Decoding the Complexity: Multiresidue Pesticide Analysis in Food

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Topics to be covered

- **Introduction**
- **Understanding Pesticides**
- **Multiresidue Pesticide Analysis**
- **Techniques for Multiresidue Pesticide Analysis**
- **Conclusion**

Introduction

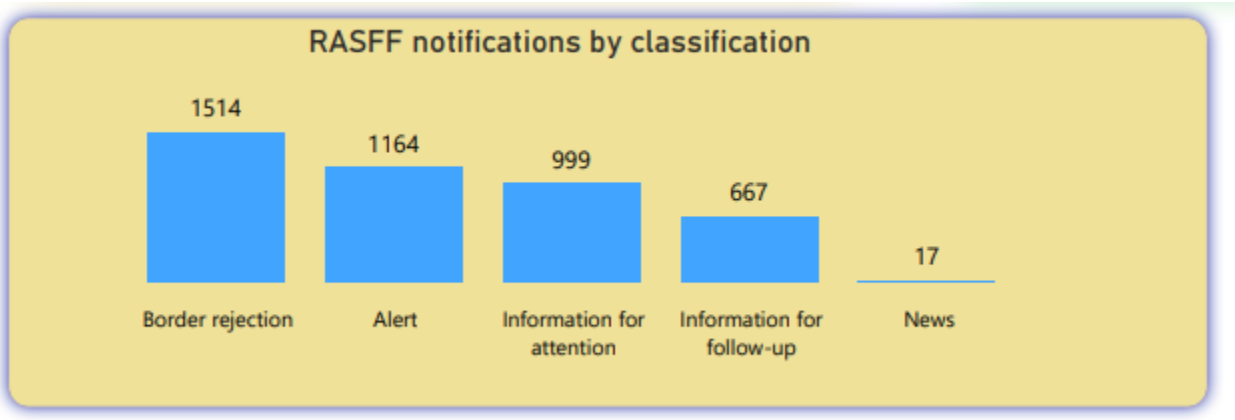
Why Pesticides Important?

Pesticide analysis in food safety is of paramount importance for several reasons:

1. **Protection of Consumer Health:** Pesticides, while beneficial for **crop protection**, can leave **residues** on food, leading to health issues ranging from immediate symptoms like nausea to long-term problems such as cancer and hormonal imbalance
2. **Compliance with Regulations:** Countries enforce **Maximum Residue Limits (MRLs)** for pesticides in food, necessitating pesticide analysis to ensure regulatory compliance
3. **Trade Facilitation:** Pesticide residue analysis certificates are often required for food imports, making such analysis vital for international trade
4. **Environmental Impact:** Pesticide residues can harm the environment by contaminating soil, water, and air. Food analysis for these residues aids in monitoring the environmental impact of farming methods.

RASFF in 2022

- The European Commission published its 2022 report of the Alert and Cooperation Network in June 2023
- For RASFF, 2022 ranked second after 2021 regarding the number of original notifications circulated
- In line with 2020 and 2021, pesticide residues was the most important hazard for health-related cases, with 990 RASFF notifications



The top ten most recurrent RASFF notifications in 2022

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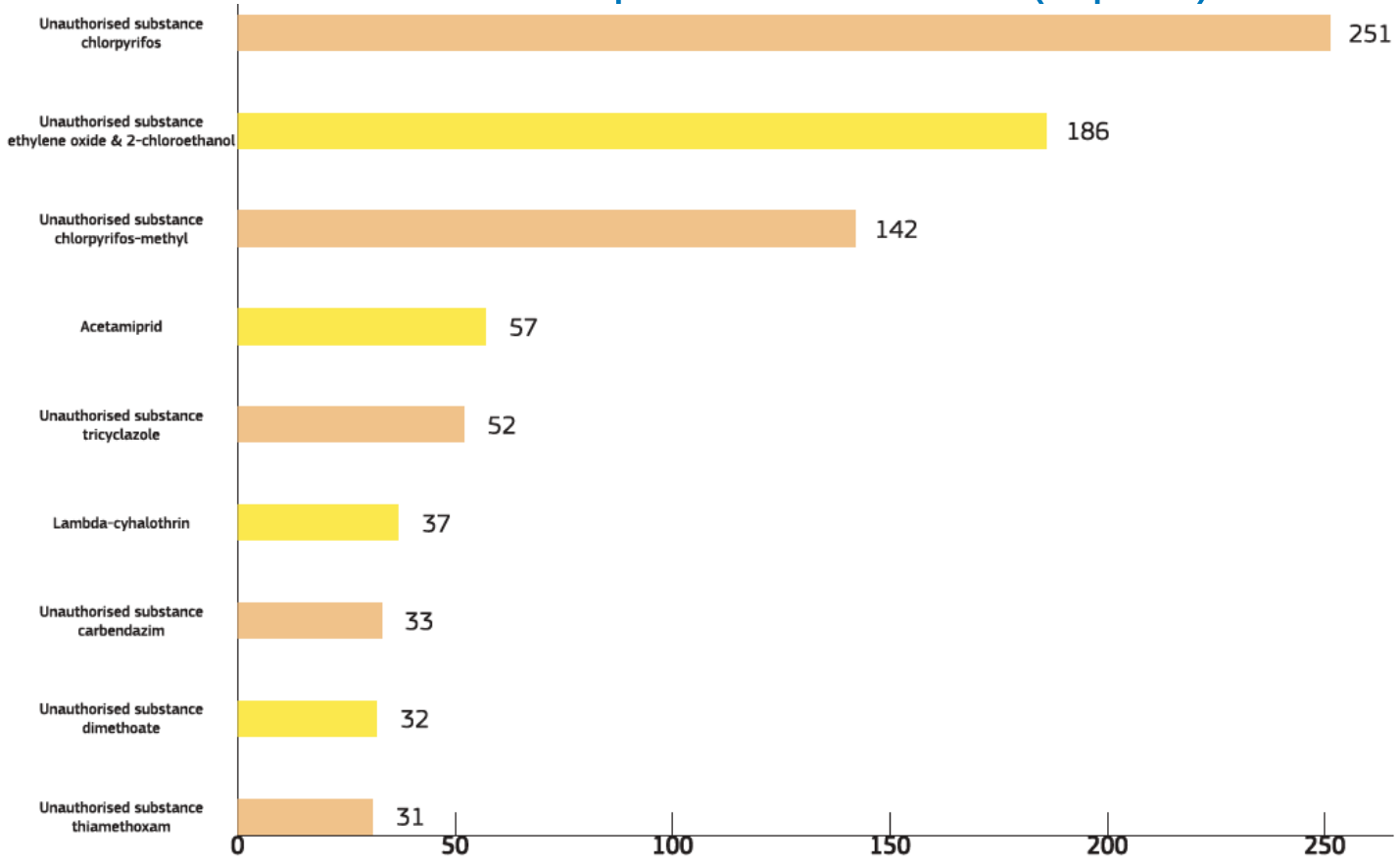
Hazard	Product category	Origin	Total
Pesticide residues	Fruits & vegetables	Türkiye	299
Salmonella	Poultry meat & poultry meat products	Poland	190
Composition	Food contact material	China	76
Salmonella	Nuts, nut products and seeds	Nigeria	66
Aflatoxins	Nuts, nut products and seeds	United States	53
Salmonella	Herbs & Spices	Brazil	46
Aflatoxins	Cereals & bakery products	Pakistan	42
Aflatoxins	Nuts, nut products and seeds	Egypt	40
Aflatoxins	Nuts, nut products and seeds	India	39
Aflatoxins	Nuts, nut products and seeds	Türkiye	39

Number of RASFF notifications on pesticides in food (top 10)



Chlorpyrifos and chlorpyrifos-methyl

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- Chlorpyrifos (sometimes referred to as chlorpyrifos-ethyl) and chlorpyrifos-methyl are insecticides used to control insect pests on a range of crops
- In **2008**, chlorpyrifos products were **authorised** for use in more than 88 countries
- The ecotoxicological and genotoxic properties of chlorpyrifos and chlorpyrifos-methyl lead to adverse effects for human health and the environment
- In **2019**, the approvals of chlorpyrifos and chlorpyrifos-methyl were **withdrawn** and the default **MRL of 0.01 mg/kg** for all commodities became applicable in November 2020 and applies to food produced in the **EU and to imports**

Chlorpyrifos and chlorpyrifos-methyl

- Non-compliant residues of chlorpyrifos and chlorpyrifos-methyl have also been determined during monitoring for official control in the EU
  - Chlorpyrifos in oranges from Egypt, in grapefruits from Turkiye, in tea leaves from China, in sesame seeds from India and in grape leaves from Egypt
  - Chlorpyrifos-methyl in grapefruits, sweet peppers/bell peppers, mandarins and lemons coming from Turkiye
- These insecticides may still be approved for use in these countries and residues are detected in commodities imported in the EU due to the use of non-approved pesticides for which no import tolerance is in place

Method for the determination of residues of chlorpyrifos and chlorpyrifos-methyl in fruit and vegetables

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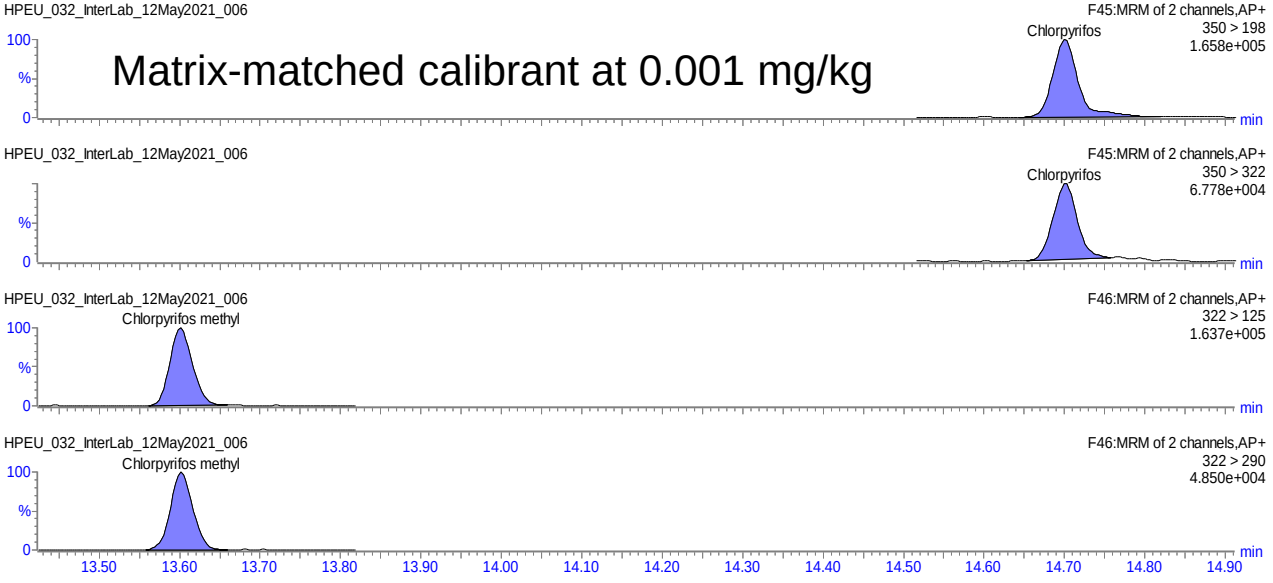
- These analytes are amenable to multi-residue methods such as QuEChERS
- The obtained extracts are typically analysed by either GC-MS/MS or LC-MS/MS
- Chlorpyrifos and chlorpyrifos-methyl were included in Waters app note for high sensitivity analysis of baby food by GC-MS/MS using APGC



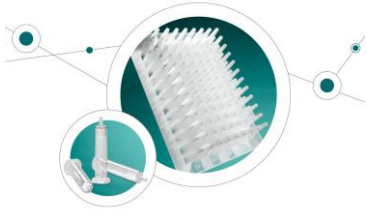
Understanding Pesticides

Are all Pesticides same for a chemist?

Differentiating pesticides for analytical testing involves several steps and techniques. Here's a general overview:

- **Sample Preparation:** In pesticide analysis, the initial step is sample preparation. This includes extraction, cleanup, and concentration of the sample. Depending on the complexity of the matrix, different techniques are used. For complex matrices, SPE and QuEChERS are employed, while direct injection is used for cleaner matrices.

- **Use of Analytical Methods:** To register their products, pesticide manufacturers are required to create and submit analytical methods for their pesticides. These methods frequently utilize chromatographic techniques like gas chromatography (GC) or liquid chromatography (LC), combined with mass spectrometry (MS) for detection



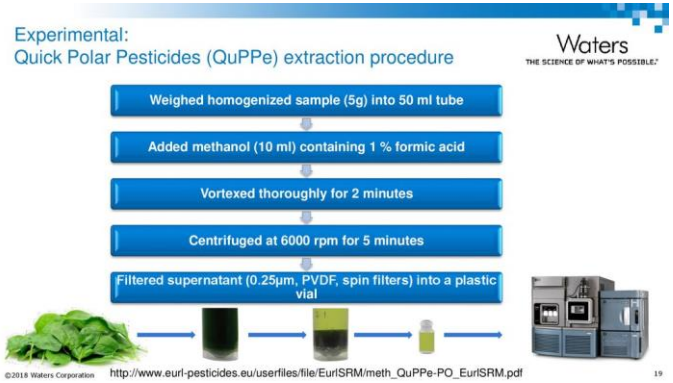
Understanding Pesticides

Are all Pesticides same for a chemist?

Differentiation Based on Chemical Properties: Pesticides have distinct chemical properties like **polarity**, **volatility**, and **stability**. These characteristics influence their behavior during chromatographic separation, enabling the identification and quantification of each pesticide

Use of Multi-Residue Methods: Multi-residue methods, designed to be broad-spectrum, are utilized for detecting and quantifying various pesticides in one analysis, accommodating pesticides with diverse chemical properties

<u>The methods and techniques for pesticide analysis can differ based on the analysis requirements, sample type, and pesticides under test. Always adhere to pertinent guidelines and regulations during the analysis</u>



Multiresidue Pesticide Analysis

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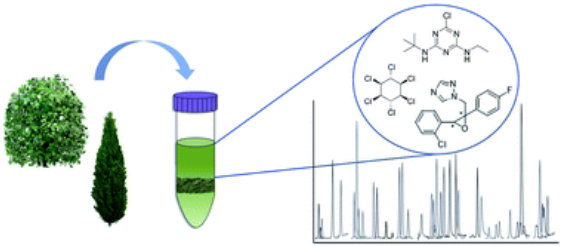
Why the methods should be for Multiresidue Pesticide analysis?

- Multi-residue pesticide analysis is a method used to **detec**t and **quantify multiple pesticide** residues in crop samples



- The term “**pesticides**” describes a broad class of crop-protecting chemicals, including **insecticides, herbicides, and fungicides**

- In response to these issues, governments have started monitoring programs to enforce regulations and guarantee food safety. However, the extensive workload involved in testing numerous pesticide and commodity combinations can be daunting

- Multi-residue analysis methods are crucial as they swiftly analyze numerous compounds, resulting in a greater number of food samples being tested for a wider array of pesticides

Multiresidue Pesticide Analysis

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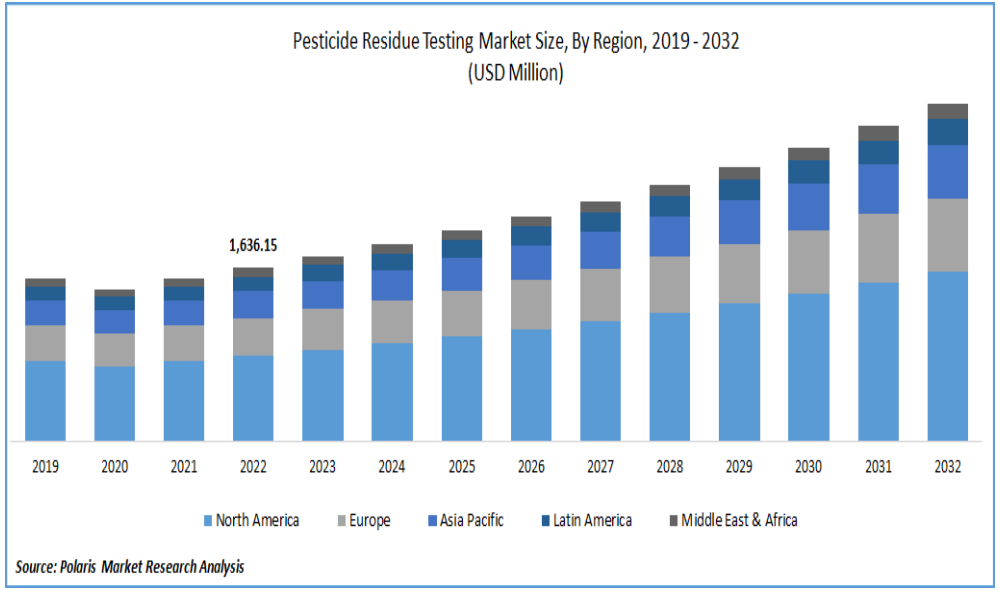
Why the methods should be for Multiresidue Pesticide analysis?

- Over 1,100 chemicals are registered as pesticides and are screened using multi-class, multi-residue protocols. Gas Chromatography (GC) and Liquid Chromatography (LC) are frequently used techniques for this multi-residue pesticide analysis



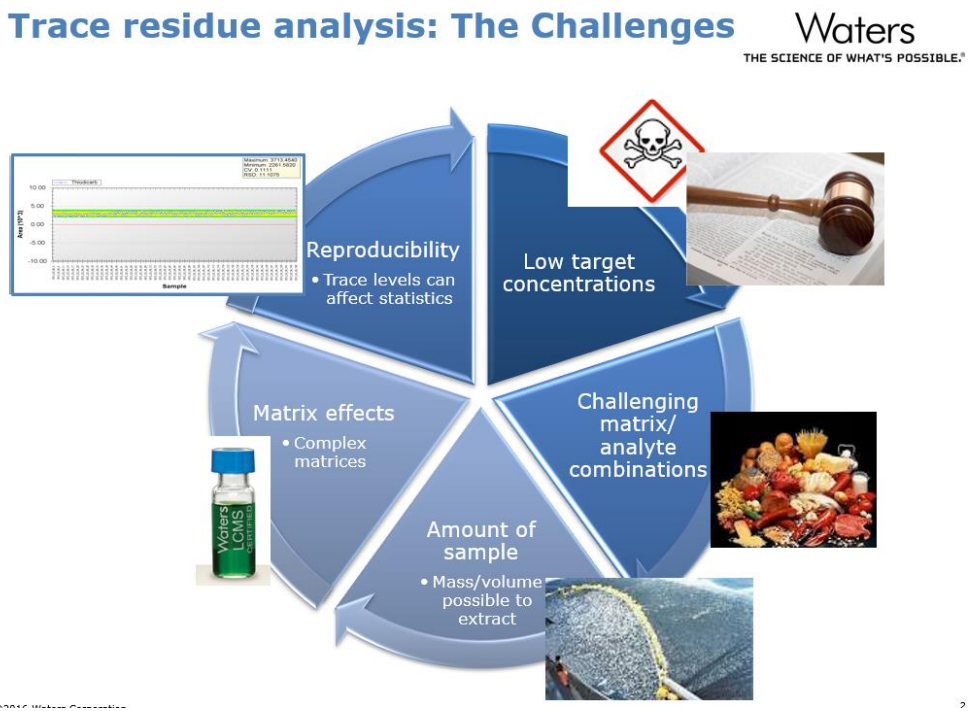
In summary, multi-residue pesticide analysis is a critical tool in modern agriculture for ensuring food safety and regulatory compliance. It allows for the rapid and efficient screening of a large number of samples for a variety of chemical compounds.

Multiresidue Pesticide Analysis

Challenges in Multiresidue Pesticide Analysis

- **Complexity of the Matrix**: The food or crop sample matrix is complex, comprising various substances like organic acids, polyphenols, natural pigments, catechins, flavonols, and metallic/non-metallic elements, among others. This complexity can hinder the detection and quantification of pesticide residues

- **Trace Levels of Pesticides**: Pesticides, often found in food samples at ultra-trace levels, pose a significant challenge to detect and quantify due to the complexity of the sample matrix



Multiresidue Pesticide Analysis

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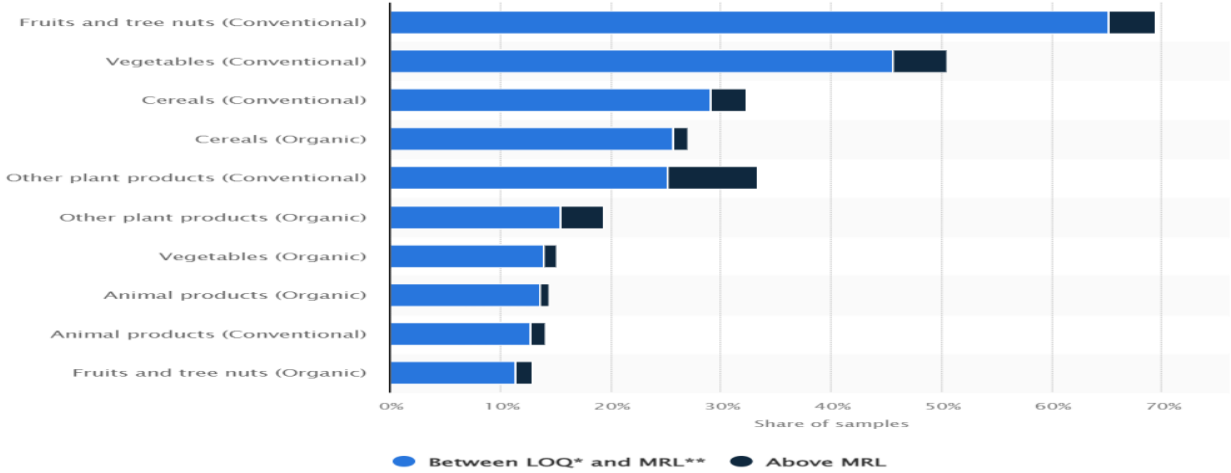
Challenges in Multiresidue Pesticide Analysis

- **Regulatory Compliance**: Due to growing concerns about pesticides' effects on human health, governments have launched monitoring programs to enforce regulations and safeguard food safety. However, complying with these regulations and ensuring adherence can be challenging



The report released in April 2023 presents data on residues of pesticides found in selected organic and conventional food products in Europe.

Techniques for Multiresidue Pesticide Analysis

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Is it possible to have a universal technique for Analysis?

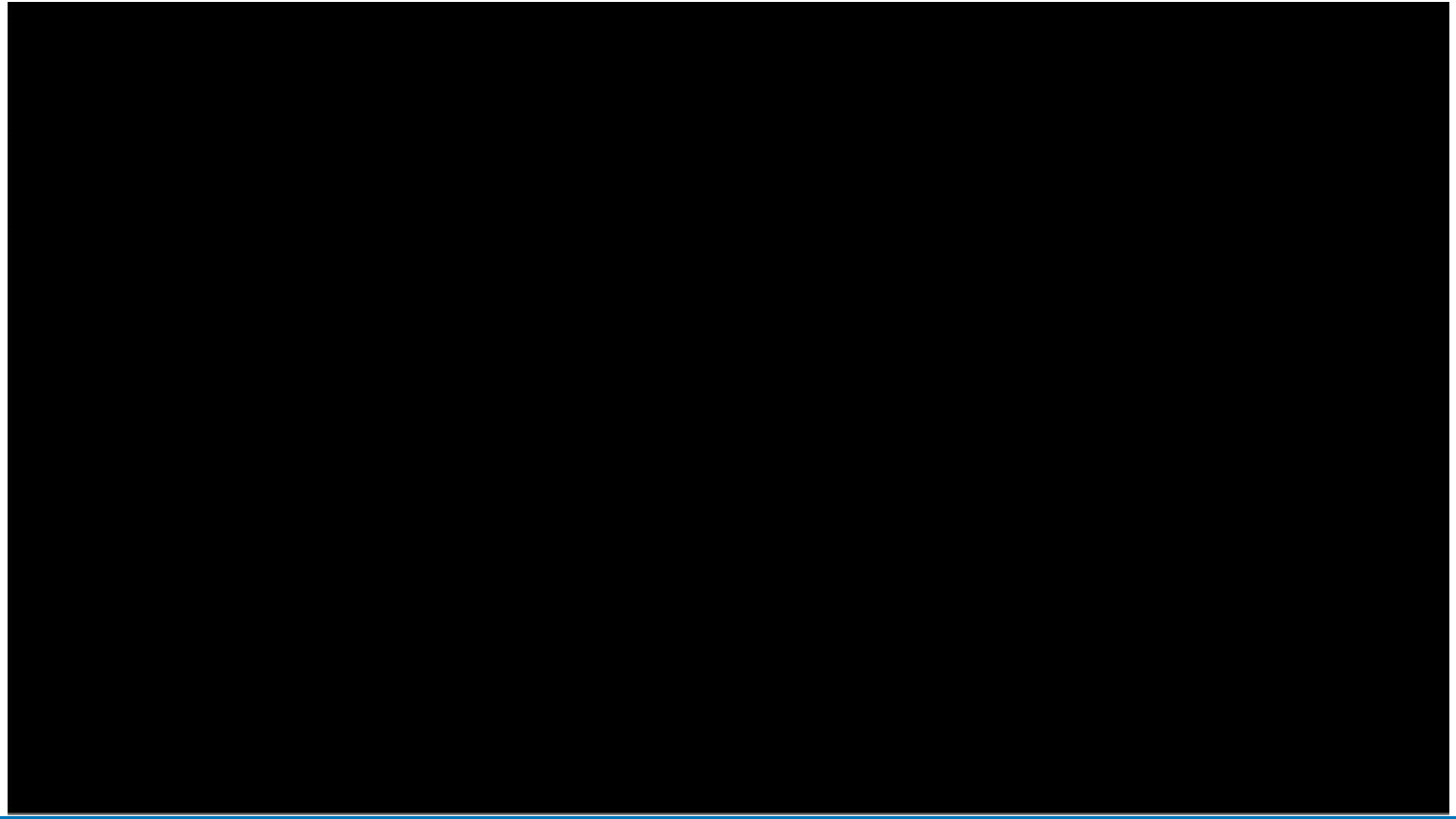
Sample Preparation Technique:

- **QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) Procedure**: This refers to a popular, fast technique for multi-residue pesticide analysis. This method allows for the examination of numerous compounds, enabling a larger number of food samples to be tested for a wide array of pesticides

Selection of d-SPE Product for Sample Cleanup

Commodity Type	Cleanup Provided	Size	AOAC Method 2007.01	CEN Method 15662
General Fruits and Vegetables (Celery, Head Lettuce, Melon)	Removes polar organic acids, some sugars and lipids	2 mL Tubes	50 mg PSA, 150 mg $MgSO_4$ Part #186004572	25 mg PSA, 150 mg $MgSO_4$ Part #186004831
		15 mL Tubes	400 mg PSA, 1200 mg $MgSO_4$ Part #186008072	150 mg PSA, 900 mg $MgSO_4$ Part #186004833
Fruits and Vegetables with Fats and Waxes (Cereals, Nuts, Dairy, Avocado)	Removes polar organic acids, some sugars, more lipids and sterols	2 mL Tubes	50 mg PSA, 50mg C_{18}, 150 mg $MgSO_4$ Part #186004830	25 mg PSA, 25 mg C_{18}, 150 mg $MgSO_4$ Part #186004832
		15 mL Tubes	400 mg PSA, 400 mg C_{18}, 1200 mg $MgSO_4$ Part # 186008073	150 mg PSA, 150 mg C_{18}, 900mg $MgSO_4$ Part #186004834

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)



Techniques for Multiresidue Pesticide Analysis

Is it possible to have a universal technique for Analysis?

Here are some of the techniques used for multi-residue pesticide analysis:

- **Tandem Mass Spectrometry (LC–MS-MS and GC-MS-MS)**:
  Gold Standard techniques for high sensitivity and selectivity.

Tandem Mass Spectrometry (LC–MS-MS and GC-MS-MS)

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Group	LC1-Q	LC2-Q	GC1-Q	LC3
Group Details	•QuEChERS compounds •LC amenable •Amenable to standard dSPE cleanup •May also be GC amenable	•QuEChERS compounds •LC amenable •Not amenable to standard dSPE cleanup	•QuEChERS compounds •GC amenable •May be LC amenable •Cleanup will depend on technique	•Non-QuEChERS compounds (too polar) •Req different extraction •May req. different methods
Additional Notes	Compatible with cleanup by PSA, C18 and GCB	Compounds adsorb to PSA, C18 or GCB, giving low recovery %	Natural and synthetic pyrethroids can be done via LC (esp. with APCI)	e.g., 70:25:5 Methanol-Water-Formic acid
Extraction	QuEChERS	QuEChERS	QuEChERS	Methanol-Water
Cleanup	YES - 1x dSPE w/ good MS - 2x dSPE w/ avg MS	YES/MAYBE - modified dSPE sorbents - no cleanup (if MS can handle)	YES - same as LC1-Q for LC - SepPak (PSA/carbon) for GC	MAYBE
Analysis by LC/MS	YES	YES	SOME (pyrethroids)	YES
Analysis by GC/MS	SOME	NO	YES (esp. organochlorines)	NO
Examples	Abamectin Bifenazate Etoxazole Fenoxycarb Imazalil Imidacloprid Myclobutanil Paclobutrazol Spinosad A and D Spiromesifen Spirotetramat Trifloxystrobin	Acequinocyl (loss to PSA)	Pyrethrins Synthetic pyrethroids (Bifenthrin, Cyfluthrin)	Chlormequat chloride Daminozide

Pesticides

Substance classes and technologies

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Pesticides

Neutral Pesticides
QuEChERS extraction

Polar pesticides
QuPPe exctraction

Acidic herbicides
QuEChERS extraction

LC amenable

[Multiresidue Method for the Quantification of Pesticides in Fruits, Vegetables, Cereals and Black Tea using UPLC-MS/MS]

[UPLC and APGC Multi Residue Pesticide Analysis on a Single Tandem Quadrupole Mass Spectrometer Platform]

GC amenable

[Determination of Pesticide Residues in RiceBased Baby Food Using GC-MS/MS with APGC™ After Extraction and Clean Up Using QuEChERS]

Glyphosate & Co

Oxyanions

Applications Notes can be found on the [FEMS SharePoint] or dedicated Waters [website]

Anionic Polar pesticides column

Cationic Pesticides

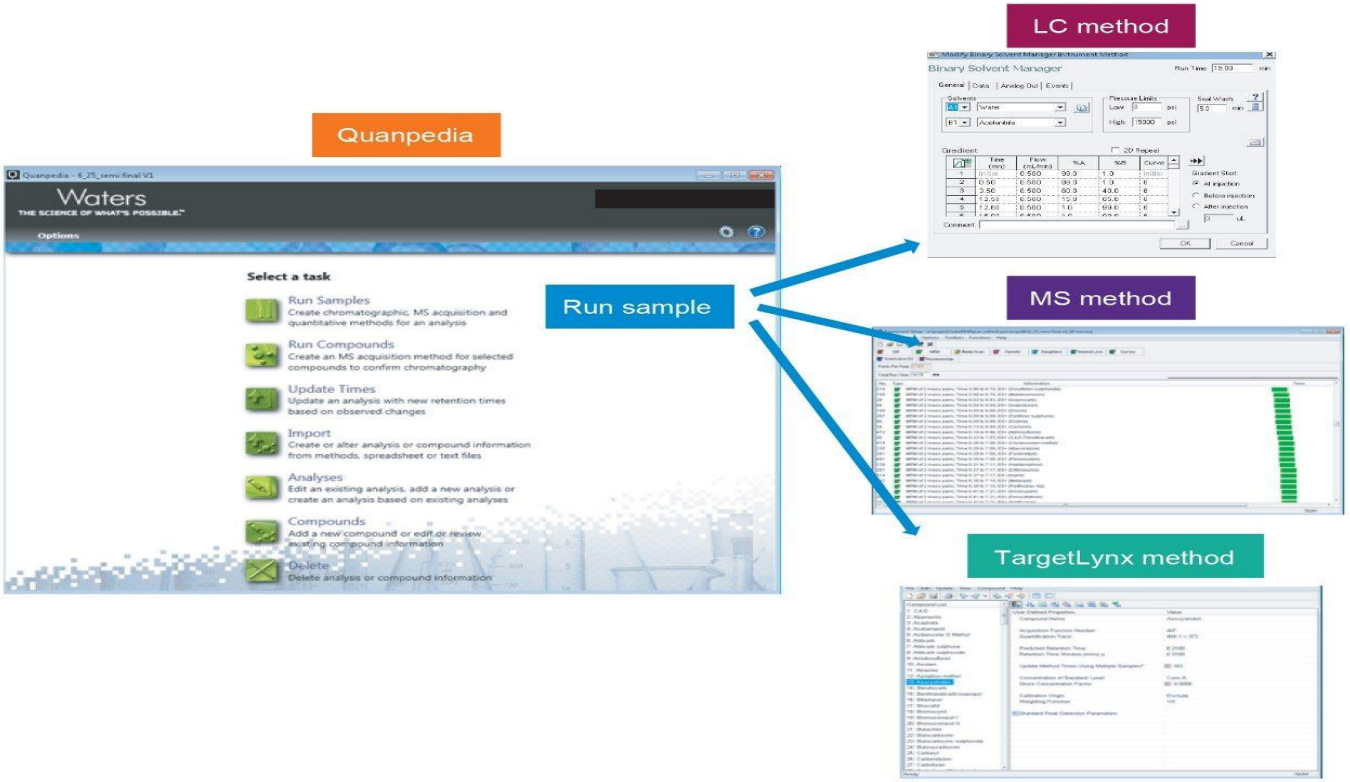
ACQUITY UPLC BEH Amide Column

ACQUITY UPLC HSS T3

[Oasis PRiME HLB Cartridge for Clean-up of QuEChERS Extracts of Soybean Pods Prior to UPLC-MS/MS Determination of Free Acidic Herbicides]

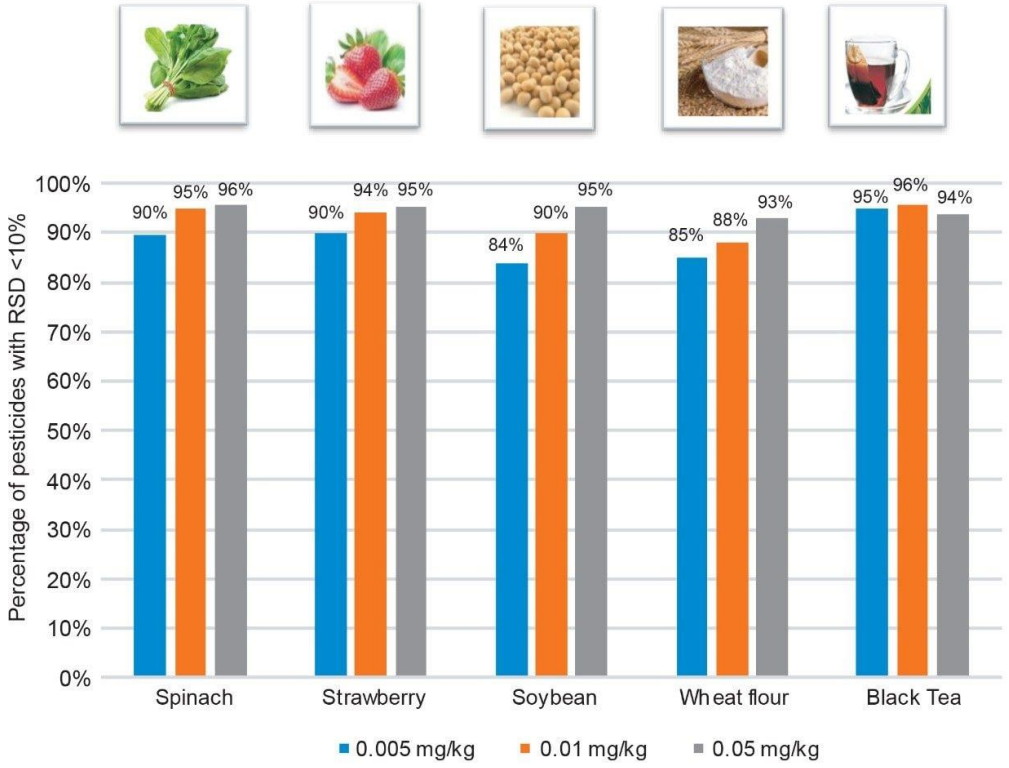
Multi residue method for LC amenable Pesticides

552 Pesticides detected bellow EU default MRL of 0.01 mg/kg



Multi residue method for LC amenable Pesticides

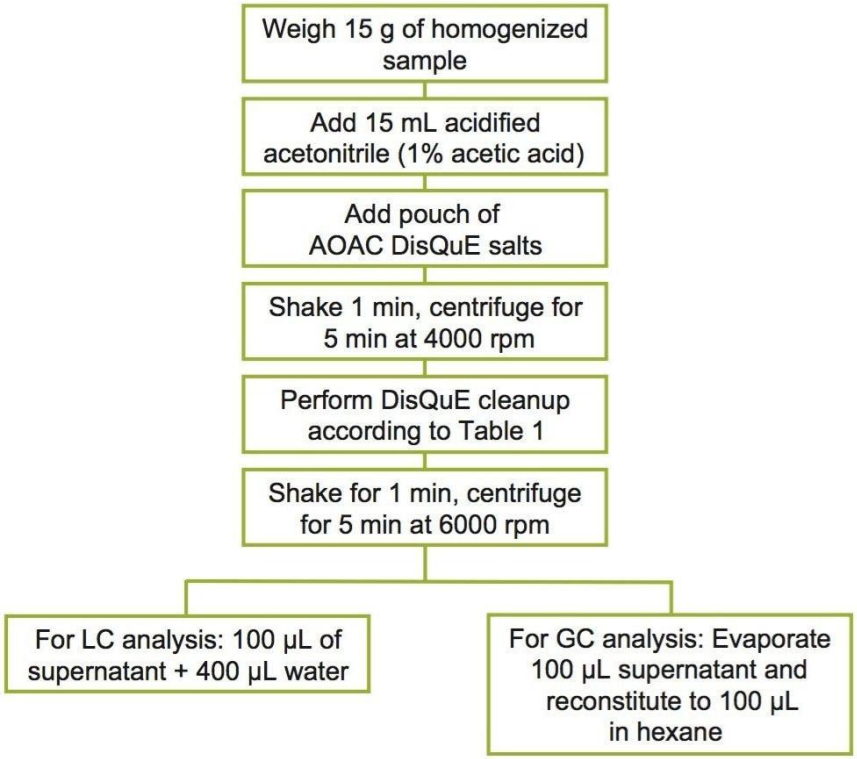
The percentage of pesticides with RSDs <10% in various matrices at 0.005 mg/kg, 0.01 mg/kg, and 0.05 mg/kg



Multi residue method for GC amenable Pesticides

Analysis of fruit and vegetable matrices at legislatively relevant levels of 0.010 mg/kg

QuEChERS extraction was performed according to the official **AOAC method 2007.01** using the DisQuE QuEChERS, AOAC Method Sample Preparation Kit (p/n: 176002922)



Multi residue method for GC amenable Pesticides

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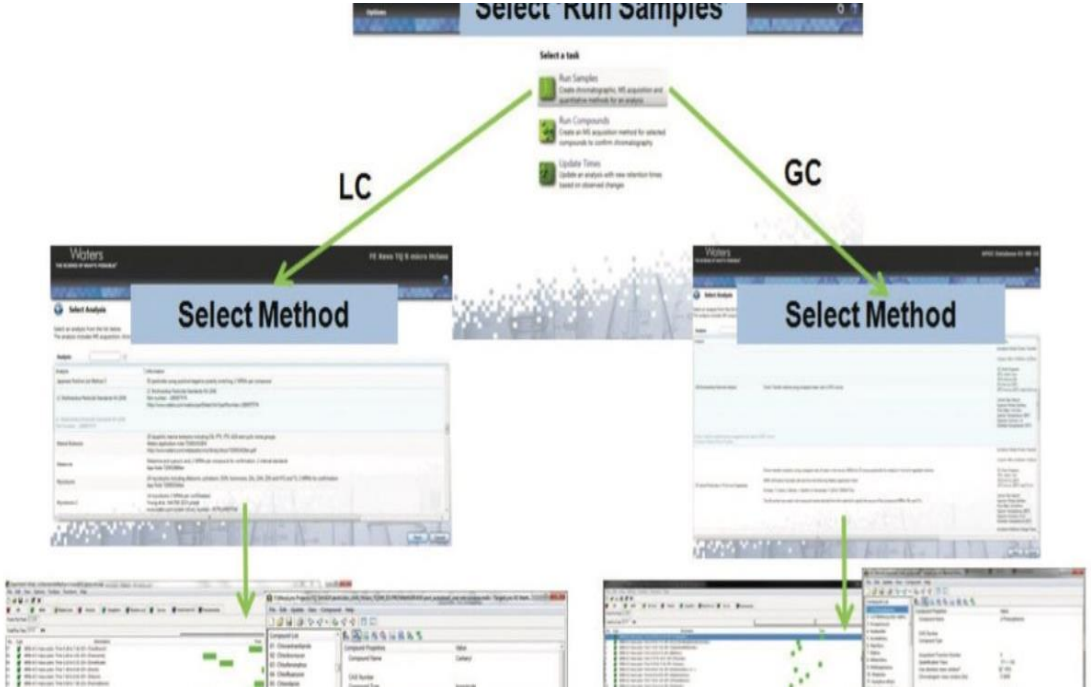
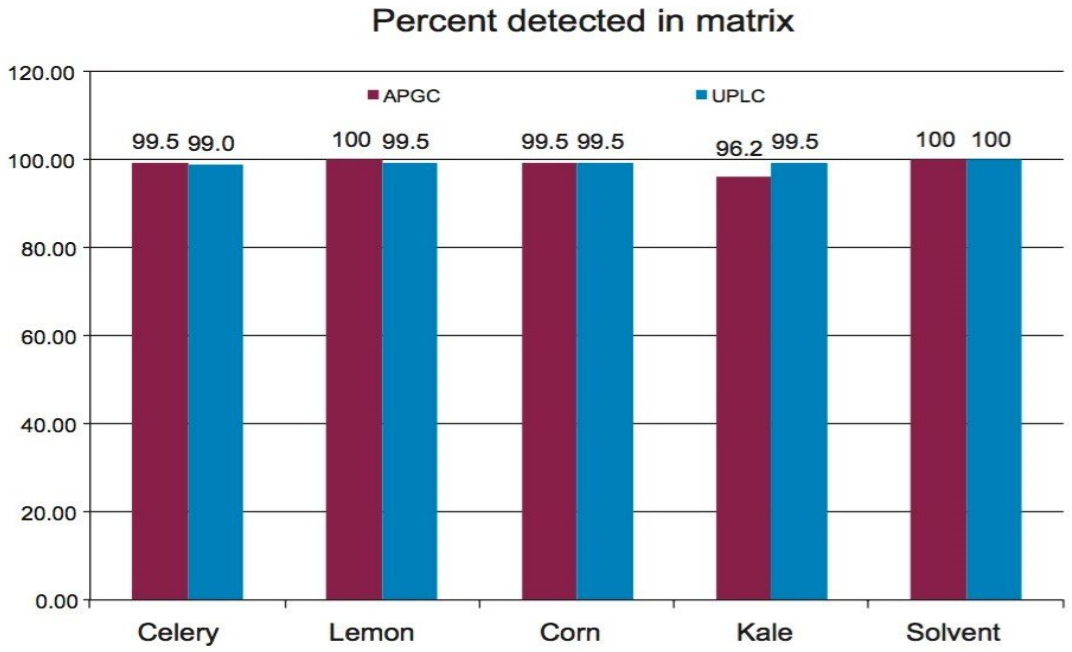


Multi residue method for GC amenable Pesticides

Matrix matched standards were prepared in celery, lemon, corn and kale over a range of 0.001 to 0.050 mg/kg



Percentage of compounds detected at 0.010 mg/kg in each matrix and associated RSDs.

Food Safety Applications

PESTICIDES

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Multi-residue Pesticide Methods

Performance, workflow optimisation and dedicated methods

GALAB
Analysis – to be sure

APGC-MS/MS for Pesticide Testing

Highest sensitivity for baby food analysis

PRIMORIS
YOUR RELIABILITY IN FOOD ANALYSIS
NOFA GROUP
Part of the Cotecna Group

Special on highly polar Pesticides

Solutions for cationic and anionic polar pesticides

eurofins
EURL-SRM

Request your resource kit

Techniques for Multiresidue Pesticide Analysis

Is it possible to have a hybrid technique for Analysis?

- **Tandem Mass Spectrometry (APGC)**

APGC uses a ‘soft’ ionization technique similar to Atmospheric Pressure Chemical Ionization (APCI). This means the fragmentation of compounds is very low, especially when compared to Electron Ionization (EI). As a result, sensitivity and selectivity are increased, and MRM precursor selection is simplified

These techniques have been developed to address the challenges of screening a large number of samples for a variety of chemical compounds, and their implementation into regular testing programs has become more widespread in recent years.

Techniques for Multiresidue Pesticide Analysis

Is it possible to have a universal technique for Analysis?

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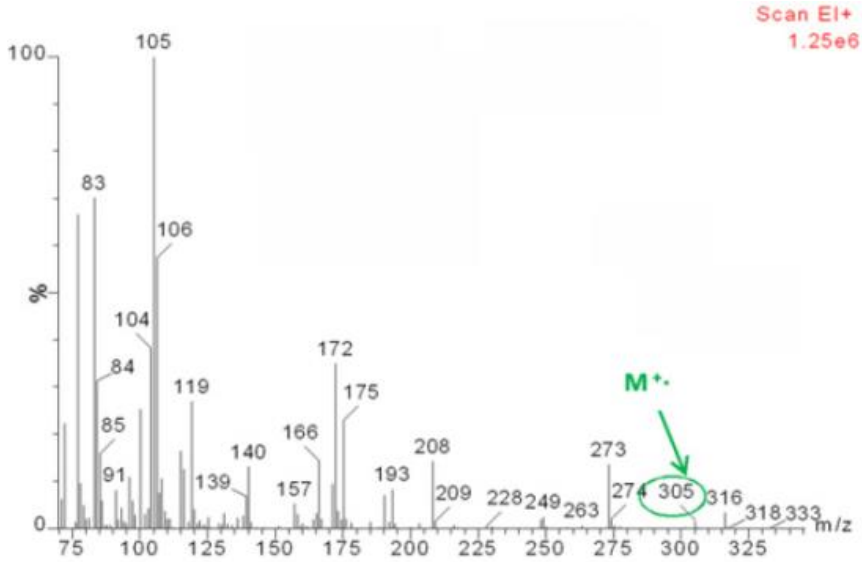
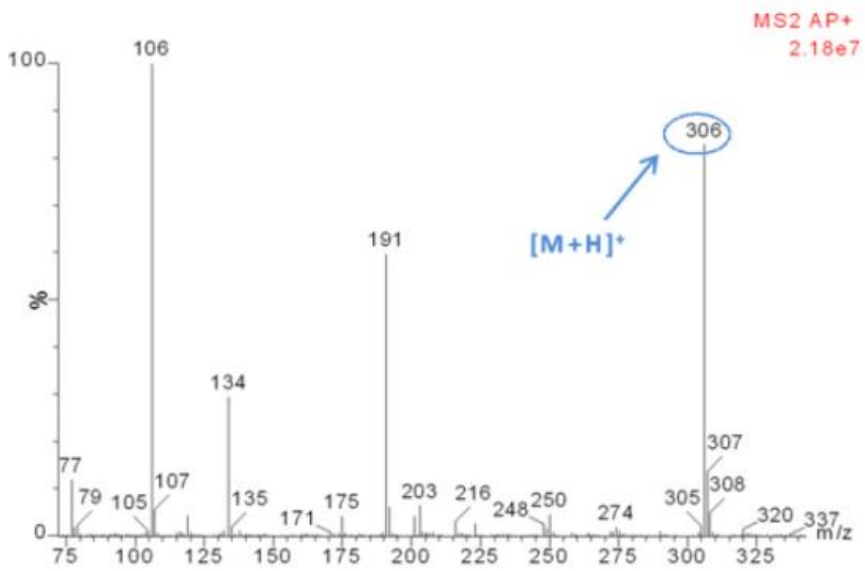
Tandem Mass Spectrometry (APGC)

APGC employs a ‘soft’ ionization method akin to Atmospheric Pressure Chemical Ionization (APCI), resulting in minimal compound fragmentation compared to Electron Ionization (EI). This enhances sensitivity and selectivity and simplifies MRM precursor selection





Atmospheric Pressure Gas Chromatography (APGC)

Comparison of sensitivity: EI vs. APGC

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tR	Compounds	Molecular formula	M	EI	APGC		APGC+H_2O
				M•+	M•+	MH+	MH+
4.70	Dichlorvos	C4H7Cl2O4P	220			+++	+++
5.97	Mevinphos	C7H13O6P	224		++	+	++
6.96	Molinate	C9H17NOS	187	+		+++	+++
8.00	Dicrotophos	C8H16NO5P	237	+	++	++	+++
8.24	Monocrotophos	C7H14NO5P	223	+	++	++	+++
8.95	Terbufos	C9H21O2PS3	288				+
9.80	Phosphamidon	C10H19ClNO5P	299		+	++	+++
9.76	Endosulfanether	C9H6Cl6O	340	+	+	++	++
9.94	Chlorpyriphosmethyl	C7H7Cl3NO3PS	321			++	+++
10.77	Chlorpyriphos	C9H11Cl3NO3PS	349		+	+++	+++
10.85	Aldrin	C12H8Cl6	362		+	+	++
11.39	Isodrin	C12H8Cl6	362		++	++	++
11.56	Chlorfenvinphos	C12H14Cl3O4P	358			++	+++
11.56	Oxychlordane	C10H4Cl8O	420		+	+	++
11.56	HeptachlorepoxideB	C10H5Cl7O	386		+	+	++
12.23	EndosulfanI	C9H6Cl6O3S	404			++	++
12.72	Buprofezin	C16H23N3OS	305	+	++	+++	+++
12.73	Dieldrin	C12H8Cl6O	378	+	++	++	++
13.10	Endrin	C12H8Cl6O	378		++	++	++
13.36	Ethion	C9H22O4P2S4	384		+	+++	+++
14.01	Endosulfansulfate	C9H6Cl6O4S	420		++		++
15.63	Azinphosmethyl	C10H12N3O3PS2	317				+
15.66	Pyriproxyfen	C20H19NO3	321			++	+++
16.04	Fenarimol	C17H12Cl2N2O	330	+	++	+++	+++
16.17	Azinphosethyl	C12H16N3O3PS2	345				+

Conclusion

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Pesticides are widely used in the cultivation of crops to safeguard against harmful insects and promote crop yields. However, the extensive use of pesticides represents a risk to human health. Therefore, legal frameworks have been established to ensure food safety, including control programs for pesticide residues

Mass Spectrometry (MS) Current targeted MS methods allow the simultaneous determination of hundreds of pesticides, whereas non-targeted MS methods are now applicable to the identification of pesticide metabolites and transformation products

A typical analysis involves a simple sample extraction and dSPE cleanup where the resulting extract is analyzed by **UPLC-MS/MS** and/or **GC-MS/MS** or Hybrid MS/MS (APGC) for rapidly monitoring pesticides and mycotoxins. This method is sensitive, reproducible, and meets or exceeds action levels for pesticides and mycotoxins testing for regulatory requirements

The number of regulated pesticides that are required to be monitored varies by state as do the action limits which range from 0.01 µg/g (10 ppb) to above 1 µg/g (1 ppm).

Thank you!

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