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Review

Liquid chromatography–mass spectrometry in food safety

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ABSTRACT

The use of powerful mass spectrometric detectors in combination with liquid chromatography has played a vital role to solve many problems related to food safety. Since this technique is especially well suited for, but not restricted to the analysis of food contaminants within the food safety area, this review is focused on providing an insight into this field. The basic legislation in different parts of the world is discussed with a focus on the situation within the European Union (EU) and why it favors the use of liquid chromatography–mass spectrometry (LC–MS). Main attention in this review is on the achievements that have been possible because of the latest advances and novelties in mass spectrometry and how these progresses have influenced the best control of food allowing an increase in the food safety and quality standards. Emphasis is given to the potential and pitfalls of the different LC–MS approaches as well as in its possibilities to address current hot issues in food safety, such as the development of large-scale multi-residue methods and the identification of non-target and unknown compounds. Last but not least, future perspectives and potential directions are also outlined highlighting prospects and achievements.

Contents

1. Introduction

Over the past decade, food safety, always an important issue, has gained a higher profile following a number of highly publicized incidents all around the world, including bovine spongiform encephalopathy in beef and benzene in carbonated drinks in the UK, dioxins in pork and milk products from Belgium, contamination of foods with pesticides in Japan, tainted coca-cola in Belgium and France, pesticides in soft drinks in India, melamine in dairy products from China and salmonella in peanuts and now pistachios in USA [1,2]. Such incidents, together with the continuing controversy about genetically modified crops, have combined to leave the general public in many countries widely distrustful of their food supply [3]. In an attempt to counter this suspicion, the governments of several countries have re-organized their management of food safety issues and, in many cases, have increased the amount of food safety-related legislation [4,5]. In today's global marketplace, the safety and quality of food products are of growing concern for consumers, governments, and producers alike. Issues relating to food safety and the public's perception of wholesomeness have become increasingly important for all food products [6]. Current

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good manufacturing practices (GMPs) are a primary basis by which food manufacturers and processors prevent, reduce, control, or eliminate food borne hazards. In addition, the Hazard Analysis and Critical Control Point (HACCP) system provides the means to analyze and target specific steps in food production (critical control points) for prevention, mitigation, or control of food contamination [7,8]. Analytical information, including surveillance data for both recognized and newly identified contaminants, is also essential. However, the information about their occurrence in food is still (very) limited [9].

Against this background, liquid chromatography–mass spectrometry (LC–MS), traditionally an important part of the medical laboratory, found a growing market from a new application – food safety testing [10]. LC–MS is particularly suited for the analysis of food contaminants, since it provides a large amount of information about a complex mixture, enabling the screening, confirmation and quantitation of hundreds of components with one analysis [11,12]. These instruments are used to test other food safety issues, such as food authenticity and labeling accuracy [13,14]. However, this review will be focus on chemical contaminants because their relative importance within the field. In order to give an idea of the wide range of applications covered, Table 1 illustrates examples regarding major classes of chemical contaminants in food determined by LC–MS.

Triple quadrupole (QqQ) mass spectrometry has been the cornerstone technique for screening and confirmation of food contaminants and residues [45]. The majority of current liquid chromatography–tandem mass spectrometry (LC–MS/MS) based contaminants and residue analysis relies on the high sensitivity and selectivity of the selected reaction monitoring (SRM) mode of QqQ-MS/MS [11,19,46,7]. LC–time-of-flight (TOF)-MS has also been established as a valuable technique for the routine control of the wholesomeness of food. In this sense, TOF techniques can record an accurate full-scan spectrum throughout the acquisition range and have resulted an excellent tool for the unequivocal target and non-target identification and confirmation of food contaminants [12,48,49]. Recently introduced tandem mass spectrometers, having both features, such as quadrupole linear ion trap (QqLIT, LTQ or Q-trap), quadrupole time-of-flight (QqTOF), LTQ-Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), and LTQ-Orbitrap, etc., have allowed the development of several new methods for contaminants detection [50,51].

This review addresses the contribution of the different LC–MS techniques to different hot issues in food safety with selected examples that have been published mainly during the past 3 years, with particular emphasis on the most recent advances in applications of LC–MS/MS for the detection and characterization of food contaminants.

2. Legislative framework

The Food Safety legislative framework is a critical determinant of whether reliable analytical methods can be developed. It stipulates (i) sampling and monitoring plans, (ii) definition of maximum residue limits (MRLs) for tolerate food contaminants and residues and minimum required performance limits (MRPLs) for some of the testing procedures to detect banned substances, and (iii) the performance characteristics of analytical methods [5,7,52]. The development, optimization and validation of suitable analytical methods are important elements of assuring reliable food contaminants and residue testing [3]. Because of this, a short description of the situation and aims of this legislative set up is mandatory. Food Safety legislation is not harmonized through the world [53]. However, well-known international bodies, the most representative of which is the *Codex Alimentarius Commission* established by FAO and WHO develops science and risk-based food safety standards that are a reference in international trade and a model for countries to use in their legislation [54]. Table 2 provides a short overview of the International and governmental bodies in charge of maintaining food safety in each different country including the most essential web sites were information is available.

As one of the world's largest food importers, the European Union (EU) exerts a major influence on food safety testing globally. The EU Commission has designated food safety a top priority, and published a White Paper on Food Safety to ensure safe products along every step "from farm to fork" [55]. This includes feed production, primary production, processing, storage, transportation and retail sale. There are increasingly stringent import standards in other countries like Japan where exporters, such as, the EU, China and the USA must comply to export food there. Countries in Asia are also increasingly establishing quality regulations for food produced for in-country consumption [56–58].

For a number of food contaminants, European legislation establishes the MRLs in different food commodities and also lays down the methods of sampling and analysis that should be used (e.g. for dioxins and dioxin-like PCBs [59] and ethyl carbamates [60]). For other compounds, detailed performance criteria to be fulfilled by the methods of analysis used by the laboratories are laid down (e.g. benzo[a]pyrene, cadmium, lead and mercury, pesticide residues) [61,62]. In this way, Commission Decision 2002/657/EC [63] is probably the key document of legislation to be consulted by analytical laboratories in control food safety. This includes definitions and descriptions of how to assess trueness, recovery, repeatability, ruggedness, and detailed requirements for MS detection and identification of targeted substances. Although it lists performance criteria and other requirements for analysis of food contaminants and residues in animal food products, this Decision has been applied in many cases, in which there are not well-established criteria (for example for a number of newly emerging contaminants). The established MRLs and/or MRPLs determine the required sensitivity and generally, compelled to improve the limits of detection. The values ranged from a few $\mu g\,kg^{-1}$ to more than $10\,mg\,kg^{-1}$ depending on the combination contaminant and food (Supplementary Table S1).

3. Applications of LC–MS in food safety

3.1. Pesticide residues

The analysis of pesticide residues is complex because there are a large number of these substances authorized or forbidden that can be applied for that purpose. Since 10 years ago, LC–MS is applied in pesticide residue analysis and its use has been increased exponentially in the last years [12]. Analytical methods for post-registration and monitoring control should fulfill the performance requirements detailed in the Doc. SANCO/2007/3131 [62]. This field is one of the most evolved areas with regards to the applied analytical methods. Several reviews on the subject help to interpret the recent trends within the field [1,12,45,48,49,51,67–71]. Furthermore, Table 3 summarizes the most recent methods established for that purpose.

The analysis may be targeted or non-targeted but always using a multi-residue procedure as generic and simple as possible, reducing to the maximum the clean up steps, Ethyl acetate with anhydrous sodium sulphate or acetonitrile with dispersive solid-phase extraction (QuEChERS method) are good examples of tendencies within sample preparation [1,11,12]. Target analysis is a conventional analysis based on developing a method with standards prior to analysis and monitoring real samples that do not detect compounds not defined in it. The standards are selected

Table 1
Common classes of chemical contaminants in food determined by LC–MS.

Chemical contaminants in food	Examples	Reference
Residues		
Agrochemicals		
Pesticide residues (>800 compounds)	Herbicides (carbaryl, diuron, monuron) Insecticides (malathion, parathion) Fungicides (imazalil, carbendazim)	[1,12]
Pharmaceuticals		
Veterinary drug residues	Aminoglycosids (kanamycin, neomycin) β-lactams (amoxicillin, chloxacillin) Macrolides (tylosin, tilmicosin, spiramycin) Nitrofurans (ronidazole) Quinolones (ofloxacin, norfloxacin, ciprofloxacin) Sulfonamides (sulfacetamide, sulfaquinoxaline) Tetracyclines (tetracycline, chlortetracycline) Amphenicols (cloramphenicol)	[15–17]
Growth promoters	Stilbenes (Dienestrol, Diethylstilbestrol) Antithyroid agents (Mercaptobenzimidazol, Methylthiouracil) Steroid androgens (ethylestrenol, methandriol) Resorcylic acid lactones (α-zeranol) β-agonists (clenbuterol, terbutaline)	[15,16]
Natural toxins		
Mycotoxins	Aflatoxins, ochratoxin A, patulin, trichothecenes, fumonisins	[18,19]
Phycotoxins	Domoic acid, okadaic acid, saxitoxine, microcytins, azaspiracids, pectonotoxins, yessotoxins	[20,21]
Phytotoxins	Ergot alkaloids (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine)	[22,23]
Environmental contaminants		
Industrial chemicals		
Perfluorinated compounds (PFCs)	Perfluorooctane sulfonate (PFOS) Perfluorooctanoic acid (PFOA)	[24,25]
Hexabromocyclododecane (HBCB)	Parent compound including diasteroisomers and enantiomers	[26]
Nanomaterials	Carbon nanotubes Fullerenes	[27,28]
Contaminants in food processing		
Heating		
Acrylamide	Acrylamide	[29,30]
Biogenic amines	Histamine, putrescine	[31,32]
Heterocyclic amines	2-amino-1,6-dimethylimidazo[4,5-b]pyridine 2-aminodipyrido[1,2-a:3′,2′-d]imidazole	[33–35]
Semicarbazide	Semicarbazide	[36]
Fermentation		
Ethylcarbamates	Ethylcarbamates	[37]
Materials in contact with food		
Melamine	Melamine, ammeline, ammelide, and cyanuric acid	[38–40]
Phthalates	Dibutyl phthalate (DBP) Benzyl butyl phthalate (BBP) di-2-ethylhexyl phthalate (DEHP), di-'isononyl' phthalate (DINP) di-'isodecyl' phthalate (DIDP)	[41]
Photoinitiators	2-isopropyl thioxanthone (ITX) 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB)	[6,42]
Bisphenol diglycidyl ether residues	BADGE, BADGE·H_2O, BADGE·$2H_2O$, BADGE·H_2O·HCl, BADGE·HCl, BADGE·2HCl, BFDGE, BFDGE·2HCl	[43,44]
Bisphenol A	Bisphenol A	[44]

Table 2
Characteristics of the current legislation and recommendation of the International organizations on food safety.

International organizations and governmental bodies	Web site
World Health Organization (WHO)	http://www.who.int/about/en
Food and Agriculture Organization (FAO) of the United Nations (UN)	http://www.fao.org/
Codex Alimentarius Commission	http://www.codexalimentarius.net/web/index_en.jsp
World Trade Organization (WTO)	http://www.wto.org/
USA	http://www.fda.gov/
European Union (EU)	http://europa.eu/
UK	http://www.food.gov.uk/
Australia and New Zealand	http://www.foodstandards.gov.au/
Canada	http://www.hc-sc.gc.ca/
Japan	http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/eng.h-page
China	http://eng.sfda.gov.cn/eng/
India	http://foodsafetyindia.nic.in/

Table 3
Selected LC–MS procedures to determine pesticide residues in food.

Pesticides	Matrix	Extraction method	*R* (%)	LC–MS	LOD ($\mu g\,kg^{-1}$)	Comments	Reference
Target methods							
Acetamiprid Imidacloprid Thiacloprid Thiamethoxam	Apricot, peach, pear, celery, courgette	Acetone extraction, SPE on Extrelut-NT20 and analyte's elution with CH_2Cl_2	75–105	LC–MS	20–100	Analytical procedure simple, rapid and specific	[72]
11 organophosphorus	Honey	Solid-phase microextraction PDMS/DVB 60 μm	19–92	LC–MS	0.001–0.005	Bromophos ethyl, diazinon, fonofos, pirimiphos ethyl, pyrazophos, and temephos were at concentrations from 6.2 to 193 $ng\,g^{-1}$.	[73]
1 carbamate				On-line SPME desorption			
23 pesticides (different classes)	Tomato, cucumber, pepper	Hollow fiber supported liquid membrane (HFSLM)	–	LC–MS	0.06–0.2	Imidacloprid was the most frequent pesticide. Cyprodinil, methomyl and carbendazim were also detected. Concentrations were below MRLs.	[74]
Amitraz and 3 metabolites (DMA[a], DMF[b], DMPF[c])	Pears	Ethyl acetate/Na_2SO_4	70–106	LC–QqQ-MS/MS	0.5	Comparison of ethyl acetate extraction and hydrolysis to 2,4-dimethylaniline	[75]
43 pesticides of insecticides, acaricides, fungicides, herbicides and plant growth regulators and 9 pesticide metabolites	Tomato, lemon, raisins, avocado	Methanol:water (80:20, v/v) 0.1% acetic acid	70–110	LC–QqQ-MS/MS	10	The method validated according to SANCO European Guidelines for representative samples.	[76]
28 pesticides (different classes)	Tomato, peas, lettuce, apple puree	Ethyl acetate/Na_2SO_4	65–94.4	LC–QqQ-MS/MS	0.002–0.007	Method for the analysis of pesticides at low concentrations.	[77]
160 pesticides (different classes)	Tomato, pear, orange	QuEChERS (acetonitrile and NaCl)	97–98	LC–QqQ-MS/MS	0.1–5	The validation follows DG SANCO/2007/3131 Thirty-three compounds were detected in 50 samples.	[78]
9 N-methyl carbamate	Spinach, tomato, potato, apple, cucumber, mandarin	Ethyl acetate/Na_2SO_4	56–119	LC–QqQ-MS/MS	5	Direct sample injection into a short column.	[79]
64 pesticide residues and their toxic metabolites	Apples, apple-based baby food	QuEChERS (1% acetic acid in acetonitrile. Mg_2SO_4 and $C_2H_3NaO_2$)	–	UPLC–QqQ-MS/MS	0.25–20/0.5	Distinctly reduced analysis time (10 min in this particular case).	[80]
53 multi-class pesticides, which are commonly used in southeast Spain	Cucumber, orange, strawberry, olive	QuEChERS (1% acetic acid in acetonitrile. Mg_2SO_4 and $C_2H_3NaO_{2)}$	70–109	UPLC–QqQ-MS/MS	0.1–0.4	Applied to the analysis of 200 samples. Imidacloprid was the most frequent pesticide.	[81]
Phenoxy acid residues	Rice	QuEChER (acetonitrile and DSPE clean up)	45–104	UPLC–QqQ-MS/MS	0.5	Difficult matrix	[82]
8 pesticides (different classes)	Orange, strawberry, cherry and apple	SPME, PDMS/DVB and CW/TPR fibers	25–82	LC–IT-MS/MS On-line coupling with the SPME extraction	0.005–0.05	Sophisticated extraction procedure	[83]
47 pesticides	Rice, wheat flour	Acetonitrile and NaCl	70–140	LC–QqLIT-MS/MS	0.2–8.0	Official method the Korea Food and Drug Administration. Tricyclazole and fenobucarb were found in polished rice samples	[84]
Organochlorines and their transformation products	Brands of juices	SPE Oasis HLB, methanol eluting solvent	71–109	LC–TOF-MS	0.08–0.45	Method successfully applied to the analysis of 23 fruit juice samples collected from different European countries and the United States. Over 50% of the samples tested contained pesticide residues at low concentration levels	[85]
297 Pesticides (different classes)	Fruits and vegetables	QuEChERS (acetonitrile, NaCl and $MgSO_4$)		LC–TOF-MS	<50	Wide range of pesticides is covered	[86]

Table 3 (*Continued*)

Pesticides	Matrix	Extraction method	*R* (%)	LC–MS	LOD ($\mu g\,kg^{-1}$)	Comments	Reference
100 pesticides (different classes)	Soft drink bottles and cans of different brands.	SPE HLB cartridges (Oasis) eluted with methanol	70–110	LC–TOF-MS	0.006–0.03	The pesticide concentrations in the raw extract of fruit used to flavor the soft drink (5–8% of the total product) was high (i.e. 300–800 $\mu g\,l^{-1}$).	[87]
100 pesticides (different classes)	Strawberry	Ethyl acetate/Na_2SO_4/$NaHCO_3$	70–120	UPLC–TOF-MS	20	Reduces the analysis time	[88]
Carbosulfan and its metabolites	Oranges, rice, potatoes	PLE with CH_2Cl_2 at 40 °C and 2000 psi for 2 cycles of 5 min	55–93	LC–QqTOF-MS	3–20	The information on elemental composition with very low mass errors constitutes faster and higher order of identification	[89]
Nitenpyram, isocarbophos, isofenphos-methyl	Peppers	QuEChERS: (acetonitrile, NaCl and $MgSO_4$)	76–100	LC–TOF-MS LC–QqLIT-MS/MS	0.1–5/0.03–1.5	Three insecticides no authorized in the European Union	[90]
12 pesticides	Multi-fruit jars and juices for infant consumption	Acetonitrile and SPE clean up	78–105	LC–QqQ-MS/MS LC–TOF-MS	LOQ/0.1–4	Applied to a total of 33 baby food samples from Spain and UK. Imazalil, thiabendazole and carbendazim were detected 60% of baby food samples.	[91]
17 pesticides	Apples	ACN	–	UPLC–QqQ-MS/MS HPLC–QqQ-MS/MS	0.5–8.0 2.0–8.0	Comparison of LC and UPLC	[92]
Non-target methods							
Amitraz and malathion and six degradation products	Pepper	(a) QuEChERS: acetonitrile, NaCl, $MgSO_4$ (b) Methanol	–	LC–TOF-MS	–	–	[93]
Approaches including both systems							
Unknown pesticides (post-harvest)	Pears	Ethyl acetate/Na_2SO_4	76–77	UPLC–QqTOF-MS/MS	0.4	Carbendazim, imazalil, and ethoxyquin were identified.	[94]
12 pesticides (different classes)	Oranges, strawberries, cherries, peaches, apricots pears	PLE with ethyl acetate and acid alumina	>70	LC–QqQ-MS/MS LC–IT-MS/MS LC–QqTOF-MS/MS	0.01–0.3	Comparation method PLE extraction with traditional extraction with ethyl acetate	[95]
Buprofezin, hexythiazox	Orange peel and flesh, banana skin and flesh, strawberry, pear	Acetone	51–130%	LC–QqTOF-MS/MS	–	The LOD estimation can be ambiguous because the non existence of chemical noise in the chromatogram	[96]
Amitraz and 4 degradation products (DMPF[c], BDMPF[d], DMF[b], DMA[a])	Pears	Ethyl acetate/Na_2SO_4	83–101	UPLC–QqTOF-MS/MS	<0.03	Identification and confirmation of a non-reported metabolite	[97]
30 target pesticides and non-target	Wheat, lettuces, avocado, oranges	Matrix solid-phase dispersion on C18 and analyte elution with Cl_2CH_2	87–102	LC–QqQ-MS/MS	1.0	Identification and confirmation of several non-target compounds	[98]
Target pesticides and non-target metabolites	Orange, lemon, grape, olive oil	methanol/water (80:20, v/v)	–	UPLC–QqTOF-MS/MS UPLC–QqTOF-MS/MS	–	Discovery of two metabolites of imazalil and two of chlorpyrifos. MetaboLynx application to TOF-MS data was helpful to detect phosmet-oxon.	[99]
Fenthion and its metabolites	Oranges	Ethyl acetate/Na_2SO_4	72–94	UPLC–QqTOF-MS/MS LC–IT-MS/MS	1–6	The degradation studies on fenthion applied to orange orchards revealed that fenthion is mainly degraded to its sulfoxide.	[100]

[a] DMA = 2,4-dimethylaniline.
[b] DMF = 2,4-Dimethylphenyl formamidine.
[c] DMPF = N-2,4-Dimethylphenyl-N-methyl formamidine.
[d] BDMPF = N,N′-bis(2,4-dimethylphenyl)imidoformamide.

based on the toxicity and/or frequency of detection. The trend within the target analysis is the development of large-scale multi-residue methods (able to determine more than 80 compounds). These methods applied LC–MS/MS, using QqQ, QLT and TOF-MS (see Table 3). LC–MS/MS using QqQ has as a serious limitation the number of compounds that can be simultaneously determined (up to 100–150 depending on the scan speed/dwell time). The conventional QqQ instruments (with moderate scan speed), allows a maximum simultaneously recording of about 10–20 transitions with appropriate sensitivity. In this case, a large-scale method required a careful distribution of the chromatographic run in several windows. For example, Hernández et al. [76] developed a multi-residue method for the screening, quantification and confirmation of 52 pesticides and metabolites in four fruit and vegetable matrices. Regarding to LC separation, the gradient was optimized in order to render a rough separation between the 52 selected analytes, obtaining less than 10 compounds monitored simultaneously per a 3–4 min time window. The confirmation performed in this way usually requires an additional injection because double number of transitions is needed for the simultaneous quantification and confirmation. This would lead to a method monitoring more than hundred transitions, which would reduce the number of points per peak leading to unsatisfactory peak shapes. This limitation might be overcome by the use of new generation instruments, which can reduce the dwell time for each transition without any loss in sensitivity, allowing to increase the number of transitions acquired simultaneously up to 100 or 150 [78,80–82,92]. These fast-switching QqQ-MS instruments significantly enlarge the number of analytes that can be detected in one run. Indeed, modern instruments produce high signal-to-noise (S/N) ratios even when relying on short SRM dwell times and can be properly combined with ultra-performance liquid chromatography (UPLC). Conventional LC with C_{18} columns plays a dominant role for the determination of pesticide residues, whereas UPLC along with sub-2 μm particle C_{18} columns reduces run time and improves sensitivity. Comparative analyses of 17 (semi)polar pesticide residues in crude apple extract, which were analyzed in two alternative LC–MS/MS systems, documented the potential of UPLC to replace "classic" LC separation strategy [92]. The data generated in optimized systems employing either Acquity UPLC (Waters) or Alliance LC (Waters) hyphenated with Quattro Premier (Waters) MS detector (tandem quadrupole), showed that (i) the number of theoretical plates was for most analytes higher in system employing LC, and with lower variability compared to UPLC, (ii) the values of height equivalent to the theoretical plate obtained in UPLC were mostly higher, however, their variability was also rather high, (iii) the analysis time in system employing UPLC was reduced by more than 50% with similar analytical output and (iv) UPLC provided significantly improved S/N followed by decreased LOQs for majority of compounds. Chromatograms illustrating LC–MS/MS and UPLC–MS/MS analysis of 17 (semi)polar pesticides are shown in Fig. 1. The reduced analysis time consequently resulted into significantly lower consumption of organic solvents.

The recently introduced hybrid QLT instrument has also been used to perform MS/MS. This instrument retains classical QqQ modes for quantitative and qualitative analysis (SRM mode and neutral loss scan) and combines them with sensitive ion trap scan modes for the confirmation of analytes or characterization of unknowns, including enhanced product ion mode, time delayed fragmentation, and MS^3 with an ion accumulation capacity higher than a conventional three dimensional ion trap analyzer. The QqLIT analyzer can provide an improved sensitivity in these MS/MS studies and up to 200 compounds can be analyzed in a unique LC–MS/MS run, with 2 SRM transitions [101]. The working modes (enhanced product ion and MS^3) are useful for the unambiguous confirmation of pesticides with poor fragmentation at low

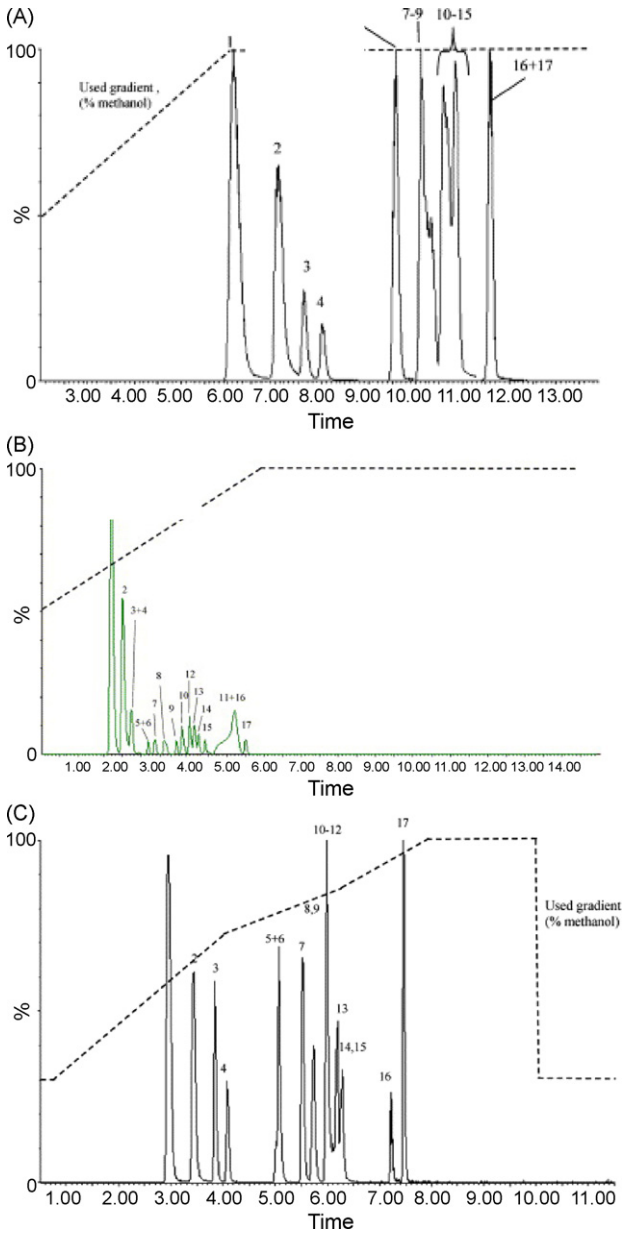

Fig. 1. Chromatogram of apple crude extract spiked with 17 (semi)polar pesticides based on the quantifying MS/MS transitions obtained by (a) HPLC–MS/MS, (b) UPLC–MS/MS when using the same gradient as for HPLC–MS/MS and (c) UPLC–MS when using a optimized gradient. For illustration, used gradient (% of methanol) is also shown. Peak identification: 1 = Carbendazim, 2 = Thiabendazole, 3 = Carbofuran, 4 = Carbaryl, 5 = Linuron, 6 = Methiocarb, 7 = Epoxiconazole, 8 = Flusilazole, 9 = Diflubenzuron, 10 = Tebuconazole, 11 = Imazalil, 12 = Propiconazole, 13 = Triflumuron, 14 = Bitertanol, 15 = Prochloraz, 16 = Teflubenzuron, 17 = Flufenoxuron. Adapted with permission from [92]

concentration levels, which cannot be easily confirmed by QqQ instruments due to the high SRM ratio between the two transitions (or absence of the second transition) for confirmatory purposes. This is the case of the pesticide spinosyn A, with a SRM ratio of 25. A possible alternative is the use of information-dependent acquisition experiments to generate additional survey scans, so that it is plausible to combine a survey scan acquired in the SRM operation mode (QqQ) with another survey scan in the enhanced product ion mode or in the MS^3 mode (using the instrument as a linear ion trap) in one single run. Thus, one SRM transition can be used for quantification, and the structural information obtained using the

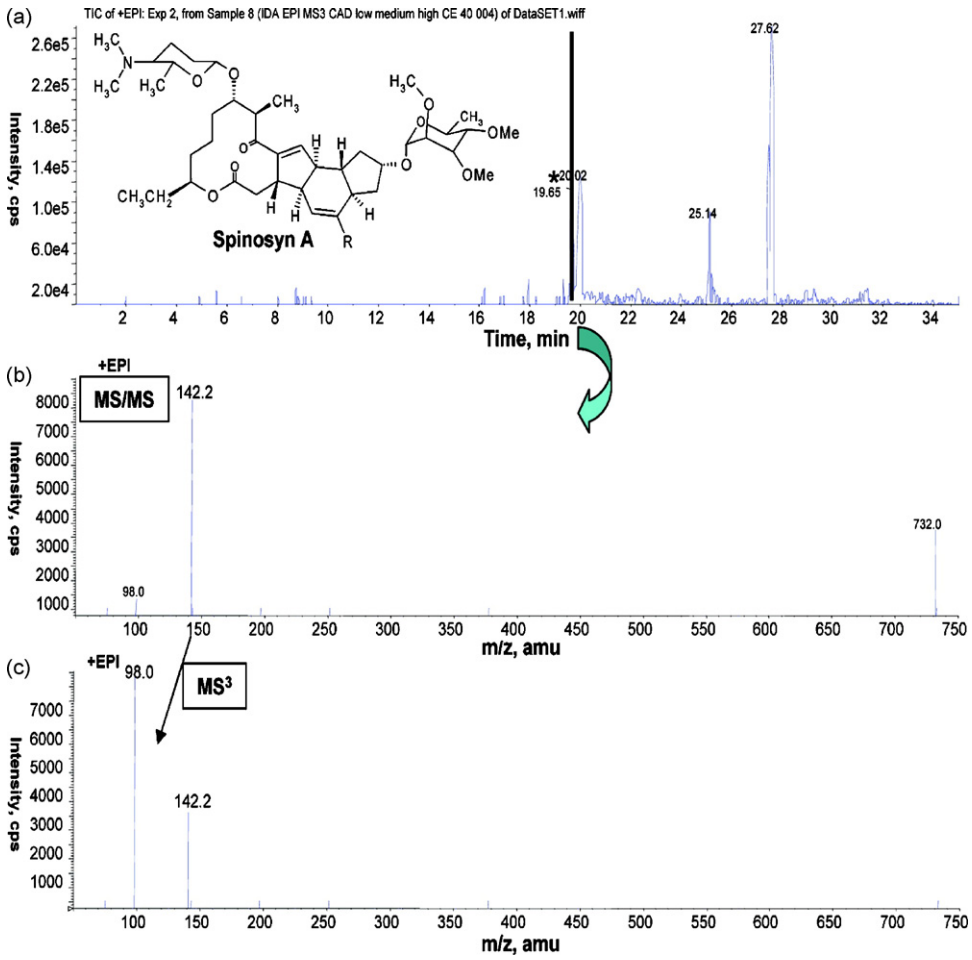

Fig. 2. Application of LC–MS/MS for the confirmation of spinosyn A, a pesticide with low CID fragmentation in a triple quadrupole, using a hybrid triple quadrupole linear ion trap instrument: (a) total ion chromatogram; (b) enhanced product ion scan mode; and (c) MS3 mode. The combination of survey scans using the SRM, enhanced product ion scan mode, and MS^3 modes by means of information-dependent acquisition experiments is very useful for confirmatory purposes. Reproduced with permission from [101] copyright © 2007 American Chemical Society.

instrument as an ion trap (IT) can be successful for the confirmation of analytes. As an example, the data obtained from the information-dependent acquisition experiments performed on spinosyn A are shown in Fig. 2.

The TOF has the ability to record unlimited number of compounds because it operates in full-scan mode. Accurate mass measurements are almost specific and universal for every target analyte regardless the instrumentation used, making possible to develop searching libraries comparable to those already existing for gas chromatography (GC–MS). In this sense, UPLC–TOF-MS is a cost-effective technique very convenient for the development of screening strategies and for performing routine accurate mass analysis based on target databases [86,102]. The other main feature of TOF-MS instruments is their high sensitivity in "full-scan" acquisition mode, so that pesticides can be detected in complex matrices at low picogram levels and, although not as sensitive as QqQ instruments operated in the SRM mode, they provide the sensitivity needed to meet current food safety regulations. Unambiguous identification is accomplished by means of accurate mass measurements from (de)protonated molecules, in-source collision induced dissociation fragment ions, and isotope signature matching. In addition, LC–TOF-MS provides satisfactory analytical performance for quantitation purposes, as has already been demonstrated so far in the literature [85,86,88]. It offers the possibility to simultaneously analyze a virtually unlimited number of compounds. Furthermore, the retrospective "post-targeted" evaluation of old data offers the possibility to detect non-a priori selected analytes (i.e. no analyte specific transitions have to be defined before injecting the sample) [90] (An example is illustrated in Supplementary Fig. 1S.)

Another trend in target LC–MS is the development of methods for particularly difficult matrices, such as olive oil (high fat content), soft drinks including fruit juices (levels of pesticides in these products are low) or baby food (MRLs are <0.01 $\mu g\,kg^{-1}$ for some pesticides). Both systems, LC–MS/MS (QqQ or QqLIT) and LC–TOF-MS, have been successfully applied in several analytical methods [83,85,87,91,103]. The concentration levels detected were of the micrograms per liter level, low when considering the European MRLs set for fruits, but very high (i.e. 300 times) when considering the MRLs for drinking or bottled water.

After look at the main features of LC–MS/MS and LC-TOF-MS several methods proposed the use of both equipments together. García-Reyes et al. [101] propose a combined approach based on LC–TOF-MS and LC–MS/MS analysis (Supplementary Fig. 2S) for the comprehensive screening of priority (target) and nonpriority (low frequency and misused) pesticides. The method is based on three steps. First, a fast comprehensive automated screening method is performed with a unique LC–TOF-MS run. Prior to the analysis of real samples, this screening procedure is compiled with appropriate software. The screening method is created using a single standard solution containing all the pesticides, by assigning for each target pesticide its corresponding peak (retention time)

Table 4
Selected LC–MS procedures to determine veterinary drugs and growth promoting agents.

Analytes	Matrix	Extraction method	*R* (%)	LC–MS	LOQ (ng g^{-1})	Comments	Reference
Approaches for target analysis							
31 antimicrobials β-lactams, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, nitroimidazoles, trimethoprim	Cattle, pig	Homogenization with Na2-EDTA washed sea sand. PLE with H_2O in 10 min (static time) at 1500 psi, 70 °C; 60% of flush in 1 cycle	75–99	LC–QqQ-MS/MS	10–50	Confirmatory, quantitative, results in samples from the market	[114]
25 antibacterials β-lactams, sulfonamides, tetracyclines, fluoroquinolones, macrolides	Milk and dairy products	Acetonitrile and 1% formic acid SPE with Oasis HLB 3 cc (60 mg) extraction cartridge and elution with acetonitrile	50–70	LC–QqQ-MS/MS	0.02–25	Screening, confirmatory and quantitative	[115]
51 veterinary drugs nitroimidazoles, sulphonamides, quinolones, ionophores dinitrocarbanilide.	Animal tissues	QuEChERS (DSPE with NH_2 sorbent)	8–95	LC–QqQ-MS/MS	3	Optimization of the extraction, confirmatory and quantitative	[116]
22 anabolic steroids natural and synthetic	Meat samples	Enzymatic digestion with *Subtilisine*, extraction with methanol, defatted with hexane and SPE using NH_2 and C_{18} cartridges in parallel and analytes' elution with methanol	Not reported	LC–QqQ-MS/MS	0.1–10	Screening and qualitative confirmatory method	[117]
22 Anabolic steroids natural and synthetic	Bovine, pork and poultry muscle tissues	Enzymatic digestion followed by extraction with methanol. SPE combining C_{18} and –NH_2 and elution with acetonitrile.	Not reported	LC–QqQ–MS/MS	<0.5	Screening and qualitative confirmatory method CCα and CCβ < MRPL	[118]
19 antibiotics tetracyclines, sulfonamides, quinolones, β-lactams, macrolides	Porcine and bovine muscle	Direct extraction with 70% methanol	68–95	LC–QqQ-MS/MS	1–30	Screening, confirmatory and quantitative method	[119]
60 antibacterials aminoglycosides, β-lactams, sulphonamides, fluoroquinolones, quinolones ionophores, dinitrocarbanilide, tetracyclines	Beef kidney juice and serum samples	*Aminoglycosides* 10 mM KH_2PO_4, 0.4 mM EDTA, 2% TCA BakerBond SPE and elution with 10% acetic acid in methanol *Other antibiotics* acetonitrile:water (4:1, kidney juice) or acetonitrile (serum) and DSPE with C_{18}	61–109	LC–IT-MS/MS (aminoglycoside screening) LC–QqQ-MS/MS (other antibiotics)	QqQ < 10 IT <500	Screening, confirmatory and quantitative analysis of 235 carcasses. Compares LC–MS/MS with three rapid antimicrobial screening test	[120]
42 veterinary drugs tetracyclines, macrolides, aminoglycosides, β-lactams, amphenicols, sulfonamides	Honey	Acetonitrile extraction, with and without acid. Hydrolysis step to broken the bonds between sugars and antibacterials (optional)	26–297	LC–QqLIT-MS/MS	27–81	Screening, confirmatory and quantitative method. Analysis of several honey samples	[121]
10 β-lactam antibiotics penicillins and cephalosporins	Milk	Automated SPE coupled on-line with the LC system	80–116	LC–QqLIT-MS/MS	0.09–1.44	Screening and qualitative confirmatory Offers high sensitivity and accuracy with minimum sample pre-treatment. Uses for the first time an automated on-line SPE offering a high throughput analysis.	[122]

Table 4 (*Continued*)

Analytes	Matrix	Extraction method	*R* (%)	LC–MS	LOQ (ng g^{-1})	Comments	Reference
18 veterinary drugs quinolones, sulphonamides, macrolides, anthelmintics, tetracycline	Milk	QuEChERS (only acetonitrile without clean up)	70–110	UPLC–QqQ-MS/MS	1–4	Screening, confirmatory and quantitative method to determine Tylosin and fenbendezol in milk	[123]
29 veterinary drugs non-steroidal, anti-inflammatory drugs, corticosteroids, anabolic steroids	Bovine muscle and kidney tissue	1% formic acid buffered with Tris, digested with protease and extracted with isopropanol-H_2O. Defatted with hexane and SPE using tandem C_{18} OASIS MAX –IRISH. Then, steroids and corticosteroids retained on the IRIS cartridge were eluted with methanol and the NSAIDs retained on the OASIS MAX, were eluted with 2% formic acid in ethyl acetate.	38–92 (n = 18)	UPLC–QqQ-MS/MS	0.1–2.2	Screening, confirmatory and quantitative method	[124]
21 veterinary drugs tetracyclines, quinolones e	Pig muscle, kidney, and liver	EDTA–McIlvaine buffer solution SPE with Oasis HLB SPE cartridge and analytes' elution with methanol	80–117	UPLC–QqQ-MS/MS	0.03–10	Screening, confirmatory and quantitative method	[125]
30 target drugs β-blockers, 11 sedatives	Animal tissues	Na_2SO_4 and acetonitrile SPE with NH_2 cartridge	76.4–118.6	UPLC–QqQ-MS/MS	0.5–2.0	Screening, confirmatory and quantitative method	[126]
100 veterinary drugs nitroimidazoles, sulphonamides, quinolones, ionophores, dinitrocarbanilide, macrolides	Egg, fish, meat	Acetonitrile/Milli-Q water (6:4, v/v) to effect protein precipitation followed by StrataX SPE and analytes' elution for egg samples with methanol/ethyl acetate (1:1, v/v) and for fish and meat samples with methanol/acetonitrile (1:1, v/v)	47–145 70–120 (for 90% of compounds)	LC–TOF–MS	4	Screening, confirmatory and quantitative method This study shows multi-compound and multi-matrix capabilities	[127]
100 veterinary drugs benzimidazoles, macrolides, penicillins, quinolones, sulphonamides, pyrimidines, tetracyclines, nitroimidazoles, tranquillizers, ionophores amphenicols, NSAIDs	Milk	Protein precipitation by acetonitrile and dilution with water SPE with StrataX and analytes elution with methanol	80–120	UPLC–TOF-MS	5–25	Screening, confirmatory and quantitative method. 100 samples of raw milk were screened. No suspected (positive) results were obtained except for the included blind reference sample containing sulphamethazine (88 μg l^{-1})	[128]
150 veterinary drugs avermectines, benzimidazoles, β-agonists, β-lactams, corticoides, macrolides, nitroimidazoles, quinolones, sulfonamides, tetracyclines	Raw milk	Protein precipitation by acetonitrile, centrifugation of an aliquot of the extraction and evaporation of the acetonitrile.	25–805	UPLC–TOF–MS	0.5–25	Screening Contaminants identification by accurate mass UPLC–TOF showed proper quantification performance Important matrix effects	[129]
100 veterinary drugs	Muscle, kidney, liver	Acetonitrile extraction with addition of $MgSO_4$ and NaCl. The acetonitrile is evaporated and the remaining aqueous solution is passed through a Oasis HLB and the analytes eluted with acetonitrile	< 50 (11% of compounds) >80 (60% of compounds) >120 (0–5% of compounds)	UPLC–TOF–MS	0.1–20	Quantification Screening Identification Validated according to the Commission Decision 2002/657/EEC.	[130]

Approaches applicable to non-target analysis							
Fluoroquinolones	Chicken	SPE elution with methanol/acetonitrile (8:2, v/v)	Not reported	LC–TOF–MS	Not reported	Screening The system identifies known fluoroquinolones and possible discovers unknown chemicals of similar structure, which show activity in the dual bioassay.	[131]
Fluoroquinolones	Chicken	On-line nanoscale coupling of SPR-MU for the screening of low molecular weight molecules with nano-LC ESI TOF-MS	Not reported	Nano-LC–TOF–MS	0.005	Screening Identification of known metabolite Possibilities for identification of unknown compounds	[132]
Stanozolol, Clenbuterol-R	Feed samples	Methanol/acetonitrile (50:50)	Not reported	LC–QqTOF-MS LC–LTQ-FTICR-MS LC–LTQ-Orbitrap-MS	Not reported	Screening Confirmation criteria Identification and elucidation of unknown compounds	[133]
Hormone and veterinary drug residues	Bovine hair and feed samples	Hair samples shaken with Tris (2-carboxyethyl)phosphine hydrochloride and NaH_2PO_4 solution followed by Bond Elut SPE Feed samples with methanol/acetonitrile (50:50).	Not reported	UPLC–TOF–MS UPLC–Orbitrap-MS	2.5–25	Screening and identification. When sample preparation for LC–MS/MS is applied to UPLC accurate mass MS, false results can be obtained if the mass resolving power of the MS is insufficient.	[134]

and m/z accurate mass window. Samples are then analyzed and the method provides a preliminary identification of the species first by retention time and m/z mass window, followed by subsequent identification (if positive results) by LC–TOF-MS accurate mass measurement (second step). Final confirmation and reliable quantitation is accomplished in the final step by LC–MS/MS analysis with two SRM transitions.

Non-target analysis covers the possibility to detect any compound related or not with pesticides present in the sample. The non-target analysis offers the possibility of identifying unexpected pesticides, transformation products and/or impurities, or even untargeted compounds that can be toxics. This analysis is more complicated because it requires the identification of unknown compounds. Several years ago, the potential of LC–TOF-MS for non-target analysis was shown by the identification of the imazalil and prochloraz degradation products [104]. This identification was accomplished basically by combining the information provided by LC–TOF-MS accurate mass analysis with that deduced from the fragmentation pathway of the parent compound and carried out by LC-ion trap MS^n experiments (typically MS/MS or MS^3). Further, an elegant analytical methodology to study and elucidate degradation products of pesticides in food was developed by using "fragmentation-degradation" relationships. From a given parent species, the fragmentation patterns occurred in-source (by collision induced dissociation) could be used as a reference or model to predict possible degradation products. Examples of this strategy were illustrated for the identification of six degradation products of amitraz and malathion on different food extracts, showing the unique potential of LC–TOF-MS. However, the accurate mass measurements, high full-scan sensitivity and MS/MS make the QqTOF analyzer more suitable for this purpose. It provides additional features for confirmation, such as the accurate mass product ion spectra after performing MS/MS experiments. Several studies comparing the advantages and pitfalls of several mass analyzers highlight the ability of QqTOF to identify metabolites and unknowns against other mass analyzers [95]. The QqTOF was recently applied to the determination of pesticide residues in food [89,94–99,105]. Studies of fenthion metabolites in oranges and amitraz degradation products in pears were performed by QqTOF [97,100]. Several metabolites were identified and were finally confirmed by the accurate product ion mass spectra without reference standards. Other interesting study [96] identified an oxidized metabolite of buprofezin in banana skin samples. In this case, the use of a QqTOF instrument was crucial for correct elucidation of this metabolite. Pico et al. dealt with the use of UPLC–QqTOF-MS to identify the pesticide residues present in complex extracts [94]. In this way, carbendazim, imazalil, and ethoxyquin were successfully identified in pear extracts because of the accurate mass determination of their protonated molecule and their major fragments in the product ion mass spectra (see Fig. 3). A few plastic and latex additives were also found, most of them probably coming from the packaging transfer to the fruits [94].

A critical review on the potential of LC–(Qq)TOF-MS in investigating the presence of pesticide metabolites in food and water samples, pointed out several pitfalls of the technique, like the difficulty to deconvolute the signal of the analyte in a chromatogram that can contain a number of endogenous compounds from the matrix [102]. Future improvements are expected in deconvolution software in order to increase the success in detecting components, especially at low levels of concentration. The use of specialized software is crucial in for a rapid and successful comparison between blank and treated full-scan data because the manual inspection of total ion chromatograms to look for visible peaks can easily fail when matrices are complex. In any case, LC–QqTOF-MS is at present one of the most advanced and efficient approaches for screening and identification of non-target pesticides and their metabolites in

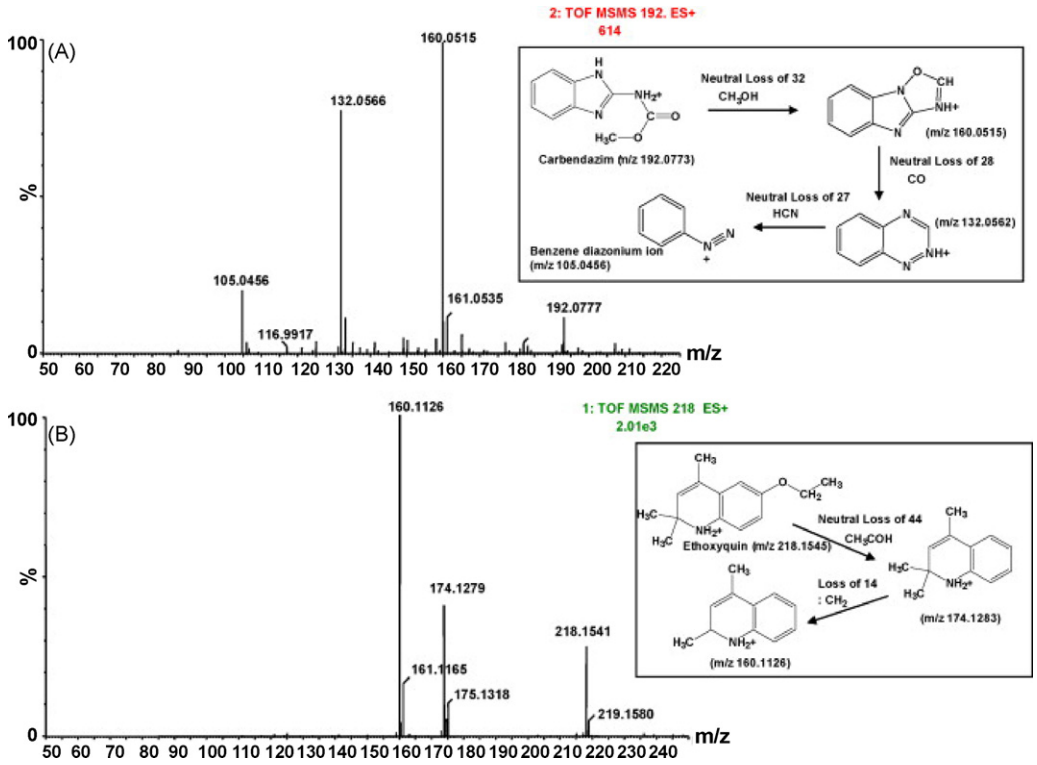

Fig. 3. Accurate product ion mass spectrum and proposed fragmentation of (a) carbendazim, (b) ethoxyquin. Proposed fragmentations are shown as an insert. Reproduced with permission from [94] copyright © 2007 Elsevier B.V.

food. This technique is now becoming a well-established approach in the field, as demonstrated by the number of published papers.

3.2. Veterinary drugs and growth promoting agents

The animal drugs utilized in food production are generally antibiotics and growth promoters for the control of various disease outbreaks and are also given to animals to increase the efficiency of feed conversion. In the EU the use of growth promoters is forbidden and the antibacterials are regulated by MRLs. Technical guidelines and performance criteria for residue control are established in the framework of Directive 96/23/EC and described in Commission Decision 2002/657/EC [63–106]. All this background has been the reason why LC–MS is the preferred technique within this field of analysis. The first reviews describing the role of LC–MS within the field of veterinary drugs and drug promoting agents in food-producing animals were published in 2005 [47,107]. The conclusion of both reviews was that LC–MS/MS, either with triple quadrupole or ion trap multiple stages, was the preferred technique applied through methods developed to cover a few members of a specific class of antibacterials and growth promoters. Since then, many LC–MS methods have been developed for veterinary and growth promoting groups such as sulfonamides, tetracyclines and quinolones. Several reviews [1,15–17,108–113] have been published showing what the main problems are and how the techniques have evolved to solve then. The major trends observed within the area are also outlined in Table 4, in which a selection of most recent and innovative methods with regards to the advances in mass spectrometry is listed. The current strategy relies on targeted analytical approaches focusing on the detection of residues of the administered compounds or their metabolites in different kinds of feed, food or biological matrices.

There is increasing interest in methods for the simultaneous analysis of various classes of veterinary drugs and growth promoters. LC–QqQ-MS (utilizing SRM, i.e. two transitions, to provide the required degree of confidence) has been the starting point for the development of such methods. Of the methods based on tandem mass spectrometry reported in Table 4, most of them used QqQ. In fact, only one applied conventional ion trap to determine aminoglycoside residues but the other selected antibacterials were quantified by QqQ. The study does not compare the performance of both mass analyzers. However, detection limits obtained by ion trap are an average of 50 times higher than those reported by QqQ [120]. This study also compares three rapid antimicrobial screening tests with LC–MS/MS. One of the first highlighted information was that the great majority of carcasses sampled (196 out of 235) did not contain any antibiotic residues by LC–MS/MS. A potential point this work considered was that the positive responses provided by the rapid screening tests but not confirmed by LC–MS/MS can be caused by an uninvestigated antibiotic or metabolite. While the list of antibiotics included in the LC–MS/MS analysis is quite lengthy, inclusion of all antibiotics known to exist is not a practical option. Although the above issues should aware, LC–MS/MS remains the standard for antibiotic residue analysis and provides a significant advantage for identification and quantitation of samples containing several antibacterials, as mixtures can be problematic to interpret with microbial inhibition screening assays.

Although, there are still few procedures suitable for analyzing compounds from unrelated classes of drugs, these procedures have become realistic. One of the advances that have made these analyses possible has been the development of fast-switching QqQ-MS/MS and QqLIT instruments, which significantly increase the number of transitions to be simultaneously acquired avoiding the use of different retention time windows. Because of its speed, the combination of UPLC and fast-switching QqQ-MS/MS is gaining considerable attention bringing a substantial saving in time (a factor of 2–8), which was needed for method optimization [123–125]. LC–MS/MS has been successfully applied for simultaneous analysis of veterinary drug residues, spanning different drug classes, such as non-steroidal anti-inflammatory drugs, corticosteroids, anabolic

steroids, β-lactams, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, nitroimidazoles, benzimidazoles, levamisole, avermectins, tranquillisers, trimethoprim, ionophores and dinitrocarbanilide in animal tissues [116,118–120,125,126,135,136], milk [115] and honey [121]. Within this field as well as within each field of residues and contaminants, there is a clear trend toward the use of multianalyte methods. Consequently, the next step forward would be to develop more generic methods to cover any type of residues and contaminants. As a first step, Mol et al. [135] compared four existing multianalyte procedures and three newly proposed methods for a test set of 172 pesticides, 82 veterinary drugs and 36 natural toxins spiked to different feed and food matrices. The extraction procedures involved extraction/dilution of the sample with water and an acidified organic solvent (methanol, acetonitrile, or acetone) and the determination was performed by UPLC–MS/MS. Application of the method in routine monitoring programs would imply a drastic reduction of effort and time.

An elegant solution for throughput increase using conventional LC and QqLIT, is automation and on-line combination of the extraction procedure. An interesting example reported a simple and inexpensive automatic procedure for rapid on-line assessment of β-lactam antibiotics, including six penicillins and four cephalosporins, in bovine milk samples as an alternative to the presently available methods, which are either time consuming or require costly instrumentation [122]. Target compounds were concentrated from 500 μl of centrifuged milk samples using an on-line SPE procedure with C18 HD cartridges. Target analytes were eluted with a gradient mobile phase (water +0.1% formic acid/methanol +0.1% formic acid) at a flow rate of 0.7 ml min^{-1}. Chromatographic separation was achieved within 10 min using a C_{12} reversed phase analytical column. These automatic methods are applicable and deemed necessary within the field of food control and safety.

The use of full-scan MS approaches (e.g. TOF, LTQ-FTICR or orbitrap) has also strongly emerged as attractive alternative for these analyses. The most common approach favored is TOF-MS. In one run, such multi-residue analysis can deal with more than 100 compounds but only by using full-scan MS techniques (e.g. TOF-MS). Several examples have already been published with UPLC–TOF-MS probably being the more powerful measurement tool. Starting with meat, fish, milk, eggs or honey, all relevant veterinary drugs – irrespective of the class to which they belong – can be detected in analysis [128]. Furthermore, this system combines high resolution for both LC and MS with high mass accuracy, which is very powerful for the multi-compound analysis of veterinary drugs. The technique seems to be powerful enough for the analysis of not only veterinary drugs but also organic contaminants like pesticides, mycotoxins and plant toxins in one single method [135].

The use of these full-scan MS approaches for non-target analysis seems also very meaningful within the field of veterinary drugs and growth promoting compounds. For instance, the feasibility of coupling the simultaneous screening of several fluoroquinolones using a dual surface plasmon resonance biosensor immunoassay, in parallel, with LC–TOF-MS for their identification. Six fluoroquinolones were simultaneously screened at or below their MRL in chicken muscle using a double or triple chip [131,132]. The samples non-compliant during the screening with the dual biosensor were further concentrated and fractionated with gradient LC. The effluent was splitted toward two 96-well fraction collectors resulting in two identical 96-well plates. One was re-screened with the dual biosensor to identify the immunoactive fractions and direct the identification efforts toward the relevant fractions in the second well plate with high resolution LC–TOF-MS. The system not only showed possibilities to screen and identify known fluoroquinolones, but also the potential for discovering and identifying unknown compounds.

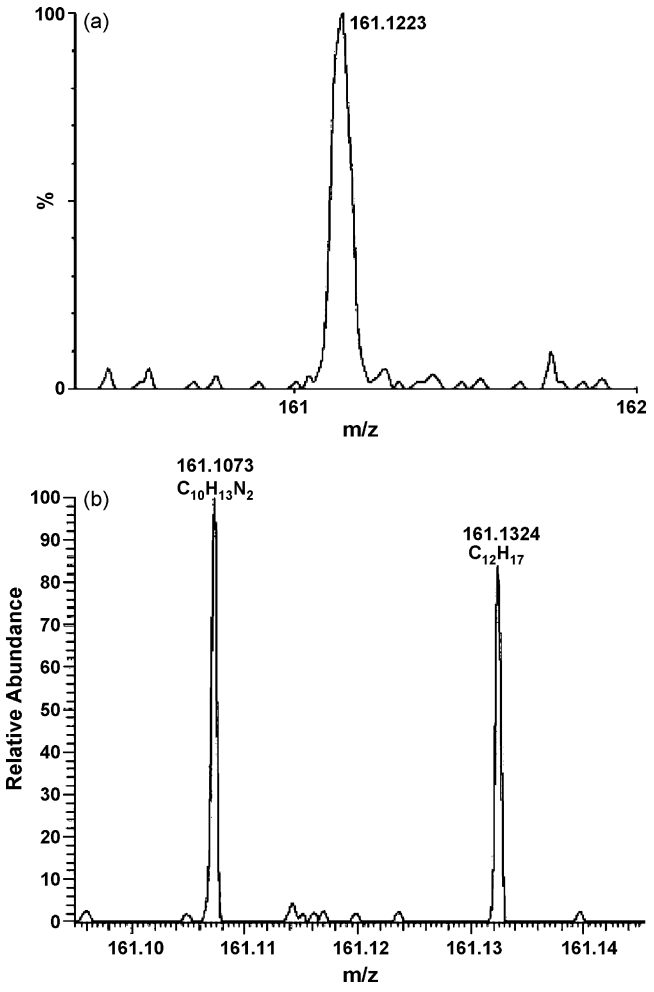

Fig. 4. Characteristic details of (a) the QTOF-MS/MS and (b) LTQMS2/FTICR product ion mass spectra of stanozolol representing one example of the doublet product ions. Reproduced with permission from [133] copyright © 2006 Elsevier B.V.

The application of higher mass resolving power mass analyzers for the determination of hormones and veterinary drugs also envisage their potential and possibilities to identify non-target analytes. Accurate mass alternatives such as LC–LTQ-FTICR-MS or LTQ-orbitrap are an emerging trend within this field [133,134]. Nielen et al. [133] discussed mass resolution and accuracy for LC–MS screening and confirmation of targeted analytes and for the identification of unknowns using the anabolic steroid stanozolol and the designer β-agonist "Clenbuterol-R" as model substances. Thanks to the exceptional mass resolution of LTQ-orbitrap-MS, the origin of strange accurate masses in the QqTOF–MS/MS analysis of stanozolol became soon apparent: as can be seen in Fig. 4b most of the product ions of stanozolol are actually doublets of distinct product ions having only minor exact mass differences. In LTQ-FTICR and in LTQ-Orbitrap-MS these doublets are easily resolved but they will overlap at least partially at 5000 (FWHM) mass resolution for *m*/*z* 161, as shown by the QqTOF-MS data in Fig. 4a. As a result, an artificial single product ion is obtained showing an accurate but wrong average mass value upon mass measurement. This will be a typical result for bench-top QqTOF-MS instruments. The applicability of UPLC combined with full-scan accurate mass TOF and LTQ-Orbitrap-MS to the analysis of hormone and veterinary drug residues was also evaluated [117]. UPLC–LTQ-Orbitrap-MS performed at a resolving power of 60,000 (FWHM) enabled the detection and accurate mass measurement (<3 ppm error) of all 14 steroid esters at low ng g^{-1} concentration level,

Table 5
Selected LC–MS procedures to determine natural toxins in food.

Analytes	Matrix	Extraction method	*R* (%)	LC–MS	LOQ (ng g^{-1})	Comments	Reference
Mycotoxins							
8-prenylnaringenin, zearalenone, -zearalenol and β-zearalenol	Beer	Dilution with water (1:1, v/v).	72.8–107	LC–MS	0.8–38.6	Quantitative and confirmatory method	[139]
Fusarium mycotoxins, beauvericin and enniatins (A, A1, B, B1)	Eggs	Acetonitrile extraction and silice SPE. Sample passed through the cartridge was collected onal acetonitrile rinse	48–79	LC–QqQ-MS/MS	0.01–22.4	Quantitative and confirmatory method High throughput determination	[140]
Aflatoxins (AFs) B1, B2, G1, and G2	Olive oil	MSPD with C_{18} and analytes elution with methanol:water (80:20, v/v)	92–107	LC–QqQ-MS/MS	0.04–0.12	Quantitative and confirmatory method	[141]
10 fusariotoxins	Wheat, maize	Extraction with acetonitrile–water (84:16; v/v). Optional clean ups (i) through the trichothecene EP column or (ii) with C18 cartridges and then, the fusariotoxins were eluted with methanol	9–90	LC–QqQ-MS/MS	0.2	Quantitative Confirmatory High throughput determination	[142]
31 selected Aspergillus, Fusarium, Penicillium, and Claviceps mycotoxins	Wheat, barley, oats	ASE using 90% acetonitrile at 100 °C and 1500 psi (10.3 MPa)	17–106	LC–QqQ-MS/MS	1–10	Quantitative and confirmatory method High throughput determination	[143]
Multi-mycotoxin, trichothecenes, aflatoxins, *Alternaria* toxins, fumonisins, ochratoxin A, zearalenone, beauvericin, sterigmatocystin	Sweet pepper	Ethyl acetate/formic acid (99:1, v/v) and clean up by aminopropyl column followed by an C_{18} column	–	LC–QqQ-MS/MS	0.32	Quantitative and confirmatory method High throughput determination	[144]
Multi-mycotoxin	Moldy food samples	Rotary extraction with acetonitrile/water/acetic acid 20:79:1, v/v/v)	–	LC–QqQ-MS/MS	0.02	Quantitative and confirmatory method High throughput determination	[145]
Enniatin H, I, and MK1688 and beauvericin	Cultures in cereal substrates: rice, barley, maize, wheat, and Indian millet kernels	Acetonitrile/methanol/water (16:3:1, v/v/v). The extract was defatted with heptanes. The bottom layer was evaporated to dryness. The residue was dissolved in methanol:water (55:45, v/v) and re extracted with CH_2Cl_2.	–	LC–IT-MS/MS	–	Structural elucidation Determination of optimal conditions for the production of toxins on maize by *F. oxysporum* KFFC 11363P and other *F. oxysporum* stains	[146]
Method applicable to 68 multi-mycotoxins and ergot alkaloids	Bread, fruits, vegetables, cheeses, nuts, jam	Acetonitrile/water/acetic acid (79 + 20 + 1 v/v)	–	LC–QLT-MS/MS	1–160	Screening, semiquantitative and confirmatory method	[147]‘
12 mycotoxins Deoxynivalenol, Aflatoxins B1, B2, G1, G2 and M1, Fumonisins B1 and B2, Ochratoxin A, HT-2 and T-2 toxin, Zearalenone	Cereals	Acetonitrile/water (80:20)	70–108	UPLC–QqQ-MS/MS	0.03–6.30	Quantitative and confirmatory method High throughput determination	[148]

17 kinds of *Aspergillus*, *Fusarium* and *Penicillium* mycotoxins	Baby foods feed stuffs	SPE clean up with Mycosep 226 Aflazon + Multifunctional cartridges Analytes' elution with acetonitrile	71–119	UPLC–QqQ-MS/MS	0.01–0.70	Quantitative Used double sample injection in positive and negative ionization	[149]
11 mycotoxins	Maize kernels, dry pasta (wheat), and eight-multicereal baby food	Acetonitrile/water (80:20, v/v) 0.1% HCOOH and, after a two-fold dilution with water	70–110	UPLC–QqQ-MS/MS	0.5–20	Quantitative and confirmatory method High throughput determination	[150]
Aflatoxin-B1 Ochratoxin	Fungal cultures and dried figs	Ethyl acetate	–	LC–TOF-MS	25 in culture media	Quantitative and confirmatory method High throughput determination	[151]
Several mycotoxins and other food contaminants	Fruits and vegetables	Acetonitrile 1% acetic acid	45–100	LC–TOF-MS	1.0–10	Detect multiple food contaminants Generic extraction methods	[135]
Multi-mycotoxin	Wheat and maize	Acetonitrile/water/acetic acid, 79:20:1, v/v/v	71–112	LC–QqQ-MS/MS μLC–Orbitrap–MS/MS	1.0	Quantitative and confirmatory method High throughput determination Compares two different LC–MS platforms	[152]
Biotransformation products of zearalenone	Plant *Arabidopsis thaliana*	Acetonitrile	80–87	LC–QqQ-MS/MS	1–50	Discuss the occurrence of masked mycotoxins in food	[18]
Phycotoxins							
Azaspiracids	Shellfish	Methanol	–	LC–QqQ-MS/MS	–	Outlines a rugged LC–MS/MS methods for the detection of Azaspiracids in shellfish Compares with the mouse assay	[153]
Azaspiracids	Mussels	Extracted with methanol and ultrafiltrated through a Microcon YM-3	–	LC–QqQ-MS/MS	–	Explain increased Azaspiracid3 concentrations upon heating of fresh mussel tissues	[154]
Azaspiracids	Mussels	90% aqueous methanol extraction	–	LC–QqQ-MS/MS	–	Confirmation of Azaspiracid2 as a dominant compounds Total levels of these toxins ranged from 1.6 to 6.1 $\mu g\,kg^{-1}$.	[155]
Microcystins	Cyanobacteria, Spirulina food products	Extracted with 75% (v/v) methanol in water, SPE on ODS cartridge and elution with methanol	–	LC–QqQ-MS/MS	0.5	34 samples (94%) contained microcystins ranging from 2 to 163 $ng\,g^{-1}$	[156]
Tetradotoxin	Marine gastropod	Boiling with acetic acid and water, SPE on Sep-Pak C18 and analyte elution with acidified methanol	–	LC–IT-MS/MS	100	Identification of tetradotoxin A moderate amount of tetradotoxin was detected in the Nassarius	[157]
Tetradotoxin	Marine gastropod	Boiling with acetic acid and water, SPE on Sep-Pak plus C18 and analyte elution with acidified methanol	–	LC–QqLIT-MS/MS	–	Contained tetradotoxin 42–60 $mg\,g^{-1}$, whereas along with minor PSP it was 3–6 $mg\,g^{-1}$. MS^3 experiments	[158]
Domonoic acid	Shellfish	PLE with methanol/acetone (9:1), florisil clean up purification inside the PLE extraction cell	81–95	LC–QqQ-MS/MS	0.2	Suitable method for regulatory use	[159]
Odakaic acid, dinophysistoxin-2	Mussels	Methanol extraction	–	LC–QqQ-MS/MS	–	Studies the cooking effect on the toxins' concentration	[160]

Table 5 (*Continued*)

Analytes	Matrix	Extraction method	*R* (%)	LC–MS	LOQ (ng g^{-1})	Comments	Reference
Odakaic acid, dinophysistoxin-2s, pectenotoxins, azaspiracids, spirolides, gymnodimines	French shellfish	Acetone/methanol and partitioned with Cl_2CH_2. Additional hydrolysis step to determine dinophysistoxin-2s and odakaic acid	Not reported	LC–QqQ-MS/MS	–	Reports on the first recorded occurrence of PTX-2, spirolide-A and their isomers in French shellfish.	[161]
Odakaic acid, dinophysistoxin-2s, pectenotoxins, azaspiracids, yessotoxins, spirolides, gymnodimines	French shellfish	Acetone/methanol and partitioned with Cl_2CH_2. Additional hydrolysis step to determine dinophysistoxin-2s and odakaic acid	–	LC–QqQ-MS/MS	–	Azaspiracids, odakaic acid and its acylesterderivatives (dinophysistoxin) were detected	[162]
Lipophilic marine biotoxins	Mussels and processed shellfish	Methanol. Then, optional hydrolysis using NaOH and heating in a heat block to 76 °C for 40 min	22–99	LC–QqLIT-MS/MS	1.0	Determine hydrolyzed and non-hydrolyzed extracts Possible decrease of regulatory limits	[163]
26 phycotoxins spirolide, yessotoxins, pectenotoxins	Mussels	Methanol acetone	–	LC–QqQ-MS/MS	–	Mussels accumulated the three major spirolides, YTX and pectenotoxin-2 seco acid	[164]
Alkaloids							
Pyrrolizidine alkaloids and their N-oxides	Honey	Methanol or diluted sulfonic acid and then SCX-SPE	–	LC–IT-MS	1	Validates against standard pyrrolizidine alkaloids and their N-oxides Supports a need for a more widespread survey of honeys.	[165]
Pyrrolizidine alkaloids (lycopsamine, echimidine and lasiocarpine)	Comfrey	Dynamic PLE using methanol-water (50:50, v/v)	–	LC–IT-MS^n LC-Orbitrap-MS^n	0.5	Quantitative lycopsamine, symviridine and their N-oxides were confirmed MS^3 experiments	[166]
Ergot alkaloids	Cereal samples	Acetonitrile/$(NH4)_2CO_3$ and PSA	94–96	LC–QqQ-MS/MS	0.2	Storage over a period of 14 days at 4 °C resulted in significant epimerization	[167]
Ergot alkaloids	Cereal products	CH_2Cl_2/ethyl acetate/methanol/NH_3 (50:25:5:1, v/v/v/v)	62–97	LC–QqQ-MS/MS	0.1–1.0	Among 66 examined products, rye breads showed the highest percentage of samples with ergotalkaloids	[168]

despite the complex matrix background. A 5 ppm mass tolerance window proved to be essential to generate highly selective reconstructed ion chromatograms, having reduced background from the hair matrix. UPLC–Orbitrap-MS at a lower resolving power of 7500 and UPLC–TOF-MS at mass resolving power 10,000 failed both to detect all of the steroid esters in hair extracts owing to the inability to mass resolve analyte ions from co-eluting isobaric matrix compounds. All these systems showed promising possibilities to be applied in non-target analysis. Much new research was presented, and much more remains to be done because their capabilities for unknown identification have only been demonstrated in target analysis.

3.3. Natural toxins

A natural toxin can be defined as a substance that is synthesized by a plant specie, an animal, or by microorganisms, that is harmful to another organism. In this way, microorganisms are the primary cause of food spoilage and foodborne illness. The ability to detect their toxins is imperative to ensure the safety and quality of our food supplies. Although LC–MS offers interesting prospects for the characterization and detection of these toxins [137,138], to our knowledge, applications within food safety have not been reported yet. Effective food control and monitoring for different groups of mycotoxins, marine biotoxins and, most recently, as emerging contaminants, alkaloid biotoxins have already been achieved by LC–MS. However, this detector still shares screen with the UV–visible and fluorescence detectors and methods are less evolved than those for pesticide, veterinary drugs or growth promoter residues and they are mainly restricted to the determination of one or a few members of similar chemical structure. Table 5 summarizes the most recent methods reported for the determination of these natural toxins distinguished into mycotoxins, phycotoxins and alkaloids. There are two common trends within these groups: the development of multi-class toxin methods and the application to structural characterization and elucidation of toxins and degradation products from cultures.

There are some reviews on the mycotoxins [23,46,169–175], but only those by Turner et al. [170], Cigic and Prosen [171], Shephard [46] and Hoerger et al. [175] dealt with analyzing methods for determination and sample preparation published in the last 10 years for the most often encountered mycotoxins in different samples, mainly in food. Mycotoxins represent a quite wide spectrum of chemical compounds as a result of the numerous species of fungi responsible of their production. The introduction of LC–MS instrumentation has made possible the development of multi-toxin methods suitable for a range of structurally diverse toxins in a single chromatographic run. The need for such multi-toxin techniques lies in the fact that a single fungal specie can produce different toxins and that a single agricultural commodity can be contaminated with different fungal species resulting in the co-occurrence of a number of different toxins. The main instrument used is the QqQ coupled with LC. With this system, multi-toxin methods have been developed for the simultaneous determination up to 86 analytes including trichothecenes (nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, neosolaniol, fusarenon-X, diacetoxyscirpenol, HT-2 toxin, T-2 toxin), aflatoxins (aflatoxin-B1, aflatoxin-B2, aflatoxin-G1 and aflatoxin-G2), Alternaria toxins (alternariol, alternariol methyl ether and altenuene), fumonisins (fumonisin-B1, fumonisin-B2 and fumonisin-B3), ochratoxin A, zearalenone, beauvericin and sterigmatocystin in sweet pepper, eggs, olive oil, cereals, fruit, vegetables, cheese, nuts, jam and baby foods [140–145]. The UPLC coupled with QqQ-MS/MS has also gain popularity because it can resolve the analysis in up to 15 min [148–150] (as an example, Supplementary Fig. 3S shows a chromatogram from the study of Garrido-Frenich et al. [148]).

LC–TOF-MS has been much less applied in the field of mycotoxins than LC–QqQ-MS/MS, probably because the low concentrations, at which these contaminants are present in food, make the difference in sensitivity crucial for a reliable analysis. However, Senyuva et al. [151] developed a LC/TOF-MS method for profiling fungal metabolites based on the correct identification of mycotoxins by searching a specially constructed database of 465 secondary metabolites. Levels of these metabolites could be monitored daily in sterilized figs. In the same way, a generic extraction method compatible with UPLC–TOF-MS was proposed for the determination of several organic contaminants, including mycotoxins [135]. The limits of detection were between <0.01 and 0.05 mg kg^{-1} and in most cases low enough to verify compliance of products with the legal tolerances.

As an interesting work that represents the culmination of the different applications of LC–MS to these compounds, Herebian et al. [152] compared two different LC–MS platforms for the analysis of mycotoxins in wheat and maize. The first one was based on LC–MS/MS technique using a QqQ operating in SRM mode with two transitions monitored for each compound. Recoveries obtained for target analytes ranged from 73 to 152% for maize and from 87 to 131% for wheat. The validation results demonstrated high sensitivity (LOD and LOQ), repeatability and linearity of the above mentioned method. The second platform was based on microcapillary (μ) LC–LTQ-Orbitrap. The application of this method in full-scan mode is a time-saving method allowing rapid identification and quantification of mycotoxins at the very low picogram level. As a disadvantage, only positive ionization was applicable to μLC–LTQ-Orbitrap, resulting in the detection of only 30 out of 32 compounds. This study demonstrates that the LTQ-Orbitrap also provides a powerful platform for the quantitative analysis of mycotoxins on account of its high sensitivity and selectivity as well as for generating metabolic fingerprints within a few minutes.

LC–MS is also used to determine the reduction of mycotoxins' concentrations and the structures of its-transformation products by microorganisms. These studies used cultures and mycotoxins at high concentrations that are not related with those that can be in food. In this way, microbes from fish guts, capable of transforming trichothecenes into less toxic compounds, were screened for their ability to transform 4-deoxynivalenol [176] and the ability of chicken intestinal microbes to degrade 12 trichothecenes has also been assessed [177]. These studies, even through not performed in food, provide interesting information to assess safety aspects.

Although in marine toxins progress has been slow, there has been an explosion of technical options in the past few years, which make the outlook promising in terms of future developments as reviewed by Botana et al. [178]. These toxins are produced mainly by the marine dionoflagellates. However, the shellfish, especially, mussels, scallops, oysters and clams, accumulate very large amounts of toxins in their digestive tracts, so marine toxins can be a food safety threat of great relevance, as the toxins may appear at any time of the year and anywhere in the world. This may include amnesic, diarrheic, paralytic and neurotoxic shellfish poisonings and azaspiracid shellfish poisoning. The accumulation of toxins in shellfish and fish has led to serious human toxic poisoning. LC–MS/MS has been recognized as the most suitable technique for the identification of the phytotoxins including domonoic acid [159], azaspiracids [153–155], okadaic acid, dinophysistoxin-2, microcystins and tetradotoxin [156–158]. Both, the limited availability of pure analytical standards of phycotoxins and the co-occurrence in shellfish of various types of these toxins complicate their determination. Table 5 demonstrated that for most of the methods recoveries and detection limits are not even reported. As with the previous group of mycotoxins, there are several interesting multi-

Table 6
Selected LC–MS procedures to determine environmental contaminants, contaminants in food processing and contaminants from packaging materials in food.

Analytes	Matrix	Extraction method	*R* (%)	LC–MS	LOQ (ng g^{-1})	Comments	Reference
Environmental contaminants							
Hexabromocyclododecane	Human breast milk	PLE with CH_2Cl_2/hexane (2:1), temperature of 100 °C, pressure of 14 MPa, two static extractions of 5 min	–	LC–QqLIT-MS/MS	–	Enantiomeric patterns are studied	[26]
PCFs	Edible fish	Liquid extraction with methyl tert-butyl ether (MTBE)	–	LC–QqQ-MS/MS	5	Confirmatory studies the accumulation of PCFs in different parts of the fish	[179]
PFCs	Raw and cooked foodstuffs	35% methanol and cleaned up clean up with Envi-Carb	–	LC–QqQ-MS/MS	0.2	Confirmatory Quantitative and applicable to packaged food	[25]
FFCs	Fish, seafood, meat, poultry, frozen entrees, fast food, microwave popcorn	Methanol extraction	64–120	LC–QqQ-MS/MS	2–6	Confirmatory and quantitative Full diet study	[180]
PFCs	Fish	PLE with water at 90 °C and 14 MPa and SPE on Oasis Wax. The PFCs were eluted with NH_4OH in methanol	85	LC–QqLIT-MS	0.003–0.05	Confirmatory Quantitative for the analysis of different parts of several fish species	[24]
C_{60} and C_{70} fullerenes and N-methylfulleropyrrolidine C_{60}	Drinking water	Water filtration and extraction of the filters with toluene	58–84	LC–QqLIT-MS	0.002–0.012	First report on the occurrence of fullerenes in water	[28]
Contaminants formed during food processing							
Heterocyclic amines	Meat extracts	PLE with methanol at 80 °C and 1500 psi, and three static cycles	45–79	LC–QqQ-MS/MS	0.2	Confirmatory, and quantitative, high extraction efficiency and sensitivity.	[33]
Heterocyclic amines	Griddled beef steak	CH_2Cl_2 and clean up with Bond Elut PRS SPE and analyte's elution with methanol–NH_3 (9:1).	5.5–61.9	LC–IT-Qq-MS/MS	0.15	Confirmatory and quantitative	[34]
Heterocyclic amines	Meat extracts	CH_2Cl_2 and clean up with a propylsulphonic acid on C18 cartridge. Analyte elution with methanol–NH_3 (9:1, v/v).	–	UPLC–QqQ-MS/MS	0.23 pg	Confirmatory, quantitative and analysis in 2 min possible	[35]
Acrylamide	Food stuffs (beef, chicken, biscuits, etc.)	Hexane and filtration through 0.45 μm syringe filter	–	LC–MS	–	Confirmatory and quantitative. Study the formation of acrylamide during cooking	[181]
Acrylamide	Roasted chestnuts and chestnut-based foods	Water and cleaned with multimode ENV+® SPE and eluted with methanol	–	LC–QqQ-MS/MS	4–9	Confirmatory, quantitative and study formation of acrylamide during roasting	[182]
Acrylamide	Spanish products, potato crisps, pastry products, sweet fritters ("*churros*") Spanish omelette	Homogenized with water, clean up with Strata-XC SPE and a ENV+ SPE and elution with MeOH:H_2O (60:40)	–	LC–Qq-IT-MS/MS or LC–QqQ-MS/MS	2–6	Confirmatory and quantitative	[183]
Acrylamide	Potato, coffee, cereals	Homogenized with water, clean up with ENV+ and elution with methanol	–	LC–QqQ-MS/MS	0.5	Confirmatory and quantitative	[184]
Acrylamide	Chinese traditional carbohydrate-rich foods	Ethyl acetate clean up with SPE with Bond Elut Accutat mixed mode SPE column consisting of a strong cation and strong anion exchanges into one bed and analyte elution with methanol	35.4–97.5	LC–QqQ-MS/MS	4	Confirmatory, quantitative $^{13}C_3$-labelled acrylamide internal standard solution	[185]

Acrylamide	Processes food (rice, bread, corn chips, potato chips)	C18 SPE and analyte elution with water	97–102	LC–QqQ-MS/MS	2	Confirmatory and quantitative	[186]
Acrylamide	Potato and cereal-based foods	0.01 mM acetic acid in a vortex mixer and clean up with Oasis MCX SPE	99.7	LC–APCI-MS	–	Confirmatory and quantitative	[187]
Ethyl carbamate	Korean soy sauce products	CH_2Cl_2 and Extrelut-NT20	82.7	LC–QqQ-MS/MS	0.5	Confirmatory, and quantitative method. Comparison with GC–MS proved that LC–MS method is better	[37]
Materials in contact with food							
Phthalates (DBP, BBP, DEHP, DINP and DIDP)	Milk and milk products	Hexane and MTBE and clean up with C_{18} and analyte elution with ethyl acetate in hexane	92–105	LC–QqQ-MS/MS	5–9	Confirmatory and quantitative	[41]
Primary aromatic amines	Aqueous food	Migration studies with 3% acetic acid (w/v) in distilled water	–	LC–QqQ-MS/MS	0.27–3	Confirmatory and quantitative	[188]
Alkylphenol and bisphenol A	Eggs and milk	Matrix solid-phase dispersion with C18 and a clean up step with aminopropyl SPE and analyte elution with CH_2Cl_2/hexane (50:50, v/v)	70–103	LC–QqQ-MS/MS	0.05–0.1	Confirmatory and quantitative Results in market samples	[189]
Bisphenol A	Milk	C18 SPE and eluted with methanol water	97–104	LC–MS	5	Sample preparation losses, the matrix effects and the instability of the LC–MS instrument are emphasized	[190]
Alkylphenol and bisphenol A	Meat	PLE with acetone, and subsequent clean up using aminopropyl SPE cartridges	89–101.3	LC–QqQ-MS/MS	0.40–1.0	Confirmatory and quantitative. Results in samples from the market	[191]
Bisphenol diglycidyl ether	Canned foods	PLE with hexane/acetone using two cycles of 5 min at 14 MPa at 100 °C, followed by liquid–liquid partition with hexane and acetonitrile and purification by SPE using C18 and aminopropyl bonded to silica (NH_2) combined and elution with acetonitrile/methanol (1:1, v/v)	82–101	LC–APCI-MS-MS	5	Confirmatory method for simultaneous analysis of BADGE, BFDGE and some derivates in solid canned food	[43]
Estrogens and bisphenol A	Milk	SPE using C_{30} material	71.4–97.1	LC–MS	0.05–0.30	A high speed and robust on-line SPE-HPLC–MS method	[192]
ITX and EHDBA	Milk	PLE extraction with ethyl acetate using two cycles at 100 °C of 5 min at 14 MPa	70–90	LC–QqQ-MS/MS	0.1	Confirmatory Quantitative and Comparison with GC–MS	[42]
ITX	Packaged food	SPE and elution with acetonitrile	85	LC–QqQ-MS/MS	0.8 pg	Confirmatory and quantitative	[6]
ITX	Milk (milk, soy milk, baby milk)	Sample preparation is limited to the addition of a deuterated ITX solution in acetonitrile that serves both as internal standard and to precipitate proteins	≈100	LC–QqQ-MS/MS	6	Confirmatory and quantitative. Isomeric analysis is possible	[193]
Cyanuric acid	Catfish, trout, tilapia, salmon shrimp	Hexane clean up with SPE (Envi-Carb) and analyte elution with methanol	67–91	LC–QqQ-MS/MS	3.5	Confirmatory and quantitative	[194]
Melamine and cyanuric acid	Animal feed	Aqueous formic acid	95–100	LC–QqQ-MS/MS	–	Confirmatory and quantitative	[195]
Melamine and cyanuric acid	Catfish, pork, chicken, pet food	Aqueous methanol, liquid–liquid extraction and cation exchange SPE clean up	–	LC–QqQ-MS/MS	10	Confirmatory and quantitative and labelled internal standards	[196]

Table 6 (*Continued*)

Analytes	Matrix	Extraction method	*R* (%)	LC–MS	LOQ ($ng\,g^{-1}$)	Comments	Reference
Melamine, ammeline, ammelide, cyanuric acid	Kidney tissue	Acetonitrile/water/diethyl amine (50:40:10, v/v/v)	103–120	LC–QqQ-MS/MS	50	Confirmatory and quantitative	[38]
Melamine	Catfish trout, tilapia, salmon, shrimp	Acidic acetonitrile, defatt with CCH_2Cl_2, and clean up using Oasis MCX SPE and analyte elution with NH_4OH in methanol	87.6–97.8	LC–QqQ-MS/MS	3.2	Method was validated for the determination of melamine in fish and shrimp muscle	[39]
Melamine	Milk-based products, food and beverage	Aqueous 1% trichloroacetic acid	79–110	LC–QqQ-MS/MS	0.01–0.1	Direct extraction without SPE clean up	[197]
Cyanuric acid	Catfish, trout, tilapia, salmon shrimp	Hexane clean up with SPE (Envi-Carb) and analyte, elution with methanol	67–91	LC–QqQ-MS/MS	3.5	Confirmatory and quantitative	[194]
Melamine, cyanuric acid	Animal feed	Aqueous formic acid	95–100	LC–QqQ-MS/MS	–	Confirmatory and quantitative	[195]
Melamine and cyanuric acid	Catfish, pork, chicken, pet food	Aqueous methanol and ion exchange SPE clean up	–	LC–QqQ-MS/MS	10	Confirmatory Quantitative. Application of labelled internal standards	[196]
Melamine	Catfish, trout, tilapia, salmon, shrimp	Acidic acetonitrile, defatted with CCH_2Cl_2, and cleaned up by Oasis MCX SPE and analyte elution with NH_4OH in methanol.	87.6–97.8	LC–QqQ-MS/MS	3.2	Method was validated for the determination of melamine in fish and shrimp muscle	[39]
Melamine	Milk-based products and other food and beverage	Aqueous 1% trichloroacetic acid	79–110	LC–QqQ-MS/MS	0.01–0.1	Direct extraction without SPE clean up	[197]
ITX	Packaged beverages	SPE Oasis HLB cartridges and elution with acetonitrile	97–103	LC–IT-MS/MS	0.5	Confirmatory and quantitative	[198]
ITX	Fruit juices	PLE with acetone/hexane (50:50) at 100 °C and 10.4 MPa for 5 min of static time, in one cycle, preheated for 2 min	68 and 73	LC–MS, LC–QqQ-MS/MS, LC–IT-MS/MS	0.05–0.78	Confirmatory, and quantitative. Spanish and Italian fruits juices were analyzed. Only LC–QqQ-MS/MS provided appropriate sensitivity	[199]

class, multi-toxins residues methods [161–164]. As a curiosity, the mouse bioassay was the accepted detection method in official monitoring of shellfish toxins, Hess et al. [153] directly compared the mouse bioassay and the LC–MS/MS test in two laboratories, and results suggest that the two tests can be considered equivalent in their effectiveness in implementing the current regulatory limit.

There is a growing interest in alkaloid biotoxins because they are widespread and can be found in food, feed and herbs, up to the point to be one of the targets of the European Food Safety Authority (EFSA). The data on tissue distribution and residue concentration in edible tissue, milk and eggs are scarce. Ergot, tropane and pyrrolidizine alkaloids are the most common. The first group can also be included in the group of mycotoxins, however, since their determination have emerged within the interest on alkaloids, they are included here. Methods based on GC–MS and LC–MS have been reported for the detection of pyrrolidizine alkaloids due to their sensitivity and specificity. However, GC–MS is not very suitable for the analysis of pyrrolidizine N-oxides due to their thermal instability that is the reason why LC–MS is the preferred method [165,166]. Liu et al. [166] applied LC-IT and LTQ-orbitrap-MS with electrospray ionization interface to determine pyrrolidizine alkaloids in comfrey showing that accurate identification and quantitation is possible with both mass analyzers. The danger of ergot alkaloids has been well-known for centuries. However, in the last years, ergot alkaloids in both raw cereals and cereal-based processed food extracts have been studied by LC–MS/MS [167,168].

3.4. Environmental contaminants, contaminants in food processing and materials in contact with food

This section groups three different groups of contaminants: the environmental ones, those formed in food processing and those coming from materials in contact with food, which have no connection among them. However, they are grouped in this part because there are similarities in the LC–MS/MS methods developed for their determination as outlined in Table 6.

The common point is that these compounds have been much less studied than the pesticides, veterinary drugs and natural toxins and the reported analytical methods are almost exclusively developed to determine a specific compound or a reduced number of closely related ones. In recent decades, pollutant compounds in the environment have been increasingly studied because of their toxicity and bioaccumulation. Hundreds of compounds have been identified, many of which have been qualified as priority by the USA-EPA and EU. The environment is subject to such excessive strains as farming, industry and densely populated areas, which inflict serious damage on the ecological balance. Among these compounds PFCs, hexabromocyclododecane (HBCD) or nanomaterials are the recently emerging problems. The most analyzed contaminants have been the PFCs and the state of the art within the field has been the subject of several reviews [200–203]. PFCs have been mainly analyzed by LC–MS/MS using QqQ and QqLIT instruments [24,25,179,180] (Supplementary Fig. 4S displays typical extracted ion chromatograms of seven PFCs from an extract of spiked anchovy muscle). On the contrary, determination of HBCD and nanomaterials in food is at the embryonic state. Only one study determined HBCD diastereoisomer levels in human breast milk from mothers of Spain and established the enantiomeric fractions in order to investigate potential selective enantiomeric enrichment in human bodies. An idea of the interest is shown by the data-HBCD was detected in 30 out of 33 human milk samples, at concentration levels ranging from 3 to 188 $ng\,g^{-1}$ [26]. Even more unknown is the situation with regards to nanomaterials, only one method using LC–QqLIT-MS has been reported for trace quantification of C_{60} and C_{70} fullerenes, and N-methylfulleropyrrolidine C_{60} in suspended water solids [28]. This work constitutes the first report on the occurrence of fullerenes in suspended solids of wastewater effluents highlighting the need of nanotechnologies residues assessment for risk evaluation of nanoparticles in the environment.

The contaminants formed during food processing may be present in food as a result of the various stages of its production, packaging, transport or holding. Some of the process contaminants that are of particular interest are heterocyclic amines, acrylamide and ethyl carbamate. The two first are the result of cooking practices and the third of fermentation processes. LC–MS/MS has been widely used as quantification technique for heterocyclic amines [33–35], and product ion scan mass spectra provided by the IT mass analyzer were used to confirm the identity of the analytes [34]. A fast new UPLC–ESI-MS/MS method for the determination of 16 HAs in food samples demonstrated that UPLC improves the quantitative response and shortens the analysis time by raising the flow rates to allow a higher sample throughput [35]. Acrylamide concentrations in a wide variety of processed foods have been analyzed by LC–MS/MS using a QqQ mass analyzer [182–186]. Interestingly, LC–IT-MS^n and TOF-MS were evaluated for use in the determination of acrylamide in typical Spanish food because using LC–MS/MS, in some potato samples an intense peak appeared near acrylamide, making it difficult to quantify, especially at low concentrations [183] (Supplementary Fig. 5S). GC/MS has been generally used for determining ethyl carbamate in alcoholic beverages. However, a sensitive and specific LC–MS/MS method was applied for the first time to quantitatively determine ethyl carbamate in traditional Korean soy sauce, which differs from alcoholic beverages in its complexity [37]. The developed method showed satisfactory validation parameters in terms of linearity, limit of detection, and accuracy. It had higher recovery and repeatability than the LC-fluorescence and GC–MS methods.

Food contact materials are all materials and articles intended to come into contact with foodstuffs, including packaging materials but also cutlery, dishes, processing machines, containers etc. Many types of materials can be used for food packing ranging from plastic to papers. The complex composition of plastics and coatings for cans (that can have additives to modify their properties) are the core of the problem. Monomers, plasticizers, antioxidants, inks, solvent varnishes and lakers are potential migrating substances.

One current example that deserves to be highlighted is the 2-isopropylthioxantone (ITX), which is an initiator of the UV curing. The first alert to the presence of this substance in several types of packed foods took place in September 2005, when the Italian authorities detected ITX in baby milk. Since then, ITX has been found in food samples in various European countries. Traditionally, ITX is analyzed by gas chromatography coupled to mass spectrometry (GC–MS). Liquid chromatography (LC) has also been used and most methods published in the literature for the determination of ITX in food samples used reversed phase columns (C_8 or C_{18}). An LC–MS method, which compares different mass analyzers – single quadrupole, IT, and QqQ – was developed for the quantitative determination of ITX in fruit juices [199]. This method gave detection limits of 3, 3, and 0.01 $\mu g\,l^{-1}$ and quantification limits of 10, 10, and 0.05 $\mu g\,l^{-1}$ using single quadrupole, IT, and QqQ, respectively. The sensitivity obtained by single quadrupole and IT are clearly insufficient to detect levels reported in food that were in the 0.05–0.78 $\mu g\,l^{-1}$ range.

Plasticizers such as Bisphenol A, aromatic amines and phtalates have also been analyzed by these methods. Sensitive LC–MS/MS determination of the phthalic acid esters DBP, BBP, DEHP, di-'isononyl' phthalate (DINP) and di-'isodecyl' phthalate (DIDP) has been achieved in milk and milk-based products [41]. Twenty primary aromatic amines were also simultaneously quantified in food simulants [188]. Bisphenol A, Bisphenol A diglycidyl ether (BADGE) and its reaction products with water and hydrochloric acid have recently been subjected to new regulations concern-

ing their migration from food packaging materials into foodstuff. LC–MS/MS detection enables the enforcement of the specific migration of 1 mg kg^{-1} for all BADGE derivatives as set by the regulatory body of the European Union. The developed methods were successfully used to monitor the contaminant exposure originating from different meats, eggs and milk [189–191].

Finally, melamine can be considered as a contaminant to include in any of the three groups. It can reach the food through environmental pollution, by migration from melamine poly-sulfonate used as superplasticizer and by illegal adding to food products in order to increase the apparent protein content. Melamine had not routinely been monitored in food, except in the context of plastic safety or insecticide residue. Following the 2008 health scare in China over powdered milk, new methods have proliferated for the analysis of melamine and its metabolites (ammeline, ammelide and cyanuric acid) in infant formulations, meat, animal feed, milk and other processed products based all of them on LC–MS/MS detection after hydrophilic interaction liquid chromatography (HILIC) separation [38,39,194–197].

4. Conclusions

The application of advanced LC–MS technologies to food contaminants and residues has attained the determination of a broader range of compounds with higher sensitivity, selectivity and specificity. Consequently, the application of LC–MS permitted more comprehensive assessment of food safety with the determination of food contaminants and residues at trace level. Detection and characterization of emerging food contaminants with demonstrated detrimental effects on human health is a major topic in modern Food Safety fully supported by the application of LC–MS/MS. One of the most important trends is to develop generic methods able to extract as many contaminants as possible and to detect all of them simultaneously. Generic procedures for the simultaneous extraction of various classes of pesticides, mycotoxins, plant toxins, and veterinary drugs in various matrixes have already envisaged its potential within this field.

Mainly two types of approaches are applied to control food safety—those that give nominal masses and perform MS/MS (QqQ or QqLIT) and those able to obtain accurate mass measurements – by MS or MS/MS – (TOF, QqTOF, orbitrap). Both are considered, as complementary tools to develop large-scale screening methods. Special features of accurate mass measurement approach are also useful in laboratory or field experiments on metabolism and degradation because their capacity to identify unexpected or unknown metabolites and degradation products of food contaminants and residues. New food safety applications showing the unambiguously confirmation of several metabolites of pesticides in fruits and the potential for discovering and identifying unknown compounds promisingly emerge. However, some limitations, such as the lack of identification libraries and the need of more evolved deconvolution softwares, have until the moment prevent a successful explosion of this challenging task.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.03.015.

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