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"Analysis of *veterinary drugs and pesticides* in food using liquid chromatography-mass spectrometry"

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ABSTRACT

In recent years, the growing interest in liquid chromatography-mass spectrometry (LC-MS) in the food safety area has resulted in various innovative and promising applications. This comprehensive review aims to examine both sample preparation and LC-MS strategies within the field of chemical contaminants in food. The exploration entails an extended investigation of peer-reviewed literature over the past decade, specifically focusing on methodologies tailored for the determination of veterinary drugs and pesticides. Furthermore, relevant development and applications of LC-MS screening methods are overviewed, accentuating their strengths and limitations. Prospective directions for advancing LC-MS methodologies in this field are included to help researchers select the most appropriate screening analytical method.

1. Introduction

Food safety is a critical factor of food chain systems, and contamination represents one of the main risks to food safety [1]. Contaminants are generally categorized into three main groups, including chemical, biological and physical hazards (Fig. 1) [2–6]. The chemical contaminants are the most representative group and involve, among others, veterinary drugs (i.e. antibiotics) and pesticides (i.e. organophosphate, carbamate), representing this review's focus. In particular, chemical contamination can arise during different stages, including processing, packaging, transportation, and storage of food. Additionally, environmental pollution, food contact materials, and intentional use of chemicals like veterinary drugs and pesticides contribute to chemical contamination.

Veterinary drug residues are considered emerging contaminants and represent one of the substantial concerns for food animal-derived products (such as milk, cheese, eggs, meat, and honey). In particular, veterinary drugs are essential for preventing or treating animal diseases. Nonetheless, the employment of unauthorized veterinary drugs can significantly endanger human health and pollute the environment. Consequently, monitoring and measuring these substances in food of animal origin is crucial for ensuring safety, protecting human health, and preserving the environment. Furthermore, pesticides may find their way into the food chain during application, leading to common, albeit low-dose, exposure through food. Intriguingly, some chemicals become more toxic as they build up in the food chain, posing significant health risks when concentrations hit a critical threshold. Pesticides, in particular, pose a heightened risk to the nervous system. While pesticides play a crucial role in protecting crops from pests and diseases, their widespread misuse has led to concerning levels of residues in vegetables, fruits, water, and soil. One of the challenges of analysing pesticides in food is compounded by the complex nature of food matrices, which often contain higher concentrations of other substances than the pesticides themselves.

To minimize contaminated foodstuffs, the European Commission (EC) has implemented measures to control the levels of harmful chemicals and ensure customer safety [7]. The basic principles of EU legislation on contaminants in food were laid down first in 1993; subsequently, the Decision 2002/657/EC regarding the performance of the analytical methods and the interpretation of the results was issued. Specifically, the most recent guidelines for analytical quality control and method validation procedures for pesticide analysis in food have been established and collected in EU SANTE/11312/2021 guidelines, version 2 of which applies from January 1, 2024.

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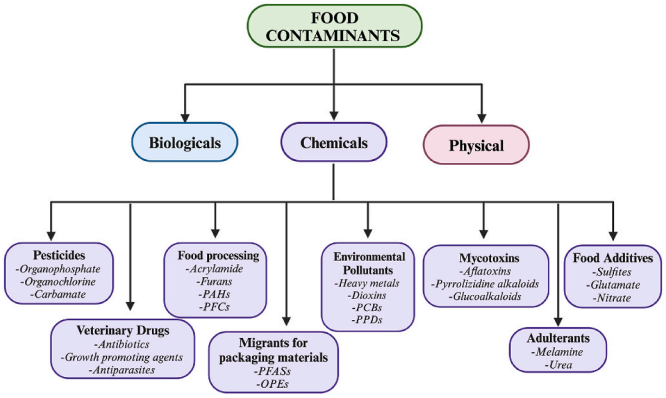

Fig. 1. Classification of food contaminants, showing in detail the different types of chemical contaminants.
Abbreviations: polycyclic aromatic hydrocarbons (*PAHs*), perfluorinated compounds (*PFCs*), perfluoroalkyl substances (*PFASs*), organophosphate esters (*OPEs*), polychlorinated biphenyls (*PCBs*), *p*-phenylenediamine compounds (*PPDs*). Created with BioRender.com.

This document describes the requirements to support the validity of data reported within the framework of official controls on pesticide residues, including monitoring data sent to the European Food Safety Authority (EFSA) and used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure. The EC has fixed the MRLs, i.e. the highest level of a contaminant residue that is legally tolerated in food, which varies based on the commodity, the toxicity, and environmental occurrence. Two main groups of substances must be monitored to guarantee the protection of human health: prohibited substances for which no MRLs could be established and contaminants (such as veterinary drugs and pesticides) having an MRL for which quantitative screening and/or confirmation methods must meet the minimum required performance criteria. In particular, Regulation (EU) No 37/2010 establishes MRLs for veterinary drug residues in animals raised for food and their products, while MRLs for pesticides in food and feed of both plant and animal origin are outlined in Regulation (EC) No 396/2005 with implementing rules introduced annually by the EC. Specific directives are provided for different groups of analytes. For example, MRLs have been specified for all sulphonamides (SAs) at a level of 100 μg/kg for muscle, fat, liver, and kidney from all food-producing species, as well as for bovine, ovine, and caprine milk. However, their use is prohibited in animals producing eggs for human consumption. If more than one SA analogue is present, their combined residue levels should not exceed the provided MRL value. Similarly, for tetracyclines (TCs), different MRLs have been established for various matrices, ranging from 100 μg/kg for muscle and milk to 600 μg/kg for kidney of all food-producing species. Other veterinary drugs with specific MRLs included, among those also mentioned in this review, bacitracin (100 μg/kg for bovine milk, 150 μg/kg for rabbits), and colistin (ranging from 50 μg/kg for milk to 300 μg/kg for eggs of all food-producing species). When pesticide use is not authorised at the EU level, MRLs are set at appropriately low levels to safeguard consumers from unauthorized or excessive pesticide residue intake. A default value of 10 μg/kg is set for pesticides present in products unless otherwise specified. For example, some pesticides have higher MRL values, reaching up to 50 μg/kg in certain specific matrices like coffee beans, herbal infusions, and spices.

Analytical methods used to determine residues of veterinary drugs and pesticides in food primarily rely on liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) detection. Regarding veterinary drugs, GC-MS offers fast detection speed, high sensitivity, and low detection costs but is mainly suitable for small drugs that can be vaporized or derivatized and then vaporized. On the other hand, LC-MS offers a wide application range, high separation efficiency, good selectivity, and sensitivity, making it the preferred method for different classes of veterinary drugs. For pesticides, both GC-MS and LC-MS are powerful tools. GC-MS has traditionally been applied for the determination of pesticides, particularly for analyzing nonionic and volatile to semivolatile pesticides, ranging from a few to a few hundred pesticides. GC-MS is highly sensitive and can detect pesticides at low concentrations. Over the past decade, the use of GC has decreased compared to LC [8]. This decline is primarily due to the increasing use of less persistent, more polar pesticides, such as glyphosate and neonicotinoids, which are unsuitable for GC due to their low volatility and poor heat resistance. Consequently, LC is typically preferred for detecting ionic or polar, nonvolatile and temperature-sensitive semivolatile pesticides. Special columns may be required for separating polar pesticides, both cationic and anionic. LC-MS offers high sensitivity and can efficiently detect a broad range of pesticides [9].

Recently, other reviews regarding the screening of chemical contaminants in foods by LC-MS have been published [9,10], and some go into detail regarding different approaches to detect specific types of contaminants and/or specific types of food matrices [11–14]. However, no comprehensive review describes an update over the past decade of optimized methods for sample preparation and LC-MS analysis of veterinary drugs and pesticides. This review article presents an overview of protocols for the extraction these contaminants and an exhaustive study of the different LC-MS setups and approaches for their screening in different food matrices. The role of the optimization of sample preparation and LC-MS method is emphasized together with the results regarding method performance. Finally, the challenges associated with the application of LC-MS in this context and the prospects are outlined.

2. Sample preparation methods for the determination of chemical contaminants in foods

Extracting analytes of interest from complex food matrices is a critical step before the LC-MS analysis, and identifying a wide range of chemical compounds using a single, broad-sample preparation protocol is ideal but can be challenging because of the inherent complexity of different food matrices and the diverse physicochemical properties (mass, polarity, pH, and volatility) and concentration ranges of contaminants [15,16]. The sample preparation steps must also be tailored to the final analysis, considering the instrumentation and the degree of accuracy required, whether quantitative or qualitative. Good sample extraction and preparation methods are essential for LC-MS analysis: the impact of matrix effects on ionization efficiency, detector noise, and ultimately on LOD and LOQ, as a direct result of the sample preparation applied, must always be considered [17].

Specifically, for the extraction of veterinary drugs and pesticides from food, diverse methods have been reported in the last years, including, among others, solid phase extraction (SPE), solid phase micro-extraction (SPME), dispersive solid-phase extraction (d-SPE) and dispersive liquid-liquid micro-extraction (DLLME) [18].

In particular, SPE, a well-established method which overcomes the limitations of liquid/liquid extraction (LLE) [19] such as difficulty to extract polar pesticides, large solvent volumes and hazardous waste [20], has proven effective in achieving acceptable recovery of veterinary drugs and pesticides in liquid foods like fruit juices, wine, and milk. To enhance the enrichment of hydrophilic contaminants from food matrices, many SPE methods utilize a hydrophilic-lipophilic balanced (HLB) N-vinylpyrrolidone-divinylbenzene copolymer as a sorbent. This copolymer can extract both nonpolar and polar analytes due to its lipophilic backbone and hydrophilic groups, respectively. For example, SPE based on HLB sorbent simultaneously extracted various antibiotic residues, such as quinolones, fluoroquinolones, sulphonamides, trimethoprim, bromhexine, and tetracyclines, from bovine milk yielding recovery values between 71.96 % and 108.70 % [21]. The same sorbent was also showed to extract fifty different pesticides, including fungicides and insecticides, from wine, with recovery values ranging from 70 % to

120 % [22]. However, extracting these substances from solid foods with SPE typically requires additional steps such as homogenization, filtration, sonication, centrifugation, and liquid/liquid cleanup [23].

To reduce the use of hazardous chemicals, SPE has evolved to include microextraction approaches like SPME [24]. Various adsorbent materials have been developed for SPME to extract veterinary drugs and pesticides from food [25]. Molecularly imprinted polymers (MIPs) [26] for example, have been used in devices such as coated fibers, monolithic fibers, stir bars, and thin films [27]. In particular, many MIP-SPME methods have been developed to extract antibiotics like quinolone, sulfonamide, and tetracycline from milk samples [28]. Additionally, nanomaterials, due to their large specific surface area and high affinity for target substances, have significantly improved the selectivity and sensitivity of veterinary drug and pesticide extractions [29]. A notable example is the use of zirconia nanoparticles (ZNPs), integrated into calcium alginate hydrogel fibers, to selectively extract organophosphorus pesticides from fruit juice [30].

Another advancement in SPE is d-SPE, a simplified technique that allows for the analysis of multiple samples simultaneously with low solvent consumption [31,32]. The critical aspect of d-SPE is the selection of the sorbent, which is used in a dispersed form within the sample. Recent developments in d-SPE have explored new adsorbent materials such as MIPs, magnetic nanoparticles (MNPs), metal-organic frameworks (MOFs), and nanomaterials based on graphene or carbon nanotubes [33]. MNP-based sorbents have shown high accuracy in extracting veterinary drugs and pesticides, with recovery values near 100 %. The main advantage of using magnetic sorbent in d-SPE is its practicality and efficiency. Using an external magnet for sample preparation eliminates the need for additional centrifugation steps, saving time and energy and enhancing the greenness of the procedures. For instance, d-SPE using MNPs has demonstrated higher recovery rates for sulfonamides from goat milk compared to traditional SPE [34] and has been effective in extracting aminoglycoside antibiotics from honey [35]. In addition, a recent advancement in MOF-based d-SPE involved the development of dihydroxyl-modified UiO-66 sorbent, enabling rapid extraction of neonicotinoid pesticides from fresh tomatoes and pears, completing the adsorption in just 5 min with minimal sample and adsorbent use [36].

DLLME is another technique known for its high enrichment factor, using organic solvents like chlorobenzene or chloroform to extract analytes from aqueous solutions. DLLME is mainly applied to aqueous food samples, with solid food matrices presenting more of a challenge [37]. Recently, biodegradable deep eutectic solvents (DESs) have been introduced for DLLME, offering advantages such as simple operation, good thermal stability, low vapour pressure, low toxicity, and above all biodegradability [38]. DES-DLLME has been used to extract pesticides from various food matrices, including milk, fruits, vegetables, and honey [38]. For example, a recent application of DES-DLLME pre-concentrated and extracted multi-class pesticide residues from milk, using a two-step DES process, involving choline chloride/ethylene glycol and choline chloride/decanoic acid, that yielded good extraction recovery (64–89 %) [39]. More recently, a rapid, simple, and environmentally friendly DES-DLLME method has been developed for the extraction and pre-concentration of organochlorine pesticides from apple juice. This method utilizes a 1:1 mixture of menthol and formic acid, offering an efficient and green approach to pesticide analysis [40].

Recently, to further adhere to the principles of green chemistry, new methodologies or enhancements to traditional methods have been developed [31]. All these methods play a key role in the sample preparation in food contaminants analysis, however the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, a two-step procedure developed at the beginning of 2000 based on a salting-out solid–liquid extraction and a d-SPE cleanup, has become the method of choice for most laboratories [41].

2.1. Quick, easy, cheap, effective, rugged, and safe (QuEChERS)

QuEChERS is popular due to its simplicity, speed, and cost-effectiveness, making it ideal for multi-analyte contaminant extraction [42]. It commonly uses acidified acetonitrile (ACN) for its versatility with different polarities, $MgSO_4$ and NaCl for salting-out, and primary secondary amine (PSA) for cleanup. Modified QuEChERS protocols may include additional sorbents like graphitized carbon black (GCB), octadecyl-bonded silica (C_{18}), carbon nanotubes (CNTs), and multiwall carbon nanotubes (MWCNTs), to address other interfering substances, including pigments and non-polar compounds [33].

QuEChERS was initially developed for extracting pesticides from fruits and vegetables, but it has since been adapted for extracting veterinary drug and pesticide residues from various food products. For example, an optimized QuEChERS method based on *Na_2EDTA-Mcllvaine buffer* and ACN as extraction solution, NaCl plus Na_2SO_4 as salting agents, C_{18} as adsorbent and ACN with 0.1 % formic acid water as the redissolving solution, enabled the multi-residue analysis of 146 veterinary drugs in beef and chicken [43]. Recently, the *p*-QuEChERS method was developed using a salt mixture of potassium phosphates to extract a larger number (>200) of different veterinary drugs from food matrices such as milk [44] and solid animal-based foods like eggs, muscle, fatty fish, liver, kidney, and honey [45]. By avoiding conventional salts such as $MgSO_4$ and NaCl, the *p*-QuEChERS method improves the recovery of critical analytes, such as the ampicillin and amoxicillin, without compromising the recovery of other non-problematic QuEChERS antibiotics. This is because bivalent ions like magnesium can form highly polar complexes with some veterinary drugs, negatively affecting their partitioning from the aqueous to the organic phase [45]. Additionally, a modified QuEChERS method has been developed for the simultaneous determination of both veterinary drugs and pesticides in eggs, eliminating the need for the d-SPE cleanup step. This method uses Fe_3O_4-MWCNTs as adsorbents and allows for rapid separation using an external magnet [46].

It should be noted that while QuEChERS is the preferred strategy for most contaminants, it has poor recovery for highly polar pesticides, for which the Quick Polar Pesticide (QuPPE) method using acidified methanol is more effective [10]. Nevertheless, the great versatility of the QuEChERS method has allowed its application to a wide range of different food matrices, as reported in this review. Interestingly, a QuEChERSER (more than QuEChERS) “mega-method” that covers a broader scope of polar and nonpolar analytes in diverse sample types has been recently established [42]. The QuEChERSER involves a low amount of sample down to 1–5 g, extraction with 5 mL/g ACN: water (4:1, v/v) (instead of 1 mL/g ACN and water addition for dry samples of QuEChERS), evaporation, reconstitution in the mobile phase, and final ultracentrifugation before LC-MS (instead of salting-out and d-SPE of QuEChERS). In Fig. 2, the two workflows for QuEChERS and QuEChERSER are shown.

3. LC-MS analysis of chemical contaminants in food

3.1. Instrument setup and MS analytical techniques

The correct detection of food contamination can be accomplished with various equipment and methods. Most analytical methods used in food safety are "targeted" and focus on detecting specific compounds or predefined groups of compounds. However, "non-targeted" screening methods complement these approaches by identifying unexpected compounds in food matrices that could pose health risks to the public. Notably, according to Decision 2002/657/EC, only methods based on chromatography coupled to MS detection are suitable as confirmatory methods for organic residues of contaminants.

Among the different types of chromatography, GC is valuable for nonpolar and semi-polar, volatile and semi-volatile compounds, and without chemical derivatization, it is useful for the analysis of

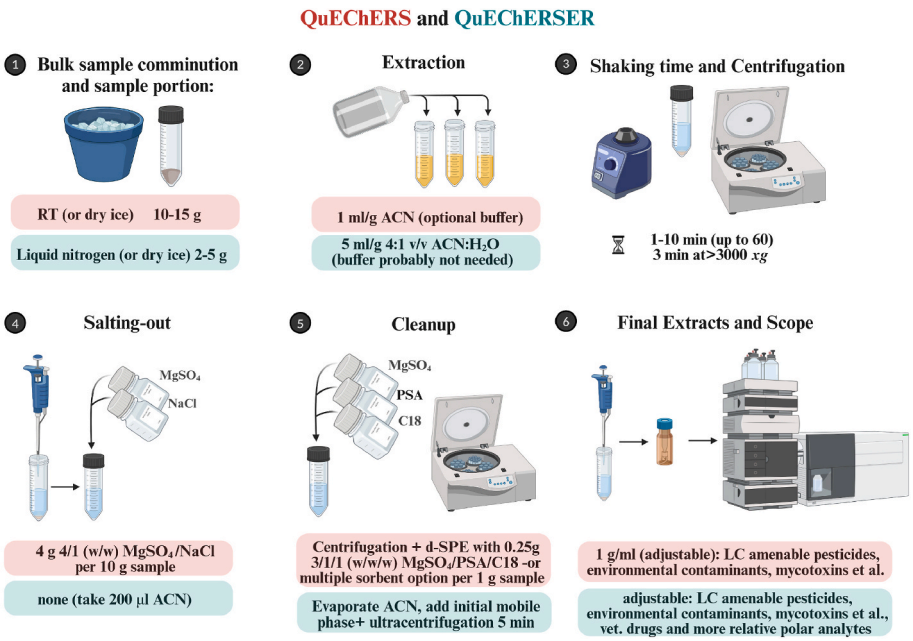

Fig. 2. Comparison of two workflows for QuEChERS and QuEChERSER. Created with BioRender.com.

contaminants, such as pesticides, industrial pollutants, and drugs in foods. On the contrary, LC is convenient for separating all types of compounds independent of polarity or volatility. Although GC and LC are complementary techniques, the growth of HPLC has led some laboratories to use HPLC primarily, even in applications for which GC are available.

The online combination of HPLC with MS is straightforward, although it requires some compromises. The most important is the need for a volatile and low ion strength (i.e., 5–50 mM) buffer. Moreover, MS analysis requires a relatively low flow rate of mobile phase: 0.05–0.2 mL/min for ESI sources and even lower rates in the range of nL/min when working with nanoESI sources to ensure increased sensitivity [47]. nanoESI offers an ideal interface for coupling with nanoHPLC, which is widely used, in combination with MS, for detecting veterinary drugs and pesticides in various food matrices [48]. Sample preparation for nanoHPLC typically involves fewer steps than other types of LC and generally includes a dilution of up to 100-fold, significantly reducing matrix effects and enhancing sensitivity. However, while nanoHPLC methods generally yield acceptable recovery of veterinary drugs and pesticides, it should be noted that the recovery values for certain analytes may not always be ideal. Additionally, only a few researchers have proposed truly multi-analyte nano-LC methods. Examples include the detection of multi-residue of veterinary drugs in honey, veal muscle, egg, and milk [49], as well as the identification of pesticides in virgin olive oil [50]. More recently, the coupling of UHPLC to MS has further improved the contaminant analysis. UHPLC requires instruments capable of withstanding high pressures (>600 bar) and chromatographic columns with particle size <2 µm, which provide higher speed, better resolution, better peak capacity, and increased sensitivity as compared to HPLC [51]. Moreover, when UHPLC is used, the ion suppression can be minimized because the co-elution of matrix interferences is avoided [52]. The coupling of UHPLC to MS analyzers has changed from traditional chromatographic approaches towards multi-class and multi-residue methods, with short injection cycle times and minimal sample preparation [52,53]. Another strategy that can potentially increase the separation performance in complex food matrices is multidimensional (MD) chromatography. MD-LC allows the sample to pass through two different separation stages, improving the degree of separation, which is especially important in food contamination analysis due to the significant sample complexity [4,54].

Regardless of the chromatographic separation, the MS detection of contaminants can involve low-resolution (LR) MS, resolution of <2000, and high-resolution (HR) MS, resolution of ≥10,000 (Table 1). The most well-established approach for targeted multi-component determination of food contaminants is instrumented with a TQ MS operating in selected (SRM) or multiple reaction monitoring (MRM), which delivers the selectivity and sensitivity required for compliance with legislative limits [55]. TQ instruments operating in MRM mode can screen many target contaminants, even in complex food matrices, when coupled with multi-residue extraction approaches. Notably, to decrease the number of concurrent MRM transitions and enhance the rate of the analysis, the dynamic MRM (d-MRM), which uses a timetable based on the t_R for each analyte, has been developed allowing high sensitivity, accuracy, and reproducibility.

HRMS instruments are now widely used to screen chemical contaminants in foods due to their high selectivity and mass accuracy [56]. They enable both targeted and non-targeted analysis through data-dependent (DDA) and data-independent (DIA) acquisitions. Target screening uses reference standards for information on t_R and fragmentation, while suspect screening relies on calculated exact mass and isotopic patterns [57]. Non-targeted screening starts with MS data to identify compounds in the sample, using exact mass, isotopic distribution, molecular species, and fragmentation information. Identified molecular formulas are matched against chemical databases, with MS/MS aiding in structure elucidation and confirmation through analytical standards if available [58]. In this regard, the TOF and Orbitrap allow consistent and accurate determination of elemental compositions suitable for pesticide and antibiotic multi-residual targeted and non-targeted screening. Moreover, by HRMS, it is possible to acquire the full-scan spectra of samples, allowing the retrospective analysis of unknown analytes using previously acquired data. This eliminates the need for reinjection of samples, providing HRMS with a noteworthy improvement over TQ MS. Interestingly, by using an HRMS instrument involving a LIT analyzer, it is possible to combine the MRM analysis with the enhanced product ion (EPI) trap scan mode [59] further improving the screening analysis. Indeed EPI scanning enhances specificity by generating comprehensive MS/MS spectra for library confirmation. Moreover, capturing MRM and EPI MS/MS scans in one injection allows high-quality quantitation and simultaneous confirmation. Recently, targeted approaches like parallel reaction monitoring (PRM) have been

Table 1
Comparison of the characteristics of Low and High-Resolution Mass Spectrometry Analyzers. Q, Quadrupole; IT, Ion Trap; TQ, Triple quadrupole; LIT, Linear Ion Trap; TOF, time-of-flight; FWHM, full width at half maximum; ppm, part per million; Hz, Herz.

Mass-analyzer type	Instrument name, manufacturer	Resolving power (FWHM defined at *m/z*)	Resolution	Mass Accuracy (ppm)		*m/z* range	Acquisition speed (Hz)
				Internal	External		
Q	ACQUITY QDa Mass Detector, Waters	/	0.7	/	/	30-1250	10
	ISQ EM, Thermo Fisher Scientific	/	1	/	/	10-2000	50/60
	LC/MSD, Agilent Technologies	/	/	/	/	10-2000	10
	LCMS-2050, Shimadzu	/	0.7	/	/	2-2000	15
3D-IT	amaZon speed ETD, Bruker Daltonics	/	0.1	/	/	5-6000	52
LIT	LTQ XL LIT, Thermo Fisher Scientific	/	0.15	/	/	15–4000	50/60
	6475 TQ, Agilent Technologies	/	/	/	/	5-3000	10
TQ	TSQ Quantis, Thermo Fisher Scientific	/	/	/	/	5-3000	5
	Xevo TQ Absolute, Waters	/	1	/	/	2-2048	10
	LCMS-8060NX, Shimadzu	/	0.5	/	/	2-2000	10
Q-LIT	QTRAP 6500+ system, AB Sciex	9200 (*m/z* 922)	0.1	/	/	5-2000	12
TOF	6230B (TOF) LC/MS, Agilent Technologies	22,000 (*m/z* 1522)	/	<1 ppm	/	25-20,000	40
IT-TOF	LC-MS IT-TOF, Shimadzu	10,000 (*m/z* 1000)	0.1	3	5	50-5000	10
Q-TOF	Revident LC/Q-TOF, Agilent Technologies	>35,000 (*m/z* 118)	/	<1 ppm	<2 ppm	20-10,000	50
	Xevo G3 Qtof, Waters	>40,000 (*m/z* 956)	/	<1 ppm	/	20-16,000	30
	impact II VIP, Bruker Daltonics	>40,000 (*m/z* 1222)	/	<1 ppm	/	50-20,000	50
	X500R QTOF, AB Sciex	>42,000 (*m/z* 956)		<1 ppm	<2 ppm	5-40,000	30
	LCMS-9030, Shimadzu	30,000 (*m/z* 1972)	0.8	<1 ppm	<2 ppm	5-40,000	100
Orbitrap	Orbitrap Exploris 480, Thermo Fisher Scientific	480,000 (*m/z* 200)	0.001	<1 ppm	<3 ppm	40-8000	40 (at RP = 7500)
Q- Orbitrap	Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Thermo Fisher Scientific	240,000 (*m/z* 400)	0.001	<1 ppm	<3 ppm	50-6000	12 (at RP = 17,500)
LIT-Orbitrap	LTQ Orbitrap XL, Thermo Fisher Scientific	240,000 (*m/z* 400)	0.0002	<1 ppm	<3 ppm	50-4000	4 (at RP = 60,000)

introduced. PRM offers high accuracy, eliminates background interference and false positives better than SRM and MRM, and improves sensitivity for complex samples. It simplifies assays by scanning all product ions, requiring no ion pair selection or optimization of fragmentation energy. Though not widely used for food contaminants yet, PRM ensures better specificity and multiplexing than SRM and MRM [60].

3.2. Screening, identification, and confirmation by LC-MS

Determination of contaminants in food by LC-MS can involve screening, identification, confirmation, and quantification. Screening methods should answer very quickly if results are compliant or suspicious; they should be very sensitive to guarantee no false compliant results and specific enough to limit the number of false suspicious results. Screening methods offer the possibility of searching for contaminants with a low probability of being present in the samples. On the contrary, commonly found contaminants should be measured using validated quantitative multi-residue methods. A contaminant can only be tentatively reported when detected using a screening method. A successive confirmatory analysis based on a validated quantitative method, including a calibration procedure, must be applied to report a quantitative result. If an analyte is not detected after a screening, it can be reported as lower than the screening detection limit (SDL), representing the lowest level at which an analyte has been detected in at least 95 % of the samples. For screening methods, confidence in detecting an analyte at a specific concentration should be established to follow the SANTE guidelines, for example, by using samples spiked at the estimated SDL. When analytes detected by screening are identified and confirmed by a confirmatory method, there is no need to check for the possible presence of false detects. Otherwise, the potential presence of false detections should be checked using non-spiked blank samples.

The confirmatory methods should lead to a compliant or a non-ambiguous non-compliant result; therefore, they should be very sensitive and very specific to forbid false results. More specific requirements about chromatographic separation and MS detection have indeed been included in Decision 2002/657/EC, and a system of identification points (IPs) was introduced to interpret the data for confirmation. The number of IPs "earned" by the detection of a precursor/product ion depends on the MS technique used (ranging from 1 IP for precursor ion detected by low-resolution MS to 2,5 IPs earned for product ions detection by high-resolution MS). A minimum of 4 IPs are required to confirm prohibited substances, while a minimum of 3 IPs are necessary for the other contaminants having a MRL. Concerning the identification, there are specific requirements for chromatography and MS methods. For example, the SANTE 11312/2021 guidelines for pesticide residue analysis specify that the chromatographic t_R of the analyte in the extract must correspond to that of the matrix-matched calibration standard with a tolerance of $\pm$0.1min for both GC and LC. Moreover, for identification based on MS spectra, it is recommended to generate reference spectra using the same MS instrument and conditions applied for analysis of the samples. Furthermore, when identification is based on selected ions, they must be selected for the analyte in the analysed matrix and the relevant concentration; in general, molecular ions or high *m/z* ions should be selected. Of course, the different MS techniques are related to different requirements for the identification of the contaminant, which are reported in Table 2.

4. Analysis of chemical food contaminants using LC-MS analysis

The conventional analysis is based on establishing an acquisition method to determine a list of known analytes, using reference standards, and methods are validated before the analysis of real samples [61]. When reference standards are unavailable, compound-specific information, such as molecular formula and structure, can be used to develop a detection method based on database searching. Here below, LC-MS

Table 2
Requirements for identification of pesticides performed with different MS techniques (SANTE/11312/2021v2).

MS detector/ Characteristics	Typical systems Single MS Unit mass resolution	MS/MS	High-resolution MS Accurate mass measurement
	Q, IT, TOF	TQ, IT, Q-trap, Q-TOF, Q-Orbitrap	Q-TOF Q-Orbitrap
Acquisition	full scan, limited *m/z* range, Selected ion monitoring (SIM)	Selected or Multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	full scan, limited *m/z* range, Selected ion monitoring (SIM), fragmentation with or without precursor-ion selection, or combinations
Requirements for identification	3 ions	2 product ions	2 ions with mass accuracy $\leq$ 5 ppm[a, b, c]
	S/N $\geq$ 3[d]		S/N $\geq$ 3[d]
	Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. The ion ratio from sample extracts should be within ±30 %(relative) of an average calibration standards from the same sequence		Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap.

[a] preferably including the molecular ion, (de)protonated molecule, or adduct ion.
[b] including at least one fragment ion.
[c] 1 mDa for $m/z < 200$.
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans.

determination, summarized in Table 3, of veterinary drugs and pesticides in different food matrices are discussed.

4.1. Veterinary drugs

Detection of veterinary drugs, including antibiotics, has seen significant progress in sample preparation, analysis methods, and MS instrumentation in recent years. Among the complex matrices for which researchers often encounter challenges in developing sample preparation protocols, there is bovine milk. In this context, Martins et al. [62] established one of the easiest, cheapest and fastest methods for confirmation and quantification of antibiotics in bovine milk by HPLC-TQ MS. They used acidified acetonitrile or acidified ethanol, followed by freezing and centrifugation, to extract 28 antibiotics, including quinolones (Qs), fluoroquinolones (FQs), tetracyclines (TCs), sulphonamides (SAs), trimethoprim (TMP) and bromhexine (BMX). The most relevant characteristics of this methodology included the small volumes of sample and solvents, the substitution, wherever feasible, of hazardous organic solvents with less harmful alternatives, such as ethanol, and the exclusion of the SPE step. The MS analysis involved establishing at least two SRM transitions for each compound, ensuring compliance with the criteria required for confirmatory analysis, and the identification of specific extracted ions for each antibiotic. This method exhibits ease of execution, affordability for routine applications, and reduced concerns regarding the generation of hazardous waste. However, although this method demonstrates strong analytical performance (Table 3), it is important to highlight that to attain higher recovery rates of polypeptide antibiotics, extraction with a basic solvent is recommended. Indeed, the basic solution improves the extraction efficiency of polypeptide antibiotics due to their basic isoelectric points, facilitating deprotonation and extraction. In this regard, Bladek et al. [63] proposed the first simple UHPLC Q-LIT MS analysis for the detection of polypeptide antibiotics, i.e. bacitracin A, colistins and polymyxins, in bovine milk, as well as in animal muscle and eggs. The strategy described the extraction of antibiotics based on a mixture of acetonitrile and water, plus ammonia solution 25 %, optimizing the proportions of solvents and the different stages of sample handling, and omitting, also in this case, the SPE step. Additionally, another relevant feature of this methodology was the rapid UHPLC separation lasting just 6 min (much faster than the 15 min gradient envisaged by the Martins et al.'s method [62]), an important advantage for high throughput analyses. Notably, even if full separation was not always achieved under the fast conditions used, the MS/MS assured high specificity. Indeed, triply charged ions were selected as precursor ions for optimal sensitivity, and two MRM transitions were established for each antibiotic: one for quantification and another for confirmation. The results showed that adding ammonia notably improved extraction efficiency, especially for polymyxins. In particular, for milk matrices, this method offers better recovery and precision (Table 3) compared to acidic extraction [62], though different types of antibiotics were analysed. Notably, both approaches [62,63] meet the criteria for confirmatory analyses and can be used for antibiotic screening in milk. Furthermore, the method proposed by Bladek et al. is also applicable for the detection of polypeptide antibiotics in other food of animal origin (i.e. muscles and eggs), while Martins et al.'s method is also adaptable to different types of LC-TQ MS equipment.

A recognized limitation of the LC-MS methods is that signals from co-eluting components can hinder analyses, especially at very low concentrations. PRM scan mode selectively monitors targeted precursor ions, addressing this issue effectively. Accordingly, the HRMS instruments in PRM mode have been used to improve the multi-class determination of veterinary drugs. In this context, Chen et al. [64] proposed a method for detecting 37 antibiotics in pork meat using QuEChERS and LC-Q-Orbitrap HRMS. The modified QuEChERS protocol exploited acetonitrile containing 10 % water to extract polar antibiotics like beta-lactams and Na_2EDTA to prevent cation-antibiotic complexes, plus a cleanup with PSA and C_{18} sorbents. This differs from the approach taken with the milk matrix where, as described in the methods above [62,63], a centrifugation step alone was enough for cleanup. The most relevant characteristic of the method proposed by Chen et al. is the PRM scan mode, which was used as an ion monitoring technique to scan only the targeted precursors, reducing interfering ion peaks. Moreover, the Q-Orbitrap HRMS assured exceptional selectivity and sensitivity. The workflow proved useful for multi-class antibiotic analysis in pork meat, meeting official requirements. However, despite its very good analytical performance (Table 3), particularly in terms of recovery and LOQ, the method does not excel from a green perspective in terms of extraction solvent volume (up to 4.5 mL of ACN). To overcome this issue, Petrarca et al. [65] advanced antibiotic analysis in animal-derived samples by utilizing miniaturized sample preparation for detecting 12 SAs in meat- and egg-based baby foods. Their modified QuEChERS method minimized solvent (1 mL ACN), salt (0.5 g), and sorbent (0.05 g) usage. Moreover, another peculiarity of this methodology lies in the instrumental setting involving HILIC coupled with Q-TOF HRMS, which enhances sensitivity via ACN's ionization properties, such as lower surface tension, higher volatility, and lower ions solvation. Full-scan mode with accurate mass measurement of precursor ion, and accurate precursor ion plus DDA MS/MS of two fragment ions, was used for quantification and confirmation purposes, respectively. A total of 4.5 IPs were assigned to each analyte (1.5 IPs from the precursor ion detected with mass errors in the range 2–10 mDa and 3 IPs from two fragment ions with mass errors >10 mDa), in accordance with the requirements of Commission Decision 2002/657/EC. The method showed satisfactory performance

Table 3
Summary of selected recent applications of LC-MS for targeted screening of chemical contaminants (antibiotics and pesticides) in food.

Sample (amount)	Analytes	Extraction solvents/salts	Clean-up step	Detection technique	Recovery (%)	LODs	LOQs	Precision (%)	Ref.
Veterinary drugs (antibiotics):									
Bovine milk (500 μL)	28 antibiotics (including quinolones (Qs), fluoroquinolones (FQs), sulphonamides (SAs), trimethoprim (TMP), bromhexine (BMX), tetracyclines (TCs))	600 μL ACN + 0.1 % formic acid (Qs, FQs); 600 μL ethanol + acetic acid (SAs, TMP, BMX); 600 μL ethanol + acetic acid + 50 μL 150 mm EDTA (TCs)	freezing −20 °C 30 min and centrifugation 12,000 × *g* 30 min	HPLC- TQ (Waters) (SRM)	62–108	0.2–10 ng/mL	2.5–25 ng/mL	15–17	[62]
Bovine milk, animal muscle and eggs (2 g)	5 antibiotics (including bacitracin, colistin A, colistin B, polymyxin B1, and polymyxin B2)	8 mL ACN/water/ammonia solution 25 %, 80/10/10 (v/v/v)	centrifugation 4500 rpm 10 min 4 °C	UHPLC-QTRAP 4500 (Shimadzu-AB Sciex) (MRM)	70–99	/	10 μg/kg	13–15	[63]
Pork meat (5 g)	37 antibiotics (including SAs, TCs, FQs, beta-lactams, and macrolides)	4.5 mL ACN, 0.3 mL water, 0.2 mL Na_2EDTA solution (200 mM) / 4 g $MgSO_4$	50 mg PSA 50 mg C_{18} 150 mg $MgSO_4$	UHPLC-Q-Exactive Orbitrap (Thermo Fisher Scientific) (PRM)	85–105.6	0.8–2.9 μg/kg	2.4–10.5 μg/kg	<15	[64]
Meat-, egg yolk- and/or vegetable-based baby foods (0.5 g)	12 antibiotics (including SAs)	1 mL ACN / 0.1 g NaCl, 0.4 g $MgSO_4$	25 mg PSA 25 mg C_{18}	HPLC-Q-TOF (Waters-Micromass) (full scan MS and DDA MS/MS)	72.9–120	2.5 - μg/kg	5–20 μg/kg	1–18.1	[65]
Honey (2g) royal jelly (1g)	42 antibiotics (including SAs, TCs, FQs, TMPs macrolides, nitroimidazoles, dapsone)	10 mL ACN with 1 % acetic acid / 4 g Na_2SO_4, 1 g NaCl Na_2EDTA and citric acid (for TCs)	50 mg PSA 150 mg C_{18} 900 mg Na_2SO_4,	HPLC-6460 TQ (Agilent) (d-MRM)	80.4–118.4	0.14–3.81 μg/kg	0.50–12.68 μg/kg	15.9–17.1	[66]
Honey (2g)	70 antibiotics (including SAs, Qs, TCs, macrolides, β-lactams, nitroimidazole)	10 mL ACN with 0.1 % formic acid / 0.05 g Na_2EDTA (for TCs, Qs, macrolides)	120 mg C_{18}	UHPLC-XevoTQ-XS (Waters) (MRM)	70.5–119.8	0.050–1.02 μg/kg	0.17–3.40 μg/kg	<10	[67]
Pesticides:									
Olives and sunflower seeds (7.5 g)	42 pesticides (including neonicotinoids, triazines, phenylureas, organophosphates, anilines)	15 mL ACN with 1 % formic acid / 6 g $MgSO_4$ and 1.5 g CH_3COONa	400 mg PSA, 400 mg GCB, 400 mg C_{18} 1200 mg $MgSO_4$	HPLC-TQ (Thermo Fisher Scientific) (SRM)	70–120	0.03–59 μg/kg	0.03–59 μg/kg	<20	[73]
Fruits (pakchoi, cowpea and pepper) and vegetables (peach, grape and watermelon) (10 g)	54 pesticides (including fipronil, thidiazuron, avermectin, carbofuran, pyraclostrobin, boscalid, and difenoconazole)	20 mL ACN with 1 % acetic acid / 3 g NaCl	100 mg PSA 100 mg C_{18} 10 mg Carb 300 mg $MgSO_4$	UHPLC-6460 TQ (Agilent) (MRM)	73.2–134.3	0.003–2 μg/kg	0.01–6.67 μg/kg	<13.8	[74]
Tropical fruits (starfruits and	50 pesticides (including insecticides, fungicides, nematicides, herbicides, and plant growth regulators)	10 mL ACN / 2.5 g NaCl	0.25g PSA 0.75g $MgSO_4$	UHPLC-Triple TOF 5600+ (Agilent- AB Sciex)	76–119	0.03–4 μg/kg	0.1–12 μg/kg	0.2–3.2	[75]

(*continued on next page*)

Table 3 (*continued*)

Sample (amount)	Analytes	Extraction solvents/salts	Clean-up step	Detection technique	Recovery (%)	LODs	LOQs	Precision (%)	Ref.
Indian jujubes (10 g)				(full scan and DDA MS/MS)					
Fruits Vegetables (15 g)	381 pesticides and herbicide residues	15 mL ACN with 1 % acetic acid / 6 g $MgSO_4$ 1.5 g CH_3COONa	/	UHPLC-QTRAP 6500+ (Shimadzu-AB Sciex) (MRM & EPI MS/MS)	/	5 μg/kg	/	<20	[76]
Honey, bee pollen, honeybees (2 g)	115 pesticides (including neonicotinoids, organophosphates, triazoles, carbamates, dicarboximides and dinitroanilines)	7 mL ACN (w/wo 2 % triethylamine), hexane / 6 g $MgSO_4$ 1.5 g CH_3COONa	50 mg PSA 150 mg $MgSO_4$	LC-6410 TQ (Agilent) (MRM)	59–117	0.03–23.3 μg/kg	0.1–78 μg/kg	4–27	[77]
Honey, jam, jelly and syrup (2 g)	694 contaminants of which mainly pesticides (including acaricides, algaecides, fungicides, herbicides, insecticides, nematicides and rodenticides)	10 mL ACN with 0.1 % acetic acid / 4 g $MgSO_4$ 1 g CH_3COONa	150 mg PSA 900 mg $MgSO_4$	UHPLC-Triple TOF 5600+ (Thermo Fisher Scientific- AB Sciex) (full scan and DDA MS/MS)	/	1–50 μg/kg	/	/	[78]

(Table 3) and enabled unequivocal antibiotic confirmation and sensitivity for baby food control (LODs ≤10.0 μg/kg, LOQs 5–20 μg/kg). Notable, while the performance of this approach is slightly lower in terms of recovery, LOD and LOQ than that of Chen et al.'s [64] method for detecting antibiotics in meat, it offers some advantages. These include the reduced sample amount (0.5 g instead of 5 g) and enhanced environmental sustainability due to a reduction in solvent and salt usage.

Another complex matrix for which multi-class antibiotic detection methods have been developed is honey, which represents a typical concentrated sugary product. Jin et al. [66] introduced the first validated HPLC–TQ MS/MS assay for quantifying 42 antibiotics in honey and royal jelly. A reduction in costs and analysis times for sample preparation, crucial parameters for routine analyses, has been achieved using ACN acidified with 1 % acetic acid and added with Na_2SO_4 and NaCl for the extraction, and a mixture of C_{18}, PSA, and Na_2SO_4 for clean-up with high recovery and minimal matrix effect. Optimized LC conditions involved using water with formic acid plus 2 mM ammonium formate as the aqueous mobile phase and methanol as the organic mobile phase. Notably, formic acid provides more protons than acetic acid, improving the ionization of analytes, whilst methanol has a relatively weak elution strength, which affects the t_R. The standout feature of this approach was the implementation of d-MRM scan mode, which enabled improved analysis rate assuring the simultaneous detection of different antibiotics, with recovery values ranging from 80.4 % to 118.4 % and precisions ≤15.9 % and 17.1 % for intra- and inter-batch precision (Table 3). The excellent analytical performances of this method have been further improved by the methodological approach developed by Yang et al. [67] based on a different instrumental setup. They performed the QuEChERS-UHPLC-Xevo TQ MS/MS multi-residue analysis of as many as 70 antibiotics in honey, using ACN added with 0.1 % formic acid and Na_2EDTA and the C_{18} sorbent, for the extraction of antibiotics and their clean up. Here, a classical MRM mode in positive ESI was applied, optimizing the conditions for precursor and product ions, fragment voltage, and collision energy. The most abundant ion was used for quantification, and the second response was used for qualitative determination. The results indicated recovery comparable to Jin et al.'s method, but improved analysis precision (<10 %) and lower detection and quantification limits. Indeed, the LODs and LOQs achieved by this methodology were about three times lower compared to the method developed by Jin et al. [66], ranging between 0.05 and 1.02 μg/kg, and between 0.17 and 3.40 μg/kg, respectively (Table 3). The improved performance in the method proposed by Yang et al. [67] as compared to that of Jin et al. [66] can be mainly ascribed to the different instrumental settings. Indeed, while both HPLC-6460 TQ MS and UHPLC-Xevo TQ XS MS are powerful analytical tools for targeted quantitative analysis, improved ionization sources and mass analyser as well as better chromatographic performance of the UHPLC-Xevo TQ XS MS, allow for shorter analysis times and increased sample throughput, as well as for increased sensitivity, resolution and dynamic range. Therefore, the combination of QuEChERS and ultra-performance LC MS/MS could pave the way for a new research direction in the analysis of antibiotic residues in honey.

4.2. Pesticides

Pesticide residues are other contaminants that can cause harmful effects on human health. For this reason, optimizing the sample preparation and developing validated LC-MS methods for their screening, identification, and quantification in food is fundamental. Regarding sample preparation, many efforts were mainly dedicated to the optimization of the QuEChERS method rather than to the QuPPe, which was effectively applied without the need for excessive modifications for highly polar pesticides [68–70]. For instance, in optimizing the QuEChERS cleanup step for pesticides in fruit and vegetable samples, PSA showed optimal performance for extracts from apple, strawberry,

and tomato matrices, whilst for high chlorophyll samples like spinach, a combination of PSA and GCB yielded better results [71]. Conversely, matrices rich in chlorophyll such as leek, leaf lettuce, and garland chrysanthemum, benefited from greater cleanup with MWNT, ensuring the removal of even trace amounts of pigments [72]. More recent studies have focused their investigation on optimizing pesticide extraction from matrices containing pigments and fats. In this regard, García-Vara et al. [73] developed QuEChERS-based analytical methods for the multi-residue determination of pesticides by HPLC-TQ MS in olives and sunflower seeds. The most relevant aspect of this study was the optimization of extraction parameters using a fractional factorial design of experiments, enabling the optimization of method conditions in a cost-effective manner. Four factors were assessed by the authors: acidification of the extraction solvent, type of extractive salts, type of clean-up salts, and acidification of the final extract. This study demonstrated that the acetate buffer QuEChERS method was more effective for sunflower seeds, while acidifying the ACN solvent with 1 % formic acid yielded the best recovery in olives and sunflower seeds. Comparison of two different sorbent mixtures (with PSA to remove saccharides and fatty acids, C_{18} for residual lipids, and, in one of the two, GCB to remove colored pigments) revealed no difference for olives, but the mixture without GCB worked better for some pesticides in sunflower seeds. Additionally, the acidification of extracts with formic acid improved the MS ionization efficiency and ensured optimal results. This fine-tuning of sample preparation enabled the development of methods which were validated according to SANTE guidelines, demonstrating linearity, repeatability and trueness with recovery values between 70 and 120 % (Table 3). This study demonstrated that by optimizing the different factors that influence extraction and cleanup steps it is possible to determine a total of 42 different pesticides in complex matrices rich in pigments and fats, such as olives and sunflower seeds. Thus, sample preparation should always be optimized to suit specific analyte/matrix combinations and experimental objectives.

Besides, great improvements in the determination of pesticides have been obtained with the use of UHPLC chromatography and the optimization of the LC-MS parameters. A simple, rapid and efficient QuEChERS-UHPLC-TQ MS approach for the detection of different pesticides in fruits (pakchoi, cowpea, and pepper) and vegetables (peach, grape, and watermelon) was developed by Xiu-ping et al. [74]. Sample preparation involved the extraction with ACN and 1 % acetic acid, and a cleanup step based on PSA, C_{18}, or Carb sorbents. A relevant aspect was the optimization of UPLC–MS/MS conditions, which involved ammonium acetate added with 0.1 % formic acid and methanol as the mobile phases to perform a 15-min gradient elution, and the ESI which was simultaneously set in positive and negative mode. Notably, the use of ammonium acetate as a buffer in the mobile phase can mitigate peak tailing and enhance the chromatographic peak shape. Furthermore, MRM optimization involved using standard solutions of target pesticides to select positive precursor ions for 45 pesticides, negative precursor ions for 8 others (such as fipronil and thidiazuron), and a positive sodium ion as the precursor for avermectin. Under the optimized conditions, the high-throughput screening and confirmation analysis of 54 pesticide residues were achieved. The LOD and LOQ were very low and ranged from 0.003 to 2 μg/kg and 0.01–6.67 μg/kg, respectively. Moreover, satisfactory recovery results were obtained in the sample analysis (Table 3). This optimized method proved to be fast, highly sensitive, accurate, efficient, and cost-effective for the routine detection of different pesticides in fruits and vegetables. However, further progress was made in the following years regarding the multi-residue analysis of pesticides in fruit and vegetables by using the different instrumental settings. For instance, Yang et al. [75] introduced a method utilizing UHPLC combined with full-scan and DDA MS/MS, employing Q-TOF MS for the screening and confirmation of multi-residue pesticides in fruits. The QuEChERS method was used to extract 50 pesticides from starfruit and Indian jujube. Also in this case the LC run involved the use of ammonium acetate to improve the peak shape and sensitivity, and most of the pesticides were eluted within 15 min. The data obtained showed that this method provides robust confirmation due to TOF high mass accuracy, enabling precise mass measurements of target ions within acceptable error limits (<5 ppm). The accurate mass measurement was a crucial feature of this methodology, allowing for the differentiation of masses and the utilization of isotopic peak intensities to determine molecular formulas and structures of pesticides. In this work the recovery, precision and linearity obtained were all good (Table 3). Compared with the method proposed by Xiu-Ping et al. [74], the analytical precision of this methodology was enhanced, while LOD and LOQ were found to be higher, although still within the necessary limits in compliance with EU Food Regulations. Interestingly, this study marks the first instance of achieving the simultaneous identification and quantification of 50 pesticides in minor tropical fruits using UHPLC-QTOF MS.

Tonoli et al. [76] subsequently introduced further enhancements in terms of performance and sample throughput for a multi-residue pesticide method in fruits and vegetables. They developed of a UHPLC-QqQ LIT MS-based method for screening 381 pesticides. The key aspect of this methodology was its improved confidence in identifying molecules, which is essential for avoiding false positives and negative results when analysing hundreds of pesticides in a single run. This represents an issue that can be encountered when using just 2 MRMs with TQ MS instruments. The high confidence in identification was achieved by the hybrid TQ MS through a scheduled MRM survey scan, utilizing transitions from either an available pesticide library or optimized in-house, conducted for screening purposes. This, in turn, triggered two EPI scans for confirmatory analysis. The recorded EPI spectra underwent dynamic exclusion after three iterations of the same precursor, enhancing both data quality and speed. Notably, this selection process ensured that only transitions with adequate intensity and distinct from the background were included for MS/MS analysis. This method was able to detect concentrations as low as 5 μg/kg and confirm 381 pesticides in a single injection. In comparison to methods proposed by others [74,75], this approach not only enabled the detection of a wider range of pesticides in fruits and vegetables but also provided several advantages. These include reduced injection volume of QuEChERS extracts to as low as 0.25 μL, improved robustness, extended lifespan of UHPLC columns, and increased confidence in pesticide identification through full MS/MS spectra.

In the last decade, some methods have also been developed for the screening, confirmation, and quantification of pesticides to be applied to products with a high sugar and low water content, such as honey. A multi-residue method was reported by Kasiotis et al. [77] for the screening of pesticides in honey, as well as in honeybees and bee pollen samples. The authors combined a modified QuEChERS method with LC TQ MS/MS analysis to detect a total of 115 pesticides in honey, bee pollen and honeybees. Extracts were obtained with ACN, deionized water and hexane, adding triethylamine for basic or weak basic compounds (such as the clothianidin, thiamethoxam, imidacloprid, and carbendazim pesticides). The LC–MS/MS screening involved 2 MRM transitions for each target analyte, except for phosphamidine and iprodione, for which only one abundant and specific MRM transition was obtained. Notably, most pesticides were analysed in a single injection, except for fipronil, fipronil sulfone, and fludioxonil, which required separate injection and analysis in negative ionization mode. The method demonstrated good performance in terms of recovery, precision, LOD and LOQ (Table 3), making it suitable as a monitoring tool for investigating pesticide residues. However, as mentioned above it is important to note that in some cases, using 2 MRMs may not be adequate to definitively identify a molecule, especially when screening hundreds of different residues. To overcome this inconvenience, more recently, a new method was introduced by Makni et al. [78] to cover a broader spectrum of compounds in honey and other concentrated sugary products (i.e., jam, jelly, and syrup). They implemented a method for the identification and the semi-quantification of 694 targeted contaminants,

mainly including pesticides, using UHPLC-Q-TOF HRMS. Sample preparation, optimized through split factorial design, involved an acetate-buffered QuEChERS method, followed by PSA clean-up and extract concentration to improve sensitivity. The key aspect of the methodology was the employment of full scan MS and DDA MS/MS to ensure robust identification and enable high-throughput analysis of hundreds of contaminants. First, a homemade compound database, including the reference MS/MS fragmentation spectra, was developed by means of standards. Then Q-TOF MS was operated in positive and negative modes, and ions to be fragmented by DDA MS/MS were selected using a precursor ion list, which included the t_R and the m/z of the targeted compounds. The authors highlighted that they established a single method for the multi-residue screening of contaminants with a wide polarity range, even at low levels in concentrated sugary products. In comparison to the method proposed by Kasiotis et al. [77], this approach enabled the screening of 6 times more analytes (694 instead of 115) with screening detection and limits of identification ranging from 1 to 50 μg/kg for most contaminants. Moreover, the concentrations of identified contaminants were estimated using three semi-quantification approaches based on calibration curves for the entire group of sugary products, family-specific or sample-specific. This method could be employed as an early-warning system for emerging contaminants, with estimated concentrations aiding in food safety improvement. This study demonstrated that using HRMS instruments expands the analytical scope significantly, aligning with the goal of enhancing hazard characterization related to the presence of chemical mixtures in food. Nevertheless, some considerations must be made regarding the ability of a single method to analyse all contaminants. Indeed, it should be kept in mind that GC may better analyse nonpolar and lipophilic contaminants, highly polar contaminants are in practice not extracted by the QuEChERS method, and PSA sorbent can retain some analytes of interest, especially carboxylic acids compounds.

5. Conclusions and future research outlook

This review provided an overview of the most essential applications of LC-MS methods for determining food chemical contaminants, specifically veterinary drugs and pesticides. Comparisons of sample preparation procedures and analytical performance were also discussed. Considering the increasing regulatory requirements for detecting contaminants within the global food supply chain, there is a strong demand for developing analytical methods capable of monitoring multi-class compounds. LC-MS analytical methodologies have notably minimized the sample-preparation procedure, enhancing selectivity for qualitative and quantitative analyses of complex matrices like food samples. Regarding targeted approaches, the multi-component determination of food contaminants with a TQ configuration, operated in SRM or MRM mode, is a well-established workflow that delivers the selectivity and sensitivity typically required for monitoring compliance with legislative limits. The challenge, however, lies in detecting non-target or unfamiliar compounds within complex food matrices. To address this, databases of chemical contaminants and ingredient compounds in food must be expanded, facilitating broader database searches for identification. In this regard, a non-targeted screening method based on LC/HRMS has become an essential and efficient means of detecting potential risk substances in food. HRMS/MS plays a pivotal role in library creation, leveraging its combination of high mass precision, resolving power, and data fragmentation. The progression of MS software and the establishment of spectral libraries for both full-scan MS and MS/MS spectra will gain attention and offer the option of fingerprint-based authentication in food quality and safety. In summary, the food safety field is considered one of the focus topics worldwide, and the presence of chemical contaminants is a prominent problem; therefore, developing high-efficiency and universal targeted and non-targeted screening strategies is significant.

CRediT authorship contribution statement

Jessica Brandi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization. **Giuliana Siragusa:** Writing – review & editing. **Elisa Robotti:** Writing – review & editing. **Emilio Marengo:** Writing – review & editing. **Daniela Cecconi:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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.

Data availability

No data was used for the research described in the article.

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