European Union Report on Pesticide Residues in Food
European Food Safety Authority

Linked to bees colony collapse disorder
The analysis of pesticides in biological samples continues to present challenges to analysts.
A number of problems crop up in the analysis of pesticide residues:
(1) the complexity and the diversity of matrices in biological materials;
(2) the low concentrations of pesticides in samples of fruit and vegetables.
Target analytes must, therefore, be isolated from matrices and then be enriched before the final determination can be undertaken.

**The main stages in analytical procedures for determining pesticides in samples of fruit and vegetables**

**SAMPLING**

**FIXING, TRANSPORT AND STORAGE**

**EXTRACTION OF PESTICIDES FROM THE SAMPLE**

**EXTRACT CLEAN UP AND PREPARATION FOR ANALYSIS**

**IDENTIFICATION AND DETERMINATION OF ANALYTES**
Multiresidue Methods (MRMs): 

**Aim of MRMs:**
Cover as many pesticides as possible from a single sample portion employing a single sample preparation procedure

But, still more than one determinative analysis run is required to cover all analytes of interest with sufficient selectivity and sensitivity...

The broader the spectrum of analytes covered by the MRM,
- The less additional methods are required to cover all analytes
- The more efficient and economical the analysis
- Less time, personnel, materials...

Michelangelo Anastasiades, Stuttgart, 2006
The first notable MRM was the Mills method developed in the 1960s by U.S. Food and Drug Administration (FDA) chemist P.A. Mills.
ACETONE EXTRACTION METHOD

In 1975, Milton Luke and colleagues at the U.S. Food and Drug Administration (FDA) introduced a new method for multiclass, multiresidue pesticide analysis of fruit, vegetable, grains, and other food samples.

1) Blend water/acetone
2) Filter

This method, which became widely known as the “Luke method,” was able to achieve high recoveries for the major types of pesticides used at that time (e.g., organochlorines, organophosphates, organonitrogens).
The use of multiple selective detectors in gas chromatography (GC), such as electron-capture detection (ECD), flame photometric detection (FPD), electrolytic conductivity detection (ELCD or Hall detector), and nitrogen phosphorus detection (NPD), allowed an expanded scope over common previous methods, which generally were effective only for single class of pesticides, such as organochlorines using GC-ECD.

For many years to follow, analytical technologies continued to improve and agrochemical companies registered many more pesticides from different classes. Although the registration process often required companies to first test the ability of the Luke method to recover the newly registered pesticides, fewer modern pesticides could be included in the FDA multiclass monitoring method, and this required the companies to develop single analyte methods in the registration process to be used for enforcement. However, monitoring labs had too few resources to use the typically very complicated methods for so many different pesticides, and little or no monitoring was done of those types of pesticides.
ACETONE EXTRACTION METHOD

In terms of analytical technology, mass spectrometry (MS) was coupled to GC in the commercial bench-top instruments during the 1980s, and they were initially used for qualitative confirmation purposes in pesticide analyses. In the 1990s, the performance features of the instruments improved to the point that detection limits were acceptably low enough that GC-MS could be used to replace selective GC detectors for quantitative as well as qualitative analysis and reduce the need for multiple injections in GC. By the late 1990s, GC-MS had become commonplace in monitoring labs.

HP 5890 (GC) coupled with HP 5972 (single quadrupole)
ACETONE EXTRACTION METHOD

Additionally, the price reduced and performance improved for high quality commercial bench-top LC-MS/MS (tandem mass spectrometry) instruments. This allowed multiclass, multiresidue analysis of many LC-type pesticides that could previously be detected only by single-analyte methods.
This European Standard contains the following methods that have been subjected to interlaboratory studies and/or are adopted throughout Europe:

**method M**: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum if necessary clean-up on Florisil®

**method N**: Extraction with acetone, liquid-liquid partition with dichloromethane or cyclohexane/ethyl acetate and clean-up with gel permeation and silica gel chromatography;

**method P**: Extraction with ethyl acetate and, if necessary, clean-up with gel permeation chromatography
ACETONE EXTRACTION METHOD

However, the Luke method, which used acetone for extraction and partitioning from water with a combination of methylene chloride and petroleum ether (and addition of salt for more polar pesticides), was not sufficiently effective, environmentally-friendly, safe, and efficient enough for “twenty-first century” standards.

Even 12393-1 is really complicated and the consumption of solvent is very high.

GPC profile from EPA 3640
QuEChERS METHOD

Generic steps of the QuEChERS technique

A) homogenized sample
B) weight 10 grams of sample
C) 10 mL of CH₃CN and shake (1 min.)
D) add extraction salts and shake
E) centrifuge
F) dSPE cleanup of an aliquot of extract
G) shake the dSPE tube and centrifuge
H) the sample is ready for analysis

Friday 31st May - Morning

10.45-11.10  Quick, easy, effective, rugged, and safe (QuEChERS) approach for the determination of pesticide residues
Steven J. Lehotay, USDA, Agricultural Research Service, Wyomissing, PA, U.S.A.
**QuEChERS METHOD**

Streamlined aspects of QuEChERS

1. reduced subsample size from a thoroughly homogenized sample
2. extraction by shaking of sample with solvent in a centrifuge tube
3. partitioning of water from the sample using MgSO4 in combination with other salt(s)
4. centrifugation to separate the extract from the water and non-soluble material rather than filtration
5. taking an aliquot of the extract rather than trying to collect the entire portion
6. use of internal standard(s) to improve accuracy and precision of the results rather than having to make calculations of extract volume depending on water content of the sample
7. injection of the same extract, preferably without solvent exchange or concentration steps, in both GC-MS and LC-MS/MS analyses.
Lehotay and Anastassiades realized that the previous work of Fillion (an effective column/cartridge-based cleanup for MeCN pesticide extracts, which had been salted out from water, with a combination of primary secondary amine (PSA), octadecylsilyl (ODS or C18), and graphitized carbon black) was a “chemical filtration” approach in which certain common matrix components in foods (e.g. fatty acids, chlorophyll, sterols, anthocyanins) remained on the sorbents and the MeCN served as the elution solvent for the pesticide analytes.

Anastassiades had the idea to dispense an aliquot of the extract into a centrifuge tube containing loose sorbent(s), and then to take a second aliquot after shaking and centrifugation for analysis.
QuEChERS METHOD

Choice of Acetonitrile as Solvent

**PROs**

- Selective (Few Co-Extractives but still broad pesticide Spectrum covered)
- Compatible with LC-and SPE-Applications
- Not Chlorinated
- Miscible with Water (Good for Initial Extraction)
- Separ. from Water-Phase by Salt-Add. (No Non-Polar Solv. Needed)
- Easier to Remove Water (with MgSO4) than from Acetone

**CONs**

- Difficult to Evaporate
- High Expansion Volume (advisable the use of solvent vent injection mode)
- Not Compatible With NPD (advisable the use of solvent vent injection mode)
- Not Compatible with GPC (But, Lipid-Co-Extraction is Low)
- Rel. Toxic (But, Method Performed in a Closed Vessel, thus minimal exposure)
- Low Lipid Solubility

*Losses of non-polar pesticides (Recov. consistent at same Lipid/solvent ratio)*
*Accessibility problems of pesticides enclosed in Lipid particles (Ultra Turrax)*

Michelangelo Anastassiades, Stuttgart, 2006
Anastassiades had the idea to dispense an aliquot of the extract into a centrifuge tube containing loose sorbent(s), and then to take a second aliquot after shaking and centrifugation for analysis.

By using dSPE, the trappings of traditional cartridge based SPE disappeared, such as needing a manifold, vacuum system, collection tubes, elution solvents, solvent evaporation apparatus, and reliance on limited commercial products.
**QuEChERS METHOD**

**NO-PSA Clean up**

For Acidic pesticides recovery drop at pH 6

Michelangelo Anastassiades, Stuttgart, 2006
**QuEChERS METHOD**

Addition of formic acid (5% in ACN):

Michelangelo Anastassiades, Stuttgart, 2006

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**Tolyfluanid stability in extract**

- **Rec. %**
- **Measured pH in extract**

**pH after PSA**

- pH 4
- pH 5
- pH 6
- pH 7
- pH 8
- pH 9
- MeCN

- 7 days
- 13 days

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**Addition of formic acid (5% in ACN):**
Some pesticides are acid labile. Sulfonylureas, Carbosulfan.

If these compounds are included in the target spectrum use an aliquot of the final extract before acidifying.

Michelangelo Anastassiades, Stuttgart, 2006
## QuEChERS METHOD

### Various versions of QuEChERS Method

#### Step 1 – extraction/partitioning

<table>
<thead>
<tr>
<th>Method</th>
<th>Original QuEChERS</th>
<th>AOAC QuEChERS</th>
<th>Buffered QuEChERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastassiades et al.</td>
<td>2003</td>
<td>AOAC 2007.01</td>
<td>EN 15662</td>
</tr>
</tbody>
</table>

- **Original QuEChERS**: Add 10 mL of MeCN to 10 g homogenized sample in a 50 mL centrifuge tube. Add ISTD, shake intensively.

- **AOAC QuEChERS**: Add 15 mL of 1% HOAc in MeCN to 15 g homogenized sample in a 50 mL centrifuge tube. Add ISTD, shake intensively.

- **Buffered QuEChERS**: Add 10 mL of MeCN to 10 g homogenized sample in a 50 mL centrifuge tube. Add ISTD, shake intensively.

- **MgSO₄ and NaCl**: Add 4 g MgSO₄ and 1 g NaCl. Shake vigorously for 1 minute. Centrifuge for 5 minutes at 5000 rpm.

- **MgSO₄ and NaOAc**: Add 6 g MgSO₄ and 1.5 g NaOAc. Shake vigorously for 1 minute. Centrifuge at >1500 rcf for 1 minute.

- **MgSO₄, NaCl, NaClO₃, and NaOH**: Add 4 g MgSO₄, 1 g NaCl, 1 g NaClO₃ · 2H₂O, 0.5 g Na₂OCl · 0.5H₂O. Shake vigorously for 1 minute. Centrifuge for 5 minutes at 3000 U/min.

#### Step 2 – dispersive SPE clean-up

- **Transfer 1 mL aliquot of supernatant to a micro centrifuge tube containing 150 mg MgSO₄ and 50 mg PSA**: Shake for 1 minute. Centrifuge for 1 minute at 6000 rpm.

- **Transfer 1 mL aliquot of supernatant to a dispersive clean-up tube containing MgSO₄, PSA (Cib, GCB or ChloroFiltr® can be added for additional clean-up)**: Shake for 30 seconds. Centrifuge at >1500 rcf for 1 minute.

- **Transfer 1 mL aliquot of supernatant to a dispersive centrifuge tube containing 25 mg of PSA and 150 mg MgSO₄ (plus 2.5 or 7.5 mg GCB to remove pigments)**: Shake for 30 seconds. Centrifuge for 5 minutes at 3000 U/min.

- **Preserve with toluene for GC/MS or 6.7mM formic acid in MeCN for LC/MS/MS**. Add TFP surrogate.

- **Preserve with 5% formic acid in ACN**. Transfer 0.5 mL to vial for GC/MS or LC/MS/MS analysis.

#### Step 3 – analysis by GC-MS(ESI) or LC-MS/MS

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*Michelangelo Anastassiades, Stuttgart, 2006*

*Open Chem., 2015; 13: 980–1010*
### QuEChERS METHOD

**Internal Standard**

<table>
<thead>
<tr>
<th>Internal Standard</th>
<th>Suitable for</th>
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<tbody>
<tr>
<td>$^{13}$C$_{12}$-Aldrin</td>
<td>GC</td>
</tr>
<tr>
<td>d$_5$-Atrazine</td>
<td>LC and GC</td>
</tr>
<tr>
<td>d$_4$-Carbendazim</td>
<td>LC</td>
</tr>
<tr>
<td>d$_3$-Carbofuran</td>
<td>LC (and GC)</td>
</tr>
<tr>
<td>d$_{10}$-Diazinon</td>
<td>LC and GC</td>
</tr>
<tr>
<td>d$_6$-a-HCH</td>
<td>GC</td>
</tr>
<tr>
<td>d$_6$-Malathion</td>
<td>LC and GC</td>
</tr>
<tr>
<td>d$_6$-Methoxychlor</td>
<td>GC</td>
</tr>
<tr>
<td>d$_{10}$-Parathion</td>
<td>GC</td>
</tr>
<tr>
<td>d$_6$-Parathion-methyl</td>
<td>GC (and LC)</td>
</tr>
<tr>
<td>d$_3$-Propoxur</td>
<td>LC and GC</td>
</tr>
<tr>
<td>Triphenylphosphate</td>
<td>LC and GC</td>
</tr>
<tr>
<td>Triethylphosphate</td>
<td>LC and GC</td>
</tr>
</tbody>
</table>

**An INTERNAL STANDARD MUST**

1. *not occur in the sample to begin with*;
2. *be stable*;
3. *give consistently high recoveries*;
4. *be readily available and inexpensive*;
5. *not interfere with any analytes*;
6. *ideally be readily detected in GC-MS and LC-MS/MS without being affected by matrix effects in either case*

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Suggested internal standards for LC and GC

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*Mass Spectrometry in Food Safety, Humana Press, 2011, S. Lehotay, Chapter 4*
QuEChERS METHOD

Dispersive SPE (dSPE)

PSA not satisfying when high contents of carotenoids or chlorophyll

**Carbon Sorbent is more Effective**

GCB (Graphitized Carbon Black) was best in handling - Used in combination with PSA at small amounts. Cleanup time (shaking) extended from 30 s to 2 min.

**Problem with GCB**

Planar pesticides have a high affinity towards GCB e.g. hexachlorobenzene, chlorothalonil, thiabendazole.

Anthracene may be used as surrogate QC standard. Recoveries > 70% will indicate that no unacceptable losses of pesticides have occurred.

Michelangelo Anastassiades, Stuttgart, 2006
QuEChERS METHOD

Simplified sample preparation challenges

Clean-up efficiency

QuEChERS
LLE
GPC
SPE
IAC

Detection selectivity

GC-HRMS-HRMS, LC-HRMS-HRMS
GC-HRMS, LC-HRMS
GC-MS/MS, LC-MS/MS
GC-MS, LC-MS
HPLC-FLD
GC-ECD, GC-NPD, HPLC-VWD
QuEChERS METHOD

Simplified sample preparation challenges

SAMPLE ➔ QuEChERS ➔ GC-MS/MS ➔ LC-MS/MS

EN 15662 defines as GC tool a simple GC-MS in SIM mode but...

For a series of gates which favor transmission of signal due to analyte over that due to chemical noise, the signal-to-noise ratio for detection of the analyte increases in spite of an attenuation in analyte signal.

Increase of Sensitivity, by the increase of Selectivity

QuEChERS METHOD

RESIDUE ANALYSIS:
BETTER BY GC-MS OR LC-MS/MS?

Distribution of limit of quantification (LOQ) data of all organophosphorus pesticides

Distribution of limit of quantification (LOQ) data of all pesticides/metabolites.

Mass Spectrometry Reviews, 2006, 25, 838–865
The better performance of LC-MS/MS is probably determined by several reasons. Among them the higher injection volume used in LC-MS/MS (20 µL vs. 1 µL) and the lower amount of fragmentation during ionization (ESI vs. EI) may explain some of these differences.

The use of GC-MS/MS introduces some little variations in the framework.

**BUT**

The high extent of the fragmentation still remain as a unfavorable factor.

Mass Spectrometry Reviews, 2006, 25, 838–865
QuEChERS METHOD

RESIDUE ANALYSIS:
BETTER BY GC-MS OR LC-MS/MS?

Infact, there is another unique feature of pesticide analysis with mass spectrometry. Relative to other contaminants, many pesticides including OCs, OPs, pyrethroids, and chloroacetanilides exhibit low intensity for the molecular ion regardless of whether EI or CI is used. Consequently in SIM mode the quantitative or qualifier ion is rarely selected as the molecular ion. In general >90% of pesticides do not monitor the molecular ion by EI or CI methods as at the working concentration ranges of trace analysis generally the molecular ion is too low in abundance to be observe.

NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

R. Raina,, Pesticides - Strategies for Pesticides Analysis
QuEChERS METHOD

RESIDUE ANALYSIS: BETTER BY GC-MS OR LC-MS/MS?

<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>Unit mass resolution</td>
<td>quadrupole, ion trap, TOF</td>
<td>full scan, limited m/z range, SIM</td>
<td>3 ions</td>
</tr>
<tr>
<td>MS/MS</td>
<td>triple quadrupole, 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>Accurate mass measurement</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 ppm&lt;sup&gt;1c&lt;/sup&gt;,&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution</td>
<td>Analyte peaks in the extracted ion chromatograms must fully overlap.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ion ratio within ±30% (relative) of average or calibration standards from same sequence</td>
</tr>
</tbody>
</table>

<sup>1</sup> preferably including the molecular ion, (de)protonated molecule or adduct ion
<sup>2</sup> including at least one fragment ion
<sup>3</sup> < 1 mDa for m/z < 200
<sup>4</sup> no specific requirement for mass accuracy
<sup>5</sup> in case noise is absent, a signal should be present in at least 5 subsequent scans
QuEChERS METHOD

RESIDUE ANALYSIS: BETTER BY GC-MS OR LC-MS/MS?

Malathion
330.358 Da

**GC-MS/MS MRM transition**

158 → 125
173 → 99

**LC-MS/MS MRM transition**
My personal advice (and experience) is to use GC-MS (better GC-MS/MS) for analysis of non API/ESI-ionizable pesticides (mainly Organochlorine pesticides) and use LC-MS/MS for the other class.
**QuEChERS METHOD**

**RESIDUE ANALYSIS:**

**CHOICE OF ANALYSIS TECHNIQUE**

**TECHNICAL REPORT**
**RAPPORT TECHNIQUE**
**TECHNISCHER BERICHT**

**CEN/TR 16468**
March 2013

ICS 65.100.01; 67.050

**English Version**

Food analysis - Determination of pesticide residues by GC-MS - Retention times, mass spectrometric parameters and detector response information

**TECHNICAL REPORT**
**RAPPORT TECHNIQUE**
**TECHNISCHER BERICHT**

**CEN/TR 16699**
July 2014

ICS 67.050

**English Version**

Foodstuffs - Determination of pesticide residues by GC-MS/MS - Tandem mass spectrometric parameters
QuEChERS METHOD

RESIDUE ANALYSIS:
CHOICE OF ANALYSIS TECHNIQUE

Contains for approx. 500 pesticides:

- CAS-Number
- Ionization method
- Structure of quasimolecular ion
- Mass of parent ion
- Declustering potential
- Mass of two main fragments
- Appropriate collision energies
- Relative retention times
- Classification of response
QuEChERS METHOD

Case Study: Olive Oil

Original Paper

Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives

Variation of 1) QuEChERS-Method for Vegetable Oil Samples (fatty matrix without water)

Analysis of multiple pesticide residues in olives and olive oil using QuEChERS and LC-MS/MS

Sara C. Cunha, Steven J. Lehotay, Katerina Mastovska, José O. Fernandes, M. Beatriz P.P. Oliveira
New Development
LC-HRMS: ORBITRAP

Characteristic frequencies:
Frequency of rotation $\omega_\phi$
Frequency of radial oscillations $\omega_r$
Frequency of axial oscillations $\omega_z$

Hyper-logarithmic potential distribution in the Orbitrap:
“ideal Kingdon trap”

$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2 / 2 + R_m^2 \cdot \ln(r / R_m) \right\}$

$\omega_\phi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left\{ \frac{R_m}{R} \right\}^2 - 1}$

$\omega_z = \sqrt{\frac{k}{m / q}}$

Only this frequency does not depend on energy, angle, etc. and is used for mass analysis

In this configuration, the apparatus is capable to operate in full scan mode with a resolution up to 100000 (1Hz) and an accuracy up to 2 ppm (positive).

- No MRM timetable
- Identification of the analytes on the base of molecular ions

IF NEEDED

Total fragmentation in HCD cell allows the record of an MS/MS spectra (with some limitations)
Glyphosate, is a broad-spectrum herbicide and, without doubts, is the world’s biggest-selling chemical used for weed control in agricultural, silvicultural and urban environments.

Chemical name IUPAC: N-(phosphonomethyl)-glycin

Common Name ISO: GLYPHOSATE

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility</th>
<th>Solvent</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>pH 2: 10.5 ± 0.2 g/l, 20 °C, 995 g/kg</td>
<td>Methanol</td>
<td>0.231 g/l</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.078 g/l</td>
<td>n-Octanol</td>
<td>0.020 g/l</td>
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<tr>
<td>Dichloromethane</td>
<td>0.233 g/l</td>
<td>Propan-2-ol</td>
<td>0.020 g/l</td>
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<tr>
<td>Ethylacetate</td>
<td>0.012 g/l</td>
<td>Toluene</td>
<td>0.036 g/l</td>
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<tr>
<td>Hexane</td>
<td>0.026 g/l</td>
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</table>
Analysis of Glyphosate and AMPA

- Small molecules
- High Polarity
- Zwitterionic Form
- Lack of Chromogenic groups
- Low vapour pressure
- Low Organic solvents solubility
- High Water solubility
Derivatization of Glyphosate and AMPA with FMOC-Cl

Glyphosate

\[
\text{HOPOCNENH}_{\text{OH}}
\]

\[\text{ClO}_{\text{H}}\]

\[
\text{HOPCNENH}_{\text{OH}}
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\[\text{ClO}_{\text{H}}\]

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\text{HOPOCNENH}_{\text{OH}}
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Analysis of Glyphosate and AMPA

Water samples: Stored frozen in plastic bottle

1 ml of HCOOH and filter + 4 ml EDTA Na₄ 1 M + 100 ml H₂O

Thawing

Sub sample of 80 ml + 1600 µl HCL 6M after 2 hours + 1600 µl KOH 6M

Overnight

80 µl ILSs 100 ng/ml + 10 ml Borate buffer 5% + 10 ml FMOC-Cl in CH₃CN 6,5 mM (daily prepared)

SPE

• 9 ml of Methanolic eluate
• Evaporate to dryness
• Reconstitute with 500 µl of HPLC Mobile Phase

HPLC-HRMS analysis
Positive ionization
**LC-HRMS: Full scan**

*Mineral water spiked with Glyphosate / Glyphosate ILS 0,2 µg/l e.a.*
PESTICIDES IN FOOD/FEED: Choice of the Method

Official methods described or recalled in binding EU rules

Methods published on international, regional standards (Supranational) or national

Methods published by technical organizations (ie widely recognized at the level International or national)

Methods published in specialized scientific journals

Methods specified by the manufacturer of Equipment

Methods designed or developed by the laboratory

Legal basis, Directive 85/591, Preamble and Article 2
Why and when we should use standardized methods

- Methods are based on widely accepted methods with sufficient validation data.
- Standards are available in three languages (EN, DE and FR).
- Clear description with all details including calibration and calculation.
- Checked by experts from many member states.
- More easy to convince accreditation bodies

- If analytical results cause international trade barriers.
- As starting point for new laboratories
Main problems in standardization of methods

- Validation requirements not easily to fulfill.
- Editorial process very laborious, because many comments have to be considered.
- Official character of “old” methods may hinder analytical progress

Whenever possible, standardized methods should offer the flexibility to apply methods in a changing “analytical world”, e.g.
In accordance with Article 12 of Regulation 882/2004, laboratories designated for official control of pesticide residues must be accredited to ISO/IEC 17025.

1. The key objectives are:
   (i) to provide a harmonized cost-effective quality assurance system in the EU
   (ii) to ensure the quality and comparability of analytical results
   (iii) to ensure that acceptable accuracy is achieved
   (iv) to ensure that false positives or false negatives are not reported
   (v) to support compliance with ISO/IEC 17025 (accreditation standard)
Validation Model

Fulfilment of legal requirements

Pesticide concept

List of all pesticides analysed in routine

Which pesticide is detected how?

LOD’s/LOQ in different types of matrix reproducibility

Recoveries, 70 – 120 %

Repeatability

Reproducibility
Thanks