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GUIDE 2.

METHODS FOR THE DETECTION AND QUANTIFICATION OF CONTAMINANTS IN CEREALS AND RELATED PRODUCTS

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CHAPTER 1.

INTRODUCTION

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- Cereals and cereal-based foods occupy a central place in the European diet and contribute substantially to daily energy and nutrient intake across all age groups.
- Their importance in food security and public health makes it essential to monitor the presence of chemical contaminants that may accumulate throughout the agricultural and processing chain, especially in case of unwanted natural or anthropgenic (wars, etc.) activities.
- Among these contaminants, trace elements, specially toxic heavy metals such as cadmium, lead, arsenic and mercury and pesticide residues represent two major areas of concern for regulators, researchers and consumers.

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- ☐ Trace elements can enter cereal crops through natural or anthropogenic pathways. Soil composition, industrial emissions, irrigation water quality, fertilisers, and historical pollution are among the main sources contributing to their presence in grains.

- ☐ Some elements, such as iron, zinc or copper, are essential at low levels but may become harmful at elevated concentrations, while others, including cadmium, lead, mercury and inorganic arsenic pose clear toxicological risks even at trace levels.

- ☐ Chronic exposure, particularly through staple foods like cereals, can adversely affect renal function, neurodevelopment, cardiovascular health, and overall long-term well-being. For this reason, European legislation has established maximum levels for several heavy metals in cereals and related products, requiring robust analytical methodologies to ensure compliance.

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- Pesticide residues constitute a second key category of contaminants. Modern cereal production often involves the use of herbicides, fungicides and insecticides to control weeds, fungal infections and pests.
- Although these treatments play a role in securing yields and limiting post-harvest losses, they may leave detectable residues on raw grains or processed products.
- The diversity of pesticide classes covering organophosphates, pyrethroids, triazoles, neonicotinoids, and others combined with their broad range of physicochemical properties makes their accurate detection challenging.
- Furthermore, environmental factors, agricultural practices, and technological processes (such as milling or thermal treatments) can influence residue levels and distribution within the cereal matrix.
- Thirdly, this guide presents methods for detecting contamination of cereals with nitrogen compounds. The detection of nitrogen compounds in cereals involves distinguishing between nutritional nitrogen (proteins) and contaminant nitrogen (fraudulent additives or war-related residues). Because nitrogen is the "proxy" for protein, the analytical challenge lies in identifying the specific chemical form of the nitrogen

Striking a Balance: Optimal Use of Pesticides for Crops – AgroToHome

- ☐ Ensuring reliable monitoring of these contaminants requires analytical approaches capable of addressing both complexity and sensitivity. Heavy metals typically demand techniques with strong elemental specificity and low detection limits, such as flame atomic absorption spectrometry (FAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS). Each method offers distinct advantages in terms of detection capability, matrix tolerance and analytical throughput.
- ☐ In parallel, pesticide analysis relies mainly on chromatographic techniques such as gas chromatography (GC) and liquid chromatography (LC), often coupled with mass spectrometry to achieve the sensitivity and selectivity needed to quantify residues at levels consistent with European regulatory requirements.
- ☐ However, accurate quantification is not solely dependent on instrumental capacity as cereals represent complex biological matrices containing starch, proteins, lipids and minerals that can interfere with both extraction and detection.
- ☐ Sampling representativeness, sample preparation techniques, contamination control, matrix effects, calibration strategy, and method validation are equally decisive.
- ☐ Addressing these challenges requires harmonised methodologies and coordinated efforts across laboratories to ensure data comparability and reliability.

https://www.azolifesciences.com/article/What-is-Analytical-Chemistry.aspx

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- **Within this context, this European project aims to strengthen and harmonise the analytical methods used to detect and quantify heavy metals and pesticide residues in cereals and related foods.**
- **It seeks to assess current practices, identify methodological gaps, and develop or refine analytical protocols suitable for trace-level contamination monitoring.**
- **The project integrates expertise from several European institutions and promotes knowledge exchange, capacity building and methodological alignment to support regulatory compliance, risk assessment and consumer protection.**
- **By advancing robust, sensitive and harmonised methods, this project contributes to improving food safety management within the European Union and reinforces the scientific basis for monitoring two of the most critical groups of contaminants in cereal products: toxic trace elements and pesticide residues.**

https://www.pipedrive.com/en/blog/project-report

- This guide provides quality control professionals and food industry operators with a practical and structured perspective on the essential methods needed to accurately identify and measure contaminants present in grains.
- Contamination of cereals with agents such as pesticide residues, heavy metals and nitrogen compounds can have serious effects on consumers, even at very low levels (below the Maximum Residue Limits – MRLs).
- Therefore, continuous and effective monitoring is mandatory to ensure that products comply with national and international standards.

Classification of methods for the analysis of contaminants in cereals and related products

The success of the analysis depends on choosing the right method:

- **<u>Rapid screening methods:</u> Immunological tests such as ELISA and strip tests (LFAs). These are the preferred solutions for the rapid verification of large batches at the point of receipt, providing fast and cost-effective results.**
- **<u>Confirmation and quantification methods:</u> Use of advanced instrumental techniques, especially LC-MS/MS and GC-MS/MS.**

These methods are necessary for the accurate quantification and validation of the positive results obtained by screening

- Advanced instrumental methods, in particular those combining chromatographic separation with tandem mass spectrometry (MS/MS) detection, are the reference methods for the analysis of contaminants in cereals.
- They provide the sensitivity, selectivity, and robustness needed to meet stringent regulations.

- Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is the preferred method for polar, thermolabile and high molecular weight compounds that are difficult to analyze by gas chromatography (GC).
- This includes most mycotoxins and many pesticide residues.

Gas chromatography with tandem mass spectrometry (GC-MS/MS) is the ideal method for volatile, semi-volatile, nonpolar and thermostable compounds:

- Organocorrhizal and organophosphorus pesticides
- various contaminants from processing (acrylamide, furan)

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CHAPTER 2.

LEGISLATION AT NATIONAL AND EUROPEAN LEVEL REGARDING THE METHODS USED TO REDUCE THE CONTAMINATION OF CEREALS AND RELATED PRODUCTS

2.1. Argument

- ☐ Legislation plays a crucial role in reducing grain contamination, as it sets clear standards and maximum permissible limits for various contaminants, from mycotoxins to pesticide residues.
- ☐ By imposing strict rules on good agricultural practices, hygiene and monitoring, the legislation ensures a safe food chain from producer to consumer.
- ☐ Without these regulations, the risk of contaminated grains reaching the market would increase significantly, endangering public health and affecting trust in food.

2.2. National and European Legislation to Reduce Grain Contamination

- Food safety is a key priority, and cereals and related products are a fundamental component of the human diet.
- That is why there is a complex legislative framework, both at national and European level, designed to reduce and control the contamination of these products.
- The main purpose is to protect the health of consumers. European framework: a unified approach At the level of the European Union, the legislation in the field is extensive and harmonized, ensuring a high standard of safety in all member states.
- This includes a number of regulations and directives addressing various types of contaminants, such as mycotoxins (toxins produced by moulds), pesticide residues, heavy metals, nitrates and genetically modified organisms (GMOs).

- European legislation sets maximum permissible limits for these contaminants, requiring strict monitoring and regular controls throughout the production chain, from farm to plate.
- By Regulation (EC) no. 178/2002, the principle of traceability is established, allowing the rapid identification of the source of any contamination.
- Also, Regulation (EC) no. Regulation (EC) No 1881/2006 is a key example, as it defines maximum limits for certain contaminants in foodstuffs, including cereals.

2.3. National Legislation: Adaptation and Implementation

- ❑ Each EU member state, including Romania, transposes and details European legislation into its own national normative acts.
- ❑ This means that, in addition to the directly applicable European regulations, there are also specific laws and orders that adapt the requirements to the national context.
- ❑ National authorities, such as the National Sanitary Veterinary and Food Safety Authority (ANSVSA) in Romania, are responsible for implementing and enforcing these rules.
- ❑ They carry out inspections, take samples and carry out analyses to ensure that cereals and derived products comply with safety standards.

2.4. Key Measures to Reduce Contamination

The legislation requires a series of preventive and control measures:

- **Good Agricultural Practices (GAP): Growers are obliged to apply practices that minimize the risk of contamination from the production phase (e.g. crop rotation, moisture management, responsible use of pesticides).**
- **Good Hygiene Practices (GHP): During storage, transport and processing, strict hygiene conditions are imposed to prevent the development of mould and other contaminants.**

- ❑ <u>**Food Safety Management Systems:**</u> **Food industry operators must implement systems based on HACCP (Hazard Analysis and Critical Control Points) principles to identify, assess and control risks.**
- ❑ <u>**Monitoring and Testing:**</u> **Regular analyses of cereals and related products are carried out to verify compliance with the maximum permissible limits of contaminants.**
- ❑ <u>**Product Recalls:**</u> **If limits are exceeded or a risk is identified, the legislation requires the immediate withdrawal of products from the market to protect consumers.**

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2.5. Initiatives and analyses against the backdrop of the Ukrainian conflict

- The current conflict in Ukraine has generated dysfunctions in global trade in agricultural products and induced significant vulnerabilities in food supply chains. Although the fundamental regulatory framework of the European Union (EU) on food safety, transposed into import regulations, remains valid for all products placed on the EU market, the supranational entity was forced to implement specific protocols and manage complex challenges generated by grain flows of Ukrainian origin, namely:
- "Solidarity Corridors" and Autonomous Trade Measures (CSFs)
  In response to the escalation of the Russian invasion, the EU has operationalised "solidarity corridors", facilitating the export of Ukrainian agricultural products via alternative land and river routes.
- Complementary to these logistical measures, Autonomous Trade Measures (MCAs) have been established. They involve the unilateral suspension of customs tariffs and quotas for agricultural products imported from Ukraine.
- The primary objectives of these interventions are to support Ukraine's economic resilience and contribute to global food security, by ensuring the continuity of essential product flows on international markets (Source: European Commission; CSIS analysis – Center for Strategic and International Studies).

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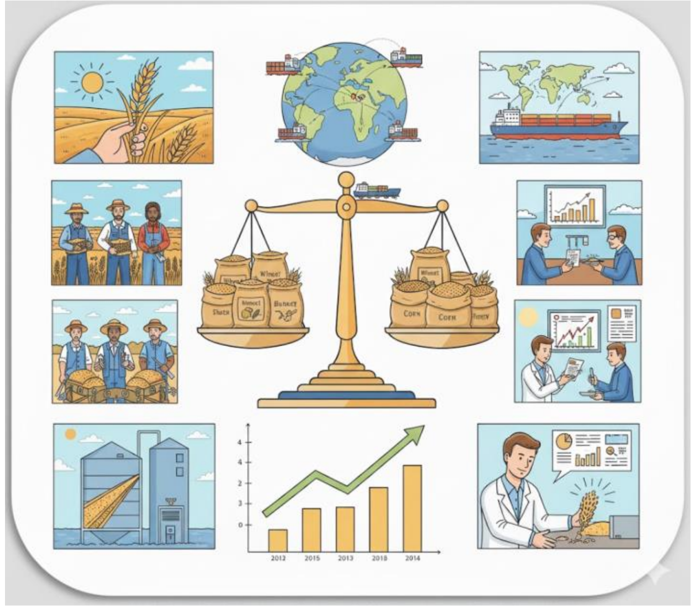
Concerns about market disruption and unilateral bans

- The significant influx of grain from Ukraine, which is essential to sustain the country's economy, has caused considerable disruption to agricultural markets in neighboring EU Member States, including Poland, Hungary, Slovakia, Bulgaria and Romania.
- Farmers in these nations have expressed their protest, citing unfair competition and, in certain situations, concerns about quality standards, such as the use of pesticides banned on EU territory. In response to these tensions, some Member States have imposed unilateral import bans.
- The European Commission initially criticized these measures, considering them a violation of the EU's competence in the field of the common commercial policy (Sources: CSIS reports, Hungary Today).



Exceptional and temporary preventive measures

- To manage the tensions generated and facilitate a compromise solution, the European Union implemented "exceptional and temporary preventive measures" in May 2023.
- They imposed a restrictive ban on the domestic trade of certain Ukrainian agricultural products – wheat, maize, rapeseed and sunflower seeds – on the territory of the 'five frontline Member States'.

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Assessment of Ukraine's food security as a candidate country

In the context of its status as a candidate country for the European Union, the European Commission has carried out a detailed assessment of Ukraine's readiness to fulfil the acquis communitaire in various sectors, including food safety.

Reports indicate that Ukraine has a certain level of preparedness in terms of consumer and health protection, having a public health law partially aligned with that of the EU. However, the assessment also highlights areas that need to be strengthened, such as:

- ☐ **administrative capacities of food safety institutions**
- ☐ **coordination and full alignment of the official food and feed control system with EU standards.**

Ukrainian laboratories are generally well-equipped and accredited, but capacity could be increased. Ukraine is active in the Food and Feed Rapid Alert System (SARAF) network, but is not a member (Sources: European Commission assessments, reported by institutions such as UGA.ua).

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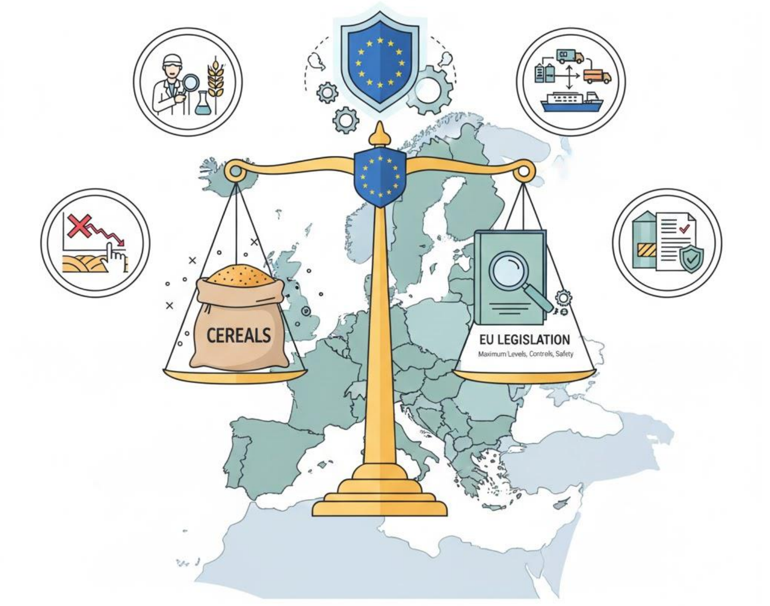
- ☐ The European Union's legislative framework on grain contaminants is rigorous and applies universally to all imports, including those from Ukraine.
- ☐ The armed conflict has significantly altered trade dynamics and required the adoption of temporary measures to manage Ukrainian agricultural product flows.
- ☐ These interventions have been complementary to ongoing efforts to support Ukraine in aligning its food safety systems with European standards.

Regardless of the geopolitical context, the fundamental principles of food safety, including compliance with maximum permissible levels for contaminants, remain of paramount importance.



EU LEGISLATIVE FRAMEWARK: CEREAL CONTAMIANTS

2.6. Legislative regulations on the import of cereals into the European Union and Romania

(A) Regulations at the European Union level:

The European and national legislation regarding cereal imports into the European Union and, implicitly, into Romania, is complex and encompasses a wide spectrum of regulations, targeting both food and phytosanitary safety, as well as customs and market aspects.

EU legislation is the foundation for grain imports from non-EU countries. The main regulated areas are:

1. Food and Phytosanitary Safety

- Regulation (EU) 2017/625 on official controls carried out by the competent authorities of the Member States to verify compliance with EU food and feed legislation. Imported cereals must comply with EU food safety standards, including maximum residue limits of pesticides, mycotoxins (e.g. aflatoxins, ochratoxin), heavy metals and other contaminants.

- Regulation (EU) 2016/2031 on protective measures against plant pests regulates phytosanitary requirements for the import of plants, plant products and other objects, including cereals, in order to prevent the introduction and spread of pests into the EU. Imported cereals may require phytosanitary certificates from the country of origin.

- Specific rules for specific products or origins: There are specific regulations for certain cereals or from certain third countries, which may require enhanced border controls (e.g. for certain mycotoxins).

2. Common organisation of the markets in agricultural products (CMO)

Regulation (EU) No 1308/2013 establishes a common organisation of the markets in agricultural products, including cereals. It includes provisions on:

- __Import tariffs:__ The EU applies customs duties on the import of cereals from third countries, which can vary depending on the type of cereal and the EU's trade agreements with that country (e.g. tariff quotas, tariff exemptions for certain countries). The EU TARIC database provides detailed information on the applicable tariffs.
- __Tariff rate quotas:__ The EU can open tariff quotas (limited quantities of products that can be imported at reduced or zero tariffs) for certain types of cereals from third countries.
- __Import/export licences:__ For certain categories of cereals or in certain circumstances, import or export licences, governed by Implementing Regulation (EU) 2016/1239, may be required.

(B) General Process of Importing Cereals from Third Countries (Non-EU) into Romania

1. Compliance with EU requirements: The economic operator must ensure that the cereals comply with all EU food safety, phytosanitary and quality standards.

2. Documentation. The following documents are required:

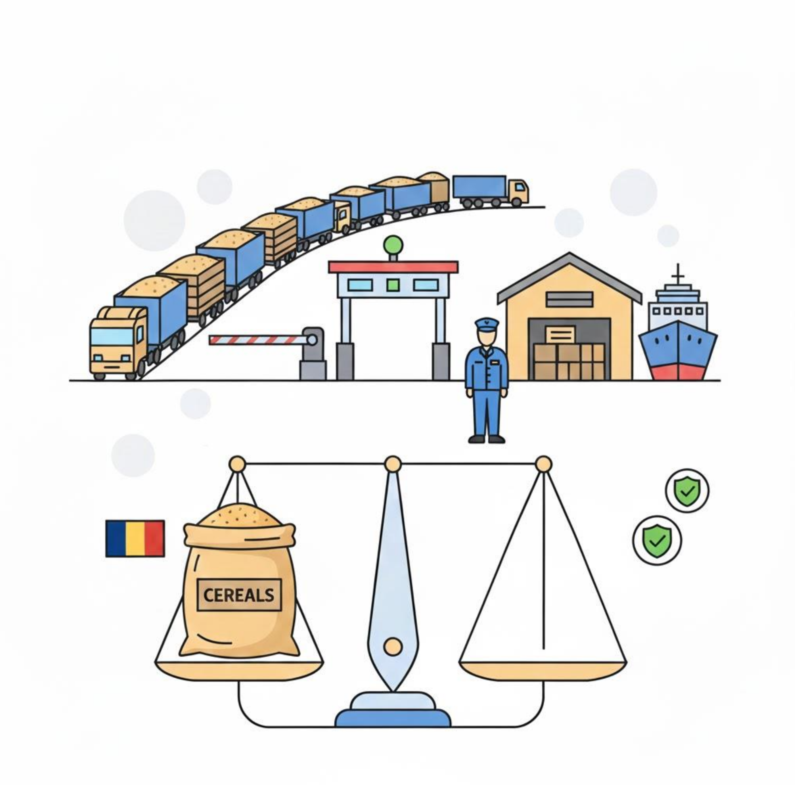
- **Commercial invoice**
- **Packing list**
- **Certificate of origin**
- **Phytosanitary certificate (if applicable) issued by the competent authority of the exporting country.**
- **Health certificate/analysis, attesting to compliance with food safety standards (e.g. mycotoxin content).**
- **Import license (if required according to GEO 84/2023 or other specific regulations).**

3. Customs clearance and border controls

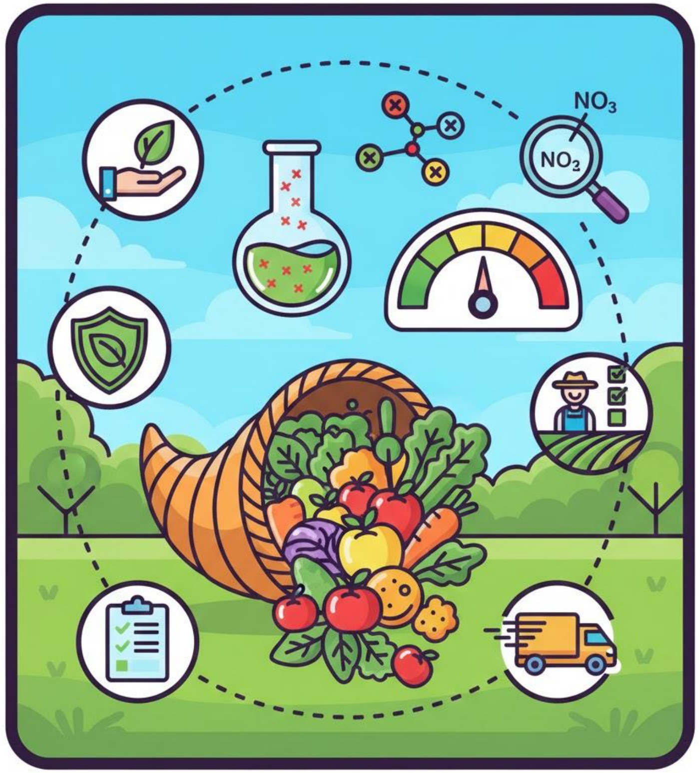
The goods arrive at an approved Border Inspection Point (BIP). Documentary, identity and physical checks are carried out by the competent authorities (ANSVSA, Romanian Customs Authority). The import customs declaration is submitted. Customs duties (if applicable) and VAT are paid.

4. Release for free circulation

Once controls have been completed and tax obligations have been paid, cereals are released for free circulation within the EU. It is crucial for importers to consult the updated specific legislation and work with customs and veterinary experts to ensure full compliance.



- In Romania, the maximum limits of the level of nitrates and nitrites in horticultural products are legislated by ORDER No. 293/640/2001-1/2002 regarding the safety and quality conditions for fresh vegetables and fruits intended for human consumption issued by the Order of the MINISTRY OF AGRICULTURE, FOOD AND FORESTRY No. 293 of August 2, 2001, Order of the MINISTRY OF HEALTH AND FAMILY No. 640 of September 19, 2001, approved by the NATIONAL AUTHORITY FOR CONSUMER PROTECTION on January 3, 2002 and published in the OFFICIAL GAZETTE NO. 173 of March 13, 2002.
- Chemical substances used in the agricultural sector are allowed on the basis of a Certificate signed by the Interministerial Commission for the Certification of Phytosanitary Products. A new CODEX - a list of certified plant protection products classifies substances into four toxicity groups - according to the legal provision published on 5 June 2004.
- The new CODEX (similar to a register) of plant protection products used in Romania has been published by MAAP. This catalog is updated every 2 - 3 years.



- By Directives 86/469 and 86/363, the Commission of the European Community established the control of residues in foodstuffs of animal and plant origin.
- The two directives cover all categories of residues (pesticides, PCBs, hormones, heavy metals, etc.).
- Directive 83/363 refers to pesticide residues and is also included in Order 825/23.05.1994 for the approval of hygiene norms regarding food and its sanitary protection and completed by Order 611/3.04.1995.
- These limits were revised in Order 356/2001 of the Ministry of Agriculture.

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CHAPTER 3.

METHODOLOGIES TO DETECT THE CONTAMINATION OF CEREALS AND RELATED PRODUCTS WITH PESTICIDES

3.1. Introduction

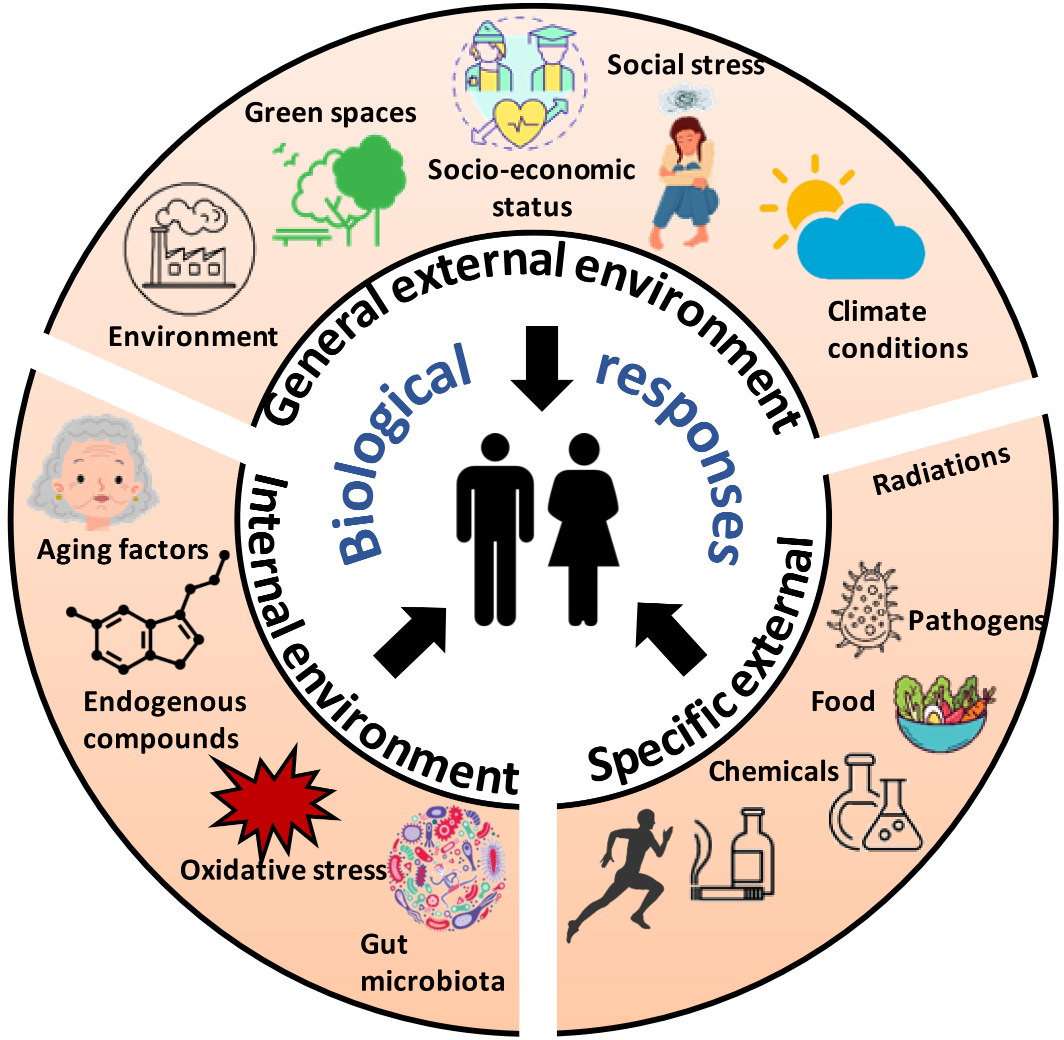
- ☐ Cereals and cereal-based products represent a fundamental component of the human diet worldwide, forming the basis of food security for billions of people.
- ☐ Their widespread consumption, combined with the intensive agricultural practices required to sustain global demand, makes the issue of pesticide contamination a major concern for public health, food safety authorities, and the agri-food industry.
- ☐ Pesticides are routinely applied during cultivation, storage, and post-harvest handling to protect crops from pests, fungal infestation, and yield loss.
- ☐ While these substances play a vital role in ensuring crop productivity, their residues may persist in the final food products. Consequently, cereals may carry residues at levels that pose potential risks to consumers, especially in vulnerable population groups such as children, pregnant women, or individuals with high cereal intake.

- **Regulatory frameworks at national and international levels—such as those of the European Food Safety Authority (EFSA), Codex Alimentarius, and various national agencies-establish maximum residue limits (MRLs) to minimize health risks.**
- **Compliance with these regulatory thresholds requires robust, sensitive, and validated analytical methodologies capable of detecting a broad spectrum of pesticide residues across diverse cereal matrices.**
- **The complexity of cereals and derived products (flours, breads, breakfast cereals, infant foods, etc.) creates analytical challenges related to matrix effects, co-extracted interferences, and the presence of both polar and non-polar pesticide residues.**
- **Therefore, modern analytical chemistry must rely on methodological strategies that combine efficient sample preparation with high-resolution detection techniques.**

https://www.fao.org/fao-who-codexalimentarius/home/fr/

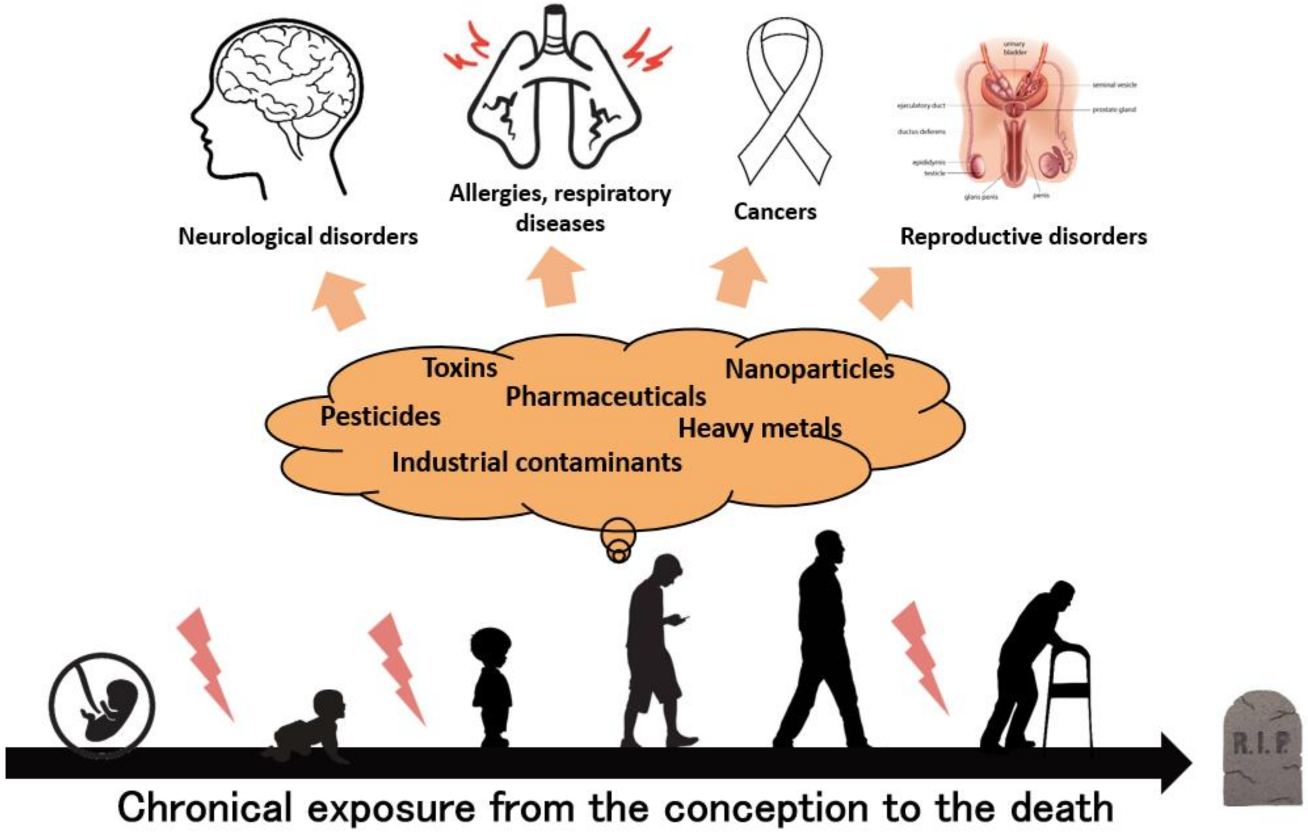
3. 2. The concept of exposome

- ❑ The exposome, a concept introduced by the Pr. Christopher Wild, refers to the totality of exposures an individual encounters throughout life, encompassing not only chemical agents but also biological, physical, social, and behavioral factors.
- ❑ Originating from medicine and epidemiology, this framework aims to better understand the cumulative impact of these diverse influences on health.
- ❑ The coexistence of thousands of exposure factors, many still poorly characterized, makes it challenging to assess their interactions and combined effects.
- ❑ Nevertheless, this complexity contributes to increased health risks, including chronic diseases, heightened vulnerability to infections, and physiological imbalances linked to repeated and varied exposures.



The chemical exposome

- The chemical exposome encompasses all the chemical substances a person is exposed to throughout their life, through air, water, food, or everyday products.
- The presence of thousands of different compounds, many of which are still poorly characterized, makes it difficult to precisely assess their combined effects.
- Nevertheless, this diversity increases health risks, notably by contributing to chronic diseases, endocrine disorders, or subtle toxic effects linked to repeated low-dose exposures.



Neurological disorders

Allergies, respiratory diseases

Cancers

Reproductive disorders

Toxins

Nanoparticles

Pharmaceuticals

Pesticides

Heavy metals

Industrial contaminants

Chronical exposure from the conception to the death

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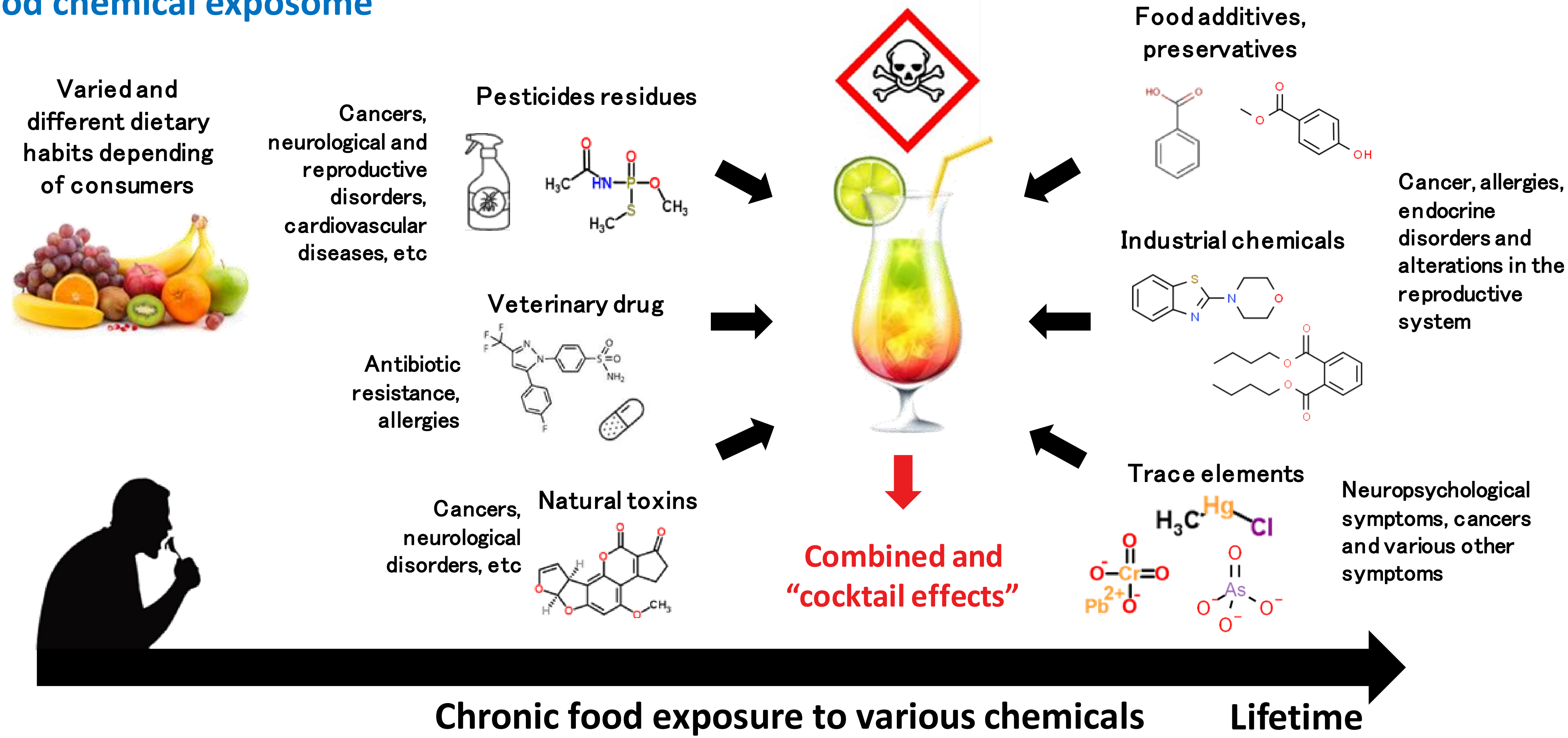
The food chemical exposome



Varied and different dietary habits depending of consumers

Pesticides residues

Cancers, neurological and reproductive disorders, cardiovascular diseases, etc

Veterinary drug

Antibiotic resistance, allergies

Natural toxins

Cancers, neurological disorders, etc

Food additives, preservatives

Industrial chemicals

Cancer, allergies, endocrine disorders and alterations in the reproductive system

Trace elements

Neuropsychological symptoms, cancers and various other symptoms

Combined and "cocktail effects"

Chronic food exposure to various chemicals **Lifetime**

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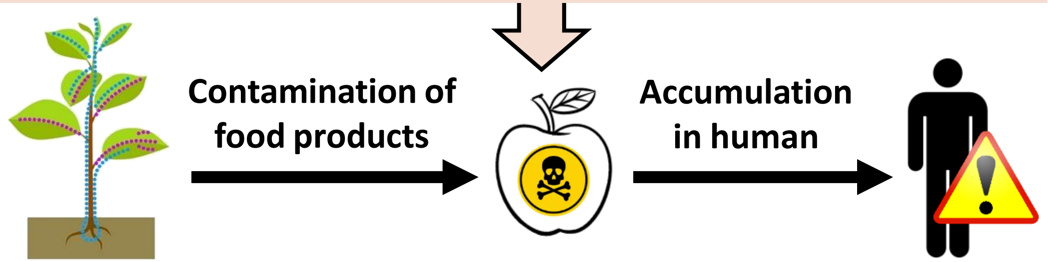
Pesticides: balancing safety and need

- Pesticides are chemical or biological substances used to protect crops from harmful organisms such as insects, fungi, weeds, or bacteria.
- Their use helps increase agricultural yields and reduce post-harvest losses, but it also carries risks for human health and the environment, including acute or chronic toxicity, endocrine disruption, and contamination of soil and water.
- To mitigate these risks, their use is strictly regulated at both European and national levels, with maximum residue limits set for food, rigorous safety assessments required, and controls on market authorization.

Widely and intensively used in modern agriculture

Modern agriculture • Protect and increase yield crops • Food availability

Accumulation of residues in soils and edible parts of plants



European Commission

Regulates the marketing and use of pesticide residues in food and feed

(Regulation (EC) No 1107/2009)

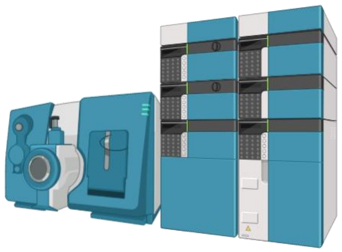
No active substance in a PPP can be used without the approval of the European Commission

Risk assessment for each individual substance

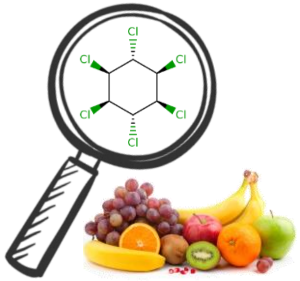
efsa
European Food Safety Authority

Maximum Residue Limit (MRL) for each individual substance
(Regulation (EC) No 396/2005)

3.3. Instrumental analysis

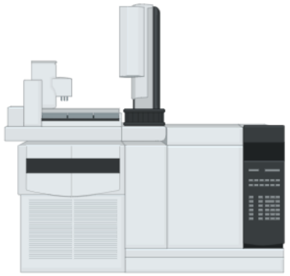


LC-MS/MS (MRM) →



Official tools for control in food

← **GC-MS/MS (MRM)**



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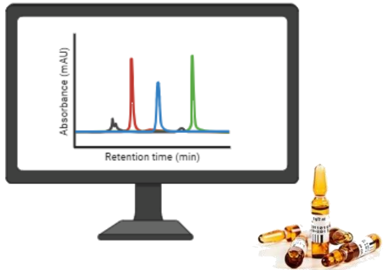


Identification & quantification of targeted chemicals

Highly selective

High sensitivity

Absolute quantification

Overlooks all chemicals non-included in the method

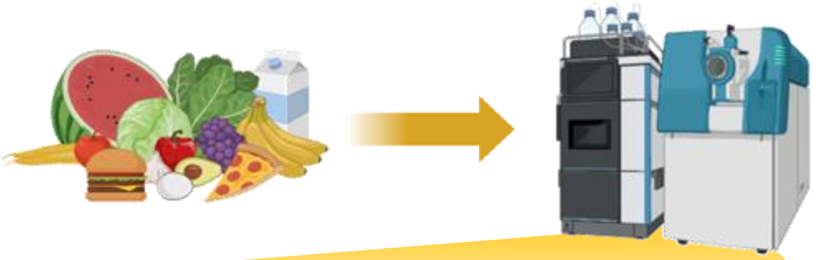
Not enough exhaustive for comprehensive characterization of hazards

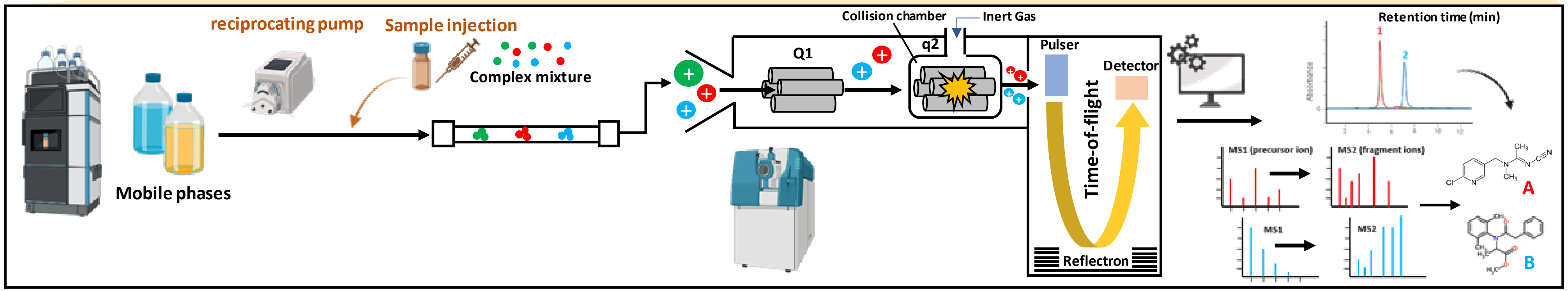
3.3.1. Official control in Europe

- ☐ In the official routine control of food, chromatographic techniques coupled with triple quadrupole mass spectrometry, LC-MS/MS (liquid chromatography) and GC-MS/MS (gas chromatography), are widely used for the analysis of pesticide residues.
- ☐ These methods allow the detection and quantification of a broad range of substances at very low concentrations, with high sensitivity and selectivity thanks to the MRM (Multiple Reaction Monitoring) mode.
- ☐ However, this mode has some limitations: it only monitors predefined transitions for known compounds, making it difficult to detect unknown or non-targeted pesticides.
- ☐ Additionally, MRM can be affected by matrix interferences, requiring careful optimization of separation and fragmentation conditions, and it offers limited flexibility for adding new analytes to established monitoring programs.
- ☐ Despite these constraints, LC-MS/MS and GC-MS/MS remain pillars of official control due to their robustness and ability to handle large and diverse sample sets.

3.3.2. A breakthrough technique : High Resolution Mass Spectrometry (HRMS)

The introduction of high-resolution mass spectrometry (HRMS) in the food safety system provides a valuable complement to conventional LC-MS/MS methods. Unlike the targeted MRM mode of LC-MS/MS, HRMS enables an exact full-scan of all ions present in a sample, allowing the detection of both known compounds and unexpected or emerging residues. This non-targeted capability is particularly useful for broad-spectrum screening of pesticides and for identifying new contaminants, metabolites, or transformation products. Furthermore, the high mass resolution and accuracy significantly reduce matrix interferences, improving the reliability of detection in complex matrices. HRMS thus complements traditional LC-MS/MS by offering greater flexibility and analytical power, paving the way for more proactive and comprehensive food monitoring.





Chromatographic separation

Detection in mass spectrometry

Data analysis

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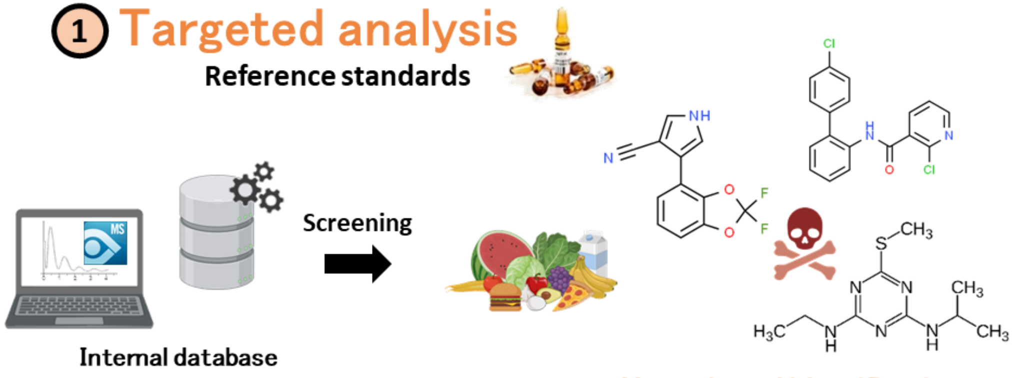
The polyvalence of LC-HRMS

In LC-HRMS, there are different types of analysis. **Targeted analysis** aims to detect and quantify analytes for which reference standards are available. **Suspect screening** allows the search for compounds for which reference standards are not available in the laboratory, significantly expanding the scope of monitoring. **Non-targeted analysis**, on the other hand, is widely used for the search for markers of interest.

LC–HRMS analysis

① Targeted analysis

Reference standards



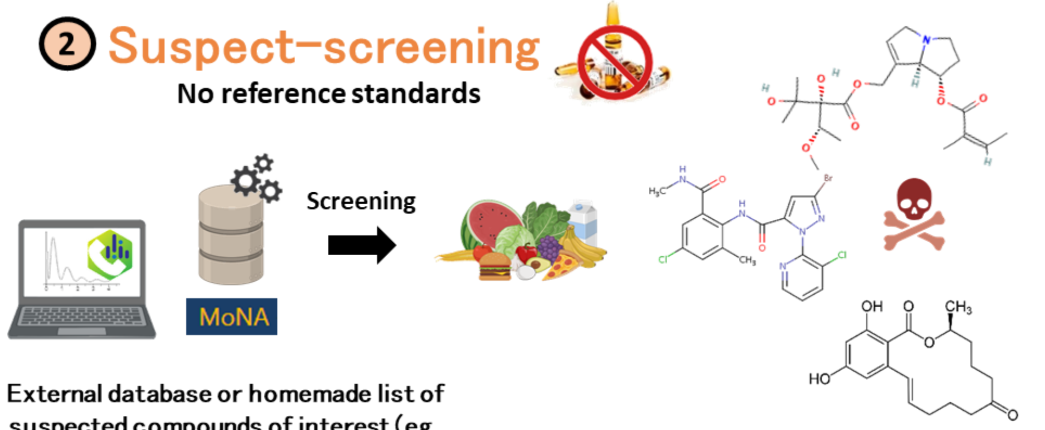
Internal database

Screening

Unequivocal identification

② Suspect–screening

No reference standards

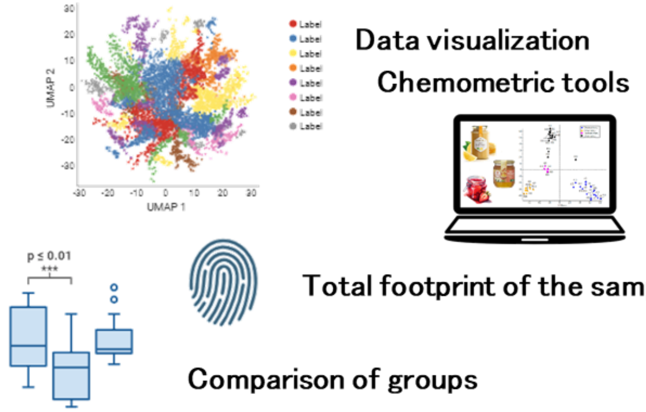


Screening

External database or homemade list of suspected compounds of interest (eg., connexion with MassBank of North America)

Tentative annotation only

③ Non–targeted analysis



Data visualization

Chemometric tools

Total footprint of the samples

Comparison of groups

Statistical comparison to find discriminant features and markers

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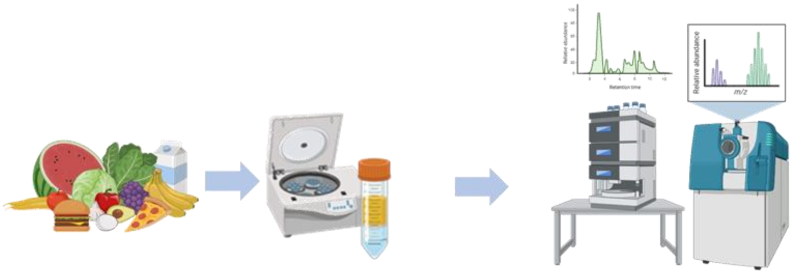
Identification of pesticides in LC-HRMS

In LC-HRMS, pesticides are usually identified by checking three key elements. First, the instrument measures **the exact mass of each molecule**, and this value is compared to the known mass of a pesticide to see if they match within a very small error margin. Second, each compound appears at a specific time during the chromatographic separation, called the **retention time**; matching this time with that of a reference standard helps confirm its identity. Third, the instrument can break the molecule into fragments, producing a kind of **"fingerprint" spectrum**. If the fragments in the sample look the same as those from the reference pesticide, the identification is considered reliable. **When a feature meets all three criteria, mass error, retention time, and fragmentation, it can be unequivocally identified and confirmed**.



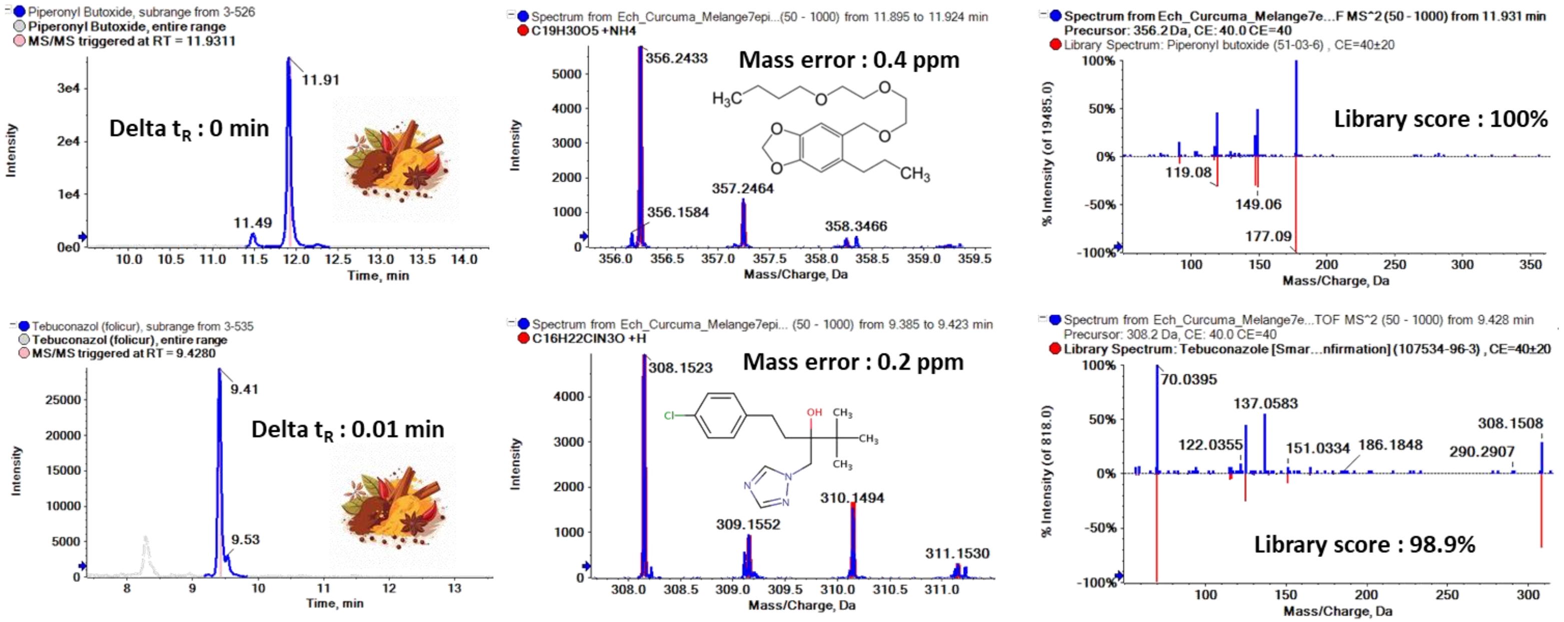
Sample preparation

LC-Q/ToF-MS analysis

Criteria & thresholds

- Delta t_R ± 0.1 min
- Mass error ± 5-ppm
- Library score MS^2 > 70%

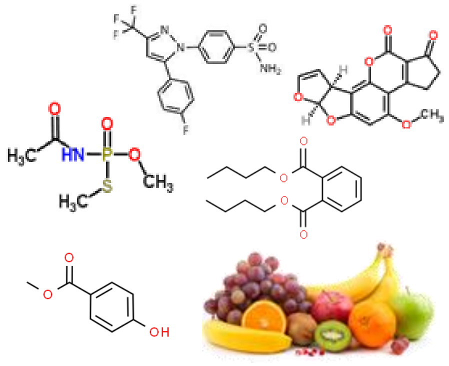
Examples : identification of piperonyl butoxide and tebuconazole in spice samples

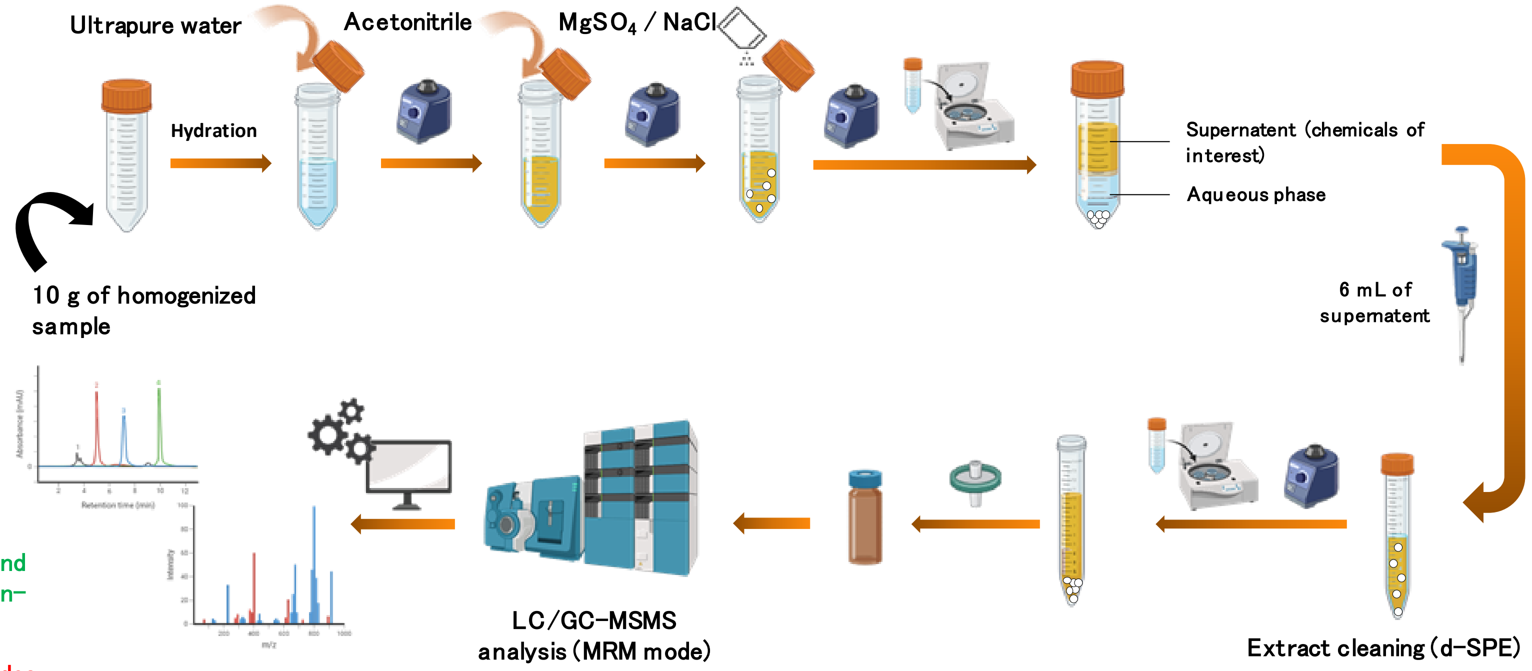


3.3.3. Sample preparation. Extraction and purification methods in food

The QuEChERS method

- “Quick, Easy, Cheap, Effective, Rugged and Safe”
- Introduced by Anastassiades et al. 2003
- The most common and first-choice sample preparation used for the analysis and control of pesticide residues in food





➡ Rapid, simple and eco-friendly

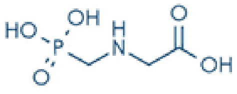
➡ Effective for “multiresidue” analysis

➡ Efficient for the analysis and quantification of medium- and non-polar analytes

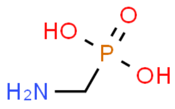
➡ Less effective for highly polar pesticides

The QuPPE method

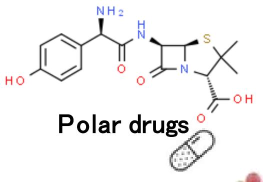
- “Quick Polar Pesticides”
- First introduced by the European Union Reference Laboratory (EURL)
- Allow to cover highly polar and ionic pesticide residues, not QuEChERS-amenable



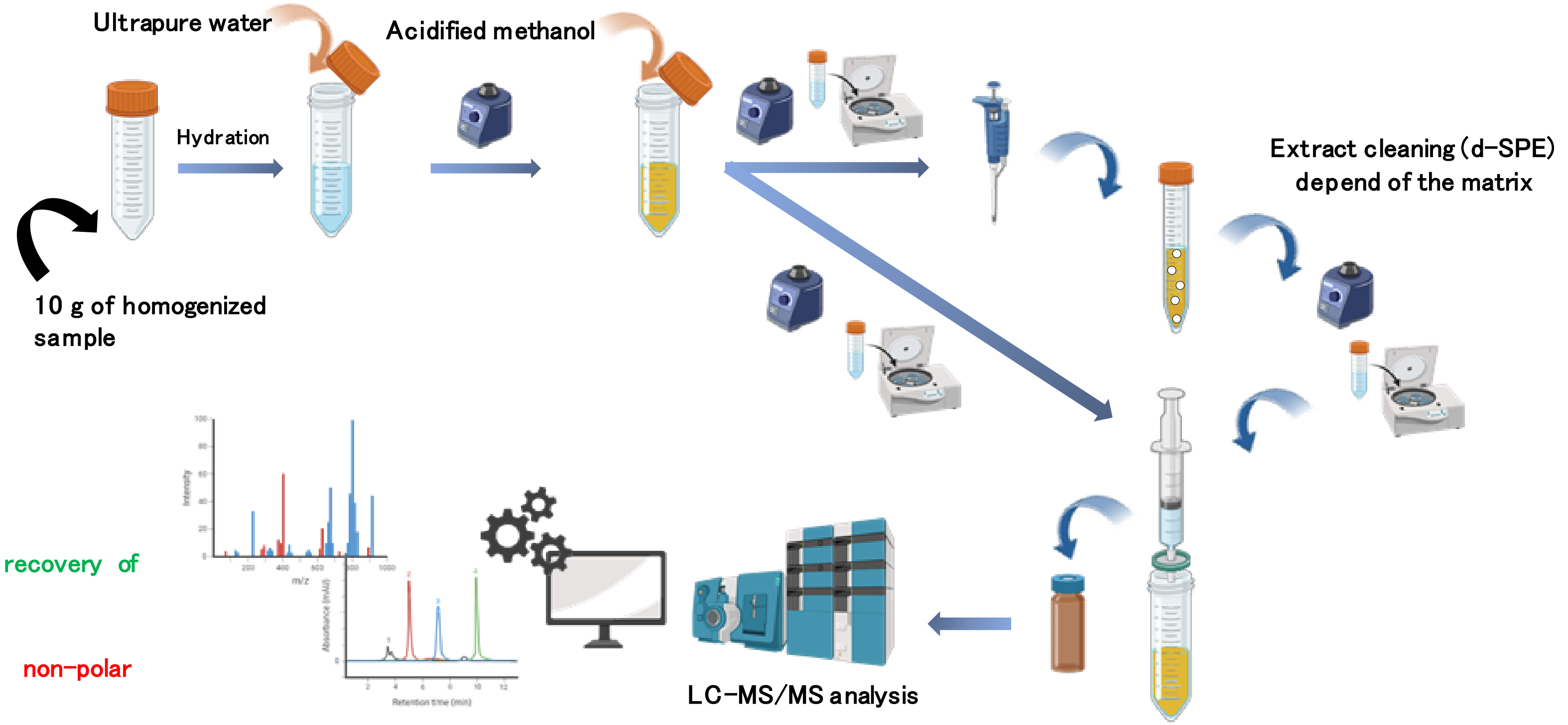
Glyphosate



AMPA



Polar drugs



Rapid and simple method

Very efficient for the analysis and recovery of polar chemicals

Less efficient for medium- and non-polar chemicals

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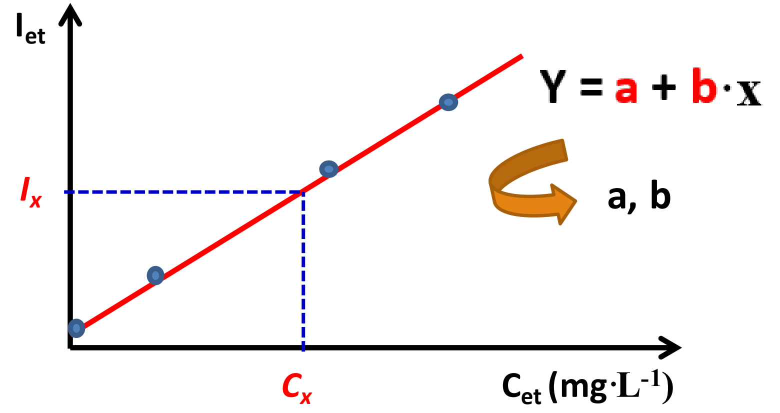
3.4. Quantification for the determination of chemical contaminants (pesticides)

$$\boxed{I = I_0 + k \times m}$$ or $$\boxed{I = I_0 + k \times C}$$ *Calibration fonction*

K, response coefficient or SENSITIVITY (to be determined by calibration)
I, intensity of analyte
I_0, average intensity of the analytical blank signal
C, concentration of analyte

External calibration method

- **This is the most common quantification method: it consists of measuring the intensity of several solutions (at least five) containing known and increasing amounts of the element to be quantified (standard solutions).**
- **The calibration curve $I_{et} = f(C_{et})$ is then plotted (I = analytical signal intensity), where I_{et} is the signal intensity corrected for the analytical blank.**
- **The intensity of the unknown solution (I_x) is then measured, and the concentration C_x is calculated using the linear regression coefficients (a and b) (I_x must also be corrected for the analytical blank).**



Step I: Calibration (analysis of the standard solutions) and determination of the parameters of the calibration function

Standard	Blank	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
$C_{standard}$ (mg·L^{-1})	0	C_{et1}	C_{et2}	C_{et3}	C_{et4}	C_{et5}
Signal intensity (I)	I_0	I_{et1}	I_{et2}	I_{et3}	I_{et4}	I_{et5}

Step II: Analysis of the unknown solution(s) (X)

Sample	X_1	X_2	X_3	…	X_n
Intensity	I_{x1}	I_{x2}	I_{x3}	…	I_{xn}

$$\mathbf{I_{xi} = a + b \times C_{x\mathit{i}}} \quad \Rightarrow \quad \boxed{\mathbf{C_{xi} = \frac{I_{xi} - a}{b}}}$$

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CHAPTER 4.

METHODOLOGIES TO DETECT THE CONTAMINATION OF CEREALS AND RELATED PRODUCTS WITH HEAVY METALS

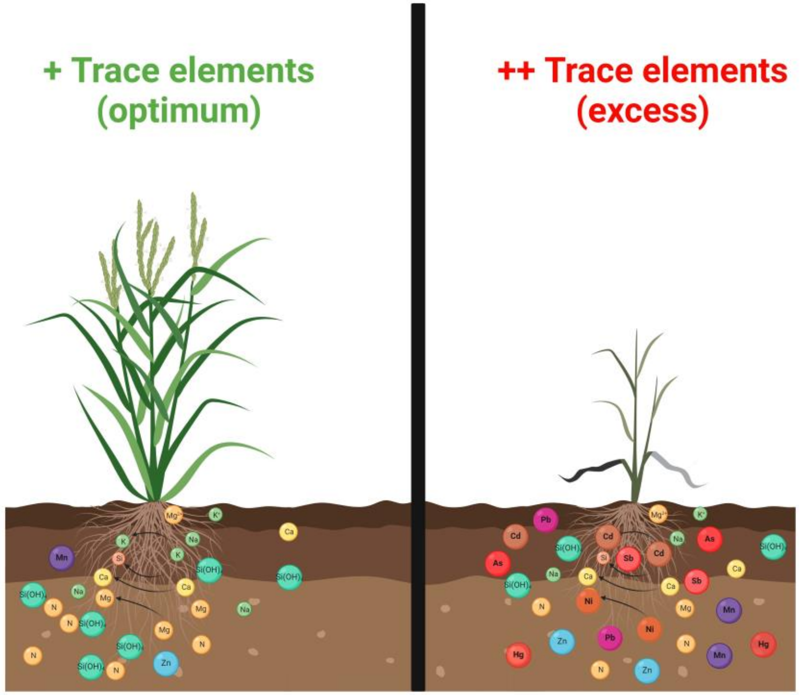
4.1. Introduction

- Trace elements are chemical elements present in a given material at average concentrations below 100 µg/g (ppm). When their levels fall below 1 ppm, they are often referred to as ultra-trace elements.
- Trace elements can be divided into two broad categories:

• Essential elements (micronutrients): Required in small amounts for biochemical and physiological processes.

• Potentially toxic elements: Harmful even at low concentrations or when present in excess.

- Historically, toxic trace elements were grouped under the term *"heavy metals"*, a designation based on their relatively high density compared to water. This terminology was misleading, as toxicity is not directly linked to atomic weight or density.
- Consequently, the term has been replaced by potentially toxic trace elements (PTTEs), which more accurately reflects their environmental and health significance.

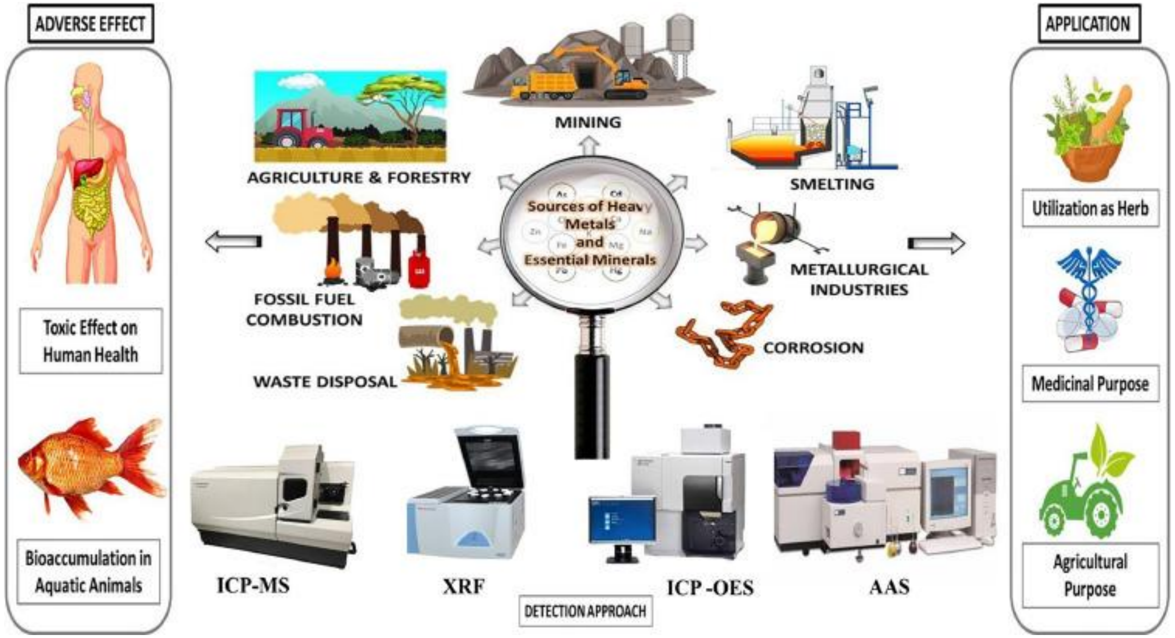


https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2024.1377964/full

- ❑ **Among PTTEs, arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd) are of particular concern due to their high toxicity, persistence, and ability to bioaccumulate in ecosystems.**
- ❑ **Conversely, several trace metals are essential micronutrients for living organisms, including iodine (I), zinc (Zn), selenium (Se), iron (Fe), copper (Cu), chromium (Cr), molybdenum (Mo), manganese (Mn), cobalt (Co), arsenic (As, in trace amounts), nickel (Ni), and vanadium (V).**
- ❑ **These elements play critical roles in enzyme function, hormone regulation, oxygen transport, and antioxidant defense. However, the distinction between essential and toxic is concentration-dependent:**

- **At optimal levels, essential trace metals support vital physiological functions.**
- **At elevated levels, they can become toxic, leading to oxidative stress, organ damage, or metabolic disruption (e.g., iron overload, manganese neurotoxicity)**

- ❑ **Trace elements are present at extremely low concentrations in natural and biological systems, yet they play a crucial role in both health and environmental quality.**
- ❑ **Because their levels often lie in the ppm (parts per million) or even ppb (parts per billion) range, quantifying them requires highly sensitive analytical methods.**
- ❑ **Traditional chemical methods, such as those based on titrimetric techniques, gravimetry or spectrophotometry are not sufficient at these scales, so modern analytical chemistry relies on advanced spectrometric and nuclear techniques.**
- ❑ **These methods allow scientists to measure trace elements with precision, distinguish between essential and toxic species, and ensure reliable monitoring in food, water, soil, and biological samples.**



https://www.sciencedirect.com/science/article/pii/S2666831924000559

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4.2. Main techniques and their main features for trace element determination in cereals

- **Atomic Absorption Spectroscopy (AAS)**
- **Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)**
- **X-Ray Fluorescence (XRF)**
- **Neutron Activation Analysis (NAA)**
- **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**

For simplicity, the most widely used techniques such as ICP-MS, ICP-OES and AAS will be discussed here. Amongst these techniques, ICP-MS is mostly wide used nowadays and hence this technique will be the main focus of the next paragraphs.

4.2.1. Atomic Absorption Spectroscopy (AAS)

Principle

The are two types of AAS, depending on the principle of the atmisation source, namely (i) flame AAS and (ii) graphite furnace AAS.

(A) Flame AAS is one of the most widely used techniques for determining trace metals in solution. In this method, the sample is aspirated into a flame (commonly air–acetylene or nitrous oxide–acetylene), where the solvent evaporates and the analyte atoms are thermally atomized. A light beam from a hollow cathode lamp, specific to the element of interest, passes through the flame.

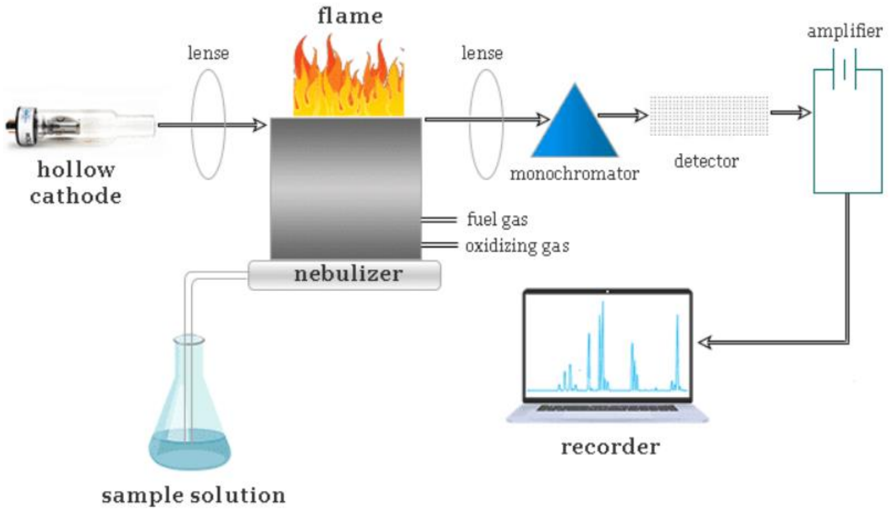
The ground-state atoms absorb this radiation at characteristic wavelengths, and the amount of absorbed light is proportional to the concentration of the element in the sample.

Advantages:

Simple, relatively inexpensive, good for routine single-element analysis.

Limitations:

- **Less sensitive (typically ppm to low ppb levels) than graphite furnace AAS or ICP-MS**
- **Mainly suited for elements present at moderate concentrations.**



(https://www.priyamstudycentre.com/2021/11/atomic-absorption-spectroscopy.html)

(B) Graphite furnace AAS (GF AAS) - in this case, a small aliquot of sample is introduced into a graphite tube (furnace). The sample is subjected to a programmed sequence of drying, ashing, and atomization at high temperature. Free atoms formed in the furnace absorb light from an element-specific hollow cathode lamp, and the absorbance is proportional to the analyte concentration.

Advantages

- Very high sensitivity, with detection limits in the pg–ng/L range.
- Requires very small sample volumes (5–20 µL).
- No nebulization → high sample introduction efficiency.
- Can handle complex matrices with optimized temperature programs.
- Well suited for trace element analysis in water, food, environmental or biological samples.

Limitations

- Single-element technique (one element measured at a time).
- Slower analysis compared to ICP-OES or ICP-MS.
- Susceptible to matrix interferences → often requires chemical modifiers.
- Graphite tube contamination and wear require maintenance and periodic replacement.
- Smaller dynamic range than ICP-based techniques.

4.2.2. Inductively coupled plasma - Optical emission spectroscopy (ICP-OES)

Principle

ICP-OES is based on the excitation of atoms and ions in a very hot plasma (≈ 6,000–10,000 K) (ICP). The plasma is generated by an argon gas flow sustained by a radiofrequency field. When the sample solution is nebulized and introduced into the plasma, the high temperature causes atomization and excitation of the elements. As these atoms and ions return to lower energy states, they emit light at characteristic wavelengths. A spectrometer separates and measures this emitted light, and the intensity is proportional to the concentration of each element.

Advantages

- Multi-element capability: Can measure dozens of elements simultaneously.
- Wide dynamic range: Suitable for concentrations from µg/L (ppb) up to mg/L (ppm).
- Robustness: Handles complex matrices (soil digests, industrial effluents, biological samples).
- Speed: Rapid analysis compared to single-element techniques like AAS.

Limitations

- ICP-OES is sensitive, but its detection limits are not as low as ICP-MS, especially for trace-level metals (ng/L–µg/L range).
- Not ideal for ultra-trace analysis
- Complex matrices (e.g. biological samples) can produce many emission lines that partially overlap with the analyte signal.

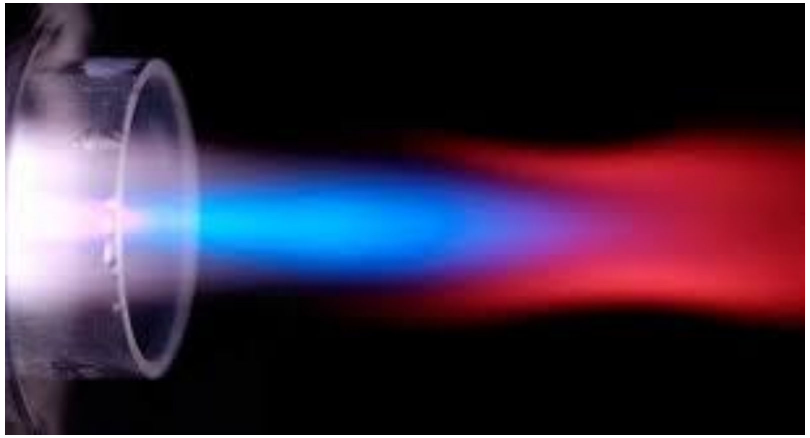
4.2.3. Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

⇒ The analysis by ICP-MS begins with the sample introduction/transport to the nebulizer by means of a peristaltic pump. The sample is nebulized and converted into a fine aerosol by the nebulizer.

⇒ The aerosol is then transported to the center of the ICP through a plasma torch, typically made of three concentric quartz tubes. This torch is positioned within an inductive coil that generates radio frequency (RF) magnetic fields that creates and sustains the plasma.

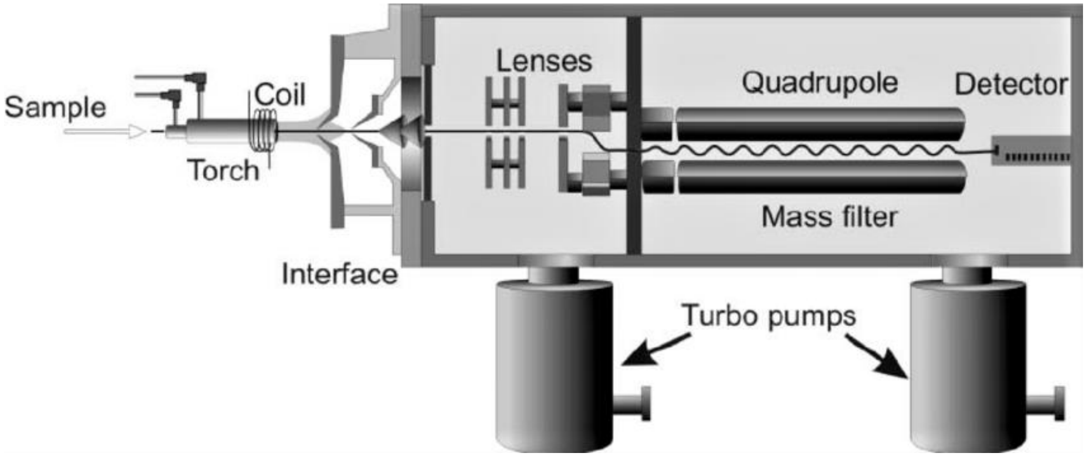
⇒ At high temperatures (6000 – 8000 K), the plasma atomizes and ionizes the analyte, producing (mostly) singly charged cations.

⇒ The ions are subsequently extracted via the interface region and directed into the ion optics, a set of electrostatic lenses that focus and guide the ions.



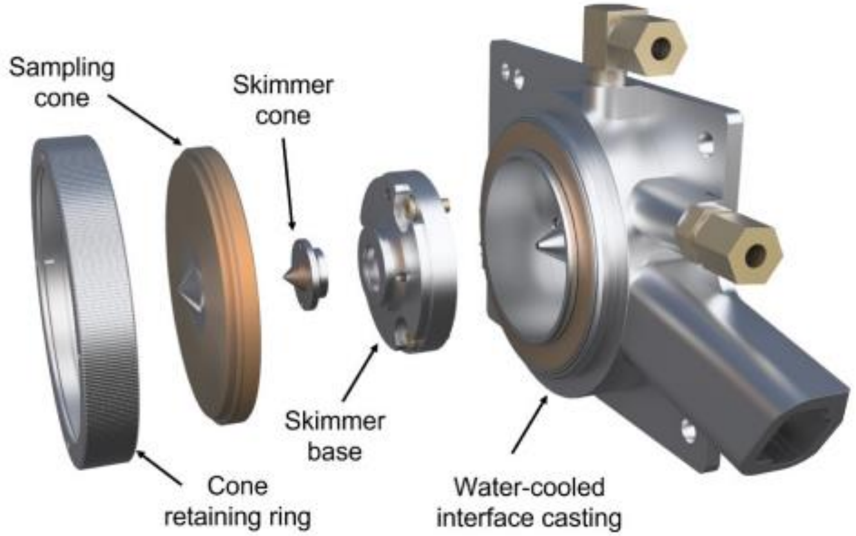
https://www.nature.com/articles/milemassspec10

- ICP-MS has seven basic compartments: the sample introduction system (nebuliser, spray chamber), a torch, the ICP, the cone interface (sampler and skimmer), the ion optics, the mass analyser and the detector.
- The standard ionisation source found in all commercial ICP-MS instruments is an Ar-based ICP. This plasma is created using radio frequency (RF) magnetic fields generated by a coil wrapped around a quartz torch.
- The standard torches found in commercial equipment are typically based on the Fassel design, characterised by three concentric quartz tubes.
- In this design, the outer tube contains the plasma gas, the intermediate tube carries the auxiliary gas and the central tube (called the injector tube) is for the carrier gas.
- Ar is often used as the operating gas for all three gas streams.



The schematic diagram showing the main part described above is provided in the figure above.

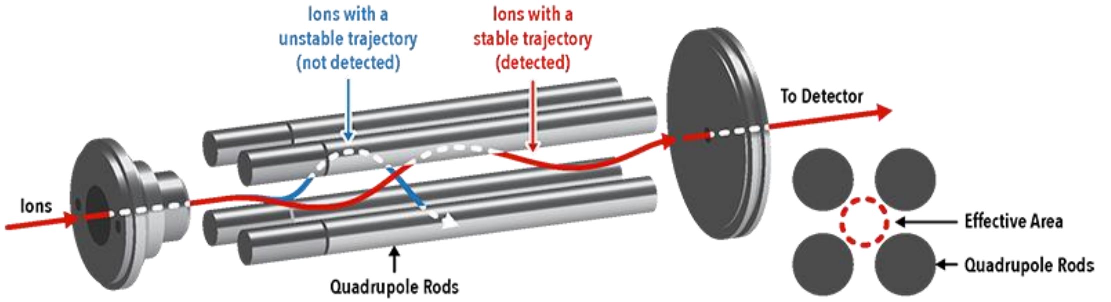
- ☐ The interface region consists of two cones: the *sampler* and the *skimmer*.
- ☐ These cones extract the ions from the plasma, with a primary pump maintaining a vacuum of approximately 101 Pa between them.
- ☐ Behind the sampler cone, a supersonic jet forms and passes through the *skimmer* cone before entering the ion optics, which focuses the ion beam and guide it to the quadrupole mass analyzer, which further processes the ions beam.
- ☐ The most common mas analyser in ICP-MS are the quadrupoles.



Interface components of an ICP-MS.

https://www.agilent.com/cs/library/flyers/public/flyer-interface-cones-icp-ms-5994-4695EN-agilent.pdf?srsltid=AfmBOooVwnfd4r67Or0-H7MOj11qAcjqQlfaixHVJ5cnoQmYrVszjBTD

- ❑ The quadrupole mass analyzer acts as a mass filter, consist of four circular rods subjected to both a direct current and a radio frequency voltage. This configuration separates ions based on their mass ratio (*m/z*).
- ❑ The selected ions are then directed to the detector, an electron multiplier, which quantifies the ions. A high vacuum, maintained by turbomolecular pumps, is essential for the analyzer and detector to function efficiently.
- ❑ Ions formed in the ICP are highly reactive and can combine with other ions, leading to the creation of polyatomic species that contribute to interference.
- ❑ For instance, the presence of chlorine (Cl) or carbon (C) can interact with the carrier gas argon (Ar), producing polyatomic species such as $^{40}Ar^{35}Cl$ and $^{40}Ar^{12}C$.
- ❑ These species interfere with the accurate measurement of elements like arsenic (^{75}As) and chromium (^{52}Cr) by overlapping with their signals.



https://www.spectro.com/inductively-coupled-plasma-mass-spectrometry-icp-ms-principle

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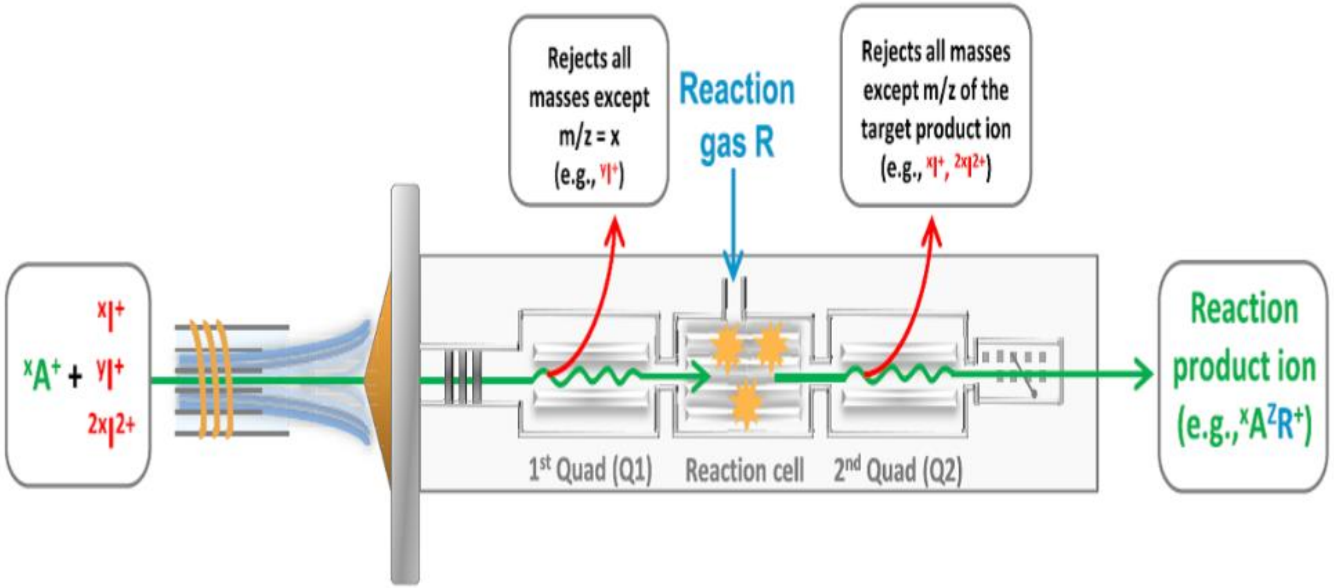
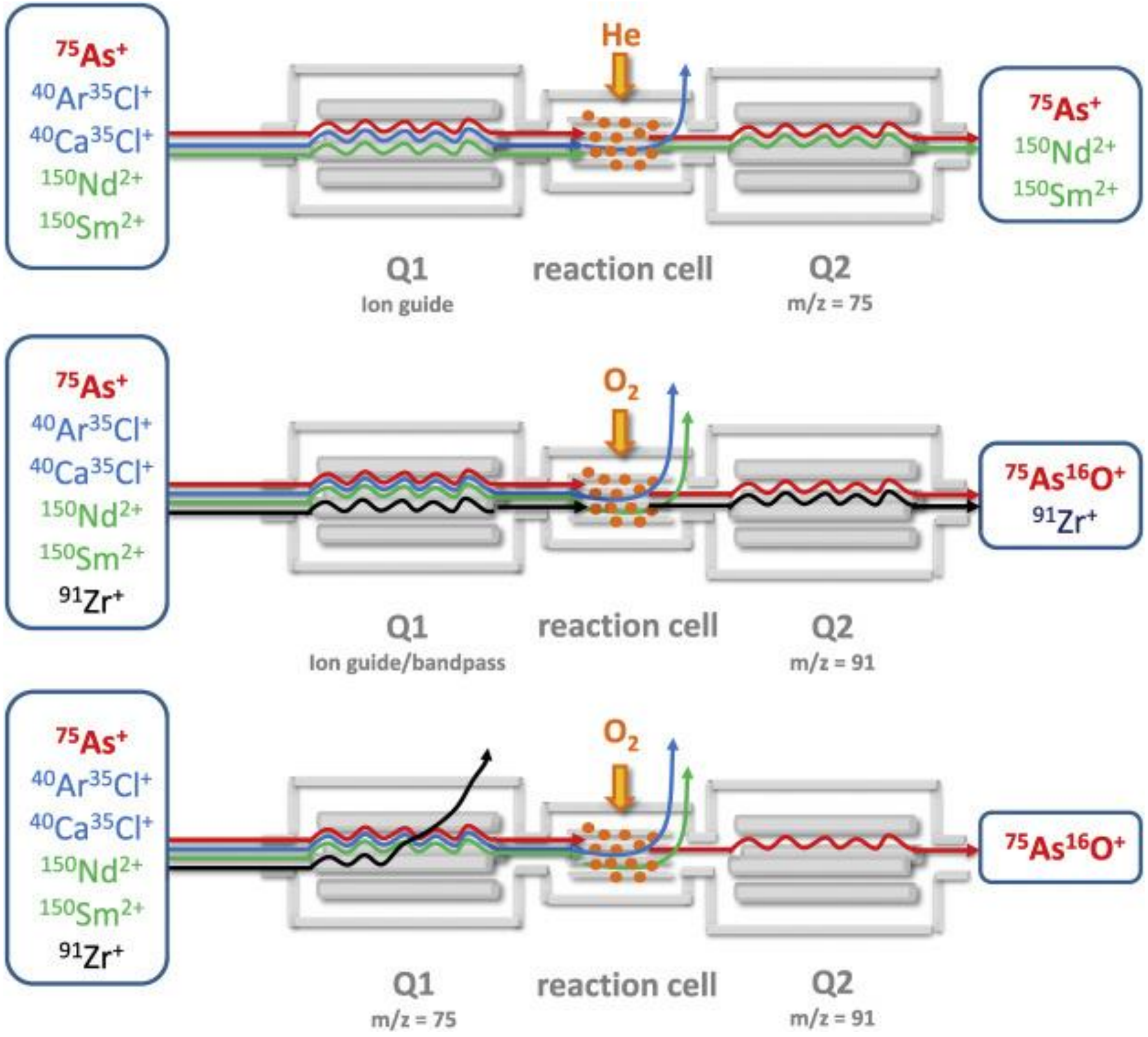
- ☐ To address polyatomic interferences, more advanced approaches include the use of collision cells, which are now standard in all modern ICP-MS systems.
- ☐ Collision/reaction cells operate based on kinetic energy discrimination (KED). In this technique, a collision gas (such as He, H_2 or Xe) or a reaction gas (such as O_2, NH_3, CH_4, H_2) is introduced into a pressurized cell.
- ☐ The collision gas interacts with both the interfering polyatomic species and the target analyte. Due to their larger size, the interfering species will lose kinetic energy more readily than the target analytes, allowing the analyte ions to be separated from the interferent ions before detection by the mass analyzer.



Schematic representation of the operating principle of the tandem ICP-MS/MS

https://www.ams.ugent.be/tandem-icp-mass-spectrometry-icp-msms

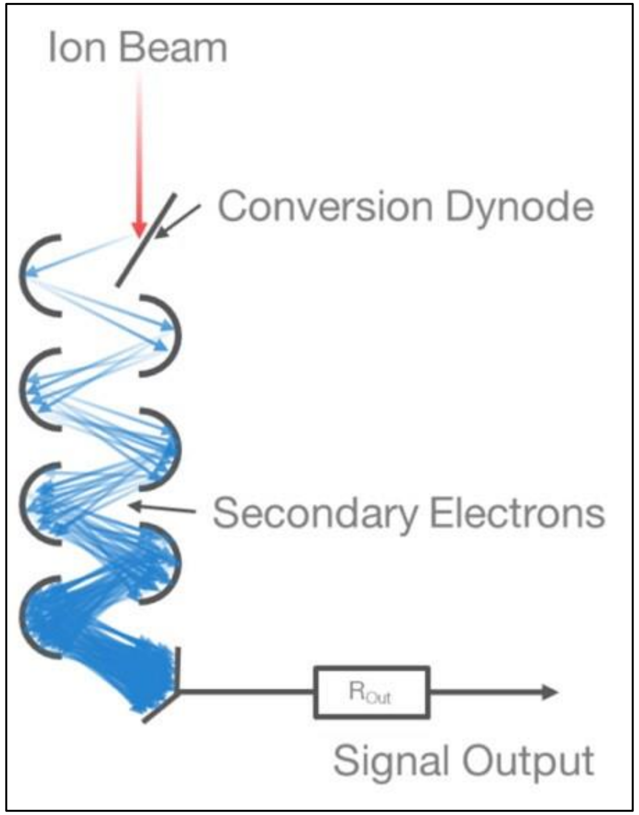


- **Alternatively, when a reaction gas is introduced, it may react with either the target analyte or the interfering species. In the former case, the target analyte forms a new species that is free of interferences and detectable.**
- **In the latter, the interfering species are converted into non- overlapping forms, ensuring they do not interfere with the analyte of interest. Those two processes significantly enhances the accuracy of element quantification.**

Example of the added value of the double mass selection mode (MS/MS) for the determination of As in complex matrices.

https://www.sciencedirect.com/science/article/pii/S000326701501065X

- ☐ Most ICP-MS systems rely on an electron-multiplier (EM) detector to quantify ion signals. An EM is made up of a sequence of charged plates called dynodes.
- ☐ When an ion hits the first dynode, it generates several electrons that are accelerated toward the next dynode, where additional electrons are released.
- ☐ This cascade continues through the entire dynode chain, progressively amplifying the signal until it becomes strong enough for the counting electronics to register it as an ion event.



The basic schematic of an electron multiplier.

https://www.thermofisher.com/fr/fr/home/industrial/spectroscopy-elemental-isotope-analysis/spectroscopy-elemental-isotope-analysis-learning-center/trace-elemental-analysis-tea-information/inductively-coupled-plasma-mass-spectrometry-icp-ms-information/icp-ms-systems-technologies.html

4.3. Sample preparation for trace elements determination

- **For the determination of trace elements, the solid samples must be digested to be compatible with the atomic spectrometry based techniques. A description of the main techniques for samples dissolution is provided below.**
- **It is worth to note that this step in a chemical analysis method is prone to introduce the highest uncertainty source (see below).**

Analytical
Methods

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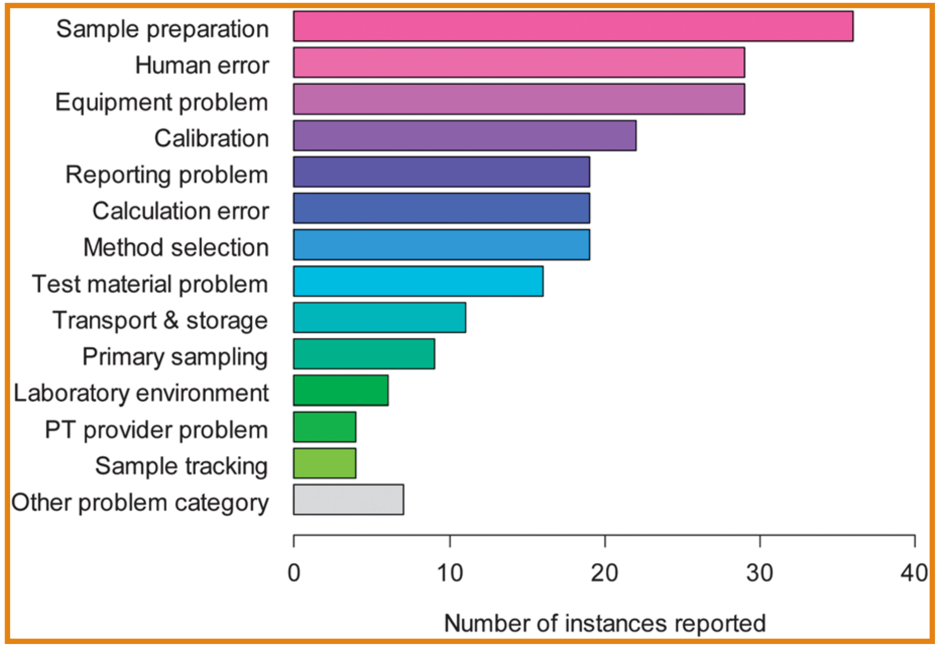
What causes most errors in chemical analysis?

Cite this: *Anal. Methods*, 2013, **5**, 2914

Analytical Methods Committee, AMCTB No 56



4.3.1. Classical dissolution techniques

Hot plate
(open-system heating)

Strong acids (HNO_3, HCl, H_2SO_4, $HClO_4$)
or
Strong acids + oxidants (H_2O_2)

Digestion bomb
(closed-system heating)

4.3.2. Modern dissolution techniques

(A) Ultrasound-assisted digestion

- This technique uses an energy source in the form of acoustic waves in the ultrasonic range (> 20 kHz) to enhance the agitation of the sample.
- Two different types of ultra-sound system are nowadays available for laboratory use, namely the ultrasonic baths and the ultrasonic probes (see below).

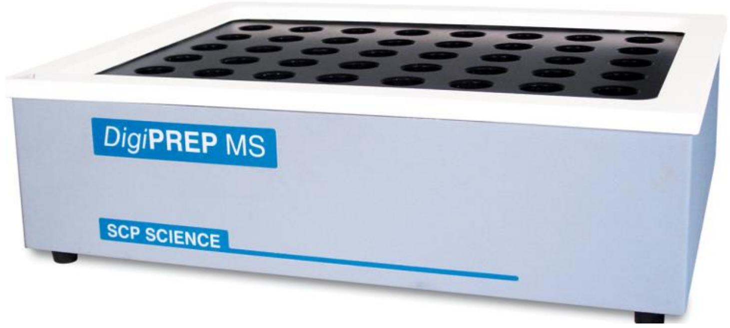
Ultrasonic bath

Strong acids (HNO_3, HCl, H_2SO_4, $HClO_4$)
or
Strong acids + oxidants (H_2O_2)

Ultrasonic probe

(B) Heating block assisted digestion

- This type of digestion systems are generally designed and manufactured with graphite block technology to offer superior performance in harsh laboratory environments.
- All have a non-metallic construction with a corrosion resistant case and a protective coated graphite block. This eliminates sample contamination from the instrument, resists aggressive corrosive attack, ensuring longevity of the system.
- The heating block systems can operate with either a simple to use key pad controller or the multi-method colour touch screen controller.



Heating block type *DigiPrep*

Strong acids (HNO_3, HCl, H_2SO_4, $HClO_4$)

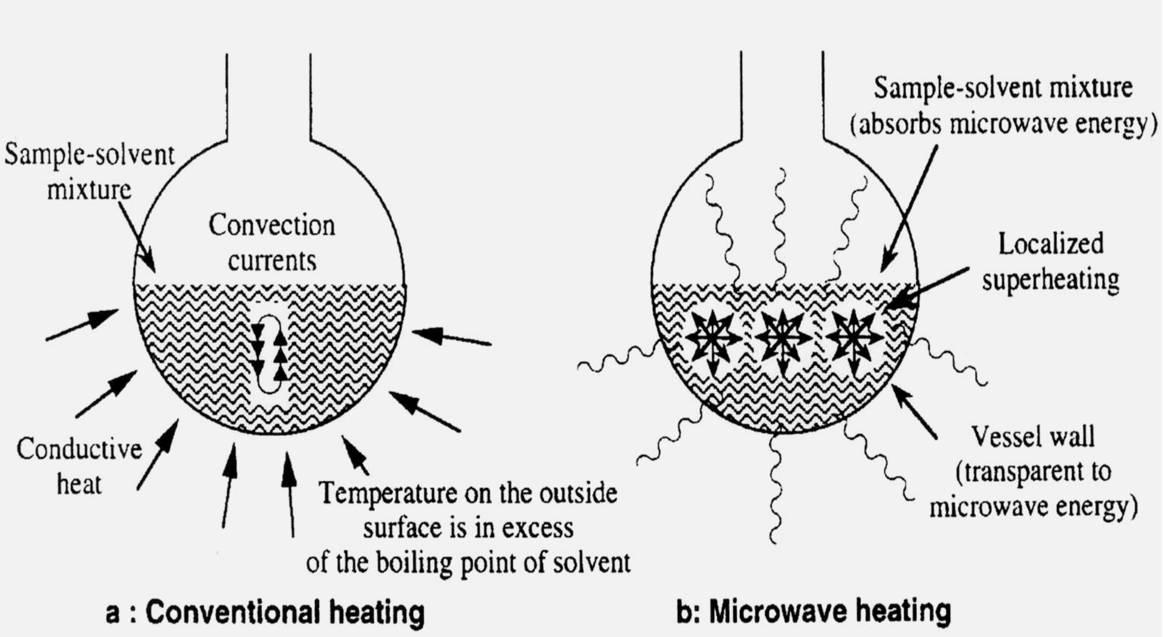
or

Strong acids + oxidants (H_2O_2)

An image of heating block for samples digestion of the brand DigiPrep is shown in the Figure to the right.

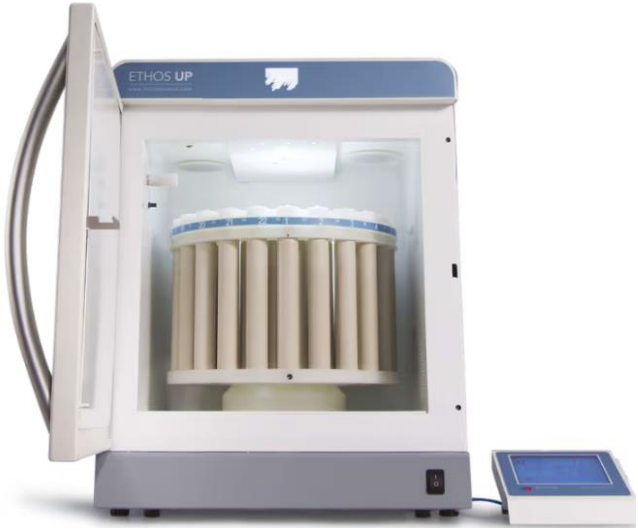
(C) Microwave assisted digestion

- Microwave energy is a non-ionizing form of electromagnetic radiation (300 MHz to 300 GHz) that induces molecular motion through ion migration and dipole rotation, but normally does not cause changes in molecular structure.
- Microwave-assisted (closed-vessel) acid digestion is a widely adopted sample-preparation technique for trace and ultra-trace elements determination by ICP-MS.
- It enables rapid mineralization of complex food matrices under elevated pressure and high temperatures, using concentrated HNO_3 alone or in combination with oxidizing or matrix-decomposing agents (e.g., H_2O_2, HCl, HF/H_3BO_3).
- The sample is mixt with the digestion reactives directly in the digestion vessel, which can be made of Teflon (in most of the case) or quartz.



Schematic representation of the difference between conventional and microwave heating.

Different types of microwave digestion systems are available on the market today (see below a picture of such a system).



Strong acids (HNO_3, HCl, H_2SO_4, $HClO_4$)
or
Strong acids + oxidants (H_2O_2)

Convention microwave oven (with Teflon vessels)

(https://www.chemeurope.com/en/products/1128777/microwave-digestion-system-ethos.html)

Microwave system type “autoclave”

4.4. EXEMPLE: Sample preparation and ICP-MS determination of trace elements determination based on the ANSES multi-elemental reference method

- ☐ The Trace Elements and Nanomaterials (TEN) laboratory of ANSES has recently published a multi-element method for trace elements determination in a large panel of food samples (including cereals) based on ICP-MS measurement and closed microwave acid digestion.
- ☐ It is worth top note that the method is also accredited by COFRAC (the French accreditation body).
- ☐ The main futures of this methods, perfectly compatible with the current project, are presented briefly below.

Sample preparation for analysis

At all stages, it is essential to ensure that contamination levels are kept as low as possible (decontamination of glassware and other materials in contact with samples/standards, good laboratory practices).

Sampling

The sampling of the sample must be adapted according to the digestion system (container capacity and potential pressure increase). It is essential to follow the instructions provided by the manufacturer of the system used for sample digestion.

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Acid addition (for sample digestion)

- ❑ The volume of acid solution required for dissolving (digesting) the sample depends on the system used and the nature of the sample. HNO_3 is generally used (~3.0 mL). The use of a small amount of HNO_3/H_2O_2 mixture (0.5–1.0 mL) is also possible to increase the oxidative capacity of the digestion and limit the formation of nitrogen oxides.
- ❑ Addition of an HCl solution is also possible, as it promotes the dissolution of certain compounds such as lead chlorides, and minimizes adsorption of some elements on the walls of digestion vessels or during transfer of mineralized solutions.
- ❑ In all cases, the digestion program must be adapted according to the recommendations of the manufacturer of the digestion system used.
- ❖ After acid (or reagent mixture) addition, gently swirl the digestion vessel to avoid sample agglomerates sticking to the walls before closing the vessel.
- ❖ Wait approximately 30 minutes at room temperature to allow for a pre-digestion step (recommended in the case of microwave mineralization systems) before starting the digestion program.
- ❖ The pre-digestion step can last several hours or even overnight, depending on the sample type and digestion system used (not required if digestion is performed using an open heating block).

https://lab-training.com/how-to-safely-carry-out-laboratory-digestions/

Digestion:

- ❑ Proceed with digestion of the sample, adding ultrapure water if necessary, using the chosen digestion system.
- ❑ The final state of sample digestion depends on the digestion temperature-the higher the temperature, the better the quality of the mineralized solution.
- ❑ The solution obtained after digestion should be clear, free of suspended particles, and have a volume equivalent to that before digestion.
- ❑ Sampling, digestion solution volumes, and heating durations should be adapted according to the type of equipment used, following the manufacturer’s recommendations.
- ❑ Allow the digestion vessel to cool to room temperature and rinse it (including the cap) with ultrapure water. Then transfer the solution into a 50 mL volumetric flask or a 50 mL polypropylene tube. Add 100 µL of internal standard solution and make up the volume with ultrapure water.

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Notes:

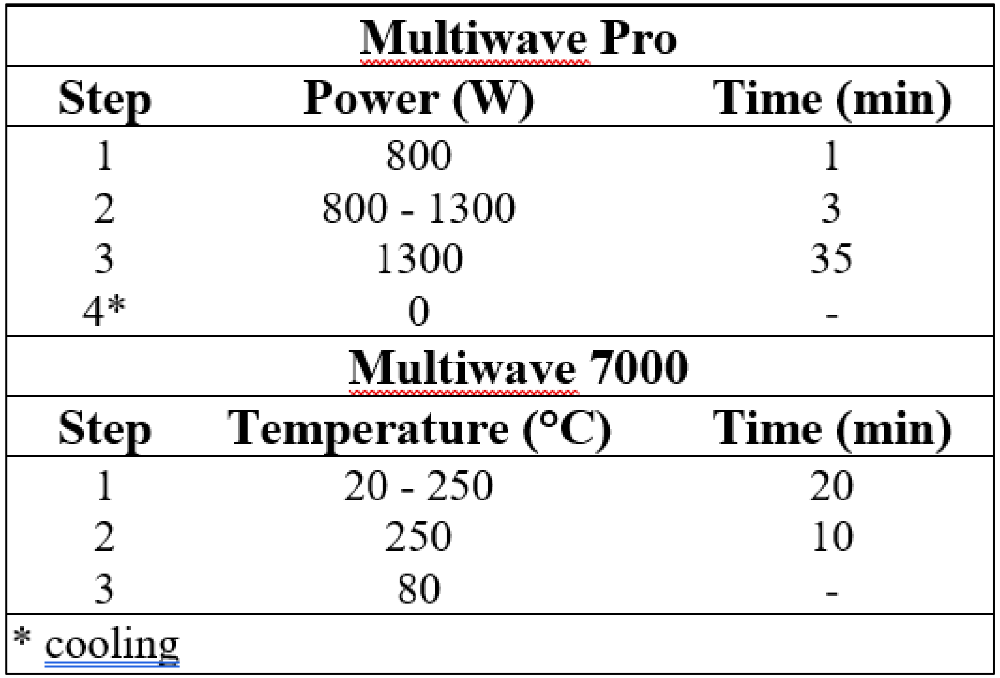
(i) Solutions can be filtered using syringe filters if necessary (especially when using a heating block system for digestion); in this case, specify the type of filters used (pore size and chemical composition). 20 mL syringes with 0.45 µm PVDF filters (Millex or equivalent), or DigiFILTER 0.45 µm PTFE (SCP SCIENCES or equivalent), can be used.

(ii) All solutions to be analyzed (including blanks and standards) must contain internal standards at the same concentration. The internal standard solution can also be added online using a peristaltic pump.

(iii) To ensure the validity (accuracy) of analytical results, it is necessary to simultaneously analyze reference materials (RMs) with known analyte contents under the same conditions. In the absence of RMs, accuracy is verified by analyzing spiked samples at known concentration levels. Blank tests must also be performed to ensure the absence of contamination. The position of the RM/spiked sample in the digestion system should be alternated to test the efficiency across all positions.

https://www.flaticon.com/free-icon/notebook_7063317

Digestion Programs for two Microwave Digestion System (Anton Paar Multiwave PRO and Multiwave 7000).



Multiwave Pro		
Step	**Power (W)**	**Time (min)**
1	800	1
2	800 - 1300	3
3	1300	35
4*	0	-
Multiwave 7000		
Step	**Temperature (°C)**	**Time (min)**
1	20 - 250	20
2	250	10
3	80	-

* cooling

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Internal Quality Control

- Several internal quality controls (IQCs) and associated criteria are used to ensure the reliability of the results. Each run includes a standard calibration, several blanks, several certified reference materials (CRMs) and different spiked sample solutions.
- The set criteria are generally follows:

calibration ($r^2 > 0.995$), blanks (values < limit of quantification (LOQ)), internal standards (values within 70 and 130% of the target value), midrange standards (values within 80 and 120% of the target value), spiked standard solutions (spike recovery within 70 and 130% of the theoretical spiked standard value), CRMs (Z-score < ± 2), and duplicates (acceptable if the relative standard deviation (RSD) ≤ 20% when mean value ≥5 x LOQ or RSD ≤ 40%when mean value ≥ LOQ and < 5 x LOQ).

When acceptance criteria were not meet, the results are discarded and the samples are re-analyzed.

https://www.educatinguk.com/what-is-internal-quality-assurance/

Complete details of the method can be found at:
https://www.sciencedirect.com/science/article/pii/S0889157525015273?via%3Dihub

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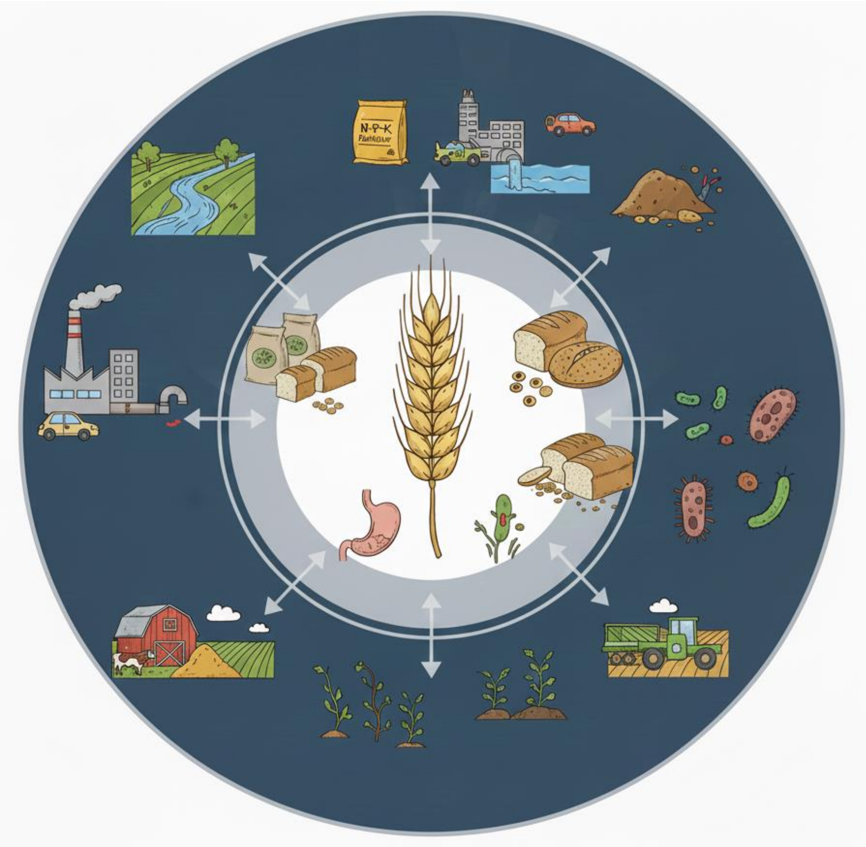
CHAPTER 5.

MEASURE TO DETECT THE CONTAMINATION OF CEREALS AND RELATED PRODUCTS WITH NITROGEN COMPOUNDS

Detecting contamination with nitrogen compounds in cereals and related products is essential for food safety and quality control. The war in Ukraine has created new contamination sources that exceed traditional monitoring. To address these risks, we must implement updated detection methods focused on explosive residues and conflict-related chemical shifts



__Relevant Forms of Nitrogen in Cereals and Related Products:__

• Total Nitrogen/Crude Protein: Protein-bound nitrogen (majority) and its role.

• Non-Protein Nitrogen (NPN): -Adulterants: Urea, Melamine, and Cyanuric Acid. These are high-nitrogen compounds sometimes added to grain or flour to make them appear to have higher protein levels than they actually do. -Natural Metabolites: Free amino acids, nucleic acids (DNA/RNA), ammonia, and peptides. -Fertilizers: Ammonium salts (like ammonium phosphate or sulfate) that may remain on crops

• Inorganic Nitrogen Compounds:

- Nitrates (NO_3^-) - Often an indicator of over-fertilization or water contamination.
- Nitrites (NO_2^-) - Can be formed by the reduction of nitrates.

In the context of the conflict in Ukraine, the contamination of cereals with nitrogen compounds has taken on new dimensions, moving from standard agricultural risks to complex chemical and toxicological threats. These forms of contamination can be classified into three main categories:

I. Residues from Munitions and Explosives

Most modern explosives are nitrogen-rich organic compounds. These are not merely pollutants; they are substances with high toxic potential.

- RDX (Cyclonite) and HMX: These are highly stable nitro-organic compounds. RDX is water-soluble and mobile in soil, being easily absorbed by the roots of wheat and corn. It accumulates in the grain and can persist into the flour.

- TNT (Trinitrotoluene): Although less mobile than RDX, its presence in the soil of explosion craters inhibits plant growth and can contaminate the harvest through dust deposited on the plants (surface contamination).

- Degradation Products: Over time, these explosives decompose into aromatic amines, which are often more toxic or persistent than the original compounds.

II. Atmospheric Contamination and "Acid Rain"

The explosion of a single missile or artillery shell releases massive quantities of nitrogen oxides

- Nitric Acid (HNO_3): Nitrogen oxides react with atmospheric moisture to form nitric acid, which falls as acid rain. These precipitations cause a sharp increase in the nitrate and nitrite content of the topsoil and leaf surfaces, disrupting the plant's nitrogen metabolism and leading to abnormal nitrate accumulation in the harvested grains.

III. Nutritional Degradation (The Nitrogen Paradox)

The war does not only bring toxic compounds; it also causes a controlled "nitrogen famine" that alters the composition of derived products.

Decrease in Protein: Due to the destruction of fertilizer production infrastructure (e.g., the Azovstal plant or factories in occupied areas), crops no longer receive the necessary fertilization.The resulting cereals have a significantly lower protein-nitrogen content. This affects baking quality (flour with weak gluten) and the nutritional value of derived products

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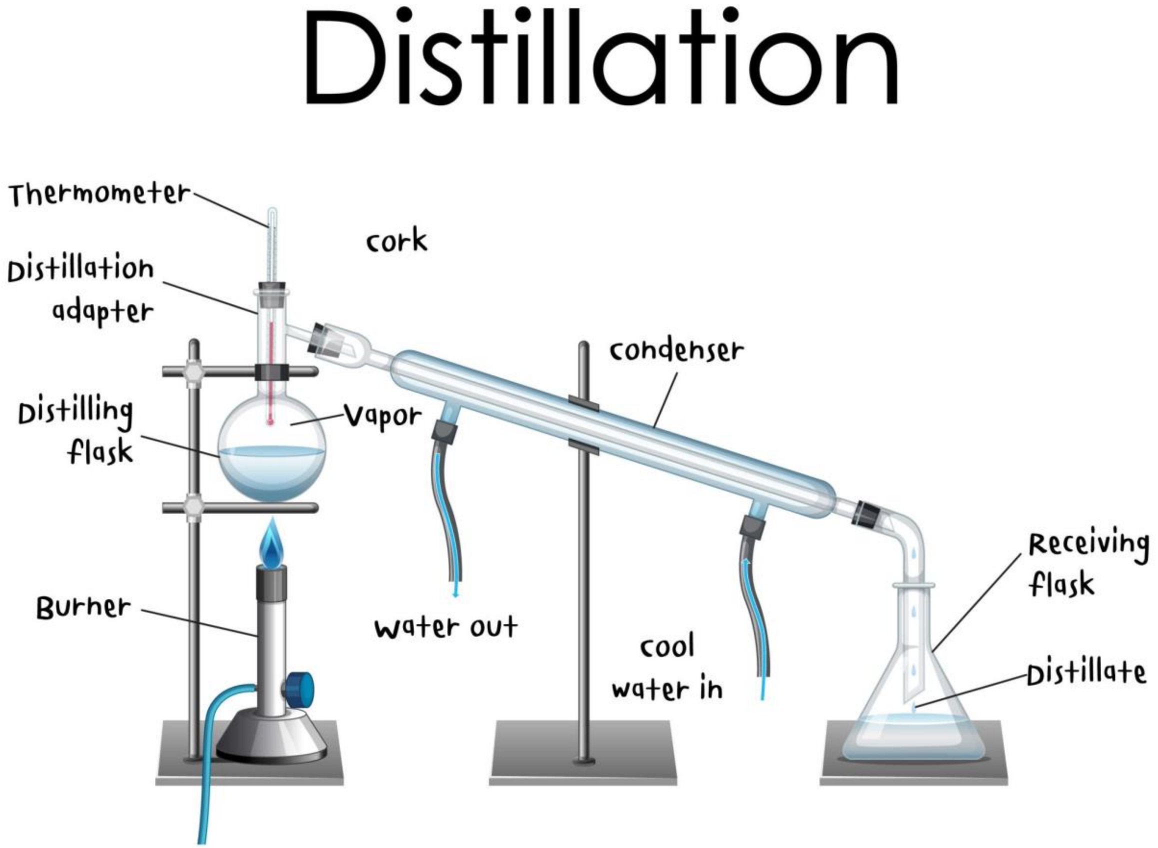
Methods for Detecting Nitrogen Compounds

1. Kjeldahl method (total nitrogen)

The Kjeldahl method is the classic method for determining the total nitrogen content of a sample, which is then used to estimate the protein content. Although it measures all organic and inorganic nitrogen (except for some rings and nitrogen in azo compounds), an abnormally high total nitrogen content that does not correspond to the expected protein levels may be a warning sign of contamination (e.g., if a non-protein nitrogen compound such as melamine is present).

***Principle*: The sample is digested with concentrated sulfuric acid in the presence of a catalyst to convert all nitrogen into ammonium sulfate. The ammonium is then liberated as ammonia via distillation with a strong base, collected in an acidic solution, and quantified by titration.**



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Kjeldahl Method Protocol

- *1. Sample preparation:* Cereals are ground to a fine particle size (approx. 0.5-1.0.mm) to ensure homogeneity. Exactly 1-2 g of the sample is weighed (depending on the estimated protein content) and introduced into the digestion flask.

- 2. *Digestion (Mineralization):* This stage converts organic nitrogen into ammonium sulfate $(NH_4)_2SO_4$: add 20 ml of concentrated H_2SO_4 and a catalyst (usually a copper sulfate or selenium tablet). Start with a low temperature to control foaming (starch caramelization), then gradually increase it to approximately 370-400°C. Digestion is complete when the solution becomes clear and light blue-green in color. Boil for an additional 30-60 minutes to ensure complete digestion.

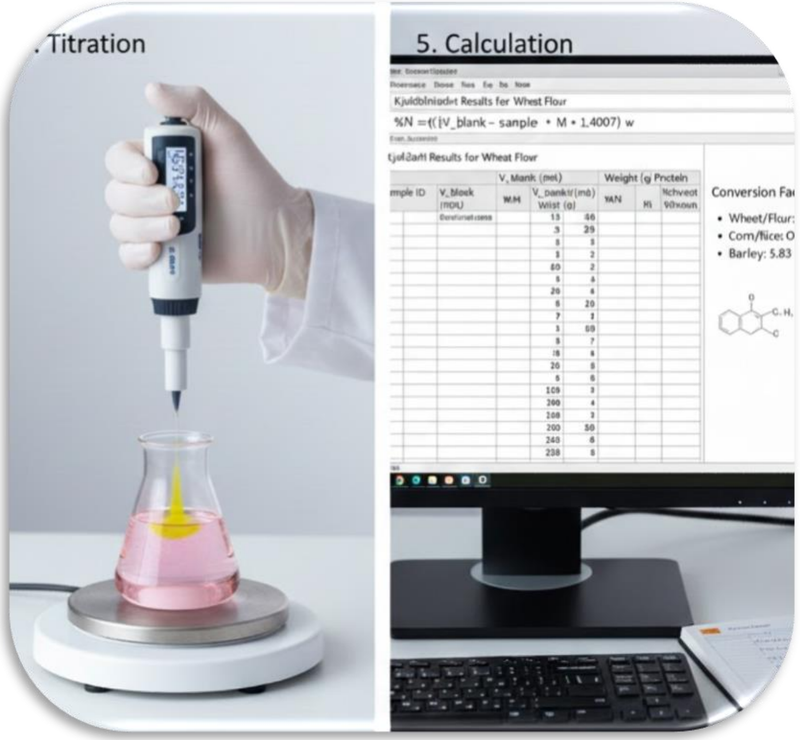
- *3. Distillatin:* After cooling, the digest is diluted with distilled water.. A concentrated solution of NaOH (4045%) is added to release ammonia in gaseous form: $(NH_4)_2SO_4 + 2\ NaOH \rightarrow Na_2SO_4 + 2NH_3\uparrow + 2H_2O$. The ammonia is steam distilled and collected in a receiving vessel containing a known volume of boric acid (H_3BO_3) or strong acid (HCl or H_2SO_4)

- ***4. Titration***: If boric acid was used, titrate directly with HCl or H_2SO_4 until the indicator changes color (from green to pink/violet). If the back-titration method was used, titrate the excess acid with NaOH.

- ***5. Calculation***: The final result is obtained by calculating the nitrogen percentage, which is then converted into crude protein using a specific conversion factor for cereals.

- ***Calculation of Nitrogen Percentage (N%):***

$$\%N=\frac{(V_{blank}-\mathrm{Vsample})\,\mathrm{x}\;M\;\mathrm{x}\;1.4007}{m}$$



Where: V_{blank}- the volume of NaOH used for the control sample (ml).

V_{sample}- the volume of NaOH used for for the grain sample (ml).

M - Actual molarity of the NaOH solution

1.4007 - Factor derived from the atomic mass of nitrogen (14.007) correlated with the units of measurement.

m - Weight of the grain sample in grams (g).

- **Calculation of crude protein (%P):**

%Protein = %N x F

Common conversion factors (F):

5.70 – for wheat and wheat flour (most commonly used in the milling industry)

6.25 – for corn, rice, oats, and most other grains or feeds

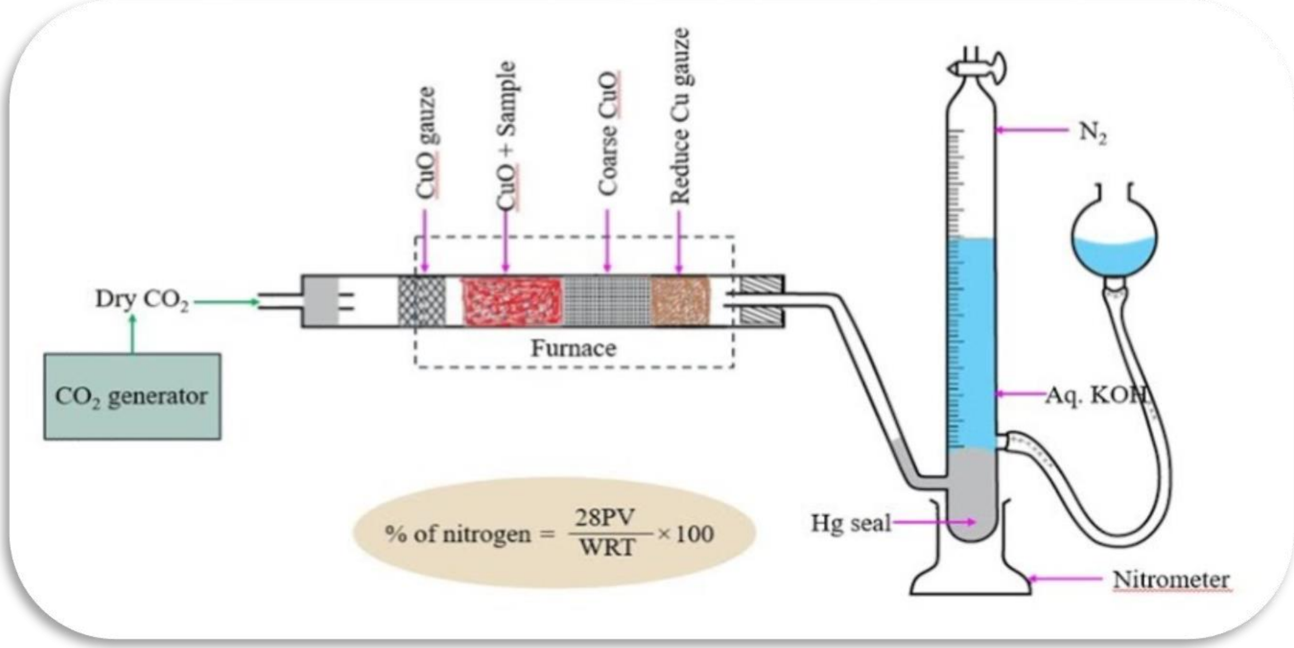
5.83 – for barley

2. Dumas Method

The Dumas method represents a modern, automated, and rapid alternative to the traditional Kjeldahl method for determining the total nitrogen content in organic samples (food, feed, soils).

The process is based on the conversion of all nitrogen within a sample into gaseous form through complete oxidation.

1. *Combustion (oxidation):* The sample is burned in a furnace at very high temperatures (typically between 800 – 1000°C) in an oxygen-enriched atmosphere. All elements are converted into gases: carbon becomes CO_2, hydrogen becomes H_2O and nitrogen becomes NxOy (nitrogen oxides) and N_2



2. Reduction: **The resulting gases pass over a bed of hot copper. Here, the nitrogen oxides are reduced to molecular nitrogen.**

3. Purification **(By-product Trapping): The gas mixture passes through absorption columns that remove water and carbon dioxide, leaving only the nitrogen and the carrier gas (usually helium or argon).**

4. Detection **(TCD): The pure nitrogen is measured using a Thermal Conductivity Detector (TCD). The generated electrical signal is converted directly into a nitrogen percentage**

Advantages and Limitations

Advantages: Beyond its speed, the Dumas method eliminates the need for corrosive acids and strong bases. It is extremely precise for homogeneous samples and allows for a high daily sample throughput.

Limitations: The instrument cost is significantly higher than that of a distillation system. It also requires rigorous sample preparation (fine grinding), as the amount of sample analyzed is usually small (milligrams).

Protein calculation:

Regardless of the method, once the nitrogen percentage (N%) is determined, the crude protein content is calculated using a product-specific conversion factor (for example, 6.25 for most foods):

$$\text{Protein } (\%) = \%N \times F$$

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3. Spectrophotometric methods (nitrates and nitrites)

These methods are specific and highly sensitive for measuring nitrate (NO_3^-) and nitrite (NO_2^-) concentrations. Nitrites are of particular concern due to their higher toxicity and potential to form carcinogenic nitrosamines.

Nitrite: *The Griess reaction* is the most common method. Nitrite reacts with a primary aromatic amine (e.g., sulfanilamide) under acidic conditions to form a diazonium salt. This salt is then coupled with another aromatic compound (e.g., N-(1-naphthyl)ethylenediamine dihydrochloride or NEDD) to produce a strongly colored azo dye, which is measured using a UV-Vis spectrophotometer at a specific wavelength (usually around 540 nm).

Nitrate: Nitrate itself has a weak color. It must first be reduced to nitrite (e.g., using cadmium column reduction) before applying the Griess reaction for quantification.

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Laboratory Protocol

Reagents Needed:

- **Griess Reagent A: 1% Sulfanilamide in 3% Phosphoric acid**
- **Griess Reagent B: 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) in distilled water.**
- **Nitrite Standard: Sodium nitrite solutions.**

Procedure:

- **Sample Preparation: If the sample is turbid, filter it or centrifuge it to ensure clarity.**
- **Reaction: - Add 100 µl of the sample (or standard) to a test tube or microplate well. - Add 100 µl of Reagent A and incubate for 5–10 minutes at room temperature (protected from light). This allows the diazonium salt to form.- Add 100 µl of Reagent B. A pink color will develop almost immediately.**

Incubation: Wait another 10 minutes for the color to stabilize.

Measurement: Measure the absorbance at 540 nm using a spectrophotometer.

Creating the Standard Curve

- To find the exact concentration of nitrite in your unknown sample, you must compare it against known values.

- Prepare Dilutions: Prepare a series of $NaNO_2$ standards (e.g., 0, 1, 2, 5, 10, 20 µM).

- Measure Absorbance: Run the Griess reaction on these standards and record the optical density (OD).

- Plot the Graph: Plot Concentration (x-axis) vs. Absorbance (y-axis).

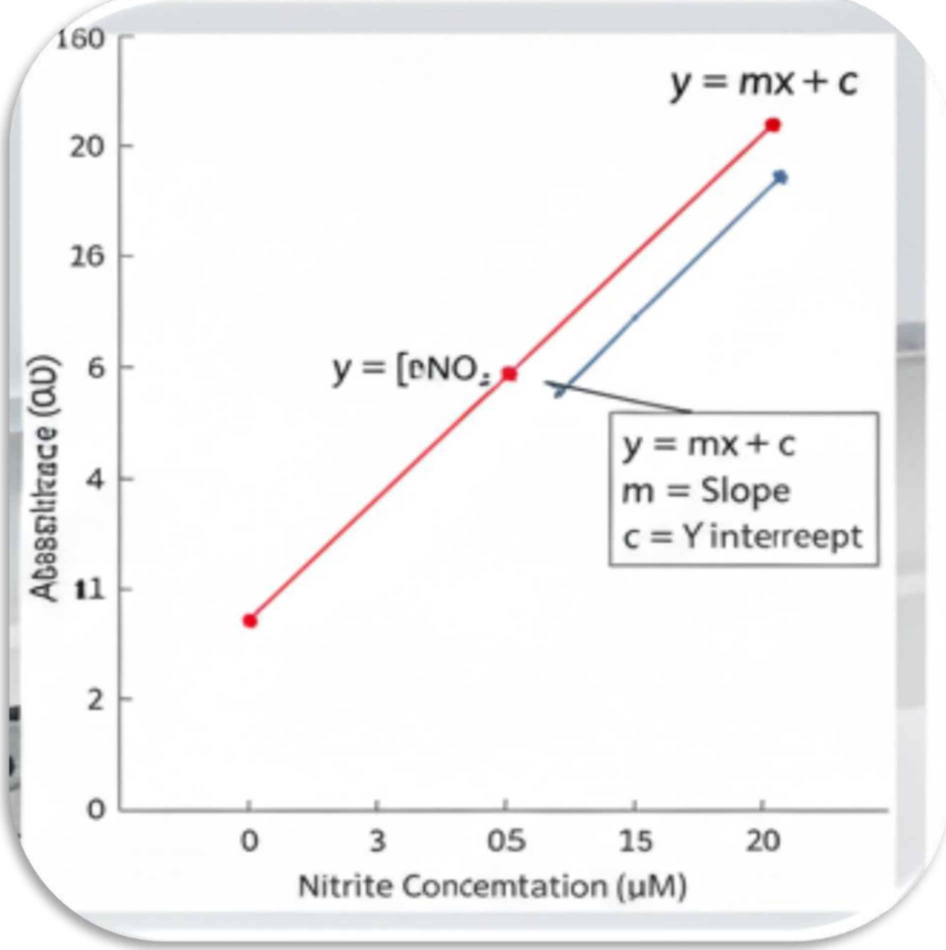
- Linear Regression: Use the formula of the line:

$$y=mx+c$$

y = Absorbance measured

x = concentration of nitrite

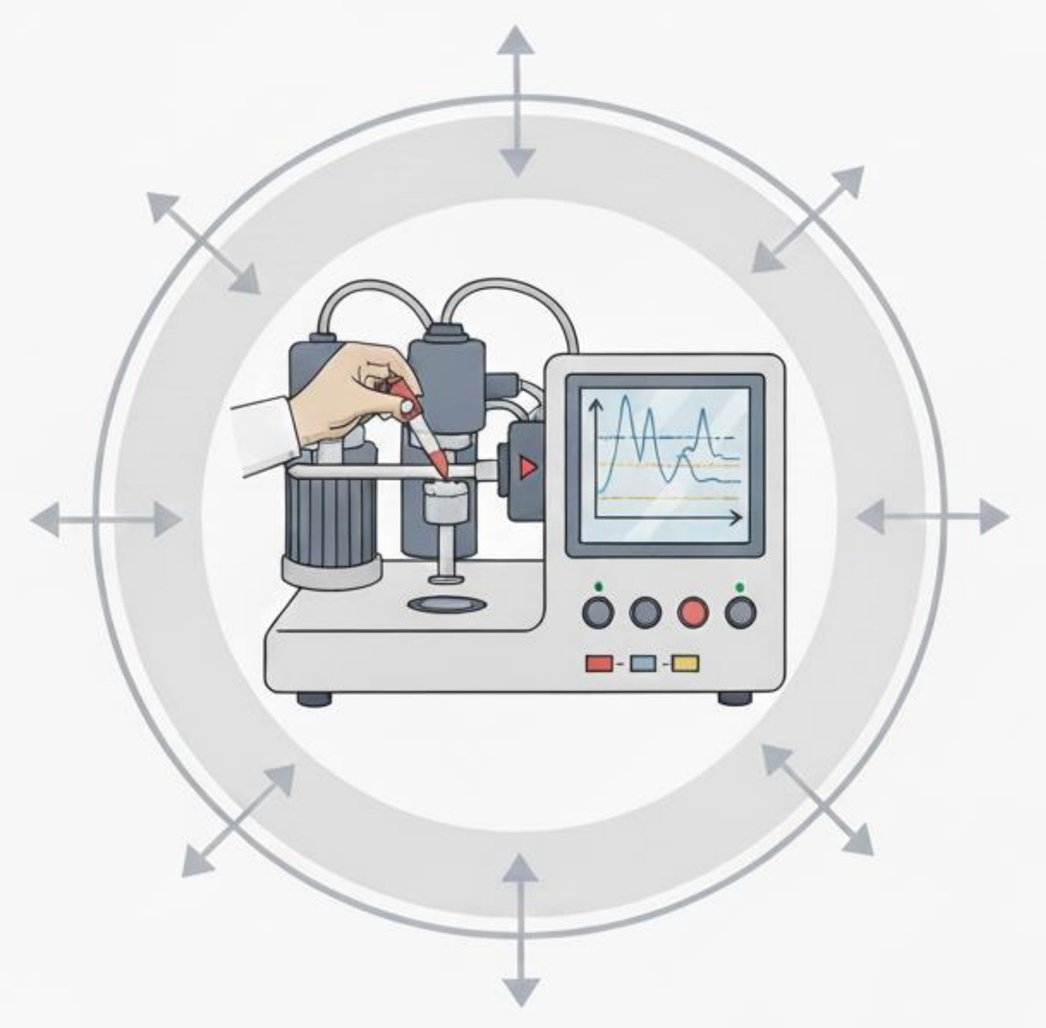
m = slope of the line



4. Chromatographic methods (specific contaminants)

For the highly specific and accurate detection of non-protein nitrogen contaminants such as melamine, the recommended method is *high-performance liquid chromatography (HPLC).*

- Principle: The sample extract is separated based on the differential affinities of the components to a stationary phase and a mobile phase.
- Detection: The separated components are quantified using a highly sensitive detector, often a UV detector or a mass spectrometer (MS).
  - HPLC-UV: Used for the detection of melamine, often around 230 nm.
  - HPLC-MS/MS: Provides the highest sensitivity and confirmation, acting as the gold standard for confirming the presence and concentration of specific contaminants such as melamine.



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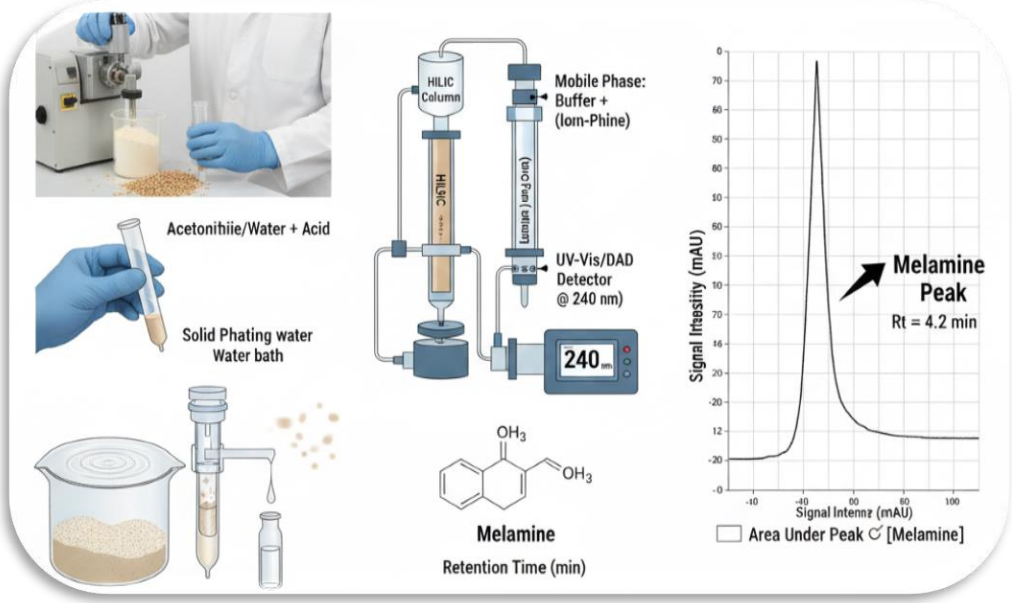
Example: Detection of melamine in grains by HPLC

- *Sample Preparation (Extraction)*: Because grains contain proteins, fats, and carbohydrates that can interfere with the HPLC column, a rigorous extraction step is necessary. The grain sample is ground to a fine powder. A mixture of acetonitrile and water (often with a small amount of acid like trichloroacetic acid or diethylamine) is added to the sample. The mixture is sonicated to ensure the melamine dissolves into the solvent. The extract is filtered and often passed through a Solid Phase Extraction (SPE) cartridge (typically a cation-exchange resin) to concentrate the melamine and remove impurities.

- *HPLC Operating Conditions:* Melamine is a polar compound, so it is typically analyzed using Reverse-Phase HPLC or HILIC (Hydrophilic Interaction Liquid Chromatography). Column: A C18 column is common, but Ion-Pair chromatography or HILIC columns provide better retention for the highly polar melamine molecule. Mobile Phase: Usually a mixture of a buffer (e.g., ammonium acetate or phosphate buffer) and an organic solvent like acetonitrile. Detection: Melamine has a strong UV absorbance. It is typically detected using a UV-Vis Detector or a Diode Array Detector (DAD) at a wavelength of 240 nm.

- *The Chromatography Process*: Injection: A small volume (e.g., 20µl) of the cleaned extract is injected into the system. Separation: As the mobile phase carries the sample through the column, melamine interacts with the stationary phase. Due to its specific chemical properties, it exits the column at a specific time, known as the Retention Time (Rt). Detection: As melamine passes through the UV detector, it produces a "peak" on the chromatogram.



- *Qualitative and Quantitative Analysis*

-Identification (Qualitative): The presence of melamine is confirmed if a peak appears at the same retention time as a known melamine standard.

-Quantification (Quantitative): The area under the peak is proportional to the concentration. By running a series of standards, a calibration curve is created.

5. Rapid screening methods

For rapid, on-site, or high-throughput screening, simpler methods are often used:

- *Test strips/kits*: These use colorimetric reactions similar to the Griess reaction, allowing a semi-quantitative or qualitative (presence/absence) result for nitrates/nitrites.

- *Near-infrared spectroscopy (NIRS)*: is the first line of defense at grain elevators and silos. It measures the vibrational energy of N-H bonds.

- An extremely rapid and non-destructive technique that measures total nitrogen/protein by analyzing near-infrared light absorption. Although it does not directly measure contaminants, sudden deviations from expected protein-NIRS correlations may indicate adulteration. While NIRS is calibrated for protein, modern software uses "Pattern Recognition" or "Adulterant Screening". If the NIRS spectrum of a wheat shipment doesn't match the standard "fingerprint" of wheat protein, it flags the sample for further laboratory testing (HPLC).

6. Gas Chromatography with an Electron Capture Detector (GC-ECD)

- is a highly sensitive method for detecting nitro-aromatic (TNT) and nitro-amine (RDX) explosives in cereals. The ECD is specifically effective here because it is extremely sensitive to electronegative functional groups, such as the nitro groups found in these contaminants.

- *Sample Preparation (Extraction)*: Cereals are complex matrices (fats, fibers, proteins), so the extraction must be precise to avoid clogging the GC column. Grains are ground into a fine powder. Acetonitrile or Methanol is typically used to leach the RDX and TNT from the cereal flour. The raw extract is passed through a Solid Phase Extraction (SPE) cartridge (often Florisil or C18) to remove lipids and pigments. The cleaned solvent is evaporated under a stream of nitrogen to concentrate the analytes before injection

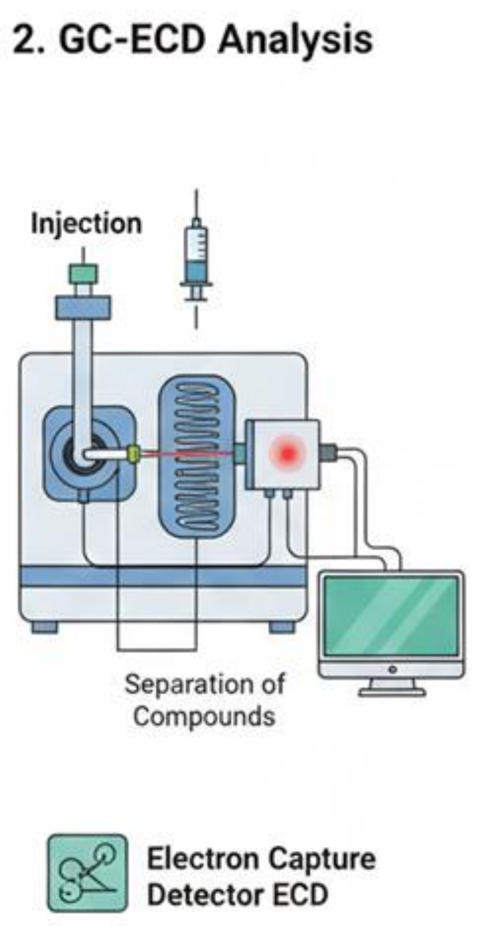
- *GC-ECD Instrumental Setup:* Because explosives are thermally unstable, the GC settings must be carefully controlled to prevent the molecules from decomposing in the injector or column.

Carrier Gas: High-purity Nitrogen or Helium.

Column: A capillary column with a non-polar or mid-polar stationary phase (e.g., DB-5 or DB-17).

Injector: Usually a "Cold On-Column" or "Programmed Temperature Vaporizer (PTV)" to protect the TNT/RDX from thermal degradation.

Detector (ECD): Contains a radioactive source (usually ^{63}Ni) that emits electrons. When the electronegative nitro-compounds pass through, they "capture" these electrons, reducing the current and creating a measurable signal (peak).



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- GC-ECD Analytical Procedure

Calibration: A standard mixture containing known concentrations of RDX and TNT is injected to establish retention times (Rt) and a response factor.

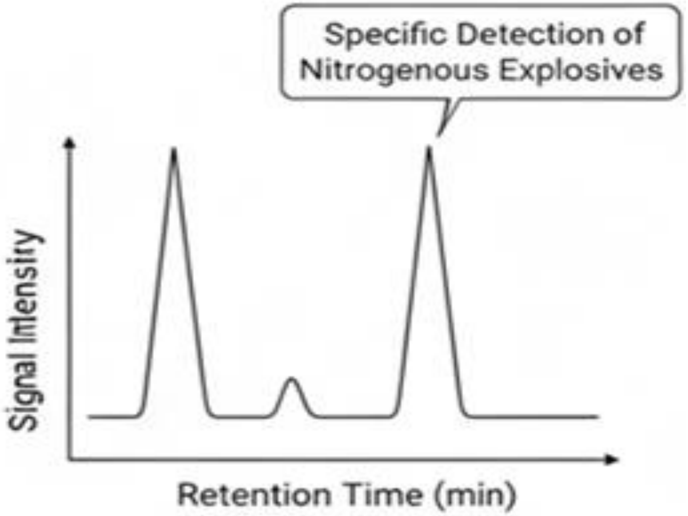
Injection: The cereal extract (1-2 μl)is injected.

Separation: As the oven temperature increases, the compounds migrate through the column. TNT usually elutes before RDX due to its lower boiling point and different polarity.

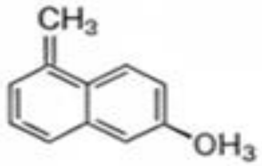
Quantification: The area of the resulting peaks is compared against a standard curve.

More modern methods for dtecting TNT and RDX: LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry), GC-IMS (Gas chromatography-ion mobility spectrometry) or High-Resolution Mass Spectrometry (HRMS)

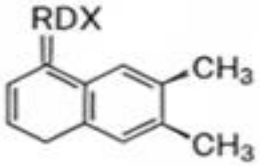
3. Chromatogram & Detection



Specific Detection of Nitrogenous Explosives



TNT



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CHAPTER 6.

CONCLUSIONS

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The detection and quantification of contaminants in cereals and related products represent a fundamental component of modern food safety strategies. As global food systems become increasingly interconnected, the need for robust, reliable, and harmonized analytical approaches is greater than ever. This chapter has presented the main principles, methodologies, and analytical tools used to identify both chemical and physical contaminants, with emphasis on trace elements (heavy metals) and pesticide residues—two major groups of hazards that can directly impact consumer health.

A key conclusion emerging from this overview is the central role of rigorous sampling procedures and appropriate sample preparation. Because cereals and derived products are heterogeneous matrices, sampling remains a critical determinant of the reliability of analytical results. Proper homogenisation, contamination control, and validated preparation techniques, uch as microwave-assisted digestion for metals or solvent extraction and clean-up for pesticides-ensure that the analytical process begins on a solid foundation.

A second essential element is the selection and optimisation of the analytical method. Modern techniques such as ICP-MS, ICP-OES, AAS, GC-MS, LC-MS/MS, and high-performance screening tools provide high sensitivity, specificity, and accuracy. Their successful application depends on adequate calibration strategies, quality controls, and the mitigation of matrix effects. The chapter highlighted different calibration approaches, including external calibration, internal standards, and standard addition methods—each adapted to specific analytical constraints and matrix characteristics.

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Equally important is the adherence to European and international legislation. Method selection, performance criteria, and result interpretation must meet regulatory frameworks that establish maximum residue levels (MRLs), maximum limits (MLs), and validated performance standards. These regulations ensure comparability of data and support risk assessment, monitoring programs, and consumer protection initiatives.

In conclusion, detecting and quantifying contaminants in cereals requires a combination of scientific expertise, reliable instrumentation, quality assurance, and regulatory awareness. By mastering these methodologies, professionals and students engaged in food science and safety are better equipped to ensure the integrity of cereal-based foods and to contribute to a safer and more sustainable food system.