



## A review of suspect and non-target screening of agriculture-related organic contaminants

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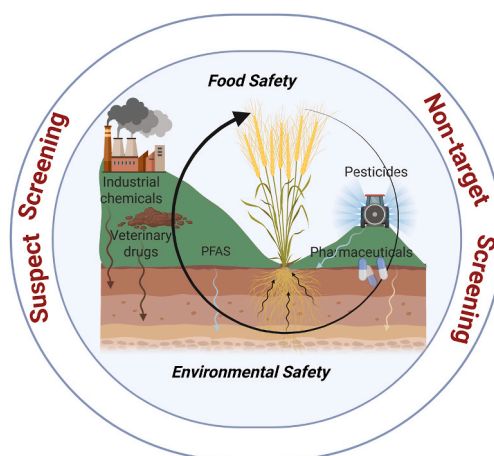
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### HIGHLIGHTS

- The principles of suspect (SS) and non-target screening (NTS) are deeply discussed.
- Advancements in SS and NTS analysis in agri-food system.
- Recent advances in data processing and analysis for SS and NTS are presented.
- Occurrence of chemical hazards in the agri-food system is discussed.

### GRAPHICAL ABSTRACT



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### ABSTRACT

**Background:** Production systems that aim to reduce waste and promote the reuse of materials contribute to more sustainable food systems. However, these approaches can also lead to the accumulation and recirculation of chemical hazards from organic waste to the soil and, ultimately, to food. To address these risks and assess the safety and effectiveness of waste reuse in agriculture, non-target screening (NTS) and suspect screening (SS)

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Crops  
Emerging compounds  
Personal care products  
Pharmaceuticals  
Pesticides  
Mass spectrometry  
Data analysis

analytical methodologies are essential, as they enable the detection of chemical residues, pollutants, and unintended by-products in soil, crops, and compost.

**Results:** A literature review covering the period 2021–2025 was conducted to identify recent analytical advances and the application of SS and NTS strategies in food production systems. The review highlights key analytical challenges associated with traditional targeted analysis and explores the improvements achieved by SS and NTS approaches. Emphasis is placed on advancements in sample treatment (extraction and clean-up), instrumentation, and data processing and analysis tools. Furthermore, the review provides an overview of chemical hazards identified using SS and NTS methodologies within the context of agri-food systems.

**Significance:** The review highlights significant progress in the development of SS and NTS strategies for the identification of known and unknown hazardous chemicals. However, it also reveals a limited number of studies applying these approaches specifically within sustainable agriculture frameworks. Despite this gap, the potential of SS and NTS to support safer and more sustainable practices in agri-food systems suggests that their broader application could be of considerable interest to the scientific community in future research.

#### Abbreviations:

AMs	antimicrobials	LOQ	limit of quantification
APCI	atmospheric pressure chemical ionization	LRMS	low-resolution mass spectrometry
API	atmospheric pressure ionization	MRL	maximum residue limit
APPI	atmospheric pressure photoionization	MS	mass spectrometry
BBF	bio-based fertilizer	HRMS/MS	high resolution tandem mass spectrometry
CFM-ID	competitive fragmentation modelling for metabolite identification	MSI	mass spectrometry imaging
DDA	data dependent acquisition	NTS	non-target screening
DIA	data independent acquisition	PCA	principal component analysis
d-SPE	dispersive-solid-phase extraction	PECs	persistent emerging contaminants
EI	electron ionization	PFAS	per- and polyfluoroalkyl substances
EIC	extracted ion chromatogram	PLE	pressurized liquid extraction
ESI	electrospray ionization	PLS-DA	partial least squares discriminant analysis
FWHM	full width at half maximum	PPCPs	pharmaceuticals and personal care products
GC	gas chromatography	PSA	primary secondary amine
GCB	graphitized carbon black	Q	Quadrupole
GNPS	global natural products social molecular networking	QqQ	Triple quadrupole
HMDB	human metabolome data base	RI	retention index
HRMS	high-resolution mass spectrometry	RS	resolution
IMS	ion mobility spectrometry	RNN	recurrent neural network
IMS-MS	ion mobility spectrometry coupled to mass spectrometry	SPE	solid-phase extraction
LC	liquid chromatography	SS	suspect screening
		TPs	transformation products
		UAE	ultrasound-assisted extraction

## 1. Introduction

There is a need in Europe to re-use residual streams to move towards a more sustainable food production system. Since 2011, the Food and Agriculture Organization (FAO) report [1] has indicated food waste (FW) and food loss (FL) (together FWL) as topics of greater concern. Data about FWL are impressive: more than 1.3 billion tons of food are wasted each year along the supply chain [2,3] and in Europe an average of 173 kg of food per person is wasted every year [4]. It is estimated that agriculture contributes to 10–35 % of the world's total greenhouse gas (GHG) emissions [5].

The EU adopted “Closing the Loop—An EU action plan for the Circular Economy” [6] and the “European Green Deal” with its core “Farm to Fork Strategy” [7], in order to ensure a lower climate footprint, new opportunities and food chains, as well as an easier and safer access to sufficient, sustainable, affordable and nutritious food.

The idea of maximising resource use is now firmly embedded in society, driven by the understanding that Earth's resources are limited. One effective approach to creating a more sustainable production system is to increase resource efficiency and waste minimisation [8]. This contributes to a more responsible use of natural resources by reducing waste generation and promoting reuse and recycling wherever possible.

Characterization and control of the materials intended to be reused is crucial for safe management. In this sense, wastewater treatment plants (WWTPs) are an integral part of this strategy as they enable the recovery of valuable resources. For instance, excess sludge from biological wastewater treatment has traditionally been disposed of by ocean dumping, landfill or incineration. Due to increasingly stringent environmental regulations, the disposal methods are being phased out and replaced by either aerobic or anaerobic digestion processes. These treatment processes remove pathogens and convert sludge into stable ‘biosolids’. Biosolids are rich in organic matter and nutrients and, depending on their quality, can be used for several land applications (e.g. fertiliser, soil conditioner, composting material, and potentially as an energy source) [9,10]. Under specific conditions, biosolids can be used in anaerobic digestion or combustion processes to produce biogas or energy.

The reuse of biosolids is a sustainable option because it has a minimal impact on the environment, enables the recovery of resources and adds economic value to what is conventionally perceived as waste [11]. However, if contaminated biosolids are used for the previously mentioned purposes, there is a risk of soil and water contamination, which could subsequently lead to an accumulation in plants and grazing animals [12], posing a food safety concern [13]. Thus, prior to re-use of these streams in a food production system, the presence of hazardous

substances needs to be evaluated in a variety of agriculture-food related matrices to safeguard food safety of such systems. For example, in recent years the presence of pesticides, industrial chemicals, components of consumer products, pharmaceuticals and personal care products (PPCPs), hormones, and other organic pollutants has been reported in different biosolids and in soils fertilized with them [14–16].

Advanced analytical methods capable of detecting contaminants at trace levels are essential for determining the extent of contamination, from the source to the matrices that act as either drivers or sinks. Although target methods are often used to detect known and regulated contaminants with high accuracy and sensitivity, the continuous changes and advances in the industry might lead to the release of unknown and unregulated contaminants which could pose adverse effects on sustainable agriculture. In this sense, the application of non-target (NTS) and suspect screening (SS) approaches using non-selective extraction protocols and high-resolution mass spectrometry (HRMS)-based techniques can fill this gap. While NTS and SS have shown to be promising strategies in environmental science for the simultaneous analysis of a wide range of chemical compounds [17], their use in food analysis applications is still growing. Indeed, NTS is particularly relevant in the context of agri-food system, where complex sample compositions demand in-depth contaminant profiling to ensure food safety.

Within this context, this review aims to highlight the significant potential of the implementation of NTS and SS approaches in agricultural systems and related scientific fields. NTS and SS have already been applied for the analysis of diverse matrices such as soil, compost, crops and plants using different analytical instrumentation for a wide variety of goals. The search for scientific literature (Web of Science) was focused on the studies published between 2021 and 2025. To focus on the topic of this review we used the keywords: i) suspect screening and non-target, ii) soil and compost, iii) crops, plants, and vegetables, and iv) circular agriculture, which retrieved a total of 882 scientific articles including 72 review articles. The exclusion keywords by automation tools were metabolomics, water and wastewater, biofluids and biomonitoring, biological samples such as urine and breast milk, and households which led us a total number of 166 papers (considering the Web of Science Categories: Analytical Chemistry, Environmental Sciences and Agriculture Multidisciplinary). All the original research papers including only target analysis or multitarget analysis of <15 compounds were excluded manually.

The results presented in terms of analytical methods and NTS and SS implemented together with the chemical hazards reported can help policy makers to provide guidance on which hazards need to be prioritized for monitoring. Also, it will help in identifying critical points where mitigation strategies need to be developed and implemented. Overall, the results of the present review will contribute to showcase the need for a fit-for-purpose and safe-by-design approach in the application of residual streams in agri-food system.

## 2. Insights of analytical challenges in target analysis

The presence of emerging compounds, including PPCPs, pesticides and industrial chemicals, has been widely reported in agricultural-related samples such as soils, sludge and crops [15]. To monitor their presence, target methods focusing on specific families of compounds have long been considered the 'gold standard' for quantitative analysis, as they provide highly specific, sensitive, and reproducible results, particularly when using chromatographic systems coupled with mass spectrometry. To broaden the scope of target analysis, nowadays more studies are focusing on developing accurate and high-throughput multi-target analytical methods to simultaneously detect all these families of compounds [18]. Low-resolution mass spectrometry (LRMS) is, by far, the preferred option for quantifying trace-level contaminants in complex matrices, including biological and environmental samples, despite the capabilities of HRMS systems. The higher sensitivity presented by target methods is particularly important in food safety related issues, specially

when it is required to know whether the concentration of a contaminant complies with regulations [19]. For example, during 2019, there were several cases of food poisoning in Uganda due to contamination of a Super Cereal (which consists of heat-treated wheat and dehulled soybeans, milk, sugar, vitamins, and minerals). An interlaboratory study revealed that while HRMS instruments successfully identified the tropane alkaloids, atropine and scopolamine, only those using LRMS instruments had the sensitivity required to accurately quantify their concentrations in the samples [20]. Moreover, Vergara-Luis and co-workers reported lower limits of quantification (LOQs) for the multi-target analysis of antimicrobials (AMs) in vegetables using a triple quadrupole (QqQ) mass spectrometer in comparison to a Q-Orbitrap system (HRMS) [21].

However, the major limitation of target analysis lies in its inherent reliance on reference standards for both the identification and quantification of specific pollutants. This can be a large handicap, as the analysis is restricted to a specific set of compounds for which commercial standards are accessible [22]. This limitation becomes particularly problematic when monitoring complex and dynamic environments such as WWTPs [23] or amended soils [24]. In these environments, a diverse array of pollutants can coexist and interact, and the potential degradation of the pollutants can lead to the emergence of transformation products (TPs) and derivatives. However, little is known about the transformation pathways of emerging pollutants in different environments [25]. Therefore, many TPs might not be correctly characterised, or reference standards may be unavailable, making them not detectable/quantifiable by target methods. Failing to detect these potentially harmful TPs could have significant consequences for both environmental and public health, as it creates gaps in the understanding of the lifecycle of the contaminant, making it difficult to conduct a comprehensive long-term risk assessment. For instance, it has been reported that AM's TPs continue to maintain their activity once they reach the environment [26], thus contributing to the spread of AM resistance, a problem classified by the World Health Organisation as one of the top 10 public health threats [25,27,28].

The identified gap can be effectively addressed by employing analytical methodologies that extend beyond conventional target analysis. High-resolution mass spectrometers (HRMS), with their superior mass accuracy, are ideal for the screening and identification of a wider range of contaminants, enabling the analysis without reliance on analytical standards by leveraging high-resolution tandem mass spectral libraries [22,29,30] due to recent improvements in hybrid instruments that combine multiple mass analysers, such as quadrupole time-of-flight (Q-TOF) and Q-Orbitrap. As a result, Thanks to the extended dynamic range provided by these innovations, HRMS now has detection capabilities comparable to those of LRMS.

However, the shift from target to NTS presents several challenges. Regarding sample preparation, most extraction and clean-up procedures used for target analysis are tailored to specific analytes or specific classes of compounds, limiting the capacity to capture the entire spectrum of compounds within a sample. This also affects the analytical determination, as broader conditions are required to achieve unbiased separation and detection of as many compounds as possible. On the other hand, the large amount of data acquired during non-targeted experiments could hinder data analysis, requiring robust workflows and computational tools to prioritize the most relevant features and ensure the compounds identified are annotated with high confidence. All these aspects could compromise the selectivity and the sensitivity of the analysis as well as the number of false positives and negatives as, in the non-target approaches, the principle of "less is more" applies. False positives can lead to unnecessary investigation, incorrect conclusions about exposure, and potential misallocation of resources, while false negatives can lead to underestimation of the chemical burden in a sample, missing potential hazards, and overlooking important environmental impacts.

### 3. Suspect and non-target strategies

SS and NTS strategies might help to extend the chemical assessment in agri-food systems in combination with the above-mentioned target approaches. Fig. 1 summarizes the main publications applying SS and NTS as a step forward to determine chemical residues in agricultural-related samples in the last five years. Soil and crops are by far the most studied matrices where SS and NTS approaches have been applied. Regarding the chemical compounds, more than 50 % of the works studied the occurrence of pesticides, pharmaceuticals or their corresponding by-products, although other families such as flame retardants, industrial chemicals, mycotoxins, musk fragrances or UV stabilizers have also been studied to a lesser extent.

#### 3.1. Extraction techniques

The choice of sample preparation methods directly influences the selectivity and sensitivity of analytical workflows, thus affecting the number and nature of compounds that can be reliably identified in complex agri-food matrices. While increased selectivity is essential in targeted analyses, sample preparation in SS and NTS approaches must be broad and non-selective in order to maximise the detection of both known and unknown compounds. However, even under these less selective conditions, some degree of purification is still necessary to minimise matrix effects that can compromise ionization efficiency and mass spectrometric detection. This inherent trade-off—known as the selectivity vs. sensitivity compromise—remains one of the central challenges in non-target analysis [31,32].

Despite recent developments, there is still a lack of standardised sample preparation protocols specifically designed for complex environmental and agri-food matrices such as soils, biosolids, and plant material. Many studies rely on adaptations of existing methods rather than developing procedures tailored to the physicochemical diversity of these matrices. For instance, extraction solvents are often selected based on previous experience or use rather than through empirical optimization, and few studies assess the efficiency of extraction across a broad range of polarities or chemical classes. This makes it difficult to compare results from different and can lead to significant bias in compound detection. In addition, the extraction yields are rarely reported in NTS workflows, which limits the ability to assess the true comprehensiveness of the extraction step. Even when recoveries are included, they are often based on a limited number of spiked standards, which may not represent

the diversity of unknowns potentially present in real samples. The number of identified compounds in real samples using different extraction protocols could be the best approximation to get the optimum extraction protocol. However, the potential false positives and false negatives in different analytical workflows can bias the results, being difficult the assessment of actual performance of extraction protocols. The implementation of high-throughput and automation-compatible sample preparation methods is another critical need. While some reviewed studies employed techniques such as QuEChERS or pressurized liquid extraction, these were not always evaluated for method robustness or reproducibility. Scalability and applicability under routine conditions remain underexplored aspects, especially for monitoring purposes in regulatory contexts. Table 1 provides a comparative overview of the extraction methodologies reported in the reviewed studies. However, rather than converging towards a standard approach, the literature demonstrates high methodological variability, with significant differences in solvent composition, clean-up steps, and extraction times. This variability underscores the urgent need for harmonisation of protocols and inter-laboratory validation efforts. Overall, while several extraction techniques have shown promise, there is still no consensus on an optimal approach for SS and NTS in agri-food matrices, and current methods often represent a compromise between practical feasibility and analytical comprehensiveness. Future research should prioritize the development of broad-spectrum efficient extraction protocols with special focus on quality control parameters (i.e., extraction yield, number of compounds extracted, false positives and negatives, matrix effect, etc.).

As summarized in Table 1, the most frequently monitored compounds by target analytical methods in soil and compost samples are pesticides although other organic compounds such as AMs, PPCPs, and per- and polyfluoroalkyl substances (PFAS) are also frequently targeted. Some of these target analyses are then expanded to screen a larger number of contaminants of the same chemical class or their TPs to increase the scope of the analytical methodology [16,21,22,25,26,28,34,36,39–41]. However, this targeted approach inherently limits the detection of emerging or less-studied contaminants, which may also be present in these matrices but remain unmonitored due to lack of standards or analytical protocols. As in target analytical methods, ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE) are the most used techniques in SS. Depending on the target compound to be analysed or the type of suspect being monitored, different solvents (e.g., methanol:acetic acid, acetonitrile, ethyl acetate,

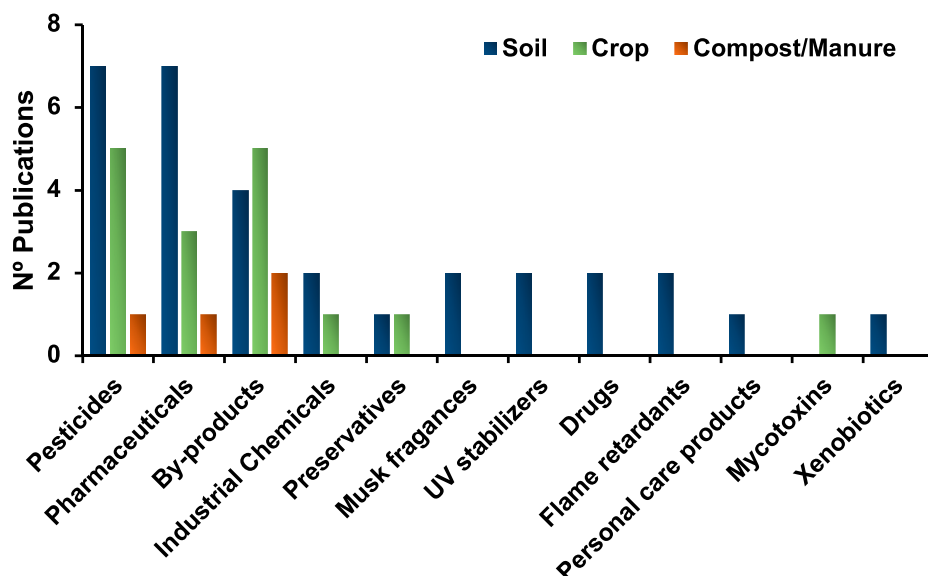


Fig. 1. Overview of scientific articles with analytical approaches including SS and NTS strategies in agricultural-related samples (2021–2025).

acetone, aqueous ammonium solution, etc.) or mixtures thereof are used to extract between 1 and 10 g of freeze-dried soil and/or compost samples (see Table 1).

QuEChERS (short for Quick, Easy, Cheap, Effective, Rugged, and Safe) is a sample preparation method proposed by Anastassiades and co-workers [42]. The original methodology had the purpose of overcoming limitations of routine pesticides monitoring in foodstuffs, for which a simple and fast method is essential. Since then, the method has been widely accepted by the scientific community and used in the extraction of several different analytes from various samples, including vortexing and centrifugation as intermediate steps. The sample (1–10 g of fresh sample), containing a certain amount of water, is treated with an organic solvent as acetonitrile for extraction. Afterwards, different salts ( $\text{MgSO}_4$  or  $\text{Na}_2\text{SO}_4$  and  $\text{NaCl}$ ) are added to increase the efficiency of the solid-liquid extraction by salting-out effect and to get a better phase separation between water and acetonitrile. The robustness of QuEChERS for the extraction of AMs, pesticides, PPCPs and PFAS in crops and vegetables (e.g., cucumber, kale grown, lettuce, onion, carrot, fruits, etc.) is extended as most of the reviewed studies used very similar protocols in both target and SS approaches [43]. QuEChERS' widespread application in SS lies in its combination of high recovery rates and its ability to extract compounds across a broad range of log P values. Indeed, good recoveries (60–120 %) have been reported for a wide range of pesticides, herbicides, and polycyclic aromatic hydrocarbons in various matrices, including highly complex ones such as soil [43].

### 3.2. Clean-up procedures

Following the extraction of organic compounds, most of the reviewed studies (fourteen out of eighteen; see Table 1) employed a clean-up step to remove co-extracted interferences, simplify the matrix, and enhance sensitivity. However, this approach carries the risk of reducing the range of chemicals extracted. While such clean-up procedures can significantly reduce matrix effects, they also pose a critical trade-off: the potential loss of analytes, particularly in the context of NTS and SS, where broad chemical coverage is essential. The search of an equilibrium between matrix simplification and analyte preservation remains one of the most pressing and unresolved challenges in SS/NTS workflows. The complexity of the matrix in agricultural samples can influence the detection of compounds [35], necessitating clean-up approaches to minimise the matrix effect during the detection, especially when energetic extraction systems such as microwave assisted extraction (MAE) or PLE are used. Based on the reviewed research works, less energetic extraction approaches (e.g. vortexing [35], shaking [40] or ultrasound sonication [33]) allow sample extracts to be analysed directly. A clean-up step was also used in most QuEChERS-based studies (six out of nine; see Table 1), but non-cleaned extracts were diluted prior to analysis to reduce the matrix effect [16,22].

Regarding the clean-up strategies solid-phase extraction (SPE) and its miniaturized version, dispersive-SPE (dSPE), are the preferred clean-up methods, mainly using  $\text{C}_{18}$ , Oasis HLB, and  $\text{C}_{18}$ -sorbents combined with primary secondary amine (PSA) or graphitized carbon black (GCB) phases, or silica in the case of non-polar compounds (see Table 1). Although these sorbents are widely used, their selection is often based on empirical knowledge rather than lack systematic evaluation of quality parameters such as selectivity, recovery efficiency, or potential for analyte loss—especially in the context of broad-scope SS and NTS applications. This raises concerns about the reproducibility and robustness of clean-up procedures when applied to chemically diverse and complex matrices. In recent years, there has been a growing trend of streamlining clean-up procedures in combination to the use of HRMS to facilitate the adoption of high-throughput analytical methods. However, the non-selective dilute-and-shoot method [44], which is commonly applied to water samples and/or some specific biological matrix such as urine, has been marginally applied to environmental solid and food samples in the case of NTS and SS strategies, due to the expected high

matrix effect and low sensitivity. As regards assessing the performance of the methods presented, the lack of standardisation of methods for SS and NTS makes it difficult to compare them. In this context, comparative studies of sample preparation techniques used for SS and NTS are needed.

### 3.3. Instrumental analysis and data acquisition

#### 3.3.1. Chromatographic separation

Among the reviewed SS and NTS studies, liquid chromatography (LC) is by far the most frequently used separation technique. Its broad applicability to a wide range of chemical hazards—including PPCPs, pesticides, industrial chemicals, and natural toxins—makes it a versatile choice, particularly for polar to moderately non-polar compounds. In contrast, gas chromatography (GC) has been applied to a lesser extent, typically for the detection of non-polar and semi-polar persistent emerging contaminants (PECs), organochlorine pesticides, and other volatile compounds [36,38,45,46]. Some studies have combined both LC and GC approaches to achieve broader chemical coverage, taking advantage of their complementary selectivity profiles.

The combined use of LC and GC is noteworthy, as no single chromatographic technique can comprehensively separate the full chemical diversity present in complex agricultural matrices. LC-based methods have limited ability to separate volatile and thermally stable compounds. Meanwhile, GC is restricted to analytes that can be vaporised and often requires derivatization for polar species. For instance, Chiaia-Hernández et al. successfully used a dual LC–GC for suspect screening in soils and sediments, enabling the detection of a wide range of compound classes, with azoles and triazines being dominant contaminants [36]. However, despite the successful of this approach, such integrated strategies remain underused in studies of agri-food chemical hazards in the agri-food field, possibly due to resource constraints or methodological complexity. In most LC-based methodologies, reversed-phase chromatography using  $\text{C}_{18}$  columns is the standard, with elution gradients composed of acetonitrile/water or methanol/water under acidic or neutral pH conditions (see Table 1). Although this setup is widely accepted and offers robust performance for mid-polarity compounds, it provides limited retention and separation for highly polar substances, which are often of highly relevant in terms of toxicology. The emerging use of hydrophilic interaction chromatography (HILIC), which is better suited for polar analytes, has attracted interest in environmental chemistry. However, its application in SS and NTS of agricultural matrices remains limited, likely due to practical challenges such as long equilibration times and poor retention time stability. The factors are particularly problematic for large-scale screening studies, where reproducibility is a key parameter.

Similarly, the use of GC in SS/NTS studies is primarily based on non-polar columns such as DB-5 ( $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ), which offer good retention of hydrophobic compounds. While some studies (e.g., Reyes Avila et al. [30]; Silva et al. [46]) have successfully applied GC-based SS for pesticide detection in crops, these efforts remain isolated. More advanced GCxGC (comprehensive two-dimensional GC) approaches, which are known to provide enhanced separation and resolution through orthogonal stationary phases, have demonstrated their applicability in environmental matrices [17] yet have not been adopted in the research of agricultural chemical hazards. Although not available in all laboratories as a specific hardware is needed, the use of GCxGC could significantly improve compound coverage and reduce co-elution issues in highly complex extracts.

In summary, although LC dominates SS and NTS applications in agri-food analysis, relying on a single chromatographic technique creates inherent limitations. Wider use of orthogonal and complementary separation techniques, particularly through combined LC/GC or multidimensional approaches like GCxGC, would improve chemical coverage and analytical resolution. Furthermore, the lack of methodological innovation in chromatographic separation for agricultural matrices

highlights the need for method development and validation tailored specifically to the complex and diverse nature of these samples.

### 3.3.2. Mass spectrometry analysis

In the field of SS and NTS analysis, MS coupled to chromatographic techniques has become an essential analytical technique for the unequivocal identification and characterization of a wide variety of compounds at trace levels. In MS analysis, ionization is a critical step because it significantly influences the intensity of the analyte signal. Regarding the studies summarized in Table 1, those methods based on LC use electrospray ionization (ESI), in most cases using both positive and negative ionization modes, which is of great advantage for SS and NTS studies to obtain greater information on the presence of chemicals in the samples. For instance, both positive and negative ionizations have been carried out for the suspect screening of agricultural samples such as compost [33], crops [19], and soil [16,22] as well as the NTS of compost [33] and crops [40,47]. In the case of GC-based methods, electron ionization (EI) is the ionization source of choice, performed at 70 eV as it provides useful chemical structure information, which can be used to characterize and confirm the detected compounds through a mass spectral library search. Additionally, although the atmospheric pressure ionization (API) sources for GC-HRMS such as atmospheric pressure chemical ionization (APCI) and a photoionization (APPI) is a growing trend in environmental and clinical analysis as they provide a soft ionization leading to the molecular or pseudo-molecular ion, they have not yet been explored in matrices related to agri-food system field [48]. These API techniques have shown a great potential to carry out SS and NTS as they provide a soft ionization leading to the molecular or pseudo-molecular ion which, in combination with hybrid mass analysers, allows to carry out HRMS/MS experiments for characteristic precursor ions, thus reducing the number of false positives results that could be generated when using low-resolution mass spectral libraries such as those employed in GC-EI-HRMS [49].

Regarding mass analysers, both LRMS and HRMS instruments have been used in SS and NTS (Table 1). LRMS instrumentation, such as a single quadrupole (Q) and QqQ, is particularly effective for SS in GC-based methods, given the availability of extensive libraries such as NIST. These libraries encompass GC-EI-MS spectra of over 200,000 analytes, which allow the identification of volatile and/or semi-volatile unknowns by matching the EI spectra as well as the retention index (RI) [30,36,38]. The most common and widely recognized RI system is the Kováts retention index [50], which is specifically designed for GC in identification purposes, and uses n-alkanes as reference compounds.

On the other hand, HRMS mass analysers such as TOF or Orbitrap allow the acquisition of high-resolution mass spectra, providing accurate mass measurements (<1–2 ppm) in SS and NTS studies. Several publications focused on the application of HRMS for SS and NTS analysis of agricultural samples used TOF [33] and Orbitrap [28,30] as mass analysers, enabling the identification of multiple families of suspect chemical hazards. Additionally, the latest advancements in HR hybrid mass analysers, such as Q-TOF and Q-Orbitrap, have allowed to perform tandem mass spectrometry with both precursor and product ions measured in HRMS. This is advantageous as it allows for the narrowing down of potential candidates based on the accurate mass. Under this scenario, both Q-TOF and Q-Orbitrap have been used in the field of SS and NTS analysis of agricultural samples to identify chemical hazards (Table 1). For instance, Q-TOF was used to analyse soil [16,22,26] and crops [33,51] and Q-Orbitrap to analyse manure [34], crops [21,40,52] and soil [24,37].

Data acquisition is a key factor for SS and NTS analysis as it conditions the data processing and analysis strategies. However, as shown in Table 1, data acquisition mode mainly depends on the analytical platform. Regarding GC-HRMS, data is mostly acquired in full scan acquisition mode. The high fragmentation achieved by EI together with the possibility to use well-established and robust mass spectral libraries (i.e., NIST, MoNA, etc.) facilitates a proper analytes identification. On the

other hand, LC-HRMS approaches generally requires the simultaneous acquisition of full scan and HRMS/MS data to collect analyte information about the (i) exact mass of the precursor ion, (ii) isotopic pattern and adduct ions information, (iii) fragmentation profile (product ions) generated from the precursor ions. As summarized in Table 1, HRMS/MS events to obtain HRMS structural information can be generally done by pre-selecting the precursor ion (data dependent acquisition, DDA) or by direct fragmentation of a mass range eluting in a time frame (data independent acquisition, DIA). DDA provides compound-specific HRMS/MS spectra, easier to interpretate, although the number of DDA spectra is usually limited to the most intense peak ions of the mass spectra (top N). In contrast, DIA allows the unbiased acquisition of all HRMS/MS information, and it requires either a previous knowledge of the expected product ions for each analyte or advanced chemometric tools to simplify data interpretation. As shown in Table 1, 56 % of the works working with LC-HRMS applied DDA while 35 % of them used DIA as acquisition strategy. For instance, Reyes-Avila et al. proposed full scan-DDA acquisition for the suspect analysis of cinnamaldehyde and limonene biopesticides and their metabolites in cucumber's crops [30] while Martínez-Piernas et al. tentatively identified transformation product of pharmaceuticals in soil and crops using a full scan-DIA acquisition [26]. It is important to highlight that several works also combined both acquisition modes as DIA was generally performed for multitarget approaches while DDA was used for SS and NTS [16,28]. Additionally, as shown in Table 1, the resolution used is usually related with the acquisition mode and the mass analyser type. Generally, hybrid Orbitrap-based mass analysers are operated at 70,000 FWHM (at 200  $m/z$ ) in full scan acquisition mode with a potentially overall larger dynamic ranges while hybrid TOF-based mass analysers operate at lower resolutions (30,000–40,000 FWHM) although they present faster scan speeds (intrinsic dynamic range). Regarding, tandem mass spectrometry experiments, the resolution is usually decreased down to 15,000–17,500 FWHM in DDA acquisition modes, as the selection of the precursor ion already increases the selectivity, while DIA acquisition modes usually operate at higher resolutions (30,000–60,000 FWHM) to ensure a high-resolution power at low and high collision energies.

### 3.3.3. Method performance characteristics

As mentioned above, most of the applications combine a multitarget analysis with a SS or NTS to broaden the chemical coverage. The reliability of the results obtained by target and SS/NTS depends on the method performance which should meet specific requirements for each approach. Regarding target analysis, a proper determination of the quality parameters is needed to guarantee the good performance of both the extraction and the analytical determination. Different specific legislations and guidelines are established for proper validation of a target methodology such as EU 2021/808 [53], 2002/657 EC [54] or the SANTE guidelines [55]. The main criteria to fulfill for target methodologies involve: (i) recoveries from 70 to 130 %, (ii) precision (RSD% values lower than 30 %), (iii) trueness (relative errors below 30 %) and (iv) detection capability at least 10 times lower than expected concentrations (ng/kg – ng/g range). Most studies in Table 1 reported results within these acceptable limits, though occasional recovery values fell below 70 % [21,22,25,34,36]. While these lower recoveries were deemed acceptable due to consistent precision (RSD  $\leq$  30 %), such justifications are rarely accompanied by full validation datasets, which undermines reproducibility and limits inter-study comparability. Another methodological issue is the heterogeneity in how detection and quantification limits (LOD/LOQ) are calculated. Although similar values are often reported, differences in calculation methods (e.g. signal-to-noise ratios, calibration curve-based estimations) make direct comparison difficult, if not misleading. Moreover, the limit of identification (LOI), which is a key quality parameter in SS and NTS analyses, goes beyond the limits often used in target analysis. It refers to the lowest concentration of a substance that an instrument can accurately identify. As in target analysis, a chemical standard is required for its



Table 1 (continued)

Sample	Compounds	Sample amount	Extraction and Clean-up	Analysis	Figures of Merit	Identified compounds (Concentration range (ng/g))	Aim	Ref		
Manure	>1600 Antibiotics & TPs	2 g (wet)	supernatant (20-fold diluted, Cit buffer (pH4)) Elute: 9 mL ACN Evaporate to 1 mL Dilute: 125 µL ACN extract: 125 µL 0.01 M oxalic acid (pH 2)	T	@ <i>m/z</i> 200)-ddMS2 ((Rs: 17500 FWHM @ <i>m/z</i> 200)) Column: ACE UltraCore XB-C18 column (2.1 mm × 150 mm, 1.7 µm) ESI+: (A) 0.1 % HCOOH; (B) MeOH 0.1 % HCOOH	8 TPs of antibiotics and/or other antimicrobial compounds (1.7–93 ng/g)	Screening of antibiotics and TPs	[34]		
Soil	~20 drugs, V-type chemical warfare agents and pesticides, toxic unknowns	4.6 g	<b>QuEChERS</b> (5 mL ACN, 2 g MgSO <sub>4</sub> , 0.5 g NaCl, 0.5 g H <sub>3</sub> Cit, 0.025 g Na <sub>2</sub> HPO <sub>4</sub> , pH 2.5) Vortex (2000 rpm, 8 min) Centrifugation (4000 rpm, 5 min, 10 °C) Dilution (2 mL of the extract to 40 mL Cit buffer pH4) <b>SPE</b> (Oasis HLB, 500 mg) Cond: 10 mL ACN, 10 mL of H <sub>2</sub> O, 10 mL Cit buffer (pH 4) Load: 2 mL supernatant (20-fold diluted, Cit buffer (pH4)) Elute: 9 mL ACN Evaporate to 1 mL Dilute: 125 µL ACN extract: 125 µL 0.01 M oxalic acid (pH 2)	SS	UHPLC-QqQ (MRM) Column: Kinetex C18 polar (2.1 × 50 mm, 2.6 µm) ESI+: (A) 0.1 % HCOOH; (B) MeOH 0.1 % HCOOH	mLOQ (ng/g)*: 0.3–5 Trueness (%): 44–120 Intraday - Interday RSD (%): <26,/n.a	UHPLC-qOrbitrap (Full MS (Rs: 70000 FWHM @ <i>m/z</i> 200) -ddMS2) (Rs: 17500 FWHM @ <i>m/z</i> 200)) Column: ACE UltraCore XB-C18 column (2.1 mm × 150 mm, 1.7 µm) ESI+: (A) 0.1 % HCOOH; (B) MeOH 0.1 % HCOOH	Spiked compounds (n.a)	Evaluation of matrix complexity in NTS	[35]
Soil	>500 Industrial and household halogenated chemicals	2 g (dry)	<b>PLE</b> (27-mm glass fiber filter, 16.2-mm cellulose filter, ~1 g of activated alumina, 30 mL CH <sub>2</sub> Cl <sub>2</sub> ) Static extraction (5 min, 80 °C, rinsing vol 60 %) × 2 Preconcentration to 0.5 mL	T & SS	GC-MS/MS Column: BR-5 ms (30 m × 0.25 mm × 0.25 µm)	mLOD (ng/g)*: <0.01–2 Trueness (%)*: 60–100 Intraday - Interday RSD (%): n.a	Esters, tertiary amines, trifluoromethyls, organophosphates, azoles and triazines, TPs, pharmaceuticals, antimicrobials and fungicides (n.a)	Screening of halogenated compounds in soils and sediments impacted by agriculture and WWTPs	[36]	

(continued on next page)



Table 1 (continued)

Sample	Compounds	Sample amount	Extraction and Clean-up	Analysis	Figures of Merit	Identified compounds (Concentration range (ng/g))	Aim	Ref	
			<b>SPE</b> (Oasis HLB, 200 mg) Load: 24 mL extract Wash: 6 mL H <sub>2</sub> O Elute: 2 x 3 mL MeOH Evaporate to 0.1 mL add 0.9 mL of H <sub>2</sub> O		(0.3 mm × 5 mm, nanoViper) and PepMap RSLC C18 (75 mm × 75 cm, 0.25 μm) ESI+: (A) H <sub>2</sub> O/ACN/HCOOH (97.9/2/0.1, v/v/v); (B) H <sub>2</sub> O/ACN/HCOOH (2/97.9/0.1, v/v)				
Soil	Pesticides	5 g (wet)	<b>QuEChERS</b> (10 mL ACN/HOAc (99:1, v/v) Vortex (20 min) & shake (5 min) Centrifugation (6000 rpm, 5 min) Centrifugation (1 mL supernatant, 12000 rpm, 4 min) Dilute 2-fold with H <sub>2</sub> O	T	UPLC-qTOF (Full Scan, Rs: 40000 FWHM) Column: ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) ESI+/-: (A) 10 mM NH <sub>4</sub> OAc (pH 5.0); (B) 10 mM NH <sub>4</sub> OAc in MeOH	mLOQ (ng/g)*: 0.9–49 Trueness (%): 64–113 Intraday - Interday RSD (%): 1–15, n.a	74 pesticides (0.5–327 ng/g)	Occurrence of pesticides in soils	[22]
	200 suspect pesticides and herbicides			SS	UPLC-qTOF (DIA, Rs: 40000 FWHM) Column: ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) ESI+/-: (A) 10 mM NH <sub>4</sub> OAc (pH 5.0); (B) 10 mM NH <sub>4</sub> OAc in MeOH	n.a	21 herbicides, 31 fungicides, 24 insecticides and 3 growth regulators (n.a)	Large-scale survey of pesticides in soil and risk assessment	
Agricultural contaminated soils	Organochlorine pesticides & TPs	1 g	<b>MAE</b> (CH <sub>2</sub> Cl <sub>2</sub> :Hex (15 mL:35 mL), 20 min) x 2 <b>SPE</b> (Silica) Load: 10 mL extract Elute: 15 mL Hex: Acetone (1:1, v/v) Evaporate to 1 mL <b>PLE</b> (1 glass fiber filter, soil mixed with diatomaceous earth (1:3), 60 mL Hex:CH <sub>2</sub> Cl <sub>2</sub> (1:1, v/v)) Concentration of the extract to 3 mL <b>SPE</b> (15 g silica +5 g Na <sub>2</sub> SO <sub>4</sub> ) Load: 3 mL extract Elute: 100 mL Hex Evaporate to 1 mL	T	GC-qTOF-MS (Full MS, n.a) Column: HP-5MS (15 m × 250 μm, 0.25 μm)	mLOQ (ng/g)*: 1–2 Trueness (%): 74–118 Intraday - Interday RSD (%):n.a	Organochlorine pesticides (77 - 21,200 ng/g)	Method development Risk assessment	[38]
				NTS			126 TPs, mainly chlorinated hydrocarbons containing benzene ring (n.a)		
Soil close to plant root	20 pharmaceuticals and >250 TPs	1 g (wet)	<b>QuEChERS</b> (10 mL 1 % CH <sub>3</sub> COOH in ACN, 5 g MgSO <sub>4</sub> , 1g NaOAc) Shake (5 min)	T & SS	HPLC-QTOF-MS (Full Scan-DIA, Rs: 30000 FWHM)	n.a	18 TPs of pharmaceuticals (0.4–18 ng/g)	Screening of pharmaceuticals and TPs	[26]

(continued on next page)





Table 1 (continued)

Sample	Compounds	Sample amount	Extraction and Clean-up	Analysis	Figures of Merit	Identified compounds (Concentration range (ng/g))	Aim	Ref
Cucumber	Biopesticides & metabolites	10 g	<b>QuEChERS</b> (10 mL EtOAc, 4g MgSO <sub>4</sub> , 1g NaCl) Vortex (2 min) Centrifugation (8170 g, 5 min) Direct injection of the extract	SNTS  mm; 1.9 μm) ESI+/-: (A) 0.1 % HCOOH; (B) MeOH	GC-qOrbitrap (DB-5ms column (30 m × 0.25 mm × 0.25 μm))  mLOQ (ug/g): 2 Trueness (%)*: 79–100 Intraday - Interday RSD (%): 1–5, 2-7	TPs of trans-Cinnamaldehyde and Limonene (n.a)	Degradation studies Toxicity studies	[30]
Vinasse	>7800 (e.g., pharmaceuticals, agrochemicals, preservatives, industrial chemicals)	5 mL	<b>Centrifugation</b> (5000 rpm, 10 min, 5 °C) 4.5 mL sample, dilute 1:1 (v/v) with H <sub>2</sub> O (pH 2.5) <b>SPE</b> (Oasis HLB, 200 mg) Load: 3 mL aliquot, add 0.045 mL Na <sub>2</sub> EDTA 0.1 M Wash: 5 mL HPLC water at pH 2.5 Elute: 2 x 4 mL MeOH Evaporate and preconcentrate to 0.5 mL MeOH:H <sub>2</sub> O (20:80, v/v)	T  UHPLC-QqLIT (MRM) Column: Acquity HSS T3 (2.1 × 50 mm, 1.8 μm) ESI+: (A) 0.1 % HCOOH; (B) ACN SS HPLC-LTQ-Orbitrap (DIA, 1st Full Scan Rs: 60000 FWHM, 2nd Full Scan Rs: 30000 FWHM) Column: Zorbax Eclipse XDB C18 (4.6 × 150 mm, 5 μm)	mLOQ (ng/L)*: 8–427 Trueness (%): >60 Intraday - Interday RSD (%): <20, n.a  n.a	19 compounds (food additives, metabolites from live organisms, pesticides and 3 additional chemicals) (<LOD)	Screening of organic compounds in vinasse from fertigated sugarcane crop areas	[25]
Cereals	Pesticides & mycotoxins	2 g	<b>QuEChERS</b> (10 mL 1 % CH <sub>3</sub> COOH in ACN, 4 g MgSO <sub>4</sub> , 1 g NaCl) Shake (1 min), Centrifugation (n.a) <b>dSPE</b> (2 mL of the extract, 100 mg Bondesil-C18, 300 mg MgSO <sub>4</sub> ) Vortex (2 min), Centrifugation (n.a)	T & SNTS  LC-QqQ (Full Scan; Rs: n.a) Column: Zorbax RRHD SB-C18 column (2.1 × 50 mm, 1.8 μm) ESI+: (A) 0.1 % HCOOH; (B) ACN Acquisition mode: Full Scan	n.a	Spiked compounds (10 <sup>-4</sup> M to 10 <sup>-9</sup> M)	Semiquantitative approach for suspect screening	[41]

**Abbreviations:** ACN: acetonitrile; CH<sub>2</sub>Cl<sub>2</sub>: dichloromethane; Cit: citrate; DIA: data independent analysis; dSPE: dispersive solid phase extraction; EtOAc: ethyl acetate; ESI: electrospray ionization; FWHM: Full Width at Half Maximum; GC-MS/MS: gas chromatography tandem mass spectrometry; HCOOH: formic acid; Hex: hexane; HPLC-LTQ-Orbitrap: high performance liquid chromatography - linear trap quadrupole - Orbitrap; LC-QqQ: liquid chromatography - triple quadrupole mass spectrometry; LOQ: limit of quantification; MeOH: methanol; mLOD: method limit of detection; MRM: multi-reaction monitoring; MTBSTFA: N-tert-butyltrimethylsilyl-N-methyltrifluoroacetamide; n.a: not available; PLE: pressurized liquid extraction; PSA: primary secondary amine; QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe; Rs: resolution; RSD: relative standard deviation; SNTS: suspect and non-target screening; SPE: solid phase extraction; SS: suspect screening; T: target; TP: transformation product; UAE: ultrasound-assisted extraction; UHPLC-QqLIT: ultra-high performance liquid chromatography coupled to quadrupole linear ion trap mass spectrometry; UHPLC-qTOF-MS: ultra-high performance liquid chromatography coupled to quadrupole time of flight mass spectrometry; WWTP: wastewater treatment plant.

calculation. LOI values provide meaningful information about method sensitivity, but few studies provide them, primarily due to the lack of standards. Greater transparency and standardisation in LOD/LOQ/LOI estimation are essential, especially when comparing studies across different matrices or laboratories. On the other hand, although there is no legislation for evaluating the performance of SS and NTS workflows for agri-food system related matrices, different publications and guidelines have been attempted in other fields such as the NORMAN guidance on SS and NTS in environmental monitoring [56] or the BP4NTA QA/QC reporting for NTS [57]. The main concern about QA/QC in SS and NTS is associated to the possibility to report false positives (type I errors) or false negatives (type II errors) results as they are hard to detect. Some of the suggestions to reduce wrong assignments are summarized in Table 2.

The evaluation of analytical methods for determining hazardous substances should not be limited solely to parameters such as sensitivity or precision. It is also essential to consider aspects such as applicability, sustainability, and overall performance of the method. In this regard, White Chemistry provides a complementary approach to traditional ecological criteria, integrating innovative tools that enable a more comprehensive assessment of the analytical process.

Among these tools, the Blue Applicability Grade Index (BAGI) evaluates the practical applicability of the method, considering criteria related to ease of use and adaptability in the laboratory [58]. The revised methods included in Table 1 obtained BAGI scores between 55 and 70, indicating that there is room for improvement, especially regarding the choice of organic solvents, automation degree, the energy expenditure generated during procedures and the use of novel miniaturized systems in high-throughput screening. To ensure the credibility and comparability of NTS analyses in agriculture, future research should prioritize the adaptation—and eventual standardisation—of QA/QC protocols, ideally through interlaboratory studies and regulatory collaboration.

### 3.3.4. Other potential hyphenated techniques

Mass spectrometry imaging (MSI) is one of several emerging visualization tools that show promise as a method for elucidating the spatial distribution and metabolic fate of xenobiotics in environmental and agricultural systems, but are underused. Although MSI is theoretically capable of detecting a wide array of surface-localised metabolites, it currently suffers from limitations in sensitivity and identification

**Table 2**  
QA/QC parameters/strategies in suspect and non-target screening methods.

QA/QC parameter/strategy	Objectives
Use of at least one internal standard per expected compound group	<ul style="list-style-type: none"> <li>• Compensate losses of compounds with similar physico-chemical properties</li> <li>• Intensity and retention time alignment</li> </ul>
Blanks (matrix, laboratory, field and instrumental blanks)	<ul style="list-style-type: none"> <li>• Identify false positives due to contamination issues or background</li> </ul>
Sequence randomization	<ul style="list-style-type: none"> <li>• Reduce systematic errors (i.e., carry over effect)</li> </ul>
Replicates and pooled samples	<ul style="list-style-type: none"> <li>• Show method repeatability</li> </ul>
Spiked quality control samples	<ul style="list-style-type: none"> <li>• Control sensitivity changes during sequence</li> <li>• Check mass accuracy and instrument precision</li> <li>• Check type I and II errors in the workflow (true and false positive rate)</li> </ul>
Standard mix calibration	<ul style="list-style-type: none"> <li>• Check target performance</li> <li>• Determine limits of identification (LOIs)<sup>a</sup></li> <li>• Check type I and II errors in the workflow (true and false positive rate)</li> </ul>
Instrument calibration (external and internal)	<ul style="list-style-type: none"> <li>• Carry out semiquantification approaches</li> <li>• Ensure mass accuracy and resolution</li> </ul>
Description of method boundary	<ul style="list-style-type: none"> <li>• Identification of potential impacts of the method (extraction, separation, detection) and the data processing in the chemical space (type I/II errors)</li> </ul>

<sup>a</sup> Lowest analyte concentration that ensures the identification of the compounds with a reliable HRMS/MS acquisition.

confidence, particularly for low-abundance or unknown compounds. Despite its potential to answer in the field of agri-food systems—such as contaminant fate within the soil-plant-food continuum—its application remains largely confined to targeted or SS workflows. For instance, MSI has been used to trace uptake routes of mycotoxins [59] and PFAS [60], and to study the translocation of fungicides, including the localization and tentative identification of metalaxyl TPs [61]. However, such examples are exceptions rather than the norm.

The absence of comprehensive NTS applications that use MSI is a significant gap, not only due to a lack of sensitivity or software limitations, but also due to a lack of methodological developments. Although existing tools, such as Lipostar MSI [62], offer semi-automated annotations, their usefulness in NTS remains limited due to the scarcity of robust workflows for compound identification, validation, and quantification. Furthermore, the high spatial resolution required ( $\leq 5 \mu\text{m}$ ) presents technical challenges, including long acquisition times and low signal-to-noise ratios. In order for MSI to make a significant contribution to NTS analyses of contaminants in the food chain, future research should focus on enhancing analytical sensitivity, integrating MSI with other techniques (e.g. LC-HRMS or IMS) and developing more reliable spectral libraries for identifying xenobiotics. Without these advances, there is a risk that MSI will remain a minority tool rather than becoming a mainstream approach in agri-food exposomics.

### 3.4. Data processing and data analysis strategies

Table 3 compiles the information related to data treatment and features annotation strategies in HRMS-based SS & NTS approaches in the reviewed scientific publications in this manuscript (i.e., papers compiled in Table 1).

High-resolution mass spectrometry (HRMS)-based NTS generates large, multidimensional datasets that remain a significant bottleneck in exposomics research. While the availability of raw data is no longer a limiting factor, the transformation of this data into meaningful chemical information is hindered by a lack of standardized and validated processing workflows. Key challenges include matrix effects, batch-to-batch variability, and the large dynamic range of analyte concentrations—often spanning several orders of magnitude—all of which can lead to inconsistent feature detection and poor reproducibility across studies. Despite the existence of processing steps such as noise filtering, retention time alignment, and mass calibration (see Fig. 2), these are often applied inconsistently, and their performance is rarely benchmarked in the context of complex environmental or food matrices.

Furthermore, variability introduced by differences in instrumentation, ionization conditions, and software tools severely limits the comparability of results across laboratories and studies. This has direct consequences for key outputs such as detection frequency and compound prioritization, potentially leading to false positives (type I error) or overlooking relevant contaminants. Existing computational strategies tend to focus on generating feature tables and annotation, but few offer robust solutions for harmonizing data processing across large-scale or longitudinal studies. As a result, the reproducibility and transparency of HRMS-based NTS studies remain areas of concern.

To advance the field, future work must focus on community-driven benchmarking of processing pipelines and datasets, clearer reporting standards, and the development of open-source tools tailored to the specific demands of NTS workflows in exposomic research. Without such standardization, the potential of HRMS-based NTS to support regulatory frameworks and environmental monitoring will remain only partially fulfilled.

To address these challenges, numerous software solutions, both commercial and open-source, have been developed, including XCMS [63], MS-DIAL [64], MZmine [65,66], MetAlign Suite [67,68], Progenesis QI (Waters, U.S.), and Compound Discoverer (Thermo Fisher, U.S.). While these tools share core functionalities, they often yield limited overlap in detected features, which possess an additional challenge in SS

**Table 3**  
Data treatment information of the overviewed SNTS studies (2021–2025).

Data treatment software	Suspect database		Spectral library	<i>In silico</i> tools for annotation	Identification assurance for prioritization strategies	Ref
	Database	Compounds included				
Data Analysis software (Bruker Daltonics)	MS Dial	All public MS/MS positive: 13303 compounds All public MS/MS negative: 12879 compounds	<ul style="list-style-type: none"> <li>• MoNA</li> <li>• MassBank Europe</li> <li>• GNPS</li> <li>• mzCloud</li> </ul>	<ul style="list-style-type: none"> <li>• MetFrag</li> <li>• CFM-ID</li> </ul>	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• Retention time prediction (quantitative structure-retention (QSRR))</li> <li>• Ionization efficiency estimation</li> </ul>	[33]
	COCONUT	Open database of 406744 natural products				
Compound Discoverer (Thermo Scientific)	In-house database	22278 TPs of pharmaceuticals generated by Biotransformer	<ul style="list-style-type: none"> <li>• mzCloud</li> </ul>	mzLogic tool	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• Retention Time Index (RTI) platform</li> </ul>	[34], [39], [21]
n.a	In-house database	18349 chemicals of product registers of the Swiss authorities (RPC, ChemPIC, MAO)	not available	not available	not available	[36]
Compound Discoverer (Thermo Scientific)	ChemSpider API	ACToR (500000 compounds), DrugBank (13857), EAWAG BBD/PPS (1396), US EPA DSSTox (738754), ToxCast (875000), US FDA UNII (800000), and LipidMaps (45,245).	<ul style="list-style-type: none"> <li>• mzCloud</li> <li>• In-house library accessed via mzVault</li> </ul>	not available	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• In-house predicted logP values over retention time model</li> <li>• Kendrick mass defect</li> </ul>	[37]
	NORMAN suspect screening lists	S2-HSWT/LfU STOFF-IDENT Database of Water-Relevant Substances (11289), S5-KWR Drinking Water Suspect List (159), S15-NORMAN Priority List (967), S27-Extended Suspect List (KWRSJERPS) (4984), S28-Biocides from the NORMAN Priority List (141).				
UNIFI Scientific Information System software platform (Waters)	In-house database	133 pesticides	<ul style="list-style-type: none"> <li>• In-house database with 133 pesticides</li> </ul>	not available	not available	[22]
NORMAN Digital Samples Freezing Platform (DSFP)	NORMAN suspect screening lists	not available	not available	not available	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• Retention Time Index (RTI) platform</li> <li>• Frequency of detection</li> <li>• Prioritization of isomeric compounds</li> </ul>	[25]
patRoom	NORMAN suspect screening lists	S15-NORMAN Priority List (967)	<ul style="list-style-type: none"> <li>• European MassBank</li> <li>• MassBank of North America</li> <li>• MoNA</li> <li>• MassBank Europe</li> </ul>	<ul style="list-style-type: none"> <li>• MetFrag</li> <li>• GenForm</li> </ul>	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> </ul>	[16]
Sciex OS software (Sciex)	In-house database	262 TPs of pharmaceuticals generated <i>in silico</i> prediction tool EAWAG-BBD Pathway Prediction System (EAWAG)		not available	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• In-house predicted logP values over retention time model</li> </ul>	[26]
Compound Discoverer (Thermo Scientific)	mzCloud In-house database	not available	<ul style="list-style-type: none"> <li>• mzCloud</li> <li>• In-house mass spectra database</li> </ul>	mzLogic tool	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• Presence of heteroatom in the molecule</li> <li>• Fold-change with blank</li> </ul>	[24]
Compound Discoverer (Thermo Scientific)	not available	not available	<ul style="list-style-type: none"> <li>• mzCloud</li> <li>• MassBank of North America</li> </ul>	not available	<ul style="list-style-type: none"> <li>• Mass accuracy threshold</li> <li>• Evaluation of MS/MS spectrum</li> <li>• Match of harmonized functions from the EPA's chemicals and products database (CPDat) to DTXSIDS</li> <li>• Frequency of detection</li> </ul>	[28]
<ul style="list-style-type: none"> <li>• Mass Frontier™ (Thermo Fisher Scientific) to confirm the fragment ions by <i>in silico</i> approach.</li> <li>• MassChemSite v(Molecular Discovery Ltd.) to identify unknown metabolites</li> </ul>	not available	not available	not available	not available	not available	[40]

(continued on next page)

Table 3 (continued)

Data treatment software	Suspect database		Spectral library	In silico tools for annotation	Identification assurance for prioritization strategies	Ref
	Database	Compounds included				
Compound Discoverer (Thermo Scientific)	EFS HRAM Compound Database	not available	<ul style="list-style-type: none"> <li>mzCloud</li> <li>mzVault</li> </ul>	not available	<ul style="list-style-type: none"> <li>Mass accuracy threshold</li> <li>Evaluation of MS/MS spectrum</li> <li>Fold-change with blank</li> </ul>	[30]
	Lipid Maps Structure Database	not available				
	Natural Products Atlas 2020.06	not available				
	LCMS Co-formulant PPP	not available				
	ChemSpider	not available				

and NTS studies [69,70]. This variability is due to differences in algorithms and processing methodologies, resulting in a different balance between false positive (type I error) and false negative (type II error) peak detections [71]. Consequently, careful selection and optimization of data processing tools are essential to improve the accuracy and reproducibility of NTS and SS workflows based on HRMS data. A practical approach to tool selection and data processing optimization involves the use of internal standards spiked at known concentrations in sample matrices. By evaluating the recovery of these compounds at their respective retention times,  $m/z$  values, and intensity ranges across different software, researchers can define QA/QC metrics to fine-tuning processing parameters and achieve results that more closely reflect expected outcomes [72]. However, a key limitation is that parameters optimized using a small, well-characterized set of internal standards may not generalize well to the chemically diverse and complex mixtures found in real samples. This is particularly critical for low-abundance compounds, which are more frequently missed (false negatives) than those present at higher concentrations [73]. To overcome these challenges, additional tools for data processing optimization have been developed based on various strategies, such as 12C/13C isotopologue pairing to distinguish true organic compounds from instrumental noise [74], and machine learning-based approaches like EVA and NeatMS, designed to filter false positive peaks [75,76].

#### 3.4.1. Data processing steps

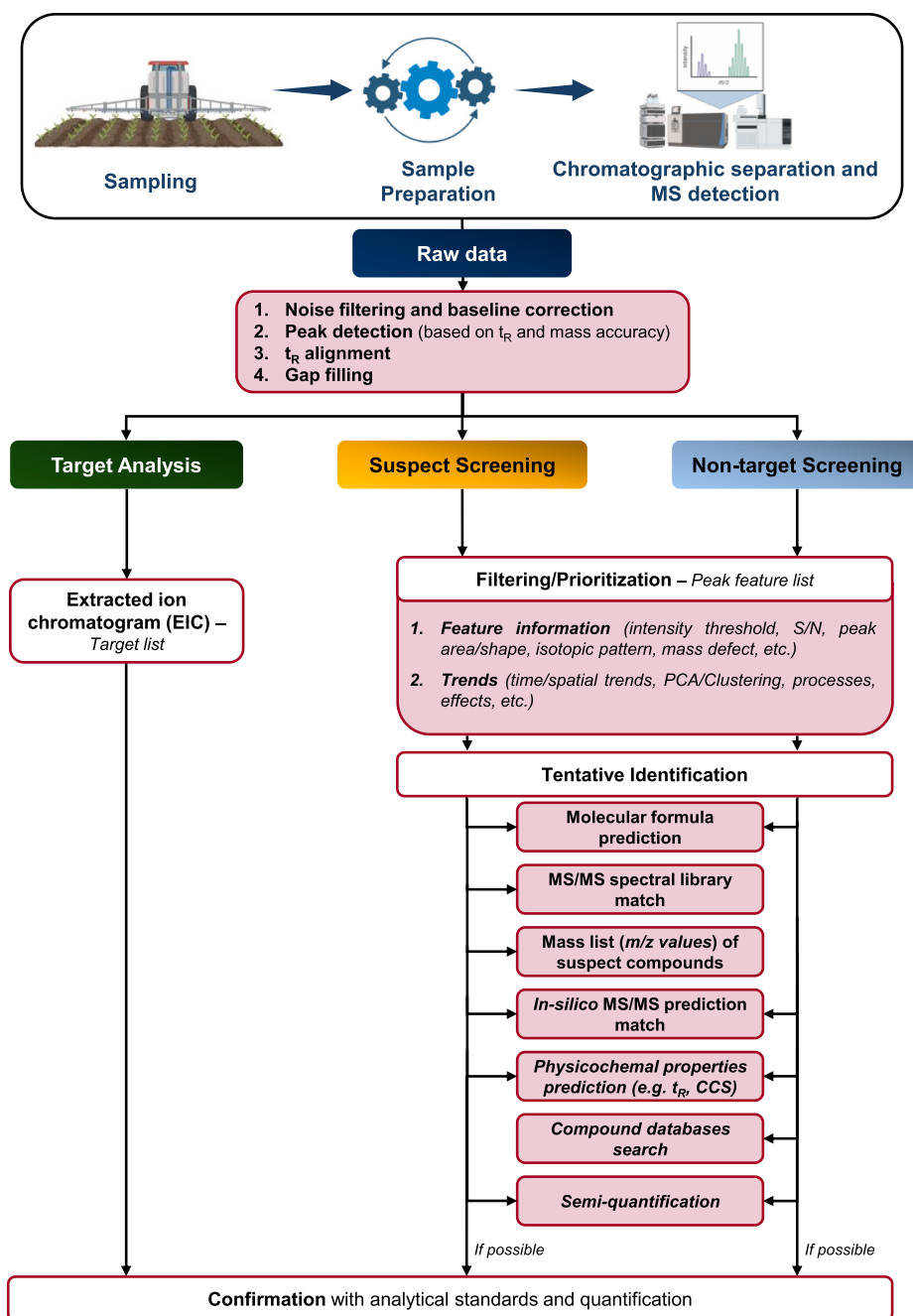
While sample extraction and data acquisition methods are usually guided by the type of sample and its chemical composition, HRMS data processing generally follows a standard sequence of procedures (see Fig. 2). These typically consist of four sequential steps: i) centroiding and threshold filtering, ii) construction of extracted ion chromatograms (EIC) and peak detection, iii) alignment of corresponding features across multiple samples, and iv) post-processing steps [77].

During the first step of the processing workflow, raw spectral data is centroided, and intensity thresholds are applied to exclude low-intensity signals, such as electronic noise, from further processing. The software MSConvert from ProteoWizard 3.0.9798 (Proteowizard Software Foundation, Palo Alto, CA, USA) is commonly used in open-access software workflows for data centroiding, format exchange, and intensity thresholding. This software has been used in numerous SS and NTS studies as the first data processing step to identify unknown contaminants, including those in agricultural settings. For example, Maini-Rekdal et al. [78] used MSConvert in a NTS study of the fungus *Neurospora intermedia* that enables waste-to-food conversion of agricultural by-products. Similarly, da Silva and co-workers [25] used MSConvert for centroiding and format exchange in a NTS study aiming to characterize organic contaminants in vinasse, a biofertilizer obtained as agricultural waste from the sugarcane processing. While MSConvert is recommended for centroiding and format exchange for data processing in open access software, proprietary software such as Compound Discoverer, implement their own centroiding algorithms which can have

an impact on subsequent data processing steps.

Distinguishing meaningful signals from background noise is a significant challenge in any LC- or GC-HRMS study. In most tools, noise thresholds are manually defined by visual inspection of the raw data [77]. Briefly, this step involves examining mass spectra in several small segments of the chromatographic run where no high-abundance peaks are eluting and then selecting a threshold value based on the intensity of persistent background ions. A complementary method is estimating the detection frequency of all ions across the LC/GC-MS run. Ions with high detection frequencies spanning the entire chromatogram are likely to be instrumental or chemical noise and can therefore be discarded [79]. While noise thresholds are a common step applied in both commercial (e.g. Compound Discoverer) and open-access tools, this step is normally defined manually as a "minimum peak intensity" value in SS and NTS-based circularity studies [19,24], which can lead to both the inclusion of noise features or the exclusion of relevant peaks. However, to the best of our knowledge, no SS or NTS study has used the detection frequency of all ions in a LC/GC-MS run to define noise thresholds in a circular agriculture context.

NTS experiments typically yield hundreds to thousands of features, with only a relatively small portion corresponding to meaningful compounds in the sample [80]. For this reason, the goal of EIC construction and peak detection, the second step in the processing workflow, is to retain all relevant features in the raw spectra. A feature can be understood as a  $m/z$  signal associated with a retention time value in LC or GC or a drift value if ion mobility spectrometry-MS (IMS-MS) is used [79]. IMS-MS is being increasingly reported in metabolomic and lipidomic studies (a type of NTS studies), as it can provide an additional dimension for isobaric and isomeric metabolite separation, as well as an additional property to refine annotation (i.e., collision cross-section). While IMS-MS was not among the techniques included in the reviewed papers (2021–2025), it has already been successfully applied to similar NTS analyses — for example, in the detection of drug-related metabolites in plants [81]. Its potential to enhance selectivity and compound identification builds on a growing body of prior research, and its absence in many studies may reflect its recent incorporation. EIC construction and peak detection represent the cornerstones of the data processing pipeline in SS and NTS studies. Ideally, different software would detect the same features in each sample. However, there is little overlap in both the number and identity of features detected by different software when processing the same data. This discrepancy, explored in detail by Myers et al. [69,70] in NTS studies, mostly arises from differences in peak detection algorithms as well as deficiencies in mass alignment and controls of feature quality implemented in different tools [82]. Selecting the most suitable peak detection algorithm for a given dataset is particularly challenging due to the absence of a "ground truth", a comprehensive set of compounds known to be present in the sample, against which algorithm accuracy can be assessed. However, in the context of contaminant detection in circular systems, at least a portion of this "ground truth" can be inferred as highlighted in Table 1, potentially



**Fig. 2.** Flowchart illustrating the workflow for target, suspect, and non-target screening using HRMS to identify and annotate agriculture-related organic contaminants. The strategy encompasses sample preparation, data acquisition, and processing steps, with increasing levels of data complexity and annotation effort from target to non-target approaches.

enabling more systematic software comparisons. Despite this possibility, no study has yet conducted such an analysis.

While the processing of LC-MS and GC-MS datasets follow similar implementations of the four core steps—centroiding, peak detection, alignment, and post-processing—spectral deconvolution is a crucial additional step in GC-EI-MS due to the extensive in-source fragmentation caused by electron ionization. Since EI-generated spectra can contain fragment ions from multiple co-eluting compounds, spectral deconvolution is necessary to computationally reconstruct fragmentation spectra for features that are not fully resolved chromatographically [79]. All major GC-MS processing software, such as MS-DIAL, MZmine, and Compound Discoverer, incorporate algorithms for spectral deconvolution. For instance, a non-target screening workflow for identifying

organic pollutants in soil samples utilized proprietary Agilent software for spectral deconvolution. These reconstructed spectra were then used during feature alignment and annotation, ultimately leading to the identification of 122 annotated organic pollutants [83]. To the best of our knowledge, no study has compared the performance of different spectral deconvolution software for GC-MS data in the context of agricultural settings.

The alignment of mass features is also crucial in SS and NTS HRMS-based studies [82]. During an analytical batch, many unexpected factors may cause the drift of retention time and  $m/z$  values, such as fluctuations in external temperature, changes in mobile phase pH and pressure (if LC-HRMS studies), column stability and lifetime, as well as sample matrix [84]. Furthermore, retention time deviations tend to be even

larger between measurements obtained from different LC- and GC-HRMS instruments, even when using the same chromatographic conditions. Therefore, sophisticated alignment algorithms able to correct large linear and non-linear shifts in retention time have been developed [85]. For example, a large-scale SS study by Ref. [86] observed significant non-linear shifts in retention time. These shifts occurred despite identical chromatographic conditions used in Orbitrap LC-HRMS instruments in different European laboratories and required the development of specialized tools (MetAlign Suite). However, in the context of contaminant detection in circular agriculture most studies have used currently available tools such as Patroon [16], and especially Compound Discoverer [24,37]. In this last tool, the retention time alignment process involves the identification of landmark features, distinct peaks present across different samples. These landmarks serve as reference points, allowing the algorithm to adjust and align non-linear deviations in retention times of corresponding peaks in other samples, thereby compensating for any shifts that may have occurred during analysis. This retention time model, named "adaptive curve", has been successfully used in NTS studies in food safety applications [19], including studies on sustainable agriculture [24].

#### 3.4.2. Data analysis strategies

While data processing can often be generalized across different datasets, data analysis strategies are typically tailored to the specific characteristics of each dataset. A crucial step in data analysis is data reduction and feature prioritization. This can be achieved using general methods (such as blank subtraction) or by applying criteria specific to certain classes of compounds such as the presence of a particular mass defect (e.g.  $\text{CF}_2$  scale for PFAS or H/Cl scale for chlorinated pesticides) or isotopic pattern (e.g., chlorine and bromine in halogenated pesticides) [10,87,88]. Other approaches employing multivariate statistical analyses to guide feature selection [89].

Among the general approaches for data reduction implemented in SS and NTS studies, blank subtraction and significant testing represent robust methods to discard irrelevant features [34,38]. According to the authors, only features missing in the control blanks (solvent blank and/or method blank), or those having a significant difference ( $p_{\text{value}} < 0.05$ ) to the peak area's controls were retained.

In addition to this general approach, several compound or class-specific strategies can also be applied. For example, in PFAS analyses, the Kendrick mass defect plot is commonly used to analyse and classify homologous series of compounds containing  $-\text{CF}_2$  monomers in the structure backbone, serving as a useful strategy to filter out non-fluorine containing compounds from potential PFAS [10,87]. This method clusters PFAS that differ only by  $\text{CF}_2$  units, allowing analysts to quickly recognize patterns, identify homologous series, and detect unknown PFAS that may be structurally related to known compounds. This approach is particularly valuable given the complexity of PFAS mixtures and the frequent occurrence of unknown PFAS with similar structural motifs in environmental samples [10,87]. For instance, a recent study on agricultural soils in Germany applied this methodology to identify several previously unknown PFAS in the same soil samples previously analysed by target methodologies [90].

The use of the isotopic patterns of certain atoms, such as Br and Cl, to guide the selection of relevant features is also a useful strategy in SS and NTS studies. These patterns, arising from the natural abundance of different isotopes, provide valuable information for identifying and quantifying molecules. In DDA, where specific ions are selected for fragmentation and analysis, the characteristic isotopic patterns of Br and Cl can be used to target and identify molecules of interest. Although DDA is widely used, it has limitations like stochastic precursor ion selection and potential for missing values. [91,92]. However, the use of isotopic patterns can help mitigate some of these limitations by providing additional criteria for precursor selection. Overall, in DDA, isotopic patterns help with precursor ion selection, while in DIA, they are crucial for fragment ion analysis and quantification. This approach

was successfully applied to characterize halogenated organic compounds in soil and sediment samples, suggesting that screening for halogenated organic pollutants in agriculture soil is necessary for environmental monitoring and risk assessment [93].

Suspect lists, which usually contain hundreds to thousands of compounds, represent a crucial strategy to effectively filter irrelevant features and ensure a more efficient annotation process. These lists typically include compound names, molecular formulas, and exact mass values, such as those found in the NORMAN database [89]. Study-specific lists tailored to certain contexts like compounds expected in specific agrifood systems or predicted metabolites generated *in silico* (e.g., using tools like BioTransformer) [34,94], can also be used. These lists can be uploaded directly into various software platforms, including Compound Discoverer, MZmine, MetAlign, and XCMS, to perform a tentative annotation by matching exact mass values. This approach helps prioritize relevant compounds and accelerates the identification process in complex samples. A practical example of this strategy is the study by Gravert et al. [37], in which a NTS approach was used to characterize organic micropollutants in agricultural soils supplemented with different fertilizers. By combining mass spectral libraries with a NORMAN list of environmental contaminants, they were able to annotate approximately 20 % of the 2306 detected features, which is significant in this type of studies. Another strategy is applying supervised and unsupervised multivariate statistical methods for identifying relevant features when multiple sample groups are present (e.g. different type of samples, temporal trends, etc.). Unsupervised methods, such as Principal Component Analysis (PCA), help revealing natural clustering patterns and visualizing the mass features responsible for the sample groupings [82]. Supervised methods, like Partial Least Squares Discriminant Analysis (PLS-DA) and decision trees, among others, use known group labels to enhance classification accuracy and highlight key features that distinguish sample groups, such as treatment versus control. Volcano plots also provide a valuable visualization for feature selection by combining measures of statistical significance and fold-change, enabling the identification of biomarkers or significant compounds [82]. A practical example of the use of multivariate statistical methods to guide feature selection is the application of differential analysis of variance to detect potential contaminants in various organic fertilizers in a circular agriculture system [37].

Beyond traditional multivariate analysis, HRMS/MS-based pattern recognition algorithms also offer powerful approaches for feature selection and structural classification. MassQL, for example, enables targeted filtering of  $\text{MS}^2$  spectra based on specific diagnostic ions, which is especially advantageous for identifying compounds within well-defined chemical classes that exhibit shared fragmentation patterns [95]. Additionally, molecular networking cluster precursor ions based on HRMS/MS spectral similarities, enabling the systematic organization of related compounds. This approach is highly effective for monitoring food quality and safety, as it facilitates the annotation of toxic compounds and their analogues through spectral matching. It thus shows great potential for the identification of food contaminants [96] and the characterization of organic compounds in soil [97].

#### 3.4.3. Annotation strategies in SS and NTS

Several strategies to support compound annotations in SS and NTS workflows are commonly applied, including: (1) comparison with reference standards, (2) mass spectral library matching, and (3) *in-silico* fragmentation and machine-learning-based prediction tools [98].

While level 1 identification [99], achieved by matching  $\text{MS}^1$ ,  $\text{MS}^2$ , and retention time data with reference standards, is the highest degree of confidence that can be achieved based on the available data, it is limited by the availability of reference substances. Consequently, identification levels of 2 and higher indicate lower levels of confidence when definitive confirmation cannot be attained.

The use of mass spectral libraries for compounds annotation allows both direct matching and, in some cases, analogue search. The selection

of a proper spectral library is crucial to increase the confidence on the annotated compounds that could be found in each sample [100–104]. Table 4 summarizes the main spectral libraries available for LC-MS and GC-MS data. On the one hand, NIST is the gold standard for compounds identification in GC-MS studies due to its reliability, extensive curation and continuous updates despite it mainly contains low-resolution mass spectra [105]. On the other hand, the range of MS spectral libraries for LC-MS data is significantly higher being most of them open access. Although most of them are universal covering a wide range of analytes, they usually present different scopes making them useful for different research fields. The application of spectral libraries for identifying organic contaminants in agricultural settings has been well documented in the literature, demonstrating their effectiveness in detecting pesticides, veterinary drugs, and other environmental pollutants [16,24,33].

The mass spectra libraries mzCloud, mzVault and Europe and North America Mass Bank, were the ones most used in the articles reviewed here (see Table 3).

In addition to spectral library matching, *in silico* fragmentation and machine-learning-based prediction tools are also important strategies for identifying unknown compounds in complex samples when reference spectra are unavailable open access [113]. In this context, open access computational tools like MetFrag [114], Competitive Fragmentation Modelling for Metabolite Identification (CFM-ID) 4.0 [115] SIRIUS [116], and MSNovelist [97] are highly relevant (see Table 5).

Among them, MetFrag and CFM-ID are two widely used *in silico* tools for aiding in the structural annotation of unknown compounds by using a probabilistic model to predict fragmentation patterns and together with mzLogic were also the most used *in silico* tools in the articles reviewed (see Table 3). For instance, Das et al. [16] demonstrated an application of these tools in the annotation of organic contaminants in agricultural soil treated with a bio-based fertilizer (BBF), utilizing MetFrag in combination with spectral libraries to improve the comprehensiveness of compound's annotation. SIRIUS expands on *in silico* fragmentation by integrating isotope pattern analysis, allowing it to determine molecular formulas with high accuracy, without requiring

**Table 4**

Summary of some of the main spectral libraries used for compounds annotations in SS and NTS studies based on GC-MS and LC-MS.

MS library	Number of spectra/ number of compounds	Useful for	Analytical platformn	Ref.
NIST 23	>390,000/ >340,000 2.5 million/ >50,000	Universal	GC-LRMS	[106]
		Universal (food and environmental contaminants)	LC-LRMS/MS and LC- HRMS/MS	[106]
MassBank EU	>120,000/ >16,000	Natural products, pharmaceuticals and contaminants	LC-LRMS/MS and LC- HRMS/MS	[107]
GNPS <sup>a</sup>	>500,000/> 30,000	Universal with emphasis in natural products	LC-LRMS/MS and LC- HRMS/MS	[108]
MoNA <sup>b</sup>	>230,000/na	Toxicological and environmental fields	GC-MS, LC- LRMS/MS and LC-HRMS/MS	[109]
METLIN	>4 million/> 850,000	Biomedical and environmental fields	LC-HRMS/MS	[110]
MzCloud	>24 million/ 39,000	Universal (Thermo Sci.)	LC-HRMS/MS	[111]
Wiley Registry	>2.7 million/ na	Environmental, food, cosmetics and metabolomics	LC-HRMS/MS	[112]

<sup>a</sup> Includes library aggregations from other sources, such as MoNA, MassBank, HMDB. Number excludes annotated spectra propagated computationally from reference MS/MS spectra (lower accuracy).

<sup>b</sup> Includes library aggregations from other sources, such as GNPS, MassBank, HMDB. Number excludes *in-silico* generated mass spectra.

**Table 5**

Summary of some of the main open access *in-silico* tools used for compounds annotations in SS and NTS studies based on GC-MS and/or LC-MS.

<i>In-silico</i> tool	Approach		Ref.
MetFrag	Rule-based	Generates theoretical product ions for the candidate molecular structure to then provide a similarity score	[114]
CFM-ID	Machine learning	MS/MS fragmentation by "learning" fragmentation pathways from empirical data, which improves accuracy for certain complex molecules	[115]
SIRIUS	Fragmentation trees	Combines fragmentation tree algorithms with isotope data to build a fragmentation pathway. It also incorporates the CSI:FingerID algorithm, which uses machine learning to predict molecular fingerprints for the unknown spectra and compare with calculated fingerprints from molecules available in structure databases	[116]
MSNovelist	Deep learning	Combines fingerprint prediction with an encoder-decoder neural network, enabling de novo generation of structures solely from tandem mass spectra	[117]

searching of structural databases [116]. This tool was successfully used in the study of Maini Rekdal et al. [78] to identify exogenous and endogenous compounds in fungal cultures growing in agricultural by-products. MSNovelist is an emerging tool that leverages deep learning to predict molecular structures directly from mass spectra [117]. It operates similarly to SIRIUS although, rather than querying a database for matching fingerprints, MSNovelist uses a recurrent neural network (RNN) to predict SMILES codes directly from the molecular fingerprints. This bypasses the need for a reference database, allowing the tool to suggest structures for novel analytes or poorly represented classes. Despite its advantages, the adoption of MSNovelist within the scientific community remains limited, likely due to the absence of a user-friendly interface.

In conclusion, the processing and analysis of HRMS data in SS and NTS studies remain challenging since is often the bottleneck in the analytical pipeline as the time spent on data processing and evaluation can substantially exceed the time for sample preparation and spectra acquisition. As discussed before, typical data evaluation comprises the molecular formula assignment process, data quality assessment, data selection, visualization, export and documentation.

Despite the rapid advancements over the past decade, many software solutions still struggle with limited overlap in detected features and persistent false peak detections. Major issues include: the performance of peak detection algorithms, the lack of community-wide standards to assess the performance of processing tools, the absence of automated parameter optimization tools, and the limited chemical space covered by existing spectral libraries. These challenges inevitably introduce variability, hinder reproducibility and limit the accuracy of data interpretation across different studies. Despite these hurdles, the development of more sophisticated tools in recent years indicate that we are moving in the right direction toward a deeper exploration of the unknown chemical space within our food systems. Standardizing processing workflows could improve reproducibility and reliability, particularly across various software platforms and laboratories. This progress is especially relevant in circular agriculture, where early hazard identification is crucial for preventing the accumulation of contaminants in the food supply chain.

#### 3.4.4. Semi-quantification approaches in SS and NTS

Although the ultimate confirmation of an annotated compound tentatively identified in SS and NTS approaches requires the comparison with an analytical standard, the large number of features usually

detected in a sample makes unfeasible this strategy. This has led to the development of the so-called semi-quantification approaches, mainly in LC-ESI-HRMS platforms, which aim to quantify detected compounds without analytical standards [118,119]. In this source, the ionization efficiency (IE) is mainly affected by the physico-chemical properties of the analyte and the mobile phase, as well as the ion source geometry, which might result on significant differences from one substance to another [120]. To overcome this limitation and ensure a reliable semi-quantification of the annotated compound different approaches have been developed: (i) use of surrogate standards for quantification [121,122] or machine learning approaches to quantify the concentration based on predicted IE [121,123,124]. Semi-quantification approaches have been already applied in food circularity matrices going a step forward in SS and NTS. For instance, Nanusha et al. [125] employed a set of labelled standards in to quantify micropollutants in sewage sludge used in agricultural fields using the semi-quantification software platform [126]. Huang et al. [38] also used a surrogate (phenanthrene-*d*<sub>10</sub>) to quantify by GC-EI-HRMS 22 tentatively identified pollutants consisting of organochlorine pesticides and transformation products in agrochemical soils from the Shandong Province (China) at concentration ranges below 10 ng/g. Additionally, Reyes-Ávila et al. [30] used calibration curves of the corresponding parent compound to semiquantify by GC-HRMS and LC-HRMS metabolites of *trans*-cinnamaldehyde biopesticide in cucumber at concentrations ranging from 6.0 to 775.3 µg/kg. These studies show the potential of this strategy to enhance the knowledge and predict the potential effect of the tentatively annotated compounds in SS and NTS.

#### 4. Occurrence of chemical hazards in agriculture

Table 1 also summarizes the occurrence of chemical residues in agricultural-related matrices. Among the matrices examined in this review (soil, compost, and crops) most studies focused on the detection and (semi)-quantification of pesticides specifically in soil (see Table 1). This is not surprising as pesticides are the most widely used, but also the most monitored chemicals.

A SS method was applied to screen over 200 pesticides in 166 soil samples collected from typical peach orchards in 12 provinces of China [22]. This survey represents, to the best of our knowledge, the first large-scale study of pesticides in soil from peach orchards. Just 5 % of the soils showed no signs of pesticide residues, while more than 86 % of the soils contained multiple residues. Herbicides, fungicides, insecticides, and growth regulators were identified at 1-2a confidence level in the tested soil samples and the total concentrations of quantifiable herbicides ranged from 1 to 327 ng/g. This study provides comprehensive and accurate information on the pesticide residue status for risk assessment.

Despite the large amount of evidence on the occurrence of pesticides in soils, no maximum residue limits (MRLs) are set in Europe. One reason for the lack of regulation in soil may be the complexity of the matrix, which makes analysis with low LOQ difficult.

In addition, pesticides may co-occur with other environmental contaminants. By expanding the scope of the SS and NTS, it is possible to uncover different chemical classes of contaminants that co-occur. As an example, a “cocktail” of 2306 compounds was found in soils [37] that had been amended at very high rates with either human urine, manure, or wastewater treatment sludge. The highest number of contaminants was added by wastewater treatment sludge, with 25 significant contaminants including blood pressure regulators, antidepressants, polypropylene glycols, synthetic steroids, and sleep medication. Pharmaceuticals contributed the largest share, followed by pesticides and natural products (see Table 1). Of particular significance is the application of Quantem to forecast the concentrations of the detected compounds, which varied from 0.2 to an impressive 10,881 ng/g of dry matter. These toxic compounds can also be masked by enzymatic or chemical reactions mediated by, for example, the soil microbiome,

resulting in the formation of TPs. This represents an additional challenge when it comes to their detection by target methods, and they may be missed. However, the parent compound may become available again after, for example, gastrointestinal digestion, making it relevant to estimate the occurrence of these metabolites as well.

Newly characterized TPs of pesticides (e.g. irgarol and fipronil) and pharmaceuticals (e.g. carbamazepine, diazepam) were reported in sediments from agricultural and urban soils impacted by WWTPs [36]. In the study of Martínez-Piernas et al., [26], 18 TPs of pharmaceuticals were identified and their potential transference from soils to crops was subsequently studied. Among the monitored pharmaceuticals, the presence of 2 AMs of the macrolide group, azithromycin and clarithromycin, was determined in the soil samples studied. In addition, 3 TPs derived from azithromycin have been detected in the same soil samples, two of them annotated at level 2b and one at level 3 of the Schymanski scale [99], and 2 TPs of clarithromycin, annotated at level 1 and 2b. Moreover, the study revealed that certain TPs were persistent, particularly in the perlite substrate. Nonetheless, there was no substantial evidence indicating the specific availability or absorption of TPs from the soil or perlite into the edible part of the tomato plant. Although it would have been interesting to know the concentration level for these TPs, only peak areas are reported in the paper.

Nevertheless, several studies involving pharmaceutical exposure experiments with vegetables have demonstrated the successful transfer of pharmaceuticals into the plants. For instance, Fučík et al. [127] cultivated lettuces in hydroponic and soil environments, both which were contaminated from the beginning of the experiment with a mixture of six pharmaceuticals including AMs (atenolol, enrofloxacin, erythromycin, ketoprofen, sulfamethoxazole, and tetracycline), each at a concentration of 10 µg/L (in hydroponic conditions) or 10 µg/g (in soil).

Lettuce samples were analysed after 14, 21 and 28 days, while contaminated soil and water were analysed at the end of the experiment, after 28 days. In total, 26 TPs were successfully identified. Most of the TPs were determined in the lettuce roots, especially in lettuce grown under hydroponic conditions, while at the end of the experiments some TPs were also detected in the water solution, suggesting that degradation of the pharmaceuticals might have already taken place before entering the plant [127]. Similarly, Tian and coworkers [128] also identified eight TPs of clarithromycin (a macrolide) and two TPs of sulfadiazine (a sulphonamide), in lettuces grown under hydroponic conditions. These studies confirm that TPs of pharmaceuticals could contaminate the food chain and contribute to the spread of AM resistance in the environment, which could have an impact on human health.

It should be noted that, in many cases, the lack of analytical standards for suspects and their potential TPs implies reporting semi-quantitative concentrations. Chen and co-workers, for example, identified 67 parent pesticides and 57 TPs in agricultural products, and they reported the semi-quantitative concentrations of the TPs in the analysed strawberries [47]. Most TPs were detected at low concentration levels, and preliminary traceability suggests that they may migrate from soil. In addition, it shows that the concentrations of parents and TPs are mostly positively correlated. Few samples exceed the MRL set by the Chinese standard for chlormequat (1 mg/kg) when only the parent compound is considered but some more would have exceeded if the TPs had also been considered.

Not only man-made chemicals, but also natural toxins can accumulate in soil and crops. To the best of our knowledge, this represents an unexplored area when it comes to NTS detection and agro-environmental matrices. Indeed, although these compounds have been extensively studied in food and feed, only little is known about their occurrence and fate in soil and manure. Our literature search did not yield any manuscripts with the selected key words (suspect and non-target, soil and compost, crops, plants, and vegetables, and circular agriculture). However, a few papers [129] have shed light on the accumulation of e.g. mycotoxins (aflatoxins, zearalenone, deoxynivalenol in the range of 6–80 ng/g in soil) using target methods,

highlighting the need to extend the scope of the NTS method and include also these molecules in the list of compounds to be monitored.

Some of the studies analysed, aimed at investigating the sustainability of vegetables by-products reuse as a BBF in crops by assessing the occurrence of organic contaminants and their potential for dissemination to soils and groundwater in fertigated areas. Da Silva et al. [25], tracked the transfer of organic contaminants from vinasse used as fertiliser in sugarcane crops. 56 compounds, mostly pesticides, food additives, industrial and naturally occurring substances were identified. Results showed no overlap between the compounds detected in vinasse (19) and environmental samples (12 in soil and 25 in groundwater), suggesting that the pollutants found in soil and groundwater might come from alternative sources other than vinasse reuse. Similar results were obtained by Das et al. [16] when investigating the residues in the soil treated with BBF. Indeed, the authors screened both soil samples from fields amended with 15 BBFs from various sources (agricultural, poultry, veterinary, and sludge). The SS results suggest that the pharmaceuticals (e.g. ibuprofen, 1-hydroxyibuprofen and lenacil) found in BBF-treated soil might come from alternative sources other than BBFs, as there is no correspondence with those found in BBF.

A similar approach, based on SS and NTS, has been applied to study the transfer of beneficial compounds such as phenolics and lipids, from the organic material to the future food [33]. In summary, the study successfully identified a range of compounds in onion and mushroom, across different confidence levels, indicating the effectiveness of the composting process in retaining beneficial compounds (fatty acids, organic acids, flavonoids) from the raw material.

Understanding the degradation and metabolism of bio-pesticides in food matrices is crucial for assessing their safety compared to conventional pesticides. This study [30] investigated the metabolism of *trans*-cinnamaldehyde and limonene in cucumber, identifying cinnamyl alcohol as a key metabolite. Its concentration peaked at 4 h, ranging from 19.9 to 94.5 µg/kg (200 µg/kg dose) and 28.1–392.0 µg/kg (1000 µg/kg dose). Toxicity evaluation revealed low risks for human consumption, with bio-pesticides showing lower persistence and toxicity than synthetic pesticides. At concentrations below 1 mg/kg in cucumber, these compounds pose minimal health risks, supporting their safer use in crop protection.

## 5. Conclusions and future perspectives

The advances on highly capable analytical methodologies, instrumentation and data processing have enabled to expand the chemical space in the agri-food system by means of simultaneous multitarget, suspect and non-target screening approaches. From a sample treatment point of view, UAE, PLE and QuEChERS are generally preferred as extraction technique to ensure a wide-scope extraction while clean-ups based on SPE are preferentially used to remove major interferences avoiding the discrimination of expected chemical residues. New trends in this field led to miniaturized procedures such as dSPE or even automated sample treatments that could help to enhance the laboratory throughput, the greenness and the practicality of the analyses. In summary, we recommend simplifying and standardizing sample pre-treatment protocols to remove impurity interference while reducing analytes loss. Regarding the analytical determination, LC-ESI-HRMS using reversed-phase columns and GC-EI-HRMS employing 5 % phenyl-polydimethylsiloxane stationary phases are generally selected to carry out these determinations, although the use of LRMS in GC-MS is also widely extended due to the availability of robust low-resolution mass spectral libraries. Further research in terms of alternative techniques to reversed-phase chromatography, such as HILIC and supercritical fluid chromatography (SFC), unified chromatography (UC), an SFC gradient in which the state of the mobile phase changes continuously from supercritical to liquid at 100 % polar co-solvent, has shown potential for the analysis of compounds in a broad range of polarity, including very polar compounds. Moreover, the use of alternative

ionization sources (i.e., APCI, APPI or atmospheric pressure plasma-base sources) as well as other promising techniques such as IMS or MSI could help to improve the performance by extending the analyte coverage, enhancing sensitivity and selectivity, as well as improving the separation or the throughput in the analysis of complex matrices. Additionally, especial attention should be given to a proper validation of SS and NTS methodologies, ensuring a reliable identification while minimizing type I and II errors. Robust validation processes coupled with specific QA/QC procedures should be developed on large sample datasets to ensure the validity of the results. Perform inter-laboratory comparisons using certified reference materials available when dealing with a wide range of compounds in complex matrices is also compulsory. Concerning data processing, most of the efforts are focused on the development of data analysis strategies to prioritize features such as KMDs, isotope pattern match or sample/temporal trends and annotation strategies to enhance the confidence of the annotations. In this sense, the use of effect directed approaches combined with semi-quantification approaches could help to link chemical exposure levels with toxicology, moving a step forward to guarantee the food safety in agrifood systems. Moreover, the development of semi- or fully automated data analysis software would be highly beneficial to reduce the bottleneck of the data treatment in SS and NTS approaches. Finally, it is important to highlight that, even SS and NTS aim to extend the chemical space of current methodologies, they generally tend to overlook certain families such as pesticides or pharmaceuticals, while other chemical residues such as mycotoxins, PFAS or industrial chemicals are residually screened. In this sense, there is a need to widen the scope of the current approaches to get a full image of the real chemical exposure that the agri-food system might be dealing with. Another particularly important point to mention is that current results from SS and NTS alone cannot provide information on toxicological effects for regulatory decisions.

## CRedit authorship contribution statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

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