33 chapter

Analysis of Food Contaminants, Residues, and Chemical Constituents of Concern

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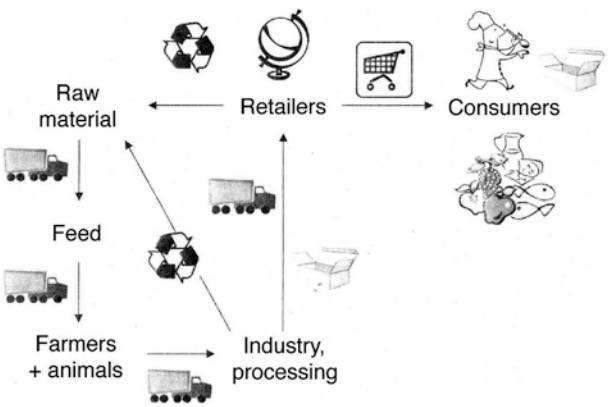
33.1 INTRODUCTION: CURRENT AND EMERGING FOOD HAZARDS

The food chain that starts with farmers and ends with consumers can be complex (Fig. 33.1, Ref. [1]). Pesticide treatment, agricultural bioengineering, veterinary drug administration, environmental and storage conditions, processing, transportation, economic gain practices, use of food additives, and/or choice of packaging material may lead to contamination with or introduction (intentionally and non-intentionally) of hazardous substances. Legislation and regulation to ensure food quality and safety are in place and continue to develop to protect the stakeholders, namely, farmers, consumers, and industry. (Refer to Ref. [2] for information on regulations of food contaminants and residues.)

Contaminants/food hazards often have threshold levels (**tolerance levels**) below which no adverse effects are observed. The US Environmental Protection Agency (EPA) establishes these tolerance levels, and the **Food and Drug Administration** (FDA) and **US Department of Agriculture** (USDA) enforce them. However, food safety incidents, microbial, biological, and chemical in nature, continue to occur. The Rapid Alert System for Food and Feed (RASFF) reported a total of 3,049 alerts/notifications in 2015, a value that is 5% higher than what was reported in 2014 [3]. Notifications were categorized as follows: chemical (36%), mycotoxins (16%), microbial (27%), and other hazards (21%). Within the chemical category, the most frequently reported hazards include allergens (e.g., histamine and sulfite), heavy metals (e.g., mercury, lead, and cadmium), pesticides (e.g., omethoate and isofenphos-methyl), and veterinary drugs (e.g., β-lactam and chloramphenicol). Microbial contaminants include molds, viruses, and bacteria (discussion and methods of analysis for this category are beyond the scope of this chapter). Examples of current and emerging chemical hazards include fraud and food adulterants (e.g., melamine), packaging chemicals (e.g., bisphenol A and 4-methylbenzophenone), degradation metabolites (e.g., acrylamide, heterocyclic amines, and furan), naturally occurring substances (e.g., cyanide and solanine), and other chemical contaminants (e.g., 3-monochloropropane-1,2-diol, benzene, and perchlorate). Another category that can be of concern includes the genetically modified organisms (GMO) and their products. Introduction and usages of GMO in food products resulted in the development of legal requirements of safety and labeling.

Given the extent of concerns with contaminants, there is a strong need for adequate and reliable methods of detection and analysis to ensure food quality, safety, and fair trade. There are several well-established and reliable methods of analysis to detect certain food hazards. Development and validation of methods to detect and analyze emerging food hazards is a continuous effort. This chapter will cover some of the screening methods and quantitative methods that are commonly used for the detection and quantification of several food hazards, in addition to some recently developed methods for the detection of newly identified and emerging food hazards. The focus in this chapter on chemical contamination is intended to compliment that of Chap. 35 on Food Forensic Investigations.



33.1 figure An illustration of the food chain (From Nielen and Marvin [1], with permission)

33.2 ANALYTICAL APPROACH

As with the analysis of any food constituent, there is an array of methodological approaches and techniques to choose from for the analysis of food hazards. Multiple factors influence the method of choice as listed in Chap. 1, Tables 1.1, 1.2, 1.3, 1.4, and 1.5, including in this case complexity of the food matrix, characteristics of the analyte (e.g., polarity, hydrophobicity, volatility, thermal stability, and chemical reactivity), and suspected level of the contaminant. The objectives for the analysis of contaminants can vary in complexity from mere detection of several suspected contaminants belonging to the same family to the determination of the exact level of a particular contaminant or, in more complex cases, detection of unknown adulterants. The general trend for regulatory institutions and industry is to implement inexpensive and rapid **screening methods**. However, depending on the objective of the analysis, **quantitative methods** that require sophisticated equipment might be needed. In this case, the industry may choose to send their samples to specialized laboratories. Once a method of analysis is chosen, appropriate consideration with regard to sampling and sample preparation needs to be made.

33.2.1 Choice of Analytical Method

The complexity of the food matrix (see Chap. 1, Sect. 1.4.4) and the characteristics of the analyte significantly influence the choice of extraction, separation, detection, and quantification techniques, as will be discussed in subsequent sections. Accuracy, precision, specificity, and sensitivity of the analytical method (see Chap. 4) are also important considerations. There are some official methods for the analysis of contaminants and residues, others are validated, and some are currently being developed and validated. Methods for the analysis of food hazards can be either qualitative, semiquantitative, or quantitative. A summary of analyses for some of the main contaminants is given in Table 33.1.

33.1 table Summary of analyses for contaminants, residues, and compounds of concern in foods

Contaminant	*Quantitative*	*Semiquantitative or qualitative (screening methods)*
Pesticides	Multiresidue (MRMs) GC (mostly) HPLC Single residue (SRMs) GC (mostly) HPLC Immunoassay	TLC Enzyme inhibition Immunoassay
Mycotoxins	HPLC (mostly) GC Capillary electrophoresis Immunoassays (mostly)	TLC Immunoassay
Antibiotics	HPLC (mostly) GC Immunoassays	Microbial growth inhibition Receptor assays Enzyme-substrate assays Immunoassays
GMOs	PCR (mostly) ELISA	LFS
Allergens	ELISA PCR LC-MS	LFS
Sulfites	Monier-Williams method Ion chromatography Enzyme method HPLC	"Ripper method"
Nitrites	Colorimetric methods Ion chromatography	Ion-selective electrode

HPLC high-performance liquid chromatography, *GC* gas chromatography, *TLC* thin-layer chromatography, *PCR* polymerase chain reaction, *ELISA* enzyme-linked immunosorbent assay, *LFS* lateral flow strip

33.2.1.1 *Qualitative or Semiquantitative Methods*

Qualitative and **semiquantitative** methods, also known as **screening methods**, are usually used to assay a large number of samples for the presence of one or more contaminants belonging to the same family (e.g., antibiotic residues; see Sect. 33.5.2.1). These methods are fast, of low cost, simple, and robust; they are often less sensitive to small changes in experimental and/or environmental conditions and are not limited to a highly controlled lab environment. While qualitative methods detect the presence of certain contaminants at or above a specified threshold, semiquantitative methods provide an estimate of the concentration of a detected contaminant. These methods include techniques such as **thin-layer chromatography** (TLC), **enzyme inhibition**, and **immunoassays** (see later sections of this chapter).

33.2.1.2 *Quantitative Methods*

For simultaneous quantitation and structural identification of chemical food contaminants and residues, **gas chromatography** (GC; Chap. 14) and **high-performance liquid chromatography** (HPLC; Chap. 13) are the two main analytical methodologies employed. The combination of GC with mass spectrometry (MS), and the availability of relatively affordable benchtop GC-MS instruments, gave preference to GC analysis for multicomponent contaminant and residue analysis, in spite of having to derivatize polar analytes. However, thermally labile and/or large analytes that cannot be easily volatilized, such as mycotoxins, polar pesticides, and most of the veterinary drug residues, must be analyzed using HPLC. Major advances in HPLC-mass spectrometry (LC-MS) have facilitated direct, selective, and sensitive analysis of the polar analytes. For example, LC-MS has largely replaced microbial and immunochemical methods used for the analysis of veterinary drugs [4, 5]. Additionally, LC-MS is being used for multiclass, multiresidue analysis of pesticides [6, 7], due to the transition from the use of persistent and less polar compounds to the more readily degradable, more polar, thermolabile pesticides.

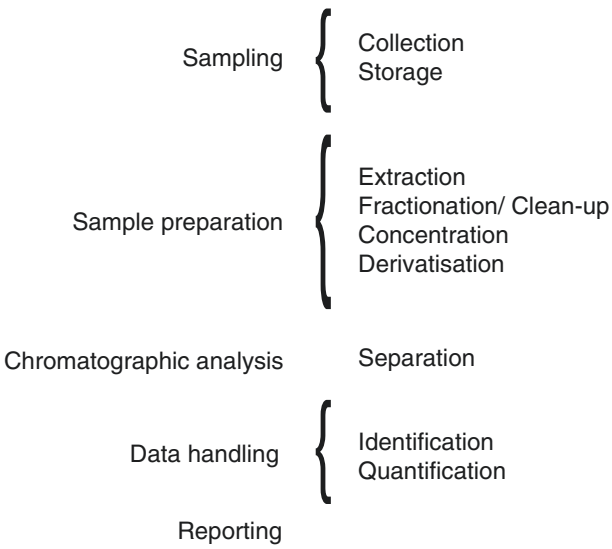
Immunoassays (Chap. 27) are rapid, simple, and cost-effective means for the detection and quantification of both single and multiple contaminants or residues, such as pesticides, antibiotics, and mycotoxins. Of the various immunoassay techniques, **enzyme-linked immunosorbent assay** (ELISA) (Sect. 27.3.2) and **lateral flow strip** (LFS) tests (Sect. 27.3.4) provide a sensitive detection of toxic analytes, whereas **immunoaffinity chromatography** (Sect. 27.4) is used for concentration and cleanup of the analyte of interest. One drawback of immunoassays is that the antibodies used in the assay may have **cross-reactivity** (affinity) for related chemical structures.

33.2.2 Sample Preparation

33.2.2.1 *Introduction*

Food samples are usually too dilute (e.g., beverages) or too complex (e.g., meat) for direct analysis of trace contaminants and residues. Therefore, sample preparation, including homogenization, extraction, fractionation/cleanup, concentration, and/or derivatization, normally precedes the analysis of food contaminants and residues (Fig. 33.2). Analysts are faced with continuous decrease of legislative limits for food contaminants and residues, resulting in the need for more sensitive, precise, and accurate measurements. Sample preparation techniques for the analysis of food contaminants and residues are continuously improved to guarantee high recovery and reproducibility (see Ref. [8] as an example). Fortunately, there also have been significant advances in analytical methods and equipment. The advanced technology of mass spectrometers (see Chap. 11), specifically, their enhanced "recognition features" when used in tandem, allows them to take over the "selectivity" of classical sample preparation methods. However, for quantification purposes, cleaner extracts are preferred. In both target and multiresidue methods, isotope dilution and addition of internal standard provide the most accurate results, compensating for sample matrix effects and ion suppression in MS. Also, faster and more efficient extraction methods are now available for the analysis of food contaminants and residues, as will be discussed in subsequent sections.

Analytical chemists often focus on perfecting the analytical technique (e.g., chromatographic analysis), overlooking the importance of sampling, sample storage, and sample preparation. Sampling and sample preparation are labor intensive and time consuming but are essential prerequisites for acquiring meaningful analytical data. There is a high probability of error and contamination during sampling and sample preparation, and these cannot be corrected at any point during the analysis. Therefore, an adequate plan for sampling, storing, and preparing samples should be implemented and validated by statistical analysis (see Chap. 5 for more details). Obtaining a sample that is representative of the level of trace substances in a heterogeneous mixture is not an easy task. Sampling for the analysis of a particular contaminant or residue will be briefly discussed in relevant sections of this chapter.



33.2 figure Flow diagram for the analysis of contaminants and residues (From Sandra et al. [10], with permission)

33.2.2.2 *Sample Homogenization*

Food contaminants and residues in many cases are unequally distributed in a food system. For instance, most pesticides are not translocated within plants and are expected to be located on the surface of fresh produce. Thus, removal of inedible parts (outer skins, stems, and cores), when applicable, and homogenization are necessary for reliable and accurate analysis. Homogenization can be achieved by chopping or grinding, followed by blending and mixing. Apart from homogenization, grinding reduces the structural features of the sample and enhances extraction efficiency. Contaminating the sample or exposing it to unnecessary heat, which can cause volatilization or degradation of contaminants or residues, should be avoided. Sometimes, cryogrinding (see Chap. 5, Sect. 5.5.2.1) is preferred for soft samples and to avoid thermal degradation.

33.2.2.3 *Extraction and Cleanup*

33.2.2.3.1 Introduction

Almost all food samples, with the exception of samples directly soluble in an organic solvent (e.g., vegetable oil), require a solvent extraction step (Chap. 12, Sect. 12.2; Chap. 14, Sect. 14.2.2.4) to isolate the target analytes from the matrix. Defatting of lipid-rich food matrices is often required, using hexane or isooctane, prior to the extraction of target contaminants. Extraction of contaminants and residues is traditionally done by solubilizing them in a suitable organic solvent, generally acetonitrile or acetone. **Anhydrous salt** (NaCl or Na_2SO_4) can be added to absorb water. In some cases, water is added so that the crude extract can be purified using a subsequent partitioning step with a second, water-immiscible solvent. When the extraction process is complete, the solvent is separated from insoluble solids by filtration.

Often, the crude sample extract is purified and concentrated before the separation/determination

steps. The degree of cleanup required depends on the method of analysis such as the chromatography mode and detector type. The objective of cleanup is to separate the target analytes from coextractives that can interfere with their detection. Often, the preliminary cleanup step is followed by a preparative chromatography step (see Chap. 12 for basic information on chromatography). Cleanup of crude water-acetone or water-acetonitrile extract, for example, can be done by partitioning with a relatively nonpolar organic solvent. The residues then can be further purified by column chromatography (either adsorption or size-exclusion chromatography). Separate fractions of column eluate can be analyzed. The choice of packing material is both analyte and matrix dependent. In some cases, such as determining aflatoxin in milk [9], monoclonal antibody affinity chromatography is used as a one-step column cleanup.

Great efforts have been made to develop extraction techniques that are faster and more efficient and require less solvent. Extraction and purification techniques have been developed based on the characteristics of the target analyte(s), which can be categorized into three classes: (1) **volatile compounds** (VC) that can be analyzed via headspace techniques, after derivatization if needed, (2) **semi-volatile compounds** (SVC) that are GC amendable (thermally stable), and (3) the **nonvolatile** (thermally labile) **compounds** (NVC), which are mostly analyzed by HPLC after extraction. Some of the widely used extraction techniques will be covered briefly in subsequent sections. References [10–14] contain more details on the various extraction techniques.

33.2.2.3.2 *Solid-Phase Microextraction*

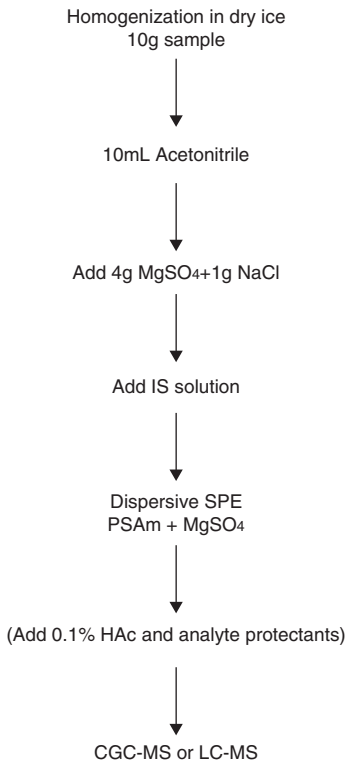
All three classes of compounds listed above can be extracted using **solid-phase microextraction** (SPME), which is a type of **solid-phase extraction** (SPE) (Chap. 14, Sect. 14.2.2.5). VCs are best analyzed by headspace techniques (see Sect. 14.2.2.2). Headspace sampling applications include the analysis of pesticides, furan, and residues from packaging materials. Headspace sampling can be done by immersing the SPME polymer-coated fibers into the headspace. SPME also can be used for SVCs and NVCs, with the polymer-coated fiber directly immersed in the aqueous samples, or placed inside a hollow cellulose membrane.

33.2.2.3.3 *The QuEChERS*

QuEChERS method [15, 16], which stands for quick, easy, cheap, effective, rugged, and safe, is a dispersive SPE (DSPE) technique that is by far the best method to extract multiple pesticide residues (Sect. 33.3.2) (see Chap. 14, Sect. 14.2.2.5, for SPE). A single sample preparation method and one chromatography method linked to MS (preferably in tandem) to determine as many pesticides as possible are very much desired. The advantages of the QuEChERS method (outlined in Fig. 33.3) include speed, ease, minimal solvent use, and lower cost as compared to conventional SPE. The types of adsorbents and solvents, as well as the pH and polarity of the solvents, can be adjusted based on the sample matrix and types of analytes. QuEChERS kits are now commercially available (e.g., Sigma Aldrich/Supelco, Restek, and United Chemical Technologies).

33.2.2.3.4 *Microwave-Assisted Solvent Extraction*

Microwave-assisted solvent extraction (MASE), which utilizes electromagnetic radiation to desorb organics from their solid matrix, can decrease significantly the extraction time and the quantity of solvent required to efficiently extract target analytes (see Ref. [17] for example application). The efficiency of MASE is attributed to the elevated temperatures that exceed the boiling point of the solvent(s) and the rapid transfer of the analyte(s) to the solvent phase. Commercial systems are available that incorporate the capacity for simultaneously extracting multiple samples within closed, lined (perfluoroalkoxy), pressurized vessels (up to 8–12), using microwave absorbing solvents. Disadvantages of MASE include lack of selectivity and loss of thermolabile analytes.



33.3 figure Flow diagram for the QuEChERS procedure (From Sandra et al. [10], with permission)

33.2.2.3.5 Accelerated-Solvent Extraction

Accelerated-solvent extraction (ASE) (Chap. 17, Sect. 17.4.4) utilizes limited quantities of organic solvents at elevated temperature (up to 200 °C) and pressure (1,500–2,000 psi) to statically or dynamically extract solid samples for short periods of time (often <10 min) [18]. See Ref. [19] for example application of ASE for pesticides and antibiotic residues. Disadvantages of ASE include diluting effect and lack of selectivity, requiring further cleanup and concentration.

33.2.2.4 *Derivatization*

The chemical structure of analytes may need to be modified to become suitable for separation and detection by specific chromatographic techniques and detected via available and/or desirable detectors. The process of structural modification through multiple chemical reactions is known as **derivatization** (see Chap. 12, Sect. 12.3.4.2.1; Chap. 13, Sect. 13.2.4.7; Chap. 14, Sect. 14.2.3). Many types of derivatization reactions have been used in the analysis of contaminants and residues, such as selected pesticide residues as discussed in Ref. [20] and the antibiotic nitrofurans as discussed in Ref. [10].

33.3 PESTICIDE RESIDUE ANALYSIS

33.3.1 Introduction

A pesticide is any substance or a mixture of substances formulated to destroy or control "pests" including weeds, microorganisms (e.g., fungi or bacteria), insects, and even mammals. Currently, there are over 1,300 registered pesticides [21], main classes of which include: (1) **herbicides** [for weed control, e.g., triazine (e.g., atrazine)], (2) **insecticides** [e.g., organochloride (OC) (e.g., dichlorodiphenyltrichloroethane (DDT)), organophosphates (OP) (e.g., malathion, dimethoate, omethoate), and methyl carbamates (e.g., aldicarb)], and (3) **fungicides** [e.g., phthalimide (e.g., captan)]. Other types of pesticides may include acaricides, molluscicides, nematicides, pheromones, plant growth regulators, repellents, and rodenticides. Reference [5] lists active substances used in various pesticides products that belong to different chemical groups, and FDA provides a glossary of pesticide chemicals [22].

Pesticide residues may occur in food as a result of the direct application to a crop or a farm animal or as a postharvest treatment of food commodities. Pesticide residues also can occur in meat, milk, and eggs as a result of the farm animal consumption of feed from treated crops. Additionally, pesticide residues can occur in food from environmental contamination and spray drift.

If pesticides were not applied, an estimated one-third of the crop production would be lost. However, pesticides may have adverse effects on human health, including, but not limited to, cancer, acute neurologic toxicity, chronic neurodevelopment impairment, and dysfunction of the immune, reproductive, and endocrine systems. Accordingly, there are strict regulations for pesticide registration and use all over the world.

Risk assessment studies, required by the EPA, are done to determine the nature and extent of toxic effects and to establish the level at which no adverse effects are observed [**no observed adverse effect level** (NOAEL)]. Based on risk assessment studies, **tolerance levels** [or **maximum residue level** (MRL)] of pesticides (the active ingredients as well as toxic metabolites, and transformation products) in food are set and enforced by government agencies, as mentioned in Sect. 33.1, to protect stakeholders and regulate international trade. In fact, tolerance levels must be established prior to registration. In general, the tolerance levels in foods range between 0.01 and 10 mg/kg, depending on the commodity and the pesticide used. Low tolerance levels have necessitated the development of more accurate and sensitive analytical methods to meet the requirements in food.

33.3.2 Types of Analytical Methods

Several factors make the analysis of pesticide residues in food a complex task. These factors include the complexity of the food matrix, the possibility of having pesticide levels as low as pictogram or femtogram amounts, and the considerable differences in the physical and chemical properties of pesticides.

Analytical methods employed for the analysis of pesticides can be categorized into either **single-residue methods** (SRM) or **multiple-residue methods** (MRM). SRMs are designed to measure a single analyte and, often, its toxic metabolites. The majority of SRMs have been developed for purposes of registration and setting tolerance levels, or for investigating the metabolism and environmental fate of a specific pesticide. Sampling, extraction, purification, and determination in SRMs are optimized for the target pesticide. The majority of the SRMs currently in use, which have undergone EPA review or have been published in peer-reviewed scientific journals, are found in Volume II of the ***Pesticide Analytical Manual*** (*PAM* II) [23]. For purposes of monitoring quality and safety, given the large number of pesticides and the considerable differences in their physical and chemical characteristics (e.g., acidic, basic, or neutral, polar or nonpolar, and volatiles or nonvolatiles), MRMs can determine various pesticides in a single run and are much preferred. Many of the MRMs are found in Volume I of the ***Pesticide Analytical Manual*** (*PAM* I) [24]. AOAC International [25] also has developed an MRM for pesticide residues, the AOAC Pesticide Screen (AOAC Method 970.52). The identification and quantification in MRMs currently used by the FDA and USDA are based on GC and HPLC analysis.

Prior to chromatographic analysis, sampling, extraction, and fractionation/cleanup in MRMs are optimized to ensure an efficient transfer of most if not all of the pesticide residues present in the sample matrix to the organic phase. To achieve efficient transfer of as much of the pesticide residues as possible from the sample matrix into the organic phase, partitioning using water-miscible solvent is performed. This is followed by partitioning with nonpolar solvent that is miscible with the polar solvent, yet immiscible with water. After the extraction step, a cleanup step is performed using adsorption columns, in particular Florisil, alumina, and silica gels, with solvent mixtures of low polarity for elution. Commercial kits based on solid-phase extraction chromatography are available for pesticide cleanup. Reference [26] includes detailed descriptions of different MRM extraction and cleanup protocols specific for pesticide analysis.

33.3.3 Analytical Techniques Used for the Detection, Identification, and/or Quantification

A wide range of analytical techniques can be used in pesticide analysis. This section highlights some of the current analytical techniques used for the detection and determination of pesticide residues.

33.3.3.1 *Biochemical Techniques*

Biochemical techniques, such as enzyme inhibition assays and immunoassays, are widely used for the detection of pesticides. Commercially available test kits include enzyme inhibition assays. The principle of these assays is based on the inhibition of a specific enzyme, essential for vital functions in insects, by pesticides present in the sample. If no pesticides are present, the enzyme will be active and act on a substrate to cause a change in color. If no change in color occurs, then the test is positive, and further confirmation can be performed following more sophisticated analysis, such as HPLC and GC, to identify and quantify the specific pesticides present.

While enzyme inhibition assays are mostly used as screening methods, with limited sensitivity and selectivity, immunoassays can be tailored to particular purposes ranging from simple screening tests (field portable [27]) to quantitative laboratory tests. Immunoassays, which can be either class or compound specific, are simple and sensitive, have high throughput, and are cost effective as compared with other conventional methods. Additionally, extensive cleanup of extracts is not necessary, unless cross-reactivity exists. ELISA (Chap. 27, Sect. 27.3.2) accounts for almost 90% of the immunoassays used for pesticide residue analysis. References [26] and [27] include more information on the application of immunoassays in the analysis of pesticides.

33.3.3.2 *Chromatographic Techniques*

33.3.3.2.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC) (Chap. 12, Sect. 12.3.4.2) can be used for screening purposes in the analysis of pesticides. Because of its low resolving capacity, low precision, and limited detection relative to GC and HPLC, it is not used as a quantitative method. However, TLC can be used as a semiquantitative method that precedes more accurate detection and quantification. An example application is the detection and estimation of pesticides that inhibit insect enzymes such as cholinesterases. Once a crude extract is separated by TLC, the plate is sprayed with a solution containing the enzyme(s), followed by a solution containing a specific substrate, which releases a colored product. The lack of color change indicates enzyme inhibition, due to the presence of pesticide residues, and the zone of inhibition is proportional to the quantity of pesticide present.

33.3.3.2.2 Gas Chromatography

With the development of fused silica capillary columns (Chap. 14, Sect. 14.3.4.2), a large number of pesticides with similar physical and chemical properties can be separated and detected. In general, GC is the preferred method for the determination of volatile and thermally stable pesticides, such as the OC and OP classes. Choice of columns and detectors is made based on the nature of the pesticides. For example, 5% diphenyl, 95% dimethylpolysiloxane stationary phase columns are commonly used in MRMs.

Pesticides often contain heteroatoms, such as O, S, N, Cl, Br, and F, in a single molecule. Therefore, element-selective detectors are often used, such as a flame photometric detector (FPD), which is suited for the detection of P-containing compounds. The FPD (see Chap. 14, Sect. 14.3.5.4) is widely used for the detection of OP pesticides in various crops, without extensive cleanup required. For the determination of OC, the electron capture detector (ECD; see Chap. 14, Sect. 14.3.5.3) is used extensively due to its high sensitivity to organic halogen compounds. With a MRM approach for multiclass detection, and using these selective detectors, several GC injections are required, which is a limitation for conventional GC analysis. Additionally, identification in conventional GC analysis is highly dependent on retention time, which is not an absolute confirmation of identity, due to matrix interferences. Coupling of fused capillary columns to MS detection enhances not only the confirmation process but also the quantitative determination (see Sect. 33.3.3.3).

33.3.3.2.3 *High-Performance Liquid Chromatography*

Development of HPLC analysis for the separation and detection of pesticides became a necessity as the number of pesticides with poor volatility, relatively high polarity, and thermal instability increased. Classes such as N-methyl carbamate (NMC), urea herbicides, benzoylurea insecticides, and benzimidazole fungicides are typically analyzed by HPLC. These compounds are often analyzed by reversed-phase chromatography (Chap. 13, Sect. 13.3.2) with C18 or C8 columns and aqueous mobile phase, followed by UV absorption, fluorescence, or MS detection (Chaps. 7 and 11). When the sensitivity of UV and fluorescence detection is poor, postcolumn derivatization can be employed [6]. However, interference from other fluorescent compounds is a major disadvantage.

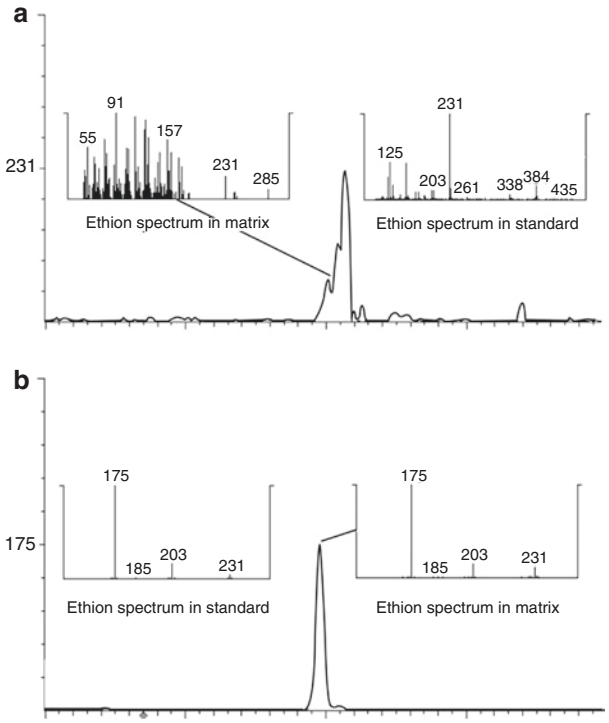
Analysis of pesticides in complex systems following conventional HPLC analysis using fluorescence or UV is often inadequate. Even the use of diode array detection might not be specific enough to resolve spectral differences, which are often too small. Utilization of MS detection widened the scope of HPLC analysis of pesticides. LC-MS is becoming one of the most powerful techniques for the analysis of polar, ionic, and thermally labile pesticides (see Sect. 33.3.3.3.2).

33.3.3.3 *Mass Spectrometry Detection*

Details on MS instrumentation, modes of ionization, and mass analyzers are provided in Chap. 11. The subsequent sections will provide sample applications of GC-MS and LC-MS used in pesticide residue analysis. References [6, 7, 16, 26, 28–31] describe more application examples and details on method development.

33.3.3.3.1 *Gas Chromatography-Mass Spectrometry*

The most common GC-MS technique for the analysis of pesticide residue involves single quadrupole instruments with electron impact (EI) ionization. The selected ion monitoring (SIM) option enhances selectivity, increases sensitivity, and minimizes interferences from co-extracted compounds. Ion-trap detectors (ITD) are also used in the analysis of pesticide residues. Using ITD, the analyzing in full-scan mode provides higher sensitivity as compared to single quadrupole analysis and allows for confirmation by library searches (NIST library spectrum search). Additionally, the ITD enables tandem MS analysis (MS/MS) by means of collision-induced dissociation (CID). The use of tandem MS improves selectivity and significantly reduces background without loss of identification capability, thus enabling the analysis of pesticides at trace levels in the presence of many interfering compounds. Figure 33.4 provides an illustration of enhanced compound identity confirmation upon the use of MS/MS vs. only MS. For the determination of multiclass residues, triple quadrupole (QqQ)



33.4 figure

Pesticide residue analysis in vegetable extract using (**a**) EI full scan, showing noisy baseline and no spectral match; vs. (**b**) Electron impact (EI) MS/MS showing clean baseline, symmetrical peaks, and excellent spectral match (Used with permission from Varian Inc., Palo Alto, CA)

MS is becoming a powerful and fast analytical tool, requiring minimum sample preparation. The chromatogram separation becomes less important when the analysis is carried out by GC/QqQ-MS/MS, since the analyzer is able to monitor simultaneously a large number of co-eluting compounds. Reliable quantitation and confirmation can be easily achieved, even at trace concentration levels. Time-of-flight (TOF) MS instruments also are gaining popularity for the simultaneous analysis of multiclass pesticides [16, 30].

33.3.3.3.2 *High-Performance Liquid Chromatography-Mass Spectrometry*

Similar to GC-MS, the use of LC-MS in the analysis of pesticide has undergone great development. Tandem MS methods, using atmospheric-pressure ionization (API), atmospheric-pressure chemical ionization (APCI), and electrospray ionization (ESI), have been developed for many pesticides such as OP, carbamate, and sulfonylurea pesticides [29]. Specifically, the ESI coupled with MS/MS was found to have high sensitivity and selectivity for a wide range of pesticides in foods. Because of HPLC solvent interferences, single quadrupole instruments are not as widely used in

LC-MS as compared to GC-MS for pesticide analysis. When using LC-MS for pesticide residue analysis, QqQ are the most widely used mass analyzers. LC/TOF-MS has also gained popularity due to its high speed, sensitivity, and selectivity [31].

33.4 MYCOTOXIN ANALYSIS

33.4.1 Introduction

Molds, which are filamentous fungi, can develop on food commodities and produce various types of chemical toxins, collectively known as **mycotoxins.** The main producers of mycotoxins are fungal species belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. Crops can be directly infected with fungal growth and subsequent mycotoxin contamination as a result of environmental factors such as temperature, humidity, weather fluctuations, mechanical damage of crops, and pest attack. In addition, plant stress due to extreme soil dryness or lack of a balanced nutrient absorption can induce fungal growth. Fungal infection and mycotoxin contamination can occur at any stage of the food chain (Sect. 33.1). The United Nation's Food and Agriculture Organization estimated that up to 25% of the world's total crops per year are affected with "unacceptable" levels of mycotoxins [32].

Mycotoxin contamination may occur in food as a result of the direct mold infection of plant-origin commodities, such as cereals, dried fruit, spices, grape, coffee, cocoa, and fruit juices (especially apple based). Mycotoxins also can occur in milk, eggs, and, to a minor extent, meat, as a result of the farm animal consumption of feed from contaminated crops. Additionally, humans and animals can be exposed to mycotoxins through heavily contaminated dust, as in the case of harbors and warehouses.

More than 300 mycotoxins, belonging to various chemical classes, are known. However, the major classes of mycotoxin with a toxicological impact on human health include **aflatoxins** (B1, B2, M1, M2, G1, and G2), **ochratoxins** (e.g., ochratoxin A, OTA), **trichothecenes** [e.g., deoxynivalenol (DON), T2, and HT-2], **fumonisins** (FBs, e.g., FB1, FB2, and FB3), **patulin** (a mycotoxin that occurs mainly in apples and apple products), and **zearalenone** (ZEA). The chemical classification and occurrence of mycotoxins (the major as well as minor ones) in raw commodities and processed foods is described in Ref. [32]. The toxic effects of the aforementioned mycotoxins include, but are not limited to, genotoxicity, carcinogenicity, mutagenicity, and immunotoxicity. Genotoxic compounds have a probability of inducing an effect at any dose; therefore, no threshold dose should be considered and they should not be present in food. For example, there is no postulated safe dose for aflatoxin B1, which is genotoxic and is the most potent naturally occurring carcinogenic substance known. However, to provide risk managers with necessary basis for making decisions, threshold doses and "safe" **total daily intake** or **total weekly intake** (TDI or TWI) have been set for other mycotoxins (Table 33.2). Based on the threshold doses, tolerance levels for mycotoxin were set in the USA (e.g., 0–35 μg/kg for aflatoxins, 2.5–50 μg/kg for OTA, 300–2000 μg/kg for DON, and 5–50 μg/kg for patulin).

While molds can be destroyed by natural causes or processing of food, mycotoxins can survive. To limit human exposure to mycotoxins, a key critical control point is avoiding the processing of raw materials with unacceptable mycotoxin levels. Therefore, implementing periodic testing is a necessity, including the adoption of reliable sampling procedures and validated methods of analysis. Organizations such as the AOAC International, American Oil Chemists' Society (AOCS), AACC International, and the International Union of Pure and Applied Chemistry (IUPAC) have method validation programs for mycotoxin analysis. References [33–37] provide detailed information on mycotoxin occurrence, health effects, control, sampling and sample preparation, and analysis.

33.4.2 Sampling

Compared to the analysis of other types of residues, the sampling step in the analysis of mycotoxins is by

33.2 table Total daily intake (TDI)/total weekly intake (TWI) for the major mycotoxins

Mycotoxin[a]	*TDI (ng/kg bw per day)*	*Organization*[b]
OTA	4	Health Canada (1989, 1996)
	5	Nordic Council (1991)
	5	EU (1998)
	14	JECFA (1996, 2001)
	120 (TWI)	EFSA (2006 [81])
FBs	2000	EU (2000)
	2000	JECFA (2000)
	400	Health Canada (2001)
DON	3000	Health Canada (1985)
	1000	Health Canada (2001)
	1000	Nordic Council (1998)
	1000	EU (1999)
	1000	JECFA (2000)
ZEA	100	Health Canada (1987)
	100	Nordic Council (1998)
	500	JECFA (2000)
	200	EU (2000)
Patulin	400	JECFA (1996)
	400	EU (2000)
	400	Health Canada (1996)

From Brera et al. [32], used with permission

[a]*OTA* ochratoxin, *FBs* fumonisins, *DON* deoxynivalenol, *ZEA* zearalenone

[b]*EU* European union, *JECFA* Joint FAO/WHO expert committee on food additives

far the largest contributor to the total error. The possible variability is associated with the level and distribution of mycotoxins in the food commodity. An unevenly distributed 0.1 % of the lot is usually highly contaminated, resulting in an overall level above the tolerance limit. Due to this heterogeneous distribution, an appropriate sampling plan is needed to ensure that the concentration in a sample is the same as that in the whole lot. An incorrect sampling protocol can easily lead to false conclusions, often false negative, leading to undesirable health, economic, and trade impacts.

The Commission Regulation 401/2006 [36] provided specifications for the sampling of regulated mycotoxins in various food commodities including cereals, dried fruits, nuts, spices, milk and derived products, fruit juice, and solid apple products. Also, AOAC International, AOCS, and the FDA in collaboration with the USDA have developed detailed sampling plans for separate commodities. In general, an acceptable plan involves obtaining a large number of samples from multiple locations throughout the lot, creating a composite sample, grinding or slurring the composite sample (to reduce particle size and/or increase homogeneity), and subsampling for laboratory analysis. The number and size of collected samples and laboratory subsamples are dependent on the matrix and the size of the lot.

An example of sampling for mycotoxin analysis is the sequential sampling of raw shelled peanuts (with a tolerance level of less than 15 μg/kg for aflatoxins); a bulk sample of approximately 70 kg is randomly accumulated (at a rate of one incremental portion per 225 kg of lot weight). This bulk sample is divided randomly into three 21.8-kg samples using a Dickens mechanical rotating divider and then ground separately. A subsample (1,100 g) is taken from one of the samples, formed into a slurry, and analyzed for aflatoxins, in duplicate. If the average of the two determinations is ≤8 μg/kg, the lot is passed and no further testing is performed. If the average is ≥ 45 μg/kg, the shipment is rejected. For averages >8 μg/kg and <45 μg/kg, a second 21.8-kg sample is analyzed in duplicate, and the average of the four results is used to decide whether to accept (≤12 μg/kg) or reject (≥23 μg/kg) the lot. If the average falls between the second set of determining values, the third 21.8-kg sample is analyzed. If the average of the six determinations is <15 μg/kg, the lot is accepted.

33.4.3 Detection and Determination

Post sampling, sample preparation commonly includes extraction, cleanup, and concentration. Sample preparation steps (discussed in Sect. 33.2.2) that are specific for mycotoxin analysis have been developed. Shaking or blending often is used for mycotoxin extraction, while SPE often is used for cleanup, especially when multi-mycotoxin analysis is needed. For a specific mycotoxin, immunoaffinity LC (IAC) is used for cleanup.

Tremendous effort has been made to develop and optimize qualitative and quantitative methods for mycotoxin analysis. A list of official methods of mycotoxin analysis is included in Ref. [32] and others in Refs. [33–35, 37]. Mycotoxin analysis kits, both for cleanup (for GC, HPLC, or TLC analysis) and for detection (e.g., ELISA, LFS, immunoaffinity with HPLC), are commercially available. The sections below briefly discuss some of the current and newly developed analytical techniques used for the detection and determination of mycotoxins in food.

33.4.3.1 *Rapid Methods of Detection*

33.4.3.1.1 TLC

A large number of TLC methods for the analysis of mycotoxins have been accepted by AOAC International, including DON in barley and wheat, aflatoxin in peanuts and corn, aflatoxin M1 in milk and cheeses, OTA in barley and green coffee, and ZEA in corn. Conventional TLC techniques are commonly used for screening purposes, with detection limits reaching 2 ng/g. When results are positive, confirmatory and more sensitive quantitative analysis follows. The overall performance of TLC for the analysis of mycotoxin is improved when used in combination with IAC. Additionally, a microcomputer interfaced with a fluorodensitometer and semiconductor-based detection improves the data handling.

33.4.3.1.2 Immunoassays

Three main types of immunoassays can be used for the analysis of mycotoxins: **radioimmunoassay** (RIA), ELISA (see Chap. 27), and **fluorescence polarization immunoassay** (FPIA). The use of RIA, where the mycotoxin (e.g., aflatoxin) is radiolabeled, has gradually been replaced by ELISA. Since all mycotoxins are relatively small molecules (MW <1,000), competitive ELISA is used (see Chap. 27, Sect. 27.3.2). In FPIA, fluorescein-labeled mycotoxin competes with unlabelled mycotoxin analyte in a sample for binding with the antibodies. FPIA has gained popularity since it involves no coating of the plate and less analysis time as compared to ELISA. Results of FPIA were comparable to that of ELISA for the analysis of DON, ZEA, and OTA [32].

Immuno-based testing methods, including membrane-based immunoassays, LFS assays, and biosensors, also have been developed for online control or field testing of mycotoxins. Membrane-based immunoassays are based on the principle of direct competitive ELISA, with the anti-mycotoxin antibody being coated on the membrane surface. For example, test kits based on membrane-based immunoassays have been validated for OTA determination in wheat, rye, maize,

and barely [38]. LFS, which is an immunochromatographic test (see Chap. 27, Sect. 27.4), is rapid and capable of detecting many mycotoxins simultaneously. However, any positive test requires confirmation by a reference method such as HPLC (see Sect. 33.4.3.2.1). Biosensors, on the other hand, are compact analytical devices that use biological components such as nucleic acids, enzymes, antibodies, or cells, associated with a transduction system. The transduction system processes the signal produced by the interaction between the target molecule and the biological component. The use of biosensors for the detection of several mycotoxins, such as aflatoxins DON and OTA, is rapidly increasing [32].

33.4.3.2 *Quantitative and Confirmative Chemical Methods*

33.4.3.2.1 HPLC

For quantitative determination, HPLC is the methodology of choice for most mycotoxins, specifically, aflatoxins, DON, OTA, ZEA, FBs, T-2 and HT-2 toxins, and patulin. Aflatoxins, OTA, and ZEA exhibit a native fluorescence and are detected directly by fluorescence detector. Pre- or postcolumn derivatization (with trifluoroacetic acid, iodine, or bromine) is required to enhance the fluorescence detection of some aflatoxins (i.e., AFB1 and AFG1), whereas precolumn derivatization with the OPA reagent is required for fluorescence detection of FBs. However, direct UV detection is used for DON and patulin. Reversed-phase chromatographic separation is normally employed for multi-mycotoxin analysis. A multifunctional column, which consists of a mixture of reversed-phase, size exclusion, and ion-exchange stationary phases, also can be used (e.g., AOAC Method 49.2.19A for aflatoxin analysis). Methods using IAC-HPLC are reported for the determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder [39]. Validated HPLC methods for the analysis of various mycotoxins are periodically reported in Methods Committee Reports published in *Journal of AOAC International*.

As is the case with pesticide residue analysis, coupling of HPLC with MS analysis, especially LC-MS/MS, provides greater sensitivity and selectivity, and allows for simultaneous analysis of multiclass mycotoxins. Additionally, the use of LC-MS/MS allows for the detection of conjugated mycotoxins (i.e., the toxin bound to a polar compound such as glucose or another sugar), which are referred to as "masked" or "modified" mycotoxins because they escape routine detection. A method has been developed for the determination of 39 mycotoxins, including conjugated DON, FBs, ZEA, and aflatoxins, in wheat and maize, using LC-ESI-triple quadrupole MS, without cleanup [40].

33.4.3.2.2 GC

GC is not widely used for the detection of mycotoxins, except in the case of trichothecenes. Trichothecenes do not strongly absorb in the UV-Vis range and are nonfluorescent; therefore, GC methods were developed for their determination. Capillary column GC is commonly employed for the simultaneous detection of different trichothecenes, e.g., DON, T2 toxin, and HT-2 toxin, using trifluoroacetyl, heptafluorobutyry, or trimethylsilyl derivatization coupled with electron capture detection. GC is often linked to MS for peak confirmation. GC-MS also can be used for the confirmation of patulin in apple juice [32]. Validated and accepted methods for the determination of trichothecenes using GC are reported as AOAC Official Methods and by the American Society of Brewing Chemists.

33.4.3.2.3 Capillary Electrophoresis

Capillary electrophoresis (CE; see Chap. 24 Sect. 24.2.5.3), generally used as a chromatographic technique, can be employed to separate mycotoxins from matrix components using electrical potential. Methods are available for the determination of patulin in apple juice [41], simultaneous determination of ochratoxins (A and B) and aflatoxins [42], and determination of ZEA using cyclodextrins (for enhancing the native fluorescence) [43].

33.4.3.3 *Other Methods of Analysis*

Other methods for the detection of mycotoxins have been reported such as **near-infrared** (NIR) and **mid-infrared** (mid-IR) spectroscopy, especially **Fourier transform infrared** (FTIR) spectroscopy (a type of MIR) (see Chap. 8, Sect. 8.3.1.2). FTIR spectroscopy was used for the detection of mycotoxins in infected corn by gathering information from mid-IR absorption spectra [44]. Rapid and accurate identification of mycotoxigenic fungi and their mycotoxins (e.g., FB1 and DON) was achieved using MIR and NIR spectroscopy [45, 46]. Calibration for these methods is based on reference HPLC or GC methods. Testing and validation of IR methods for the detection of mycotoxins are currently being pursued.

33.5 ANTIBIOTIC RESIDUE ANALYSIS

33.5.1 Introduction

Animals intended for human consumption may not only be given drugs (e.g., antibiotics, antifungals, tranquilizers, and anti-inflammatory drugs) at therapeutic levels to combat diseases, but also some countries allow use of drugs (mainly antibiotics) at subtherapeutic levels to reduce the incidence of infectious diseases and for weight gain advantages. The **Center for Veterinary Medicine** (CVM), a branch of the FDA,

regulates the manufacture, distribution, administration, and withdrawal period (time between the last drug treatment to the animal and the slaughter or use of milk or eggs by humans) of drugs given to animals, including animals from which human foods are derived. Most drug residues of concern for human food are antibiotic residues and thus are the focus of this section. Some of the major families/types of antibiotics, referred to in later sections, include β-lactams, sulfa drugs, cephalosporins, tetracyclines, and chloramphenicol.

Residual levels of any antibiotics given to animals used for human consumption are of concern for a variety of reasons, including the fact that some consumers are allergic to certain antibiotics, and the possibility that some microbes may develop antibiotic resistance due to constant exposure. Some antibiotics can be carcinogenic as well, such as nitrofuran compounds [47]. Also, on a practical level for dairy products such as cheese made with starter cultures, antibiotic residues would reduce the intended microbial growth and therefore reduce acid production. For all these reasons and more, the FDA has strict regulations on antibiotic residues in human food. Due to these regulations, antibiotic-contaminated meat and milk (including milk products) are considered to be adulterated. The FSIS monitors antibiotic residues in meat products from cattle, swine, lamb, goat, and poultry. Number of violations vary from year to year, for example, 38 antibiotics violations were reported in 2004, while 8 were reported in 2011 [48]. FDA's Center for Food Safety and Nutrition monitors antibiotic residues in milk and its products, including milk from pick-up tanks, pasteurized fluid milk, cheeses, etc. In 2014, out of four million samples tested, 703 were reported to have antibiotic residues including β-lactam and sulfonamide [49].

Samples are tested for antibiotic residues using rapid screening methods. A positive result from a screening test suggests that one or more types of antibiotic are present, so further testing is required to identify and quantify the specific antibiotics present.

33.5.2 Detection and Determination

Often, procedures such as defatting, protein hydrolysis (in case of meat or egg samples), protein precipitation (in case of dairy samples), and aqueous wash (in case of honey to remove excess sugar) precede the extraction of antibiotic residues. For the extraction of many antibiotics, liquid-liquid extraction and SPE are commonly used, followed by a partial purification step often using ion-exchange cleanup systems that take advantage of the acid/base character of the antibiotics. Sample preparation steps, including extraction and isolation (as discussed in Sect. 33.2.2) specific for drug residues, are reviewed in Ref. [50].

Tolerance levels are established for some antibiotics, while others have a zero tolerance (as is the case for nitrofurans and chloramphenicol). Chloramphenicol, for instance, is an antibiotic of considerable current concern in the USA, European Union, and other countries. It is used in some parts of the world in producing shrimp and has been found in imported seafood products (e.g., shrimp, crayfish, and crab). Because of the adverse health effects on humans, the FDA has banned the use of chloramphenicol in animals raised for food production and set a zero tolerance in human food [21 CFR 522.390 (4)]. Therefore, the analytical methods need to be as sensitive and selective as possible.

A wide variety of analytical methods have been developed and optimized for the analysis of antibiotics, categorized as screening or determinative and confirmatory. Reference [4] lists the methods commonly used in the analysis of several antibiotics, and Ref. [51] gives the process used by the AOAC to validate test kits. One excellent resource for antibiotic test methods, specific for milk and dairy products, is Ref. [52]. The sections below briefly discuss some of the analytical techniques used for the detection and determination of antibiotics in susceptible food commodities.

33.5.2.1 *Screening Methods*

Some of the major categories for rapid screening assays, some of which are quantitative, are: (1) microbial growth inhibition, (2) receptor assays, (3) enzyme-substrate assays, and (4) immunoassays. Some screening methods are specific to individual antibiotics, some are specific to a class of antibiotics, and some have no specificity. Screening assays for antibiotic residues in test samples initially relied mostly on inhibition of microbial growth, but now many use other principles for detection.

In **microbial growth inhibition** assays, turbidity, zone of inhibition, or acid production can be measured. In a **turbidity assay**, an indicator organism growing in a clear liquid culture will cause an increased turbidity; growth is inhibited, so turbidity is reduced if antibiotics are present. In a **zone of inhibition assay**, the test material diffuses through an agar-based nutrient medium that has been uniformly inoculated with spores of a susceptible organism. Any antibiotics present in the test material will inhibit the germination and growth of the organism, creating clear zones. In the **acid production assays**, the acid produced when microbes grow causes a color change in the medium. No color change means that the test sample contained an inhibitory substance. While microbial growth inhibition assays are more time consuming than many newer screening tests, they are inexpensive and applicable to testing large numbers of sample and provide some sensitivity to multiple antibiotic categories [52]. AOAC Official Methods

include a nonspecific microbiological method for antibiotics and numerous microbiological methods for specific antibiotics [25].

An example of a **receptor assay** is the Charm II® test (Charm Science, Lawrence, MA), which has different versions, designed to detect different groups of antibiotics. The assay involves a competition between labeled antibiotics (labeled using ^{14}C or ^{3}H, depending on the specific type of Charm II® test system) and antibiotic residues in a milk sample for a limited number of specific binding sites on the surface of bacteria added to the test sample. The greater the concentration of antibiotic residue in the sample, the less radiolabeled tracer will become bound to the microorganism. The method can be applied to milk, certain dairy products, honey, and meat.

Enzyme-substrate assays measure the inhibition of an enzyme acting on a substrate, caused by the presence of an antibiotic. An example used for testing raw milk is the Penzyme® III commercial kit (Neogen, Lansing, MI), which is specific for β-lactam antibiotics, and inhibits D,D-carboxypeptidase on an equimolar basis. When this enzyme acts on a specific substrate, it causes release of D-alanine, which can be measured in additional steps of the assay resulting in a color change [52].

Immunoassays, namely, ELISA and LFS (see Chap. 27, Sects. 27.3.3 and 26.3.4, respectively), also can be used for screening of antibiotic residues. The Charm ROSA (Rapid One Step Assay) MRL assay, intended for milk and cream testing, is an example that uses a lateral flow strip. Another example of an immunoassay marketed for testing milk is the SNAP® kit (IDEXX Laboratories, Inc., Westbrook, ME). The assay is based on a competition between residual antibiotics in a milk sample and enzyme-labeled antibiotics in the test kit. The enzyme acts on a substrate to cause a color change; any antibiotics in the milk will result in a decrease in color development. Competitive ELISA can be used for the detection of a specific antibody, for example, the Veratox® assay (Neogen Corporation, Lansing, MI) used to detect chloramphenicol.

33.5.2.2 *Determinative and Confirmatory Methods*

Quantitative determination of antibiotic residues in food products follows the same general steps as for other trace analytes. After sample preparation steps, the partially purified extract is subjected to chromatographic separation, detection, and quantification. The most commonly used chromatography system is HPLC (mostly using reversed-phase separation mode) coupled with UV, fluorescence, chemiluminescence, or postcolumn reaction detectors. For confirmatory and identification purposes at trace levels, LC-MS and LC-MS/MS are increasingly used in the analysis of antibiotics [5, 19].

The FDA provides regulatory LC-MS/MS methods to detect fluoroquinolones in honey [53]. For fluoroquinolones in salmon, the FDA has LC method with fluorescence detection [54] and a confirmatory LC/MS method intended for both salmon and shrimp [55]. The confirmatory FDA method for fluoroquinolones in catfish is an electrospray LC/MS method [56]. Additionally, LC-MS/MS has been used for confirmation of β-lactam residues in milk [57]. The detection of 14 different types of sulfonamides, in milk [58] and in condensed milk and soft cheeses [59], at levels below 10 ng/mL, also was achieved using LC-MS/MS. LC-MS/MS has been compared with ultra-high-performance liquid chromatography/quadrupole time-of-flight MS (UHPLC/Q-Tof MS) for analysis of macrolide antibiotic residues in a variety of foods [60]. LC-MS/MS gave a lower limit of detection and better precision, but the UPLC/Q-Tof MS provided better confirmation of positive findings. (See Chap. 13, Sect. 13.2.3.3, for UHPLC.)

33.6 ANALYSIS OF GMOS

33.6.1 Introduction

Agriculturally important plants may be genetically modified by the insertion of DNA from a different organism (**transgene**) into the plant's genome, conferring novel traits that it would not have otherwise, such as herbicide tolerance or insect resistance. The modified plant is termed a **genetically modified organism**, or **GMO**. GMO production relates to a number of food crops, including the following: corn, soybeans, cotton, canola (a cultivar of rapeseed), rice, sugar beets, and papaya. Although protection from pests and herbicide tolerance are the most common GMO traits, other GMO crops include plants that have been modified to improve postharvest quality or enhance the nutritional makeup of the food. Examples include vegetables with an extended shelf life, apples that have reduced browning rate (Granny Smith apples), and "golden rice," which produces the precursor to vitamin A.

Despite the prevalence of GMO crops, there are concerns about limiting crop variation (i.e., monocropping); unintended impacts on other plants, insects, wildlife, and nearby communities; and possible allergies. The debate about the use of GMO crops has resulted in government regulation for application and labeling. There are specific guidelines put forth by various governments regarding the analyses used. Therefore, even producers must be able to accurately test ingredients and other products.

Many companies produce high-quality, easy-to-use test kits, many of which are specific for a certain GMO protein or gene. These kits generally fall into two categories, based on the methodology: **polymerase chain reaction** (PCR) kits that specifically amplify the DNA of the GMO gene (specific or shared

by many GMOs) so that it can be detected and **immunoassays** (ELISA and LFS) that are specific for the proteins. Further reading on the GMO and GMO detection topics is listed in the Refs. [61–63].

33.6.2 DNA Methods

Detection of the transgene DNA is an effective method of testing for GMO material in a sample. The analysis involves three distinct steps: (1) **extraction** of the DNA from the sample, (2) **amplification** of the DNA by PCR, and (3) **identification and quantification** of the amplified DNA (note: for **real-time quantitative PCR analyses**, amplification and detection/quantification occur at the same time). While all three steps are important, the PCR amplification is critical to the specificity and success of the analysis.

33.6.2.1 *DNA Extraction*

Application of extreme levels of sheer and heat can damage the DNA, rendering the subsequent PCR and detection ineffective. Extraction of the DNA from the food matrix, therefore, should be performed prior to significant processing of the material, preferably on raw ingredients. DNA extraction protocols differ somewhat, but all include disrupting the matrix to release the DNA, usually by grinding the sample to a fine powder. This is followed by dispersal of the ground material into an extraction solution and removal of unwanted components. For example, lipids may be removed by solvent extraction and protein by the addition of a protease. A final step may involve the precipitation of the DNA with cold alcohol, such as ethanol or isopropanol.

33.6.2.2 *PCR Amplification*

PCR is a cyclic method that exponentially increases the copy number of a specific DNA sequence, by means of enzymatic replication. It uses thermal cycling to alternately replicate the target sequence and then melt the DNA into single strands in order to repeat the process. The method is based on the use of two synthetic DNA fragments that are complementary to opposite ends of the target sequence. These fragments are termed the **primers**, which are commonly 18–35 bases in length, and they can be produced only when the target sequence is known. If a general identification of any GMO material is desired, then the primers used would be complementary to the promoter sequence, which is common to all transgenic crop plants that are normally grown for industrial food production. If a specific GMO product is to be identified, then the primers would consist of a sequence that includes the transgene DNA and plant DNA. This is necessary to avoid the detection of DNA that originates from bacteria that may have been on or in the plants.

In addition to the specific primers, the PCR mixture also includes a heat-stable **DNA polymerase**, such as Taq polymerase (from *Thermus aquaticus*); the **nucleotide bases** that comprise DNA, in the form of **deoxynucleoside triphosphates** (dNTPs); and a buffer solution to maintain the optimal conditions for the reactions. All of these components are present in the commercial kits. The reaction vials are then placed in a **thermal cycler**. Once the PCR system is started, the mixture is first put through a high-temperature cycle to melt the DNA (separate it into single strands), then a lower-temperature cycle to allow the primers to anneal to the single-stranded target DNA, and finally an intermediate-temperature cycle to allow the DNA polymerase to synthesize a new DNA strand complementary to the target strand by adding dNTPs, starting at the primers. The process is then repeated, usually for 30–50 cycles, which is sufficient to produce millions of copies of the DNA.

33.6.2.3 *DNA Analysis*

When sufficient DNA has been generated by PCR, the sample can be analyzed by agarose gel electrophoresis. The sample and standards migrate through the gel, and after the run, the gel is stained and the presence and abundance of DNA can be identified, by comparison to the position and degree of staining of the standards.

Some of the commercial test kits contain reagents to specifically label the double-stranded DNA with tags, such as fluorescein. These kits are meant to be used with special PCR equipment that deposits the finished, labeled mixture into a capillary where the intensity of the fluorescence, which is proportional to the abundance of DNA, is determined by high sensitivity fluorescence spectroscopy, rendering the electrophoresis step unnecessary. In this case the specificity is contingent on the sequence of the primers, which directly determine the nature of the double-stranded DNA present. These kits also include standards and other reagents necessary for accurate quantification of the target DNA abundance in the sample. If real-time PCR is used, the fluorescence is read in the reaction tube after each cycle of amplification, yielding a curve containing multiple data points rather than a single data point for each sample.

33.6.3 Protein Methods

The protein methods for GMOs are immunoassays, primarily ELISA (Chap. 27, Sect. 27.3.2) and LFS (Chap. 27, Sect. 27.3.4), generally used for testing raw agricultural products and not processed products. Both qualitative and quantitative ELISA test kits for GMOs are commercially available, with the latter having typical detection limits of 0.01–0.1 %. The LFSs are generally less sensitive (0.1–1.0 %), but their speed and ease of use make them ideal for testing in the growing field, at storage areas, and at points of transport.

33.7 ALLERGEN ANALYSIS

33.7.1 Introduction

Food allergens are food proteins that trigger an allergic response. Symptoms of an allergic response include hives, face and tongue swelling, and difficulty breathing and can include the severe, life-threatening allergic reaction called anaphylactic shock. It is important to note that food allergy, which triggers an immune system reaction, is distinct from other adverse responses to food, such as food intolerance (e.g., lactose intolerance), pharmacologic reactions (due mainly to food additives such as sulfites and benzoate), and toxin-mediated reactions (due to residues such as pesticides and mycotoxins).

A significant percentage of the population has food allergies, and the prevalence is rising. More than 160 foods can cause allergic reactions in people with food allergies, but over 90% of the food allergic reactions in the USA are caused by the eight most common allergenic foods, referred to as '**the big eight**': milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans [64]. In the USA, the Food Allergen Labeling and Consumer Protection Act of 2004 targets these eight food allergens [65]. It applies to all foods regulated by the FDA (both domestic and imported), requiring that labels list all ingredients by their common names and identify the source of all ingredients derived from the eight most common food allergens. Food allergies are an issue around the world; numerous other countries have or are considering labeling regulations. Since there is no cure for food allergies, strict avoidance of food allergens is the only effective action. All of these facts point to the importance of both screening and quantitative methods for analysis of allergenic foods.

Methods available for the detection of food allergens are mainly based on protein or DNA detection, as discussed in subsequent sections. A commercially available rapid screening method that is not based on protein or DNA detection is the swab and adenosine triphosphate (ATP)-sensitive detection method. This method is based on the detection of ATP present on the surface of multiple allergenic foods, e.g., peanut butter, whole egg, soybeans, and milk. It is used mainly to prevent food allergen cross contact during cleaning of processing equipment. Test kits for food allergen analysis (protein or DNA based and others) are commercially available. A review of food allergen analytical methods is given in Ref. [66].

33.7.2 Protein Methods

33.7.2.1 *General Considerations*

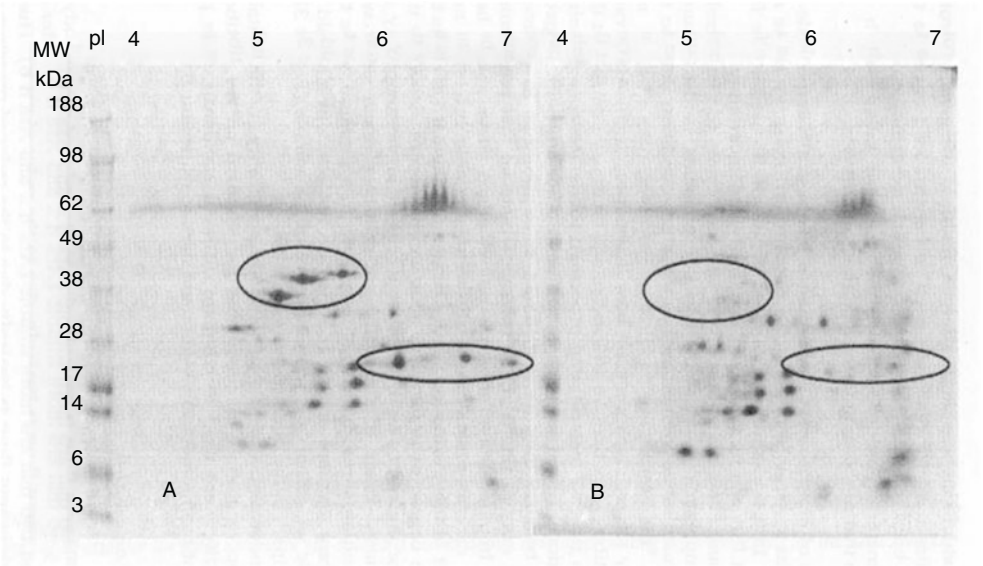
Similar to the analysis of many other food constituents of concern present in small amounts, sampling adequacy and the detection limits are of concern with analysis for allergens. Another concern is the adequate extraction of the different allergens. Unlike the trace analytes of concern mentioned thus far, since the analytes are proteins, the extraction solution is normally a buffer at various pHs and salt concentration. Extraction buffers differ in their ability to extract food allergens, with some solutions extracting the same allergens in different concentrations, while others cannot extract all the allergens. For example, phosphate buffer fails to extract the major peanut allergen (Ara h 3); however, the efficiency of extracting this allergen is greatly enhanced upon the addition of salt (Fig. 33.5). Additionally, it is crucial that the extraction solution is compatible with the assay used (e.g., immunoassay) and does not alter the chemical structure of the analyte. The choice of extraction procedure should also take into consideration the food processing conditions employed. Upon processing, the solubility of the proteins can be reduced due to denaturation and aggregation, resulting in reduced protein recovery [67]. Therefore, to obtain reliable and accurate results, it is crucial to select the right extraction solution for the target analyte(s).

33.7.2.2 *Protein-Based Analytical Techniques*

Classical protein-based methods used for the analysis of food allergens usually involve antibody-based assays (immunoassays) due to their specificity and sensitivity. Immunoassays target the offending allergen by use of monoclonal or polyclonal antibodies, or a combination of both. Most of the commercially available test kits, which differ in specificity and the number of proteins they target, use polyclonal antibodies. Immunoassay-based methods used for the analysis of food allergens include ELISA (Chap. 27, Sect. 27.3.2), LFS (Chap. 27, Sect. 27.3.4), Western blot (Chap. 27, Sect. 27.3.3.1), biosensor immunoassays (antibodies immobilized on a biosensor chip), and dot immunoblotting (Chap. 27, Sect. 27.3.3.2) [68].

Western blot and dot immunoblotting are mostly used for qualitative and screening purposes. The most commonly used immunoassays for the quantitative analysis of food allergens are ELISA methods, using a competitive or, more commonly, sandwich format (see Chap. 27, Sect. 27.3.2.3). The competitive ELISA is used for the small protein allergens, with a molecular weight less than 5 KDa. Numerous sandwich and competitive ELISA methods have been developed for several food allergens [66]. Lateral flow test strips are useful for screening purposes because they are very rapid and inexpensive and do not require instrumentation.

There are several drawbacks when using immunoassays, including cross-reactivity of antibodies with other proteins, food matrix interferences, structural changes due to food processing, destruction of epitopes upon adsorption onto solid matrices, and variability between manufactures due to use of different extraction buffers and antibodies. Often



33.5 figure

Evaluation, by two-dimensional electrophoresis, of the ability of (**a**) high salt buffer and (**b**) phosphate buffer to extract the peanut allergen Ara h 3 from raw peanuts (From Westphal [67], with permission)

times, 1-D or 2-D electrophoresis is used to isolate target protein to avoid cross-reactivity. In spite of the drawbacks, immunoassays are most commonly used for the detection of allergens. However, advanced proteomic techniques are evolving including mass spectrometry-based proteomic methods, most notably LC coupled with tandem MS, capable of detecting and quantifying multiple allergens simultaneously [66].

33.7.3 DNA Methods

There are several advantages and disadvantages for the use of DNA-based methods in the analysis of food allergens. DNA-based methods do not target the allergen in the sample; therefore, the detection of the allergen-encoding DNA does not always correlate with the presence of the allergen, especially when the food has been fortified with purified protein. Upon processing, for example, production of protein isolates, protein, and DNA could be separated resulting in false conclusions regarding the presence of the allergen in the sample. Regardless of these disadvantages, DNA-based methods are very specific and sensitive techniques with the advantage that targeted DNA is less affected by several processing and extraction conditions as compared to proteins. The choice to use DNA-based methods is dependent on the type of sample being analyzed.

DNA-based methods involve the extraction of the DNA (see Sect. 33.6.2.1) followed by amplification by PCR using a thermostable polymerase (see Sect. 33.6.2.2). The amplified sample is then visualized by fluorescence staining or by Southern blotting following agarose gel electrophoresis. This procedure normally provides qualitative data, or semiquantitative data if internal standards were used. Quantification can be achieved if real-time PCR (see Sect. 33.6.2.1 and Ref. [66]) or PCR-ELISA was used. PCR-ELISA method involves linking the amplified DNA fragment of an allergenic food to a specific protein-labeled DNA probe, which then is coupled with a specific enzyme-labeled antibody. Quantification of the DNA is based on the enzyme-substrate color producing reaction. Reference [60] includes a list of commercially available real-time PCR and PCR-ELISA test kits; for the analysis of allergens, readers are referred to Ref. [66].

33.8 ANALYSIS OF OTHER CHEMICAL CONTAMINANTS AND UNDESIRABLE CONSTITUENTS

33.8.1 Introduction

Pesticide residues, mycotoxins, antibiotic residues, and allergens in foods have been of concern for many years. However, in any given time period, there are numerous additional chemical hazards that must be addressed. Many of these substances fall into the following categories: (1) banned (e.g., coumarin) or allowed in some countries but not others, (2) legally limited (e.g., sulfites, benzene), (3) intentional contaminants (e.g., melamine), (4) approved for use but of concern (e.g., monosodium glutamate), or (5) natural constituents of concern (e.g., acrylamide, furan). This section would be very lengthy to cover in detail the screening and quantitative methods for many of these chemical hazards. Instead, only a summary (Table 33.3) is given of select current and emerging hazards, with

33.3 table Select compounds of concern and their methods of analysis

Compound	*Nature of compound and reason in food*	*Reason of concern*	*Major foods/ ingredients identified*	*Major methods*
Acrylamide	Formed in carbohydrate-rich foods cooked at high temperature	Neurotoxin and carcinogen, and other health risks	Fried and oven-based foods high in carbohydrates	LC-MS/MS
Benzene	Can form at low levels in some beverages containing both ascorbic acid and benzoate salts	Carcinogen	Soft drinks	Headspace GC-MS
Bisphenol A (BPA)	Used to make polycarbonate plastic, but can leach from plastic	Can mimic body's own hormones. Associated with multiple health problems. Banned or amounts are limited by various countries	Foods/beverages in polycarbonate plastic	GC- MS, GC-MS/MS, LC-MS, LC-MS/MS
Cyanide	Naturally found in certain seeds, fruit stones, and cassava roots	Many cyanides are highly toxic to humans	Apple seeds, cherry pits, cassava	UV colorimetry (after reflux distillation)
Furans	Volatile liquid that seems to form during traditional heat treatments	Carcinogen	Variety of foods	Headspace GC-MS
Heterocyclic amines	Formed in meat products heated at high temperatures, with creatine being a necessary precursor with free radicals formed as a result of the Maillard reaction initiating their production	Neurotoxin, mutagen, and carcinogen	Grilled/fried meat and fish to well done stage	Reversed-phase LC coupled with fluorescence detection, LC-MS
Melamine	A trimer of cyanamide (with 66% N) that has been added illegally to increase apparent protein content of foods and ingredients	Causes kidney failure. Legal limits have been set	Added previously to wheat gluten, milk, and infant formula	HPLC-MS/MS (GC-MS for screening)
4-Methylbenzophenone (4MBP)	Metabolite of a chemical component of ink used for food packaging	Concern for health risk with long exposure	Boxes of cereal	LC-MS/MS, GC-MS
4-Methylimidazole	Formed via Maillard browning during normal cooking of certain foods. Formed in manufacturing of caramel coloring	Some concern about whether it is a possible carcinogen to humans	Roasted meats and coffee, caramel coloring, beverages with caramel coloring	LC-MS/MS (ion chromatographs), GC
3-Monochloropropane 1,2-Diol (3-MCPD)	Formed when proteins (soy) ae hydrolyzed by heat and food-grade acids to create hydrolyzed vegetable protein	Carcinogen	Soups, savory snacks, and gravy mixes flavored with acid-hydrolyzed vegetable protein	GC-MS, LC-MS, SCF-MS/MS

(continued)

33.3 table (continued)

Compound	*Nature of compound and reason in food*	*Reason of concern*	*Major foods/ ingredients identified*	*Major methods*
Nitrosamines	Produced from nitrites in the presence of proteins upon processing at high temperatures, as in frying	Carcinogen	Cured meats, primarily cooked bacon, beer, some cheeses, nonfat dry milk, fish	GC-MS for volatile nitrosamines, LC-MS for nonvolatile nitrosamines
Perchlorate	Component of rocket fuel and formed naturally	Can interfere with iodide uptake into the thyroid gland, leading to hypothyroidism	Bottled water, milk, lettuce	LC-MS/MS (ion chromatograph)
Solanine	A glycoalkaloid found in potatoes that helps protect the plant, but causes bitter taste when at certain level in potato tuber. Peeling potatoes decreases content	Toxic to humans above certain levels	Potatoes	LC-MS/MS, HPTLC, GC

their major method(s) of analysis [69]. To reach the limit of detection (LOD) desired and to positively identify the compounds, many of the major analyses rely on gas or liquid chromatography, with mass spectrometry. Described in more detail below is the analysis for two compounds of concern, sulfites and nitrates/nitrites, which are not as commonly analyzed by chromatography as by other methods. There are legal limits in foods for both of these compounds, so they are monitored regularly in select foods for quality control purposes.

33.8.2 Sulfites

Although sulfites are classified as allergens, they are covered in this separate section because the nature of the chemical compound, symptoms, and methods of analysis are quite different than for other allergens. Sulfites and sulfiting agents are a group of chemical compounds that include sulfur dioxide (SO_2), sulfurous acid (HSO_3), and the following inorganic sulfite salts that can liberate SO_2: sodium (Na) and potassium (K) sulfite, Na and K bisulfite, and Na and K metabisulfite [70]. In some foods they occur naturally, but in other foods they are added for a variety of reasons, including preventing microbial growth and browning. Sulfites naturally occur to some extent in all wines but are commonly added to stop fermentation at the appropriate time and to prevent spoilage and oxidation. Dried fruits and vegetable products are sometimes treated with sulfites to reduce browning. Shrimp, lobster, and related crustaceans can be treated with sulfite to prevent "black spot." Some consumers are highly intolerant to sulfite residues in food, most commonly resulting in asthma attacks [71]. Therefore, the FDA forbids sulfites from being added to foods intended for raw consumption (e.g., salad bar foods) and requires the phrase "contains sulfites" on the label of foods that contain greater than 10 ppm sulfites, whether naturally occurring or added during manufacturing [21 CFR 101.100 (a)(4)] [72]. Many countries besides the USA have set strict limits on the residual levels of sulfites in various foods.

Reactions of sulfites with other food components make analysis challenging, often resulting in decreased levels during storage. Most methods of analysis detect free forms of sulfite plus some bound forms. However, none of the available methods, alone, measures all forms of sulfite in foods, which includes free inorganic sulfite plus the many sulfite bound forms. It is not known which forms of sulfite cause the adverse responses in sulfite-sensitive consumers, so the focus has been on measuring as much as possible of the residual sulfite, both free and bound forms [63].

One of the long-time, quantitative methods for sulfite analysis of foods is the **Monier-Williams procedure** (AOAC Method 990.28) [25], which measures "total" SO_2 (actually, free sulfite plus reproducible portion of bound sulfites, such as carbonyl addition products). The FDA refers to this method in regulations for labeling sulfite-containing foods. In this method, the test sample is heated with HCl, converting sulfite to SO_2. Nitrogen gas bubbled through the sample sweeps SO_2 through a condenser and a hydrogen peroxide solution, oxidizing

SO_2 to H_2SO_4. The sulfite content of the sample is directly related to the amount of H_2SO_4 generated, measured by either a gravimetric or turbidimetric procedure.

Other analytical methods for sulfites include the following:

1. The **"Ripper" method**, long used by the wine industry as a rapid screening method, compared to the more time-consuming Monier-Williams method; sulfite is titrated with an iodide-iodate solution, using a starch endpoint indicator; measures "free" SO_2 (APHA Standard Method 4500 – SO_3^{-2} B) [73].
2. Enzymatic method; sulfite is oxidized to sulfate, generating hydrogen peroxide that is further reacted with NADH-peroxidase, to produce NAD which is measured by absorption at 340 nm (Sect. 26.3.1.3.1).
3. Ion chromatography, using amperometric detector (AOAC Method 990.31) [25].
4. HPLC, with ultraviolet [74] or fluorometric [75] detection.

33.8.3 Nitrates/Nitrites

Sodium nitrate and sodium nitrite are both food preservatives commonly used to cure processed meat products, but there are legal limits on the level of sodium nitrite in the finished product. Nitrates (NO_3) and nitrites (NO_2) are very similar chemically, and sodium nitrate is readily converted to sodium nitrite. Sodium nitrate is naturally occurring in some vegetables, and it converts to sodium nitrite when it comes in contact with your saliva. The naturally occurring high levels of nitrates in celery and Swiss chard make powders of them common ingredients in some cured meat products. Both sodium nitrate and sodium nitrite give cured meat an appealing pinkish-reddish color and prevents it from turning brown. More importantly, sodium nitrite works with sodium chloride to prevent the growth of *Clostridium botulinum*. However, when products with high levels of residual nitrites are cooked at high temperatures (e.g., frying of bacon), the nitrites react with amines naturally present in the meat to form nitrosamines, which are suspected carcinogens. Also, high levels of nitrates or nitrites in the diet may induce the disorder methemoglobinemia, which can be fatal due to the reduced ability for oxygen transport in the blood. In addition to the residual nitrates in processed meat products, there is concern about the levels of nitrates and nitrites in drinking water, and in the water and soil used to grown vegetables, especially when consumed by infants and young children [76, 77].

Health concerns have led to the following EPA- and FDA-regulated limits:

1. Drinking water – 10 ppm nitrates, 1 ppm nitrite (EPA; 40 CFR 141) [77]
2. Bottled water – 10 ppm nitrate, 1 ppm nitrite, and 10 ppm total nitrates and nitrites (FDA; 21 CFR 165.110) [78]
3. Finished meat products – 200 ppm sodium nitrite (FDA; 21 CFR 172.175) [79]
   There are also FDA legal limits on sodium nitrate and sodium nitrite for certain cured fish products [79].

During the curing of processed meat products, processors ensure the legal limits of sodium nitrite in the finished product largely by carefully monitoring the amount of sodium nitrate used in the formulation of the product. The levels of nitrates/nitrites may be monitored throughout the production system with relatively rapid tests for nitrates/nitrites. There are both official methods and more rapid rapids of analysis used for testing both processed meat products and water, as described below.

Three of the AOAC International official methods [25] for nitrates/nitrites include the following:

1. Xylenol Method (AOAC Method 935.48, Nitrates and Nitrites in Meat)
   In the xylenol method, the sample, with sulfuric acid added, is treated with 2,4-xylenol to produce 6-nitro-2,4-xylenol, which is distilled into a water-isopropanol alcohol-ammonia hydroxide mixture. The ammonia salt of 6-nitro-2,4-xylenol is a yellow color measured at 450 nm, compared to a nitrate N standard curve.
2. Colorimetric Method (AOAC Method 973.31, Nitrites in Cured Meat)
   In the colorimetric method, sodium nitrite is extracted from the sample then reacted with two reagents, sulfanilamide (sulfa) and naphthylethylenediamine (NEDA). These compounds react with nitrite to produce a purple dye, which is proportional to the concentration of nitrite ions.
3. Ion Chromatographic Method (AOAC Method 993.30, Inorganic Anions in Water)
   This method is intended for measuring nitrate-N, Nitrite-N, and several other inorganic anions in drinking water, but is also used within the meat industry with meat extracts as the sample. Anions in the sample are separated using an ion chromatography system that includes a guard column, separator column, and suppressor device and quantitated using a conductivity detector.

Rapid methods of analysis include ion-selective electrodes (ISEs) and test strips. Regarding ISE, both nitrate and nitrite ISEs are commercially available for testing food products (liquid samples) (see Chap. 21, Sect. 21.3.4, for details of ISEs). Regarding the test strips, various companies make test strips intended for

checking the nitrate content of water. For example, AquaChek nitrite/nitrate test strips (Hach, Loveland, CO) are able to test for either nitrates or nitrites, or both, using two pad areas on the test strip, with one area measuring nitrates and the other pad area measuring nitrites. The nitrate test area contains a combination of chemicals to reduce nitrates to nitrites. Nitrites, at an acid pH, react with sulfanilic acid to form a diazonium compound, which couples with an indicator to produce a pink color. The color intensity is proportional to the concentration of nitrate. Color blocks for interpretation of results are provided for both nitrate N (0–50 ppm) and nitrite N (0–3 ppm).

33.9 SUMMARY

Consumer concerns and government regulations focused on the safety of foods dictate the need for analysis of various food contaminants, residues, and chemical constituents of concern. These compounds include pesticide residues, mycotoxins, antibiotic residues, GMOs, allergens, food adulterants, packaging material hazardous chemicals, environmental contaminants, and certain other chemicals. Both rapid screening methods and more time-consuming quantitative methods are required to meet the needs of industry and government, in an effort to ensure a safe and reliable food supply. A positive result from a screening method usually leads to further testing to confirm and quantify the presence of the compound of concern. Sampling and sample preparation can be a significant challenge due to the low levels of the chemicals and the complex food matrices. Sample preparation often includes homogenization, extraction, and cleanup and sometimes requires derivatization. Screening methods increasingly utilize immune-based techniques, such as ELISA, LFS, immunosensors, and immunoaffinity chromatography columns. Some immunoassays can be considered quantitative, rather than just screening methods. Other screening methods commonly used include enzyme inhibition assays, thin-layer chromatography, and inhibition of microbial growth. While GC is a common chromatographic technique used for the quantitative analysis of some pesticides, for many other compounds of concern covered in this chapter, the predominant chromatographic method is HPLC. Both GC and HPLC analysis are now commonly coupled with mass spectrometry detection, often using MS tandem systems. The testing for GMOs and allergens typically involves either protein-based methods (e.g., immunoassays) or DNA methods using PCR. Work continues to improve and develop various methods of analysis for chemical residues and compounds of concern, focusing largely on speed, cost, and reliability of screening methods and detection limits of quantitative methods.

33.10 STUDY QUESTIONS

1. Explain the importance of each of the following steps in sample preparation for the analysis of contaminants and residues of concern:

   (a) Grinding/homogenization
   (b) Extraction
   (c) Cleanup/purification
   (d) Derivatization

2. In the analysis of contaminants and residues of concern, compare and contrast:

   (a) GC vs. LC analysis
   (b) MS vs. MS/MS analysis
   (c) LC with fluorescence or UV detection vs. LC-MS
   (d) TLC vs. automated chromatography (LC or GC)
   (e) Microplate ELISA vs. LFS

3. The "tolerance level" for residues of the pesticide chlorpyrifos on corn grain is 0.05 ppm:

   (a) What is meant by "tolerance level"?
   (b) What federal agency sets that tolerance level?
   (c) What federal agency enforces that tolerance level?
   (d) What is "ppm" equivalent to in terms of (a) weight per volume, and (b) weight per weight units commonly used to express concentration?

   wt/vol: wt/wt:

   (e) In Volumes I and II of the *Pesticide Analytical Manual*, you find described "multiresidue" methods and "single-residue" methods. You also have found numerous screening methods. Which one of these three types of methods (i.e., multiresidue, single residue, screening) would you use to ensure your compliance with the tolerance level for this pesticide? Briefly explain the nature of this type of method, and why you chose this method over the other two types of methods.

4. Mycotoxins are of potential concern in corn, especially in certain growing and storage conditions:

   (a) Sampling is a major contributor to error in the analysis for mycotoxins. Why is sampling for mycotoxin analysis such a challenge?
   (b) Identify the most commonly used quantitative chromatographic method for mycotoxins. Justify the preference of this method.

5. Regarding antibiotic residues, briefly explain the following:
   (a) How might these get into foods?
   (b) What types of foods most likely contain them?
   (c) Why are these antibiotic residues a problem?
   (d) Why have techniques such as immunoassays largely replaced microbial growth inhibition assays for screening antibiotic residues?
   (e) What method is most commonly used for accurate quantitative determination and confirmation?

6. You want to identify GMOs in a soybean field where you do not have access to a sophisticated analytical laboratory. What food constituent would you be analyzing, and which analytical technique would you use? Explain the principle of this technique.
7. You are analyzing unwanted chemical components in the foods/raw materials listed below. Identify one possible unwanted chemical component that would likely be of concern with this specific food, and state an appropriate method for quantitative analysis. Also give one appropriate screening method for each chemical component identified. (Note: For each food, give a different quantitative likely unwanted chemical component and a different method of analysis.)

Likely unwanted chemical component	Quantitative analysis method	Screening method

   (a) Oats
   (b) Peanuts
   (c) Milk
   (d) Wine
   (e) Cured meat

8. Describe the DNA-based method and identify the two protein-based methods for GMO detection and quantitation. Describe the strengths and weaknesses of each.
9. For the analysis of food allergens, protein-based or DNA-based methods are commonly used:
   (a) Give an example of when you would choose a protein-based method over a DNA-based method. Justify your choice and provide the principle of the method of choice.
   (b) Give an example of when you would choose a DNA-based method over a protein-based method. Justify your choice and provide the principle of the method of choice.
10. Regarding the compounds described in Table 33.3:
    (a) Identify five compounds associated with the heat of cooking/frying of foods.
    (b) Identify two compounds typically analyzed by GC (vs. LC), and explain the preference for GC methods with these compounds.
    (c) Identify two compounds associated with packaging material.
    (d) Identify a compound that is an economic adulterant.
    (e) Identify two compounds naturally present in specific foods, but toxic to humans at certain levels.
11. Regarding sulfites, explain how this "allergen" differs from the food allergens described in Sect. 33.7 in the response of sensitive humans, how they differ in the nature of quantitative methods, and why quantitative determination is relatively difficult for sulfites compared to food allergens.

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