I. INTRODUCTION

A. Occurrence of Chemical Contaminants in Food

In recent years, issues related to food safety have received growing attention as the stakes, rather economical or health-related, became increasingly important. During the past 20 years, several successive crises have alarmed consumers and hence led the appropriate bodies of administration to establish new control action plans to ensure the quality and safety of food-based products. Economic stakes and the necessity to protect consumers have urged the scientific community to acquire and apply the best analytical tools to ensure the absence of toxicity linked to such issues. During this same period, a parallel development was observed between the toxicity knowledge concerning humans and the evolution of the legislation at the food safety level. The term “food contaminants” was introduced and defined in regulation 315/93 as follows: “. . . any substance not intentionally added to food which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination. Extraneous matter, such as, for example, insect fragments, animal hair, etc., is not covered by this definition” (Council Regulation 315/93, 1993).

The development of intensive production (aquaculture, poultry) and treatment for both culture or disease prevention (prophylactic treatment, birth control, flock management) can lead to the presence of chemical residues at trace levels. Thus, an increasing vigilance is mandatory for food-related companies to ensure the quality of their raw materials.

Chemical contaminants that could be present in food-based products are from different chemical categories, such as: pesticides, mycotoxins, aquatic toxins, veterinary drug residues, heavy metals, component migration from packaging, growth hormones, and environmental or processing contaminants.

They can be present at all stages of the product development process either at the beginning in plants or soil (e.g., pesticides, mycotoxins, environmental contaminants), by cross-contamination (e.g., allergens), packaging (migrant components), and even at the cooking stage (e.g., acrylamide). Figure 1 shows the number of alerts within the European Union (EU) community related to the presence of some important chemical contaminants between 2002 and 2008.

B. Legislation and Regulation

1. Veterinary Drug Residues

Veterinary drug residues include a broad range of chemical substances, the main ones being the antibiotics represented by the following chemical families: aminoglycosides, β-lactams, macrolides, sulfonamides, tetracyclines, (fluoro)quinolones, and amphenicols. Anti-coccidiostatics such as nitroimidazoles and nitrofurans can also be observed at trace levels within some food products. Certain hormones or compounds possessing growth-promoting properties such as β-agonists, steroids, corticosteroids, and thyreostats are also included within this category. These compounds are monitored by the EU community in a well-defined manner (Council Directive 96/22/EC, 1996; Council Directive 96/23/EC, 1996). These regulations define maximum residue limits (MRLs) for veterinary drugs according to the specific food matrix. The Council Regulation 2377/90/EC (1990) divides the veterinary drugs residues, which could be potentially present in food-based products, into several different groups.

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Within this regulation, these chemicals are spread into four different annexes:

Annex I includes a list of pharmacologically active substances for which MRLs have been fixed.
Annex II includes a list of pharmacologically active substances that are not subjected to MRLs. These substances can then be used within food production but only in accordance with further specific regulations.
Annex III includes a list of pharmacologically active substances used in veterinary medicinal products for which provisional MRLs have been fixed.
Annex IV includes a list of pharmacologically active substances for which no MRL can be set in relation to their toxicological effects. Therefore, these compounds are totally forbidden for usage within food production.

The European Council Directive 96/23/EC (1996) lays down measures to monitor the substances in question and proposes the classification of some of these residues into two groups. Group A includes substances having anabolic effects and unauthorized substances (referring to Annex IV of Council Regulation 2377/90/ECC), whereas Group B includes a list of veterinary drugs, contaminants such as mycotoxins, dyes, organochlorines, etc. The main residues are summarized in Table 1.

2. Mycotoxins

Mycotoxins are of great concern to health scientists. Toxic fungal metabolites such as aflatoxins, trichotheeces, zearalenone, and others are mycotoxins which can be found in our environment and have been previously reported to induce various diseases (Ueno, 1985; Pitt, 2000). Mycotoxins are secondary metabolites produced by different fungi, including Fusarium, Aspergillus, and Penicillium. Aflatoxin B1 can be converted into aflatoxin M1 (reported in milk), as well as ochratoxin (OTA), zearalenone (ZON), fumonisins B1, T-2 toxin, HT-2 toxin, and deoxynivalenol (DON) (vomitoxin); these are the most frequent mycotoxins reported within Europe. Aflatoxins and ochratoxin A are known to be carcinogenic (Hussein & Brasel, 2001). Mycotoxins develop at high temperatures and humidity levels and may be present in many foodstuffs (Fig. 2).

Although the reported presence of mycotoxins dates back to the middle ages (ergotism), it was only in the 1960s with the discovery of aflatoxins that mycotoxins were considered a health risk to both humans and animals (Asao et al., 1963). Since then, regulations have evolved for these toxins present in food and feed, as it was estimated that 25% of the world’s crop production and 20% of crop production within the EU may be contaminated with mycotoxins (Fink-Gremmels, 1999). Industrialized countries are considered to be less exposed to such risk due to technical, climatic, and educational reasons. There are more than 100 countries in the world that have set specific limits for these compounds encompassing 13 different mycotoxins or groups of mycotoxins in food and feed. Compared to other continents, Europe has the most extensive and detailed regulations worldwide.

Maximum tolerance and guideline levels have been established for several mycotoxins including aflatoxins, OTA, DON, and ZON in different food and feed products (Commission Regulation (EC) No. 466/2001, 2001); whereas for the others, respective levels are still under discussion. Moreover, the distribution of the concentration of mycotoxins in foodstuffs is an important criterion to consider (homogenization of samples). This distribution can be heterogeneous, especially for the compounds produced from Aspergillus species (Champeil, Fourbet, & Dore, 2004). Detailed studies on the variability linked to sampling have been conducted by Whitaker (2006), resulting in a series of sampling plans for different mycotoxin—commodity combinations. Consequently, EU regulations recommend to work using relatively high quantities (>30 kg) of starting material to result in good homogenized samples (Commission Directive 98/53/EC, 1998), making the implementation of monitoring plans more difficult (Spanjer et al., 2006).

<table>
<thead>
<tr>
<th>Group A: Substances having anabolic effects and banned substances</th>
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<tbody>
<tr>
<td>(1) Stilbenes, stilbene derivatives, and their salt and esters</td>
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<tr>
<td>(2) Antithyroid agents</td>
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<tr>
<td>(3) Steroids</td>
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<tr>
<td>(4) Resorcylic acid lactones including zeranol</td>
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<tr>
<td>(5) β-agonists</td>
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<td>(6) Compounds included in annex IV of council regulation 2377/90/EC</td>
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<tr>
<th>Group B: Veterinary drugs (a) and contaminants</th>
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<tbody>
<tr>
<td>(1) Antibacterial substances, including sulphonamides, quinolones</td>
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<tr>
<td>(2) Other veterinary drugs</td>
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<tr>
<td>(a) Anthelmintics</td>
</tr>
<tr>
<td>(b) Anticoccidial, including nitroimidazoles</td>
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<tr>
<td>(c) Carbamates and pyrethroids</td>
</tr>
<tr>
<td>(d) Sedatives</td>
</tr>
<tr>
<td>(e) Non-steroidal anti-inflammatory drugs (NSAIDs)</td>
</tr>
<tr>
<td>(f) Other pharmacologically active substances</td>
</tr>
<tr>
<td>(3) Other substances and environmental contaminants</td>
</tr>
<tr>
<td>(a) Organochlorine compounds including PCBs</td>
</tr>
<tr>
<td>(b) Organophosphorous compounds</td>
</tr>
<tr>
<td>(c) Chemical elements</td>
</tr>
<tr>
<td>(d) Mycotoxins</td>
</tr>
<tr>
<td>(e) Dyes</td>
</tr>
<tr>
<td>(f) Others</td>
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</tbody>
</table>

(a) Including unlicensed substances which could be used for veterinary purposes.

FIGURE 2. Number of notifications related to mycotoxins in 2008 in different foodstuffs (2008 Annual Report of RASFF). Notifications refer to a food/feed on the market for which a risk has been identified that does not require rapid action (e.g., product no longer in the market).
3. Pesticides

The total number of pesticides reported by the EU is estimated to be in excess of 1,100. Their use is particularly spread for fruit and vegetable cultures. The use of pesticides for agricultural purposes can have harmful effects, in particular to the environment and human health after ingestion of contaminated products (Maroni, Fanetti, & Metruccio, 2006). Side effects such as neurological toxicity, dysfunction of the immune system, or endocrine and tumor apparition were observed in animals (Gammon et al., 2005). Moreover, a study carried out on newborns linked the appearance of some cancers to the agricultural use of pesticides (Walker et al., 2007). Growing public interest towards potential health risks caused by the presence of pesticides in foodstuffs has strongly modified the strategies linked to culture protection, with an emphasis on food quality and safety. In addition to the sensitivity and accuracy needed for valid quantification, an evident analytical requirement is necessary to provide reliable information for confirmation of the identity of the pesticide detected to avoid reporting false-positive results. Several guidelines consider analytic confirmation a required parameter before reporting positive findings. Thus, the European SANCO Document on Quality Control Procedures for Pesticide Residue Analysis (PRA) (2006) proposes confirmation principles that are mainly based on data obtained by gas chromatography (GC)–mass spectrometry (MS) analysis under electronic ionization (EI). However, regarding the use of other MS-based approaches (e.g., liquid chromatography (LC)–MS using API ionization, high-resolution MS, chemical ionization (CI)), no detailed mass spectrometric criteria have been currently reported for analyte confirmation that takes into consideration the PRA guidelines. Therefore, several analytical laboratories have implemented rules describing the identification and quantification of organic residues and contaminants in foodstuffs of animal origin (European Commission Decision 2002/657/EC).

Recently, the EU has established more than 17,000 MRLs for 133 pesticides according to the raw material and their needs in relation to human feeding (Council Directive 76/895/EEC, 2004; Council Directive 86/362/EEC, 2004). Moreover, specific regulations were set by the EU community regarding baby food products, as it was shown that early exposure to children can induce the development of chronic disease and serious dysfunction (Daniels, Olshan, & Savitz, 1997). Thus, two directives were issued by the EU Commission regulating pesticide residues in cereals based infant food and infant formulae (Commission Directive 2003/13/EC, 2003; Commission Directive 2003/14/EC, 2003), with strict requirements on the limit of detection (LOD) and quantification (LOQ) for the analytical methods used.

In the US, the Environmental Protection Agency (EPA) in collaboration with the Food and Drug Administration (FDA) Department and the US Department of Agriculture (USDA) is responsible for setting pesticide tolerance levels in food (www.epa.gov). The subsequent list of tolerances has been compiled and published within the Code of Federal Regulations (CFR), Chapter 40, Part 180.

4. Processing Contaminants

Processing contaminants are generated during such production steps as fermentation or thermal treatment. These components are primarily absent in the raw materials, and their formation occurs via chemical reactions between naturally present compounds and/or degradation products that occur during processing (e.g., acrylamide, furan, 3-methyl chloride propene diol) or are induced during certain cooking conditions (e.g., heterocyclic aromatic amines, acrylamide) (Studer, Blank, & Stadler, 2004). These compounds cannot be entirely removed, but appropriate selection of raw materials and optimization of processing conditions can greatly minimize their formation. The main compounds already described are: nitrosamines, polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, furan, advanced glycation end-products (AGEs), acrylamide, and chlorophenols. These compounds are suspected to be human carcinogens and therefore their monitoring is of high priority. There is still no harmonized legislation for these contaminants, but due to their toxicity, many analysts are focused focusing their efforts in developing highly sensitive and selective analytical methods.

5. Allergens

Allergens are substances that trigger or favor allergic reactions, meaning a set of unexpected or excessive reactions can occur due to a response from the immune system following a contact, an infection, ingestion, or inhalation. Ingestion of allergens can cause minor dermatologic effects such as redness or blistering, or fatal effects such as anaphylactic shock (Nieuwenhuizen & Lopata, 2005). To achieve a higher level of health protection for consumers and to guarantee their right to know the relevant information, EU legislation has recently been modified. Food products should ensure transparent labeling of ingredients classified as potential allergens. The EU Directive 2003/89/EC (2003) on the indication of ingredients in food requires food manufacturers to list 12 groups of potential allergens if they are used as ingredients in pre-packed foods, including alcoholic drinks, regardless of their quantity. Food products associated with allergens include cereals containing gluten, fish, crustaceans, eggs, peanuts, soya, milk, and dairy products including lactose, nuts, celery, mustard, sesame seed, and sulfites. They are responsible for over 90% of all known allergic reactions. The list of allergenic food ingredients included in the Annex of the Directive is being constantly re-examined and updated as required, on the basis of the most recent scientific knowledge.

6. Environmental Contaminants

Environmental contaminants are chemicals present in the environment that can be transferred into food and water supplies knowing that they may potentially have an impact on human health. Many different types of chemicals have already been reported as environmental contaminants such as lead, mercury, pesticides, phthalates, polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs) that include dioxins, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). Several developments in new regulations and regulatory methods have taken place in over the last few years that have had a considerable impact on water analysis (Richardson, 2007). The US EPA’s website is a good source for obtaining details on regulations and regulatory methods (http://www.epa.gov). Currently, there are primary drinking water regulations for 92 contaminants, including 11 disinfection
by-products (DBPs), 53 organic contaminants, 16 inorganic contaminants, 4 radionuclides, 7 microorganisms, and turbidity (www.epa.gov/safewater/contaminants).

II. ANALYTICAL STRATEGIES

Analytical parameters such as method performance, acceptance criteria, and analytical limits are defined and therefore met within the methods developed by the scientist. In comparison to official methods, the EU regulation 2002/657/EC used for veterinary drugs residues affords a high degree of flexibility, and the methods can be easily and quickly adapted in the case of an emergency crisis (Commission Decision 2002/657/EC, 2002). In addition, this specific EU regulation takes into account recent technical developments since the advent of LC–MS/MS. For substances that fall within Group A, the ambiguity created by the zero-level tolerance led to the introduction of the minimum performance level (MRPL). Obviously, the zero-level tolerance will be completely different according to the type of sample preparation and technique employed (e.g., enzyme-linked immunosorbent assay (ELISA) test or MS). Therefore, MRPLs represent the minimum amount of an analyte in a sample that has to be detected and confirmed. This limit is then the minimum requirement for the detection level of a method and needs to be taken into consideration when developing an analytical technique for a banned substance.

Implicitly, the analytical approach used needs to be confirmative especially when handling analytes at zero-level tolerance. Therefore, a combined approach using a first screening step (e.g., rapid test kits, biosensors) followed or not by the use of a second confirmatory method (e.g., LC–FLD, MS) can be envisioned. In this framework, the introduction of identification points (IPs) and the ion intensities ratio (MS) concept as additional requirements for confirmatory methods was defined.

A number of IPs are required according to the group category of the analyte. Indeed, a minimum of four IPs are necessary for Group A substances while at least three are mandatory for substances belonging to Group B. The number of IPs required depends on the analytical technique employed; however, MS, infrared (IR), and nuclear magnetic resonance (NMR) spectroscopy are the only techniques currently recognized as confirmatory ones. Table 2 summarizes the number of IPs required when employing MS, this is also dependent on the type of instrument and acquisition method used.

It is noteworthy to mention that for Group A substances, LC coupled to ultraviolet or fluorescence detection contributes to a maximum of one IP provided that the relevant criteria are fulfilled. Moreover, according to the relative ion intensities (ion ratios) observed from mass spectrometric detection, certain tolerance limits have also been introduced for confirmatory methods. This feature is based on the value of the ratio between two ions originating from the same targeted analyte (e.g., protonated or deprotonated species vs. adduct ions or obtained from in-source fragmentation ions, or acquired from separate tandem MS (MS/MS) acquisitions) (Commission Decision 2002/657/EC, 2002).

In 2003, the FDA proposed a guidance document intended to provide specific directives for the development, evaluation, and application of mass spectrometric methods for confirming the identity of animal drug residues (CVM Guidance Document No. 118: www.fda.gov/cvm). This guidance document describes different confirmatory procedures such as replicate samples, the need to have real incurred samples or an equivalent to demonstrate a zero false rate, etc. It also elaborates on confirmation criteria that slightly differ from the EU guidelines. For instance, a method’s limit of confirmation is defined as the concentration where the weakest diagnostic ion no longer appears at an acceptable signal-to-noise level or where the false-negative rate becomes excessive. Standard(s) comparison should be analyzed simultaneously with unknown extract samples and the preparation and batch sequence should be fully described. On the MS side, any of the following chromatograms may be used: total ion chromatogram (TIC); reconstructed ion chromatogram (RIC); all single ion chromatograms (from full scan, selected ion monitoring (SIM) or selected reaction monitoring (SRM) acquisition modes). Criteria such as signal-to-noise (S/N), acceptability range for retention time shifts are also described (i.e., <2% for GC–MS and <5% for LC–MS). Confirmation criteria vary depending on the technique used for mass spectral data acquisition. For example, the full scan mass spectrum of a chemical should include at least three structurally specific ions. The mass spectrum obtained from a suspect compound should visually match the spectrum obtained from a contemporaneous standard. In the SRM acquisition mode, if a precursor ion is selected, and only two structurally specific product ions are monitored in the third quadrupole, the relative abundance ratio of these ions should match those obtained from the analysis of standards within ±10%, and if three or more

<table>
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<th>TABLE 2. Relationship between MS techniques and IPs required</th>
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<tr>
<td>Low-resolution mass spectrometry (LRMS)</td>
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<tr>
<td>LRMS(^{c}) precursor ion</td>
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<tr>
<td>LRMS(^{d}) product ion</td>
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<tr>
<td>High-resolution mass spectrometry (HRMS)</td>
</tr>
<tr>
<td>HRMS(^{c}) precursor ion</td>
</tr>
<tr>
<td>HRMS(^{d}) product ion</td>
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</table>

Reproduced from Stolker and Brinkman (2005) with permission from Elsevier. Copyright 2005. HRMS is defined as having a resolving power above 10,000 (full width half mass, FWHM).
structurally specific ions are monitored, these ratios should be within ±20% of standards.

Method validation is a critical point of the analytical process and the different regulatory agencies have established several requirements concerning this to be fulfilled. The setting of different parameters to ensure selectivity (IPs), sensitivity (S/N, detection, and quantification limits) is defined in all guidelines. Regarding veterinary drug residues, decision limit (CCα) and detection capability (CCβ) have been introduced by the European regulation 2002/657/EC. For banned compounds, they represent, respectively, the limit at and above which it can be concluded with an error probability of α (α = 1%) that a sample is non-compliant and the lowest concentration at which a method is able to detect a truly contaminated sample with a statistical certainty of 1 − β (β = 5%). For MRL set compounds, CCα and CCβ are, respectively, the limit at and above which it can be concluded with an error probability of α (α = 5%) that a sample is non-compliant and the concentration at which the method is able to detect permitted limit concentrations with a statistical certainty of 1 − β (β = 5%) (Commission Regulation 2002/657/EC, 2002). The accuracy is measured through the repetition of n samples analyzed at three concentration levels (n = 6 replicates, each), representing 1, 1.5, and 2 times the MRPL value; or at 0.5, 1, and 1.5 times the MRL one. In addition, inter-laboratory tests are organized to evaluate the reliability of the analytical method.

Concerning the other types of chemical contaminants, there is no regulation that describes thoroughly the analytical performance required; therefore, most of the analysts base their method validation approach on the veterinary drugs regulation (Commission Regulation 2002/657/EC, 2002).

It is important to mention that inter-laboratory tests can be performed to ensure the quality of the validated method. In this frame, the Food Analysis Performance Assessment Scheme (FAPAS) is the largest and most comprehensive analytical chemistry proficiency testing scheme in food and regularly conducts inter-laboratory tests for different analytes in food, feed, and drink using different analytical approaches (www.fapas.com).

The choice of the analytical technique for monitoring trace residues of chemical contaminants is very important. Selectivity and sensitivity are the two main parameters to guide this choice. In case of monitoring a group of analytes having a defined MRL (e.g., Group B substances), speed will also be an important factor to assess for high-throughput screening approaches. Thus, reliable rapid test kits or biosensors can be used as a fast screening approach. Most of the time, the food raw materials that are assessed are free of contamination so that only a limited number of suspected positive samples will be re-analyzed using a confirmatory method. Regarding banned compounds (e.g., Group A compounds), both selectivity and sensitivity aspects are very important; therefore, an adequate and targeted method is a prerequisite to avoid false-negative results.

Nowadays, the current trend of analytical methods developed, using MS techniques consists of two distinct analytical strategies: either targeted towards banned compounds (one specific or several belonging to the same class of analytes) or the monitoring of multi-class contaminants. For the former approach, a stringent extraction protocol will be developed to provide high recovery, selectivity, and therefore sensitivity; whereas the latter one may suffer a little in the recovery extraction of multiple compounds which will anyway be compensated by enough sensitivity given by the MS detector. Within such a strategy, highly focused and targeted methods are devoted to ensure the absence of highly regulated contaminants, while maintaining a high throughput of analysis for monitoring the others.

### A. Dedicated Approach

This approach refers to the analysis of a single analyte or class of compounds. Here, the notion of selectivity is relevant at the detection stage but also during the sample preparation steps. Indeed, a suitable and sometimes stringent extraction procedure is mandatory to selectively extract the analyte(s) of interest from the sample matrix. According to the sensitivity level required, a confirmatory method could complete this analytical scheme. Numerous review articles have reported the main sample preparation strategies adopted for the monitoring of chemical contaminants residues in food (Stolker & Brinkman, 2005; Baggiani, Anfossi, & Giovannoli, 2007; Ridgway, Lalljie, & Smith, 2007). The most widespread techniques involve liquid–liquid extraction (LLE), solid-phase extraction (SPE) (Picó et al., 2007a), and sorptive extractions such as solid-phase micro-extraction (SPME) (Beltran, Lopez, & Hernandez, 2000).

Within such approaches, quantification is performed, when possible, using the isotope dilution MS approach. Addition of a stable-isotopically labeled internal standard at the beginning of the extraction enables the control of potential variation that can occur during sample preparation (Bakhtiar & Majumdar, 2007); so that an external calibration curve built in solvent can be used (limiting the time spent by the analyst to build a matrix-matched calibration curve). An internal standard should ideally be an isotopically labeled analogue (2H, 13C) that displays similar behavior (extraction, chromatography, ionization, ion suppression/enhancement) as the monitored analyte(s). However, such approaches require specific methods to be developed and validated for each analyte or class of compounds.

### B. Multi-Screening Approach

Considering the increasing numbers of analytes to monitor, multi-screening approaches represent an elegant, high-throughput alternative even if highly challenging for the chemist. Indeed, such methodologies allow several analytes from distinct classes of compounds to be monitored during one run, therefore fitting well with an overall strong financial push of reducing the turn around time (TAT). Up until now, most of these approaches have been applied to pesticide analysis (Alder et al., 2006) in different food matrices (Lehotay et al., 2005; Pang et al., 2006). In addition, the development of mycotoxin multi-residue analysis is increasing as Fusarium, Penicillium, and Aspergillus fungi produce numerous mycotoxins frequently belonging to different toxin classes (Miller & Trenholm, 1994). The number of multi-toxin LC–MS methods is still limited, but methods to monitor various mycotoxins and their metabolites, respectively, in bovine milk (n = 18) (Sorensen & Elbaek, 2005) and in cereals (n = 90) (Berthiller et al., 2007) have been reported. Such approaches are also limited in the veterinary drug analysis field, but only a few multi-class methodologies have been reported in honey from various geographical origins and flower types (n = 42) (Hammel
et al., 2008) and in various meat samples \( (n = 130) \) (Yamada et al., 2006).

However, within our current knowledge, no attempt has been made to develop a multi-contaminant (e.g., pesticides, veterinary drugs, mycotoxins) approach within a single food matrix, mainly due to several constraints related to the different physico-chemical properties of each category of contaminants (e.g., each having a different extraction efficiency, etc.). Such an approach would represent a powerful tool in residue analysis as it will lead to a significantly reduced TAT, therefore giving more time to build trends of occurrence for distinct contaminants/food matrices.

### C. Non-Confirmatory Methods: Qualitative, Semi-Quantitative Approaches

Chemical contaminant residues in complex food matrices can be monitored by several analytical techniques depending on the sensitivity and specificity required. Thus, any well-equipped laboratory should use a broad range of analytical techniques starting from the qualitative and semi-quantitative rapid screening tests, to chromatographic methods coupled to different detectors and finally up to the most sophisticated and expensive instrumentation. However, the EU regulation for the monitoring of veterinary drug residues mentions that only nuclear magnetic resonance (NMR), IR spectroscopy, and MS can be recognized as confirmatory techniques (Commission Decision 2002/657/EC, 2002). Nevertheless, the use of rapid test kits and non-mass spectrometric coupling techniques are widely used for monitoring chemical residues in food contaminants and should be further discussed.

#### 1. Rapid Test Kits

Various rapid test kits are available for the monitoring of chemical contaminants within food. Those tests are based on different mechanisms such as inhibition, competition, immunochromistry, or receptor type. The analyte is detected via a distinct mechanism for each test and their presence is determined by a “change of color” indicator (Fig. 3).

![FIGURE 3. Tetracycline ROSA® strip test results reading (www.charm.com). Visual comparison test line (T) to the control line (C). Positive (C) line is darker than (T) line; negative (T) line is darker or equal to (C) line.](image)

For example, inhibition tests contain among other things bacterial spores which under specific conditions evolve into a vegetative form. This evolution can be inhibited by the presence of a substrate (e.g., antibiotic), which will modify the color of the indicator (Yamaki et al., 2006). These tests present several advantages such as reduced costs and rapid analysis, as sometimes no sample preparation step is necessary. However, these tests are still considered as non-confirmatory approaches and complementary analyses will have to be carried out in case of suspected non-compliant samples (Commission Decision 2002/657/EC, 2002). Furthermore, issues related to false-positive results have also been reported (Gaudin, Cadieu, & Maris, 2002; Kang, Jin, & Kondo, 2005). One of the most widespread rapid test kits is the immunological test, or the so-called ELISA. This test is widely used for the determination of pesticide residues (Brandon et al., 2002), mycotoxins (Zheng et al., 2005), or veterinary drug residues (Muldoon et al., 2000; Wang et al., 2007).

Other commercial kits such as “Charm” tests are available. Those kits are based on competition between a radioactively labeled antibiotic (\(^{3}H, ^{14}C\)) and the analyte for a specific receptor. These tests were initially developed for pesticides (e.g., organophosphorous, methylcarbamates) (Saul et al., 1996), veterinary drug residues (sulfonamides, tetracyclines, amphenicols, β-lactams, etc.) (Charm & Chi, 1982; Korsrud et al., 1994; Nouws et al., 1999) and for several mycotoxins (DON, ZON, OTA, aflatoxin, fumonisin) (Saltar et al., 2006).

Biosensors can also be considered as rapid tests. They usually possess a selective recognition layer built onto the surface of a transduction system (Logrieco et al., 2005). The interaction of the substance is followed when the recognition layer induces a change in the latter which is converted into a measurable electronic signal by the transducer (Fig. 4).

![FIGURE 4. Key components of biosensors along with examples of biological receptors/transducers. Reproduced from Terry, White, and Tigwell (2005) with permission from ACS Publications, Copyright 2005.](image)

Indeed, the specificity, selectivity, and adaptability of biosensors make them ideal candidates for use throughout the food industry. A biosensor can be defined as an integrated receptor–transducer device, able to provide selective quantitative or semi-quantitative information by the use of a biological recognition element. Several types of biosensors (e.g., electrochemical, calorimetric, optical, acoustic, etc.) exist. One of the most widely reported types is the optical-based biosensor, especially the surface plasmon resonance (SPR)-based biosensor. In SPR, a biomolecular binding event causes a change at a metal/liquid interface, usually involving a complex that includes a specific antibody that acts against a target analyte (Terry, White, & Tigwell, 2005). Those changes (in the refractive index) are recognized by a shift in the SPR signal, revealing the presence of a target analyte in a sample solution. SPR and others biosensor systems have been widely used to detect food contaminants (Crooks et al., 1998; Mohammed et al., 2001; Tudos, Lucas-van...
den Bos, & Stigter, 2003; Yuan et al., 2008; Rebe Raz et al., 2009). Biosensor formats including simple “one-shot” disposable devices conform to food safety requirements and future ongoing technical improvements will be of great benefit to the food industry.

In summary, rapid test kits can be considered as powerful tools for qualitative/semi-quantitative screening for chemical residues in foodstuffs. These techniques require limited sample preparation and no chromatographic separation is needed prior to detection. However, these tests are specific for individually targeted analytes and could therefore not be expected to cover the whole range of potential food contaminants. In this frame, the utilization of chromatographic methods coupled to a non-mass spectrometric detector can also be addressed as cost-effective alternatives to techniques such as MS.

2. GC-FID/ECD/NPD

Gas chromatography (GC) is a separative technique that can be useful in the analysis of complex samples such as food matrices. This technique is mainly applied to volatile compounds able to be vaporized without degradation.

Gas chromatography coupled to flame ionization detection (GC-FID) is a non-selective technique based on the measurement of an electrical signal generated by electrons from the combustion of the carbon atoms that constitute the molecules exiting from the GC column. This principally results to a lack of selectivity as this detector will be able to detect all the organic compounds possessing carbon atoms. Therefore, the risk of potential interferences when analyzing complex matrices is high, resulting to a limited number of applications in the food chemical contaminant field (Barden et al., 1997; Blanco-Gomis et al., 2001; Nozal et al., 2004).

In addition to FID, nitrogen–phosphorous detection (NPD) was discovered during the observation that an alkali salt in the flame of a FID system enhanced the ionization of N and P containing compounds. Therefore, GC-NPD results in a better sensitivity than GC-FID, although its high specificity towards only nitrogen and phosphorous compounds has also limited its field of application (Pigeon et al., 2005; Cardeal & Dias Paes, 2006; Pytianos et al., 2006; Likas, Tsipopoulos, & Miliadis, 2007).

Finally, GC coupled to an electron capture detector (ECD) is based on the measurement of electron absorbing compounds (usually halogenated) by creating an electrical field in which molecules exiting a GC column can be detected by the drop in current. Thus, ECD is one of the most sensitive detectors available, enabling the use of GC-ECD for extremely tough applications. It is the principal technique of choice for certain environmental chromatography applications due to its extreme sensitivity to halogenated compounds like PCBs, organochlorine pesticides (Sharif et al., 2006), herbicides (Vela et al., 2007), and halogenated hydrocarbons (Zhou et al., 2007). The ECD has been reported to be 10–1,000 times more sensitive detector than FID but with a limited linear dynamic range (Cochran & Frame, 1999).

3. LC-UV/FLD

The coupling of liquid chromatography with either ultra-violet (LC-UV) or fluorescence detectors (LC-FLD) are widely employed for the determination of chemical contaminants in food-based products (Beek & Aerts, 1985; Rupp, Munns, & Long, 1993; Kijak, Jackson, & Shaikh, 1997; Vassilakis, Tsiipi, & Scoullos, 1998). Despite their relative low cost, both techniques nowadays are increasingly less used in favor of MS. LC-UV is based on the absorption measurement of chromophore type compounds within a given wavelength range (190–400 nm). As most organic compounds absorb in the UV region, this can result in a relatively high chemical noise background leading to a lack of sensitivity and selectivity for the analysis of specific analytes at trace levels especially within complex matrices. LC-FLD gives better sensitivity and selectivity than LC-UV, as this technique is specific towards compounds exhibiting fluorescence properties (fluorophore). While some chemical food contaminants such as fluoroquinolones (McMullen, Schenck, & Vega, 2009) exhibit natural fluorescence properties, some other compounds require a derivatization step (addition of a fluorescent moiety) to enable detection in LC-FLD (Kijak, Jackson, & Shaikh, 1997; Vassilakis, Tsiipi, & Scoullos, 1998; Dall’Asta et al., 2004). Numerous chemical contaminants have been monitored by LC-FLD such as mycotoxins (Trucksess et al., 2007), bisphenol A form PVC food packaging (Lopez-Cervantes & Paseiro-Losada, 2003), or veterinary drug residues (Ali et al., 2000; Edder et al., 2002).

Both LC-UV and LC-FLD are known as non-destructive techniques, meaning that the analyzed samples can be recovered after analysis unlike MS-related techniques. However, LC coupled to MS techniques shows other advantages compared to these detectors with improved sensitivity and selectivity (Fig. 5) and without the need of a derivatization step.

In addition, the EU regulation for the monitoring of veterinary drug residues mentions that only NMR, IR spectroscopy, and MS techniques can be recognized as confirmatory methods (Commission Decision 2002/657/EC, 2002).

III. MASS SPECTROMETRIC TECHNIQUES

Mass spectrometry (MS) and MS/MS techniques represent powerful tools for the analysis of chemical contaminant residues in food. The main advantages afforded by these techniques include the possibility to perform quantitative analysis, structural elucidation, and molecular separation based on the mass to charge ratio (m/z) of analytes. Basically, mass spectrometers comprised three blocks: the ionization source, the mass analyzer, and the detector. The analysis of complex samples can be either directly realized or performed after a separation step such as GC or LC. These techniques will be reviewed subsequently with an emphasis on their applications for contaminant residue analysis.

A. Analysis of Complex Mixtures Without Chromatographic Separation

1. Matrix-Assisted Laser/Desorption Ionization (MALDI)

Since its introduction in the early 1990s, matrix-assisted laser desorption/ionization (MALDI) MS has been applied for the analysis of a wide variety of molecules (Karas, Bachmann, & Hillenkamp, 1985; Tanaka et al., 1988). Viewed as a soft ionization technique (with the ability to desorb and ionize large intact molecules), MALDI is commonly coupled with a time of
flight (TOF) mass analyzer and has been widely used in the analysis of peptides and proteins (Doucette, Craft, & Li, 2000; Shevchenko et al., 2000). However, there are limited reports on the successful application of MALDI to quantify small molecules (Cohen & Gusev, 2002; Kraj et al., 2003; Gobey et al., 2005). This is largely due to the high degree of chemical interferences below a $m/z$ of 500, caused by the co-crystallized MALDI matrices. In addition, the TOF analyzer is thought to be less suitable for quantification because of its narrow dynamic range (usually less than three orders of magnitude). Several attempts to increase the quantitative performance of this technique have been made (Duncan, Matanovic, & Cerpa-Poljak, 1993; Hensel, King, & Owens, 1997; Horak, Werther, & Schmid, 2001). Among them, high repetition rate lasers have been incorporated to improve throughput (Hatsis et al., 2003). These lasers allow a larger number of measurements to be executed within a short time range enabling rapid signal averaging and increased sensitivity. This approach has so far mainly been applied for the quantification of pharmaceutical compounds (Hatsis et al., 2003; Gobey et al., 2005). Hatsis et al. have compared the linearity obtained with a MALDI-Q-TOF instrument using high repetition rate lasers versus a MALDI-QqQ for the analysis of clonazepam and demonstrated at least two orders of magnitude for the Q-TOF compared to at least three for the QqQ. Over the last few years, the dynamic range/linearity of the TOF analyzer has been improved with the addition of dynamic range enhancement features introduced by MS suppliers.

The authors applied this methodology to quantify the amount of benzodiazepines in a commercial pill, and an error of $<5\%$ was obtained between the present method and the manufacturer’s certified values. To our knowledge, no quantitative application has been reported for chemical contaminants residue analysis in food, but this approach can represent a significant advent in terms of TAT and high-throughput analysis, as the chromatographic step may be omitted.

2. Desorption Electrospray Ionization (DESI)/Direct Analysis in Real Time (DART)

Desorption electrospray ionization (DESI) represents another new ionization approach for direct analysis. This interface was introduced in 2004 by Cooks and co-workers (Takats et al., 2004). DESI is carried out by directing pneumatically assisted electrosprayed droplets onto the surface to be analyzed at atmospheric conditions. Ions obtained from molecules originally present on the surface of the sample are produced. The contents of the solvent spray, the gas flow rate, the amount of applied voltage, the spray angle, and the ion uptake angle, as well as the various distances in aligning the spray, sample, and mass spectrometer...
are all variables which can be studied to achieve an optimal mass spectrum for a particular type of sample.

Desorption electrospray ionization (DESI) enables the acquisition of high-quality mass spectra for a broad range of molecules from different sample surfaces (Talaty, Takats, & Cooks, 2005; D’Agostino et al., 2006; Kauppila et al., 2006). The ability to rapidly analyze complex matrices with little or no sample preparation is very important and represents one of the main advantages of this technique. However, to our knowledge, no application has been reported for food analysis, mainly because the sensitivity achieved by the DESI is not efficient enough for trace analysis. Indeed, one of the major drawbacks of this methodology is the presence of ionic interferences from complex matrices that can affect the final sensitivity.

Direct analysis in real time (DART) is an atmospheric pressure ionization source allowing the analysis of different materials in open air under ambient conditions without requiring sample preparation (Cody, Laramée, & Durst, 2005). Samples are submitted to an electronic excited-state gas flow, typically helium. Ions, electrons, and neutral species are generated by an electrical discharge involving several mechanisms including Penning ionization, within which the ionization occurs via an energy transfer from an excited-state molecule or atom possessing ionization energy higher than the sample one. The mechanism postulated with the use of helium is related to the formation of charged water clusters followed by proton transfer (Cody, Laramée, & Durst, 2005). The charged and desorbed sample molecules are then introduced into the vacuum system of the mass spectrometer for detection, and mass spectrometric results are obtained within seconds. A prerequisite is the proper positioning of the cut plate with the analyte on an edge location. As for DESI, efforts should be made to try and enhance the sensitivity of this approach and hence make it amenable for traces analysis.

The use of DART ionization was reported in several applications including pharmaceutical analysis (Williams et al., 2006). Up until now, only two applications describing the use of DART have been reported in food analysis, the measurement of isopropylthioxanthone (ITX) in milk matrices (Morlock & Schwack, 2006) and for melamine in pet food (Vail et al., 2007).

### B. Coupling Techniques

Mass spectrometric techniques can be used for the confirmation of chemical residues with high sensitivity and selectivity; however, due to the complexity of different food matrices, an initial separative chromatographic step (mainly LC or GC) is required and can be mandatory in some cases (e.g., co-eluting chemical interferences). In the past, GC–MS has been widely used in both environmental and food analysis. However, many analytes such as pesticides, drugs, and toxic substances are not suitable for GC analysis as a consequence of their polarity, thermal liability, or low volatility. Moreover, chemical derivatization is often required prior to analysis, which may considerably lower the TAT before delivering the final results.

The discovery of atmospheric pressure interface ionization in the 1970s brought LC–MS to the front stage. Indeed, LC–MS has acquired a role of growing importance in food analysis, as is attested by the wide variety of applications recently reported (Stolker & Brinkman, 2005; Zollner & Mayer-Helm, 2006; Greulich & Alder, 2008).

### 1. GC–MS/(MS)

Similar to other GC-coupled approaches, GC–MS is suitable for thermally stable and highly volatile compounds. A derivatization step is often required to make compounds detectable but this is balanced by the high chromatographic resolution obtained by capillary GC columns. The most popular ionization modes in GC–MS are CI and electron ionization (EI). The latter yields vast fragmentation patterns made up of multiple molecular ions that can be useful for structural elucidation (via available MS databases). The main drawback of EI-MS/(MS) is the relatively low abundance of the molecular ion owing to the collision conditions (MS/MS experiments). However, GC–EI-MS/(MS) yield highly reproducible (tandem) mass spectra; which in addition with retention time locking features can result in very efficient structural identification thanks to the currently available MS databases (Wiley, NIST, etc.). Over the past decade, the methods employed for trace level determination have changed considerably. Most of the chemical contaminant residues are now monitored by LC–MS; however, specific pesticide residues are still largely analyzed by GC–MS (Alder et al., 2006).

Indeed, ionization of pesticides in GC–MS has been largely reported using either EI or CI (positive: PCI or negative: NCI). Although single quadrupole instrumentation is largely popular (SIM), ion trap analyzers in full scan acquisition mode have also been shown to provide adequate sensitivity with the additional advantage of analyte confirmation via library searches (Cairns et al., 1993). Alternatively, CI has rarely been reported on, this is mainly due to the higher signal intensity fluctuations produced by different pesticides compared to EI ionization. GC–MS/MS has been successfully used for monitoring many different chemical contaminants, for example, mycotoxins (D’Agostino, Provost, & Drover, 1986; Plasencia et al., 1990; Olsson et al., 2002), veterinary drug residues (Börner et al., 1995; Sánchez-Brunete et al., 2005), processing contaminants (Becalski et al., 2005; Goldmann et al., 2005), environmental contaminants (Diaz-Cruz et al., 2003; Almeida et al., 2007), and pesticides (Cook et al., 1999; Vitali et al., 1998; Pigeon et al., 2005).

In addition to classical GC–MS/(MS) applications, GC coupled to isotope ratio mass spectrometry (IRMS) has also been reported for use in food analysis. This technique aims to determine the source of an organic substance based on the measurement of the relative isotopic abundance of an element such as carbon, hydrogen, oxygen, etc. As the isotopic ratio of these elements can become locally enriched or diminished under different thermodynamic and kinetic factors, measurements of these isotopic ratios can be used to highlight differences between samples having the same chemical composition (Godin, Fay, & Hopfgartner, 2007; Muccio & Jackson, 2009). Sample introduction can be realized either with GC or LC. Several applications of IRMS related to food authenticity have been reported to establish the use of artificial sweeteners in honey samples (Cabanero, Recio, & Ruperez, 2006) or to differentiate sparkling drinks pressurized with CO2 via a fermentation process from a cheaper and easier carbonation through CO2 from an external cylinder (Calderone et al., 2007). IRMS has also been described in doping analysis for the detection of exogenous steroids (Aguilera et al., 1999, 2001) and its application towards steroids, which belong to
the Group A compounds, and can represent a real asset for food safety analysis.

2. GC × GC–MS

Compared to conventional one-dimensional (1D) GC, comprehensive two-dimensional (2D) gas chromatography (GC × GC) offers increased peak capacity, improved resolution, and enhanced mass sensitivity. Peaks are resolved by their elution from the first dimension and further separated in the second dimension. A typical GC × GC system consists of two GC columns with different retention mechanisms connected in series. During the separation on the second column, the eluate is retained and focused with a cryogenic modulator on the exit of the first column. The second column is usually shorter and narrower than the first one resulting in a fast separation. This system is usually coupled to a TOF mass analyzer as it presents advantages over a quadrupole in terms of fast acquisition and higher resolution capabilities, available on some instruments.

In addition, it generates 2D structured chromatograms, which can aid in the identification of compound classes. Sample preparation procedures can often be minimized, or even eliminated in some cases, due to the superior separating power offered by the technique. All of these advantages make GC × GC a very powerful tool in environmental analysis including the determination of toxic compounds at trace levels in complex matrices. As an example, Figure 6 illustrates the case of a carrot extract spiked with a pesticide (i.e., chlorfenvinfos).

In Figure 6a, 2D-GC increases the separation space and improves the chromatographic resolution compared to 1D-GC (Fig. 6b) where the analyte is co-eluted with the interfering matrix components. Consequently, the mass spectrum obtained (Fig. 6c) reveals characteristic ions of chlorfenvinfos at m/z 323, 267, 109, and 81 resulting in an improved matching degree compared to a database reference mass spectrum (Fig. 6d). On the other hand, the spectrum resulting from 1D-GC/MS showed fragments issued from the matrix components and the lack of characteristic ions from chlorfenvinfos (Fig. 6e) (Panic & Gorecki, 2006).

Two-dimensional (2D)-GC has been applied successfully for different categories of foodstuffs in several matrices, a comprehensive review reports the main application described in this field (Tranchida et al., 2004).

3. LC–MS(/MS)

Liquid chromatography (LC) coupled to MS is an analytical technique that combines the separation power of high-performance liquid chromatography (HPLC) or ultra high-pressure liquid chromatography (UHPLC) with the sensitivity and specificity of the mass spectrometer detector. This technique represents a powerful analytical tool for the analysis of numerous compounds

![Figure 6](https://example.com/figure6.png)

**FIGURE 6.** a–e: GC × GC–TOF-MS versus 1D-GC–TOF-MS for the analysis of a spiked carrot extract. a: GC × GC-TOF contour plot; (b) 1D-GC–TOF-MS chromatogram of the same region, upper trace TIC scaled to 1%; lower trace m/z 323 trace, (c) MS spectrum obtained after GC × GC separation, (d) library spectrum of chlorfenvinfos, and (e) MS spectrum obtained after 1D-GC separation. Reproduced from Panic and Gorecki (2006) with permission from Springer, Copyright 2006.
in complex matrices. However, in contrast with GC–EI-MS, the ability to build mass spectral databases is limited. The ionization conditions in EI remain the same according to various instrument suppliers (kinetic energy = 70 eV), whereas the optimal fragmentation conditions in LC–MS/MS vary from one instrument to another (different optimal in-source and CID conditions). However, Cappiello et al. (2005) may have settled this issue by introducing a LC–EI-MS coupling system. Different column formats (stationary phases, dimensions, etc.) are used for the separation of chemical contaminants, the most reported format being the reversed phase sorbent (Stolker & Brinkman, 2005; Alder et al., 2006; Zollner & Mayer-Helm, 2006). The use of columns filled with small particles (<2 μm) enable the use of high linear solvent velocities that lead to improved resolution, sensitivity, and speed (Plumb et al., 2004). This technology is known as UHPLC and has been successfully applied for chemical contaminant residue analysis in food (Shao et al., 2007; Wang & Leung, 2007; Kovalczuk et al., 2008). Leandro et al. (2006) described a comparative study between LC–MS/MS and UHPLC–MS/MS for the screening of 16 pesticides in some diverse fruit and vegetable matrices. The authors showed that the signal-to-noise ratio (S/N) was enhanced when using UHPLC, together with a reduced total run time (2.5 × faster than LC–MS/MS) (Fig. 7).

Among the different types of ionization interfaces, the main ones correspond to electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). These three interfaces overlap a wide range of analyzable compounds. However, ESI is the most widespread technique as it enables the ionization of polar molecules and high molecular weight compounds (multi-charged species). The alternative use of APCI and APPI for specific compounds, not easily ionizable in ESI, has also been reported (e.g., mycotoxins) (Sforza, Dall’asta, & Marchelli, 2006; Lattanzio, Solfrizzo, & Visconti, 2008). In addition, Bruins and co-workers have reported that the ionization mechanism of APPI compounds, not easily ionizable in ESI, can be unfavorable due to the formation of cluster ions from the mobile phase solvent (Robb, Covey, & Bruins, 2000). Consequently, the chemical noise typically observed under APPI ionization is significantly reduced, resulting in improved S/N (Takino et al., 2004; Mohamed et al., 2007a).

Since its arrival, numerous and continuous applications have clearly revealed the huge impact that LC–MS/MS has had on the analysis of chemical contaminant residues within foodstuffs (Fig. 8).

A large number of review articles have covered a wide range of contaminants, including veterinary drug residues (Stolker and Brinkman, 2000), mycotoxins (Zollner & Mayer-Helm, 2006), pesticide residues (Alder et al., 2006), environmental contaminants (Diaz-Cruz et al., 2003), and processing contaminants (Ruñán-Henares, Delgado-Andrade, & Morales, 2006).

Despite the sensitivity and selectivity provided by novel MS instrumentation, the direct injection of food-based extract is still perilous and could lead to several issues such as bad sensitivity or frequent maintenance of the MS interface. A sample preparation step is then required to provide as clean an extract as possible. Different sample preparation methodologies have been reported on for the extraction of chemical contaminants residues in food samples (Ridgway, Lalljie, & Smith, 2007; Marazuela & Bogialli, 2009). Within the current manuscript, a focus has been placed on SPE and especially towards its automation for chemical contaminant residues in food-based products.

IV. NEW TRENDS IN SAMPLE PREPARATION
BEFORE MS ANALYSIS

A. Solid-Phase Extraction Using Selective Sorbents

SPE is by now one of the most popular sample preparation approaches. This technique has been present since the 1970s but did not really come to fruition until the 1990s with several key improvements such as cartridge format, automated systems, and the continuous release of new stationary phases. The basic principle of SPE is similar to LC but in an extreme way. Whereas in LC the analytes are retained selectively onto a stationary phase, in SPE, analytes are blocked onto the stationary phase and eluted with an adequate solvent. Stationary phases used in SPE are similar to those used in LC such as reversed phase, normal phase, or ionic exchange phases.

Stationary phases used for off-line SPE approaches are also available for on-line systems. Although reversed phase extraction media are the most widely available, the combination of the advantages of automated SPE with the use of selective sorbents represents a powerful analytical tool, especially for low concentration analytes.

Among the selective sorbents used, immunoaffinity (Chen, Li, & Peng, 2005), molecularly imprinted polymer (MIP) (Mohamed et al., 2007b, 2008) and restricted access material

**FIGURE 7.** SRM chromatograms of standards recovered from a potato-based baby food matrix analyzed by (a) LC–MS/MS and (b) UHPLC–MS/MS. Insets show expanded peaks for terbufos sulfone (spiked at 0.01 mg/kg). Reproduced from Leandro et al. (2006) with permission from Elsevier, Copyright 2006.
(RAM) (Pereira & Cass, 2005) are widely known. The first two have their mechanisms based on selective molecular recognition (Hennion, 1999; Hennion & Pichon, 2003), whereas the latter designates a type of support that limits the accessibility of the interaction sites to small molecules only (i.e., size exclusion) (Souverain, Rudaz, & Veuthey, 2004). Regarding the selectivity afforded by these techniques, removal of interferences is quickly achieved resulting in a significant decrease of matrix effects or background noise (Mohamed et al., 2007b). The combination of these selective sorbents with the velocity of on-line SPE and the specificity of MS can result in a significantly improved analytical performance in terms of overall sensitivity, speed of analysis, and high throughput (Boos & Fleischer, 2001; Koeber et al., 2001). Although the use of such sorbent approaches is rarely described for contaminants residue analysis, their application to food quality assessment should become a very attractive one.

B. On-Line Coupling with Sample Preparation Techniques

The sample preparation step is often considered as a tedious and time-consuming process regarding several issues such as incomplete matrix dissolution, analyte evaporation, cross-contamination, etc. All these parameters can lead to bad/poor repeatability and reproducibility of data. Moreover, every additional step required by a method can lead to an increase of the final experimental error. To circumvent this issue, one possibility would be to reduce the number of pre-treatment steps, ensuring that a highly sensitive and selective detector will ultimately be used. Unfortunately, even the best analytical technologies require minimal sample preparation to avoid regular preventive or more serious maintenance of the instrument. On the other hand, automated systems can also be considered an elegant solution to minimize human intervention and therefore reduce potential sources of errors. In that context, on-line sample preparation coupled to MS detection offers several advantages such as minimizing sample and solvent consumption, improved repeatability and higher analysis throughput (therefore lowering the TAT before releasing compliant food raw materials).

1. Column-Switching Approach

The major drawbacks of classical SPE are linked to the solvent volumes used, the number of steps required for the analyte(s) pre-concentration, and also the rather limited number of analytes that can be analyzed simultaneously. The development of on-line SPE processes enables the assessment of faster methods and therefore a higher analytical throughput. Table 3 summarizes advantages and disadvantages of on-line and off-line SPE approaches.

Complete automated devices are now available which utilize a switching valve system (Hopfgartner & Bourgogne, 2003) and also commercial instruments (e.g., Spark instruments and turbulent flow chromatography (TFC)) (Ayrton et al., 1997; Bourgogne, Grivet, & Hopfgartner, 2005). Column switching valves enable the analytical chromatographic separation while re-conditioning the enrichment and/or clean-up steps prior to the next injection (Fig. 9).

The extraction support used for the analyte pre-concentration is coupled via a switching valve (6- or 10-port valve) to an analytical column to perform separation of the analytes before detection. Conditioning, washing, and elution steps can also be carried out with a high-pressure pump system, while the analytical column is being re-conditioned (Fig. 9a). The organic mobile phase elutes the analytes from the extraction support and separation is then performed by a second LC system before final detection (Fig. 9b). It is noteworthy to mention that in some systems, the high organic content elution phase can be subsequently diluted with aqueous mobile phase (separation starting gradient condition) to avoid peak broadening.

Stationary phases available in classical SPE are identical to those used for automated SPE systems. Numerous applications have been realized for bioanalysis or environmental analysis and up until now very few applications have described the analysis of contaminant residues within foodstuffs (Riediker et al., 2002;
Bacaloni et al., 2005; Juan-García, Font, & Pico, 2007; Mottier et al., 2008; Kantiani et al., 2009).

2. Automated Devices

Complete automated systems based on switching valve devices obviously present key advantages over classical column switching systems. Indeed, for method development the investigation of different stationary phases both for the extraction and the analytical column can turn out to be complex and time consuming. In this context, the use of automated column selector devices affords a plenary answer to this important issue. The company Spark Holland initially developed an on-line SPE system (named as Prospekt I), with the possibility of exchanging the SPE cartridge after each injection. This system was originally designed for the direct injection of biological fluids. At a later stage, to improve sample throughput, a system was launched with a dual cartridge column device (namely Prospekt II). This second instrument generation was applied to the quantitative analysis of chlormequat and mepiquat (pesticides) in different foodstuffs.

**TABLE 3.** Comparative features of on-line and off-line SPE configurations

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>On-line SPE</th>
<th>Off-line SPE</th>
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<tbody>
<tr>
<td>Analysis of the total amount of analytes extracted</td>
<td>Several measurements can be performed with the same extract</td>
<td></td>
</tr>
<tr>
<td>Reusable cartridges</td>
<td>Relatively non expensive equipment and consumables</td>
<td></td>
</tr>
<tr>
<td>Minimal sample handling : improved</td>
<td>Lower matrix effects due to only a part of the extract being analyzed</td>
<td></td>
</tr>
<tr>
<td>repeatability/reproducibility</td>
<td></td>
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<tr>
<td>Direct and fast elution of the sample after</td>
<td></td>
<td></td>
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<tr>
<td>pre-concentration : minimal degradation</td>
<td></td>
<td></td>
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<tr>
<td>Reduced analysis time and high-throughput</td>
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<table>
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<tr>
<th>DRAWBACKS</th>
<th>Homogenized sample issue</th>
<th>Time consuming approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment relatively expensive</td>
<td>Manipulation of the sample : cross-contamination, poor accuracy</td>
<td></td>
</tr>
<tr>
<td>As the extract is analyzed in its entirety,</td>
<td>Possible loss of analyte during evaporation/resuspension</td>
<td></td>
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<tr>
<td>matrix effect can be observed</td>
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![FIGURE 9.](image) Schematic representation of a column switching system using a six-port valve configuration: (a) the SPE extraction is performed while the analytical column is being equilibrated and (b) analytes are separated on the LC column while a SPE cartridge conditioning (or change) is occurring.
and quantification of β-lactams in bovine milk with an on-line SPE procedure. The method developed offers high sensitivity and accuracy and meets the requirements of the European legislation (Kantiani et al., 2009). This instrument also allows the performance of simultaneous sample preparation and analysis resulting in a high-throughput approach, but to our knowledge has mainly been applied for the analysis of biofluids (Alnouti et al., 2005; Koal et al., 2006).

Another commercial automated instrument called TFC was introduced in 1997 (Quinn & Takarewski, 1997) and developed for the direct injection of biofluids onto a column packed with 30–50 μm spherical porous particles. In fact, this concept appeared in the 1960s as a new tool to perform fast analysis in opened tubular columns (Pretorius & Smuts, 1966). The choice of flow rate here is considered to be the key parameter in improving the extraction efficiency and clean-up step. Subsequently, Wu et al. (2000) showed that under high flow rates, these stationary phases can tolerate several hundred injections of plasma (100 μL) without any loss in MS performance, which is in contrast to the use of conventional laminar flow rates.

Several features of this instrumentation are attractive such as the multi-column module which allows up to six TFC SPE columns along with six analytical ones to be set up in parallel. In addition, it incorporates multiplexing technology where up to four parallel systems can be synchronized to a single MS. Each system operates independently, permitting multiple methods to run simultaneously. Although most of the TFC applications are related to bioanalysis and pharmaceutical assays (Wu et al., 2000; Cegłarek et al., 2004; Sauvage et al., 2006), some recent applications reveal its high potential for food analysis (Hammel et al., 2006b; Mottier et al., 2008; Presta et al., 2009).

V. MASS ANALYZERS

As previously mentioned, the analysis of chemical residue contaminants in food requires high sensitivity (in many cases at low μg/kg level) and selectivity for a wide variety of analyte/matrix combinations and for compounds having distinct physicochemical properties. Although GC–MS is still widely used, especially for pesticide residues analysis, nowadays LC–MS has become the technique of choice for most laboratories. Mass analyzers such as the quadrupole (Q) are largely used to monitor the analyte(s) using the SIM acquisition mode (Codony et al., 2002; Wu, Wang, & Simon, 2005; Jin & Zhu, 2006). Alternatively, ion trap (IT) mass analyzers can also be used in the full scan acquisition mode (Sagratini et al., 2006; Chen & Chen, 2007). However, these methods present some limitations regarding selectivity and sensitivity. Sagratini et al. have compared the sensitivity obtained for the analysis of ITX in fruit juices using either a single quadrupole, an ion trap (in full scan mode), or a triple quadrupole (QqQ) in SRM mode (MS/MS). This latter mass analyzer was observed to reach a detection limit 300-fold lower than the two former ones (Sagratini et al., 2006). These results demonstrate the importance of MS/MS, which can be performed on an IT (MS” acquisition mode), QqQ, or hybrid instrument combining quadrupole and linear ion trap or time-of-flight mass analyzers (QqQ/LIT and Q-TOF) (Pico, Blasco, & Font, 2004). Among these various mass analyzers, QqQ was the analyzer of choice in every laboratory dealing with the quantification of chemical contaminants at trace levels (generally operating in SRM acquisition mode). However, other functionality scans can be used (precursor ion, neutral loss (NL), etc.) alone or associated in combination with other scan types throughout information-dependent acquisition (IDA) experiments (after setting a certain threshold for the survey experiment).

Another key point is the structurally rich information that could be provided with medium/high-resolution mass spectrometers such as TOF mass analyzers or hybrid quadrupole TOF (Q-TOF) instrumentations. Q-TOF instruments offer the potential of combining both the high resolution of the TOF with the capability to conduct “real” MS/MS experiments (by selection of the parent ion and not using in-source dissociations).

A. Low-Resolution Mass Spectrometry Instrumentation

1. Triple Quadrupole Instrumentation (QqQ)

Up until now, QqQ apparatus are the most widespread instruments used for the analysis and quantitation of chemical residues in foodstuffs (Stolker & Brinkman, 2005; Zollner & Mayer-Helm, 2006; Murkovic, 2007). This is mainly due to the different scanning possibilities available on these instruments (e.g., SRM, precursor ion scan (PIS), NL scan). In addition to classical SRM, the use of PIS or NL scan experiments can be very informative for the monitoring of a typical class of contaminants, giving rise to characteristic common fragment ions (e.g., m/z 92, 108, 156 for sulfonamides, Mohamed et al., 2007a; m/z 223 for ergot alkaloids, Mohamed et al., 2006; m/z 42, 70, 84, 112, and 120 (NL); and m/z 68, 71, and 79 (PI for atrazines and its transformation products), Steen, Bobeldijk, & Brinkman, 2001). Such scanning experiments allow the identification of non-targeted compounds (in case of unobtainable commercially available standards), enlarging the potential of the analytical method used. Functionalities present in QqQ instruments enable the combination of different scanning modes throughout using the so-called IDA mode. Basically, a survey experiment is set (Q1, Q3, MS/MS, SRM, PIS, or NL) and according to a certain threshold defined by the analyst, the instrument can switch automatically to another scanning experiment predefined by the user (mainly CID but without any pre-selection of the ion to collide unless an inclusion/exclusion list is generated). Mohamed et al. reported the use of such an IDA experiment for the identification of an unexpected ergot alkaloid residue (i.e., ergosine) in a flour extract sample. The authors settled a PIS...
survey experiment based on a common product ion (m/z 223) for a specific class of alkaloids, combined to a CID experiment to further confirm the presence of this analyte (Mohamed et al., 2006).

2. Ion Trap (IT)

Most of the classical ion trap mass spectrometers (IT-MS) are either based on scanning of quadrupole (Paul’s trap, 3D) or nonlinear fields (linear, 2D). IT-MS instruments have been used to determine contaminant residue in different food matrices (Hiemstra & de Kok, 2002; Royer, Humpf, & Guy, 2004; Sagratini et al., 2006; Chen & Chen, 2007). Its main advantages include the high sensitivity in the scanning mode and the possibility to perform MS^n enabling the structural elucidation of unknown compounds in food extracts, thanks to the characteristic MS/MS spectra. However, the dynamic range observed is typically lower compared to QqQ instruments. Furthermore, the presence of co-extracted compounds can introduce additional difficulties in the appropriate selection of the diagnostic ions.

3. Hybrid Quadrupole-Ion Trap (QqLIT)

These instruments present the same core as a QqQ mass spectrometer; it is to say a first quadrupole (Q1), a collision cell (Q2), and a third quadrupole (Q3). The particularity of this instrument lies in the ability of Q3 to be used either as a classical quadrupole or as a linear ion trap (Hopfgartner et al., 2004) (Fig. 10).

When scanning using Q3 as a quadrupole, a couple of radiofrequency/continuous field values are fixed, whereas a radiofrequency is applied to Q3 when operating as a linear ion trap. Therefore, it becomes possible to perform within the same run a further experiment using a quadrupole classical scanning event followed by another data-dependent acquisition using the ion trap functionalities, without affecting the sensitivity (Hopfgartner et al., 2004). In addition, it utilizes the scanning possibilities of a quadrupole (e.g., neutral loss, precursor ion, SRM) and ion trap (e.g., MS^n).

By now, this hybrid instrument was reported for the determination of various contaminant residues in different types of food matrices (Li et al., 2006; Hernando et al., 2007a,b; Mohamed et al., 2007a; Díaz-Cruz, García-Galán, & Barceló, 2008). García-Reyes et al. have described a combinatorial multi-residues approach using a LC–TOF-MS instrument for accurate mass measurement as a screening approach, followed by a second analysis using a LC-QqQLIT for quantification and analyte confirmation. This strategy allowed them to monitor 100 pesticide residues with accurate mass measurements (LC–TOF-MS) as a first screening step, whereas confirmation was performed by the SRM mode using two transition reactions for each compound (LC-QqQLIT) and quantification achieved by using a matrix-matched calibration curve model on both instruments (García-Reyes et al., 2007).

Another important aspect to point out when dealing with multi-screening approaches is one of having enough scan speed possibilities to provide at least 10 data points to define a chromatographic peak. Indeed, the time spent by the mass analyzer to monitor every SRM transition may be difficult to cope with, particularly with the capabilities of UHPLC (typical peak widths of few seconds). To overcome such an issue, several instrument manufacturers have introduced the scheduled SRM notion. Thus, the analyst will need to define precisely the expected retention times (with an allowed window for eventual peak shifts) for each individual SRM to monitor plus the total scan time required. The software will then automatically truncate...
the chromatogram in different retention time windows, giving adequate time to scan efficiently each chromatographic peak (Hammel et al., 2006a,b).

B. High-Resolution Mass Spectrometry Instrumentation

1. Time of Flight (TOF)

Interesting advantages of TOF-MS instruments include the high acquisition speed and good mass resolving power of 10,000 or more (peak width at half mass). Thus, such mass analyzers are able to perform accurate mass measurements as well as recording full scan mass spectra. Regarding the increasing number of new chemicals used within the food industry, it is of high importance to have available analytical techniques able to identify unknown analytes in complex matrices. Therefore, the use of accurate mass measurement is very useful to provide elemental composition of both parent and fragment ions (in-source CID) and also to discriminate nominal mass compounds. The accuracy of TOF mass analyzers is much higher than that of ion trap and quadrupole instruments due to the excellent ion separation in the flight tube and subsequent detection (Eckers, Haskins, & Langridge, 1997; Pergantis et al., 2000). Therefore, typical mass accuracies of around 5 ppm or even below can be expected (with internal lock mass) both in full scan and MS/MS modes; providing very useful information for structure elucidation. Table 4 summarizes the sensitivity, selectivity, accuracy, and dynamic range of various mass analyzer instruments.

In addition to some quantification drawbacks, TOF analyzers seem to have a bright future for the characterization and identification of degraded or metabolized chemical contaminants, or to highlight the presence of new processing contaminants which may be of concern for human health.

2. Hybrid Quadrupole-Time of Flight (Q-TOF)

Hybrid quadrupole time of flight (Q-TOF) instruments were introduced by Morris et al. (1996) in the mid-1990s. This technology combines the resolution and mass accuracy of a TOF analyzer with the capability to perform real MS/MS experiments (while in-source CID can be obtained on TOF instruments). Thus, the possibility to perform full scan CID experiments with accurate mass represents one of the numerous advantages of Q-TOF mass spectrometers. In addition to the sensitivity and mass accuracy, an evident analytical requirement is to provide reliable information for ensuring the presence of chemical contaminants to avoid reporting false-positive results. Several authors have reported on the utility of Q-TOF instruments as an interesting tool for elucidation and compound confirmation (Gentili et al., 2006; Weber et al., 2006; Grimalt et al., 2007) as a high number of IPs can be reached (Hernández et al., 2004). The valuable structural information provided by this technique may even allow the elucidation of non-targeted compounds (Nielén et al., 2003; Grimalt et al., 2007; Picó et al., 2007b). With respect to the increasing popularity of metabol(omics) applications and also in the field of multi-screening methods and product authenticity, we can envisage that further instrumental developments/improvements coupled with more sophisticated softwares will appear over the coming years.

Regarding quantification at trace levels, QqQ instruments permit satisfactory results at very low concentrations (sub μg/kg) with a considerable high dynamic range of typically five orders of magnitude, compared to three orders for Q-TOF instruments. Quantification of targeted analytes at ultra-trace levels can therefore be compromised and may possibly result in a specific issue when dealing with banned substances (Gentili et al., 2006; Wang & Leung, 2007). This lower dynamic range is a consequence of ion saturation at the upper part of the concentration range, and several instrument manufacturers have introduced a “dynamic range enhancement” system to help improve on this issue. The use of hybrid Q-TOF instruments remains almost unexplored for contaminant residue analysis in food and most of today’s applications are related to pesticide analysis (Nielén et al., 2003; Lacorte & Fernandez-Alba, 2006; Grimalt et al., 2007; Picó et al., 2007b).

Obviously, Q-TOF technology offers a very strong potential for chemical contaminant analysis as a screening and structural elucidation tool. Together with the MS E (MS everything) feature, introduced by the Waters Corp. (Milford, MA, USA) the analyst can simultaneously acquire two MS traces at low and high collision energies in one run. Thus, the intact molecules will be present in the first trace, whereas characteristic fragment ions of the molecule will appear in the second (MS/MS spectra) thanks to a very efficient deconvolution algorithm (to avoid problems of co-eluting substances).

**TABLE 4.** Comparison of various mass analyzer instruments

<table>
<thead>
<tr>
<th>Mass Analyzer</th>
<th>Sensitivity in full scan</th>
<th>Selectivity</th>
<th>Accuracy</th>
<th>Dynamic range</th>
<th>Unique features</th>
</tr>
</thead>
<tbody>
<tr>
<td>QqQ</td>
<td>Medium</td>
<td>High</td>
<td>Low, unit resolution</td>
<td>High</td>
<td>Neutral loss</td>
</tr>
<tr>
<td>IT</td>
<td>High</td>
<td>High</td>
<td>Low, unit resolution</td>
<td>Medium</td>
<td>MS&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td>ToF</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Accurate mass &amp; sensitivity</td>
</tr>
<tr>
<td>QqQLIT</td>
<td>Medium</td>
<td>High</td>
<td>Low, unit resolution</td>
<td>High</td>
<td>Enhanced product ion</td>
</tr>
<tr>
<td>QToF</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
<td>Medium</td>
<td>Accurate mass &amp; selectivity</td>
</tr>
</tbody>
</table>

3. Fourier Transform Ion Cyclotron Resonance (FT-ICR)

Fourier transform ion cyclotron resonance MS (FT-ICR), also known as Fourier transform MS, is a mass analyzer introduced by Alan G. Marshall and Melvin B. Comisarow in the mid-1970s (Comisarow & Marshall, 1974) for determining the \( m/z \) of compounds based on the cyclotron frequency of the ions in a fixed magnetic field. Most of FT-ICR-MS applications are related to high molecular weight molecules (Marshall, Hendricksson, & Jackson, 1998) and so far few applications were reported in the food domain (Wu, Rodgers, & Marshall, 2004).

4. Orbitrap

The basic principle of the Orbitrap was described by Makarov (2000) and consists of an inner and an outer electrode used to trap ions in a quadro-logarithmic electrostatic field, which then generates a transient ion signal related to the axial ion motion rather than the radial ion motions used in FT-ICR. Oscillation frequencies for stored ions with different \( m/z \) ratios are then obtained using the Fourier transform. The Orbitrap mass analyzer has demonstrated high resolving power, mass accuracy, and high space charge capacity with pulsed ion sources (Perry, Cooks, & Noll, 2008). Most of its applications are currently related to the bioanalytical field (e.g., proteomics and metabolomics) (Perry, Cooks, & Noll, 2008). One feature of the Orbitrap that can be used in food contaminant analysis is the high mass accuracy measurement that could provide information to determine unknown compounds (Thevis et al., 2005).

VI. CONCLUSION AND PERSPECTIVES

As the global food trade is increasing, a plethora of chemical contaminants are susceptible to being observed as trace residues in the final food product. To ensure good quality and safety of our food, stringent and rigorous monitoring plans associated with eventual follow-up are established. Over the past few years, regulatory agencies have proposed new requirements to better assess the performance of analytical laboratories, and updated MRLs for specific chemical contaminants. In this context, MS techniques play a pivotal role due to the sensitivity and selectivity of the detector. On the analytical side, the increasing number of sample/analytes led scientists to focus on fast, reliable, and reproducible approaches. High-throughput analysis should be emphasized in particular with the use of automated sample preparation tools to limit human intervention (repeatability) and to speed-up the extraction process. Direct analysis by removing the chromatographic separation step can be an answer to higher throughput analysis, providing that the sensitivity and the selectivity of tools such as DESI or DART are improved to match the required regulations. Separation time can also be reduced by using sub-2-µm particle media (UHPLC) and applying high flow rates. However, as a direct consequence there is a need to develop high scan speed MS systems that possess higher linear dynamic ranges for quantification purposes or to propose alternative solutions such as software tools to increase the number of data points to well define a peak. On the instrumentation side, the increasing potentiality to find unknown analytes should stimulate the food analyst to use accurate mass instruments along with MS/MS spectral databases. In addition to providing exact mass measurements, these high-resolution instruments may remove co-eluting chemical interfering compounds (sometimes observed in triple quadrupole instruments). Data processing is also a major key point and the integration by the different manufacturers of adapted software for food analysis (regulation criteria) can allow the analyst to save a considerable amount of time. The advent of statistical tools such as principal component analysis (PCA) could be applied to quality and food safety as a possible way to select high-quality raw materials with improved nutritional quality or to highlight product adulteration. In that context, the use of mass spectrometric techniques seems to be the best answer for the stringent requirements linked to contaminant residue analysis in foodstuffs.

VII. ABBREVIATIONS

CID collision-induced dissociation
MRL maximum residue limit
NLS neutral loss scan
PIS precursor ion scan
SPE solid-phase extraction
TAT turn around time

REFERENCES


Bakhtiar R, Majumdar TK. 2007. Tracking problems and possible solutions in the quantitative determination of small molecule drugs and metabolites.


