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Chapter

The Role of Liquid Chromatography-Mass Spectrometry in Food Integrity and Authenticity

Guillem Campmajó, Nerea Núñez and Oscar Núñez

Abstract

Liquid chromatography coupled to mass spectrometry (LC-MS), tandem mass spectrometry (LC-MS/MS), and high resolution mass spectrometry (LC-HRMS) today are among the most common techniques to guarantee food integrity and authenticity. Targeted approaches, where a family of characteristic bioactive substances in the analyzed food products are monitored, are a common practice to ensure food authenticity regarding the production region since bioactive substances content and distribution in food depend on multiple parameters such as climate conditions, water resources, agrochemical practices, etc. On the other hand, non-targeted approaches, such as metabolomic fingerprinting, are a common practice where a huge number of spectral detected variables in the analyzed foods are monitored. In both approaches, characteristic patterns are searched among the analyzed food products by means of statistical chemometric methods to address food characterization, classification, and authentication. In the present chapter, the role of LC-MS in combination with chemometrics to guarantee food integrity and authenticity will be discussed. Coverage of all kinds of applications is beyond the scope of the present contribution, so we will focus on the most relevant applications published in the last years by addressing the most interesting examples and important aspects in the food authenticity field.

Keywords: food authenticity, liquid chromatography, mass spectrometry, high resolution mass spectrometry

1. Introduction

Food products are very complex mixtures constituted by a great variability of naturally occurring compounds such as lipids, carbohydrates, proteins, vitamins, organic acids, and volatile organic compounds, among others. Moreover, they can also contain many other substances coming from agrochemical treatments and technological processes, or even migrating from the materials employed in food packaging, which sometimes are contaminants.

Food manufacturers, researchers, and society in general are also becoming very interested in the quality of food products, not only from the nutritional point of view but also in relation to food safety issues or regarding the presence of bioactive
substances with beneficial properties for consumers (functional foods, nutraceuticals, etc.). Aspects related to the cultivation production region of natural food products (fruits, vegetables, etc.), as well as the cultivation techniques employed, begin to be also of great interest to the final consumers, giving rise to the consideration of the protected designations of origin (PDO) of natural foodstuffs as important food quality attributes.

Nowadays, the food supply production is worldwide distributed and consequently a globalized issue. Although international and local regulatory bodies have established important rules in the labeling of food products, in general, it is often almost impossible to know the real origin of most of the components of a given food product, especially those that have been processed. Within this context, considering the complexity of the food chain and that many players are involved between production and consumption; food manipulation and adulteration practices are raising because it is in fact much easier to conduct fraud without being easily detected. For example, Moore et al. collected information from published articles in scholarly journals and general media, organized it into a database, and reviewed and analyzed the data to identify trends within food ingredient fraud practices from 1980 to 2010 [1]. They observed that olive oil, milk, honey, and saffron were the most common targets for adulteration reported in scholarly journals and potentially harmful issues identified include spices diluted with lead chromate and lead tetraoxide, substitution of Chinese star anise with toxic Japanese star anise, and melamine adulteration of high protein content foods.

Food adulteration practices have a long history and dates back to times when trading began. In general, food adulteration is carried out to increase volume, to mask the presence of inferior quality components and to replace the authentic substances for the seller's economic gain. However, it must be considered that the deliberate adulteration of food and its misrepresentation to deceive final consumers is illegal worldwide, having not only economic consequences, but also representing important health issues when prohibited substances are added to deceive the organoleptic properties of the final food product or when the adulterant can produce allergy episodes. Thus, the development of new analytical methodologies to guarantee food integrity and authenticity is required, also considering that food adulteration has become increasingly sophisticated, often being specially designed to avoid detection through routine analysis approaches.

The analysis of food products is difficult not only because of the complexity and diversity of sample matrices but also due to the great variability of compounds that can be present. In addition, food components differ in polarity, structures, as well as in concentration levels, going from components at grams per kilogram level to those found at trace level concentrations (low μg/kg, ng/kg, etc.). These are important aspects to consider when selecting the analytical approach to employ. Sample treatment and sample extraction procedures, separation and determination approaches, and identification and confirmation strategies need to be considered simultaneously when addressing the development of an analytical method in food integrity and authenticity analyses. Nowadays, liquid chromatography coupled to mass spectrometry (LC-MS) or to tandem mass spectrometry (LC-MS/MS) is among the most effective analytical techniques for the structural characterization and analysis of food products. The appearance of ultra-high performance liquid chromatography (UHPLC) methodologies, either using sub-2 μm particle packed columns or porous-shell columns (with sub-3 μm superficially porous particles), opened up new possibilities to achieve high throughput chromatographic analytical separations, 5- to 10-fold faster than with conventional LC methodologies, while keeping or even improving chromatographic resolutions [2]. The use of liquid chromatography coupled to high resolution mass
spectrometry (LC-HRMS) and accurate mass measurements have recently gained huge popularity due to the great ability of these methodologies to provide more comprehensive information regarding the exact molecular mass, elemental composition, and detailed molecular structure of a given compound. In comparison to classical low resolution mass spectrometry (LRMS) techniques, HRMS allows to differentiate isobaric compounds (substances with the same nominal mass-to-charge ratio but different elemental compositions). Moreover, the high resolution attainable with HRMS favors the simplification of sample treatment and preparation procedures, leading to faster analytical methodologies with less and simple sample manipulation. HRMS allows to perform both screening and quantitation in a single run, including targeted, suspect, and non-targeted analyses. Another important advantage of HRMS, especially when data is stored in full-scan mode, is the possibility of later stage retrospective analysis, allowing the identification and determination of new unknown or suspected compounds in a previously analyzed food sample.

An important aspect in food products, especially those of plant origin, is that the presence, distribution, and content of many bioactive substances is related to many food features such as the variety and species of the products, the degree of maturation in the fruits and vegetables employed, the geographical production areas, the growing and manufacturing practices used, etc. A similar consideration can be mentioned for food products of animal origin, where many substances present in the final product will be related to the animal species, the farming practices employed, the animal stress, etc. Therefore, food chemical profiling, for instance of amino acids, biogenic amines, alcohols, aldehydes, esters, acids, terpenes, polyphenols, etc., can be exploited as sample data descriptors to achieve the characterization, classification, and authentication of food products.

Regarding chemical profiling in food integrity and authenticity by LC-MS and LC-HRMS methodologies, two main approaches are typically employed: targeted and non-targeted analyses. Targeted approaches can be performed by both LC-MS(\textit{MS}) and LC-HRMS techniques and are based on the specific determination of a given group of known selected chemicals, or a group of chemicals belonging to the same family or with a similar structural feature. The concentrations (or peak signals) of these targeted compounds are then used as food features (markers) to address food integrity and authenticity. This approach requires, in general, a previous quantitation step using standards for each targeted component. However, when dealing with food products, which as previously commented are very complex matrices, the quantitation of some chemicals may be a difficult task, especially due to the possibility of unknown interfering compounds. In contrast, non-targeted approaches (based on metabolomic fingerprinting) are mainly employed with LC-HRMS techniques. These fingerprinting approaches are based on untargeted analysis of instrumental responses without assuming any previous knowledge of relevant or irrelevant food components. In the case of LC-HRMS, food sample fingerprinting information consists, in general, of peak intensity values recorded as a function of \textit{m/z} and retention times [3].

Due to the complexity of food sample matrices and the variability of chemical components that can be present, the amount of chemical data that can be extracted, especially when dealing with non-targeted LC-HRMS fingerprinting approaches, is huge. As a consequence, in order to extract (bio)chemical information from the sample data sets able to characterize, classify and authenticate food products, chemometric data treatment methodologies are necessary. Multivariate methods such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) are among the most employed chemometric methods for exploratory and classification purposes in food integrity and authenticity [4].
In the next sections, several examples dealing with targeted and non-targeted strategies based on LC-MS(/MS) and LC-HRMS methodologies, in combination with chemometrics, to guarantee food integrity and authenticity will be addressed.

2. Targeted approaches

2.1 LC-MS(/MS) methodologies

LC-MS and LC-MS/MS are among the most common techniques used in the literature to obtain qualitative, quantitative, and structural information in the determination of low molecular weight compounds in a great variety of sample matrices, including foodstuffs. The low sensitivity typically achieved when LRMS is employed, especially with some analyzers such as triple quadrupole (QqQ) and ion trap (IT) instruments, makes them ideal to be employed when targeted approaches are intended. This strategy is based on the specific determination of a given group of compounds (i.e., some selected chemicals, a group of chemicals belonging to the same family, etc.) that can then be used as biomarkers to address food integrity and authenticity. Although this approach typically requires the quantitation of these chemicals by using adequate standards for each targeted component, in some cases targeted profiling is also possible by means of employing only the peak area signal of a given set of compounds, without the requirement of knowing the concentration values.

Polyphenols, aromatic secondary metabolites ubiquitously spread through the plant kingdom, are among the most common biomarkers employed to address food integrity and authenticity when targeted LC-MS(/MS) methodologies are employed in the analysis of plant-related foodstuffs [5] and some selected applications found in the literature are summarized in Table 1.

As can be seen in the table, reversed-phase liquid chromatography (RPLC), mainly employing C18 columns [6–12] and gradient elution with an acidified aqueous solution and methanol or acetonitrile as mobile phase components, is usually proposed. For example, Seraglio et al. [7] described the development of a reproducible and sensitive method for the simultaneous determination of 32 phenolic compounds in bracatinga (Mimosa scabrella Bentham) honey samples using HPLC-ESI-MS/MS. The separation was performed with a C18 reversed-phase column in less than 17 min, using gradient elution with water and acetonitrile, both acidified with 0.1% formic acid. Other stationary phases have also been proposed for the separation of polyphenols in food products. For instance, Alakolanga et al. [13] described the use of a C18 amide reversed-phase column for the determination of 35 phenolic compounds in fruits of Flacourtia indica (Burm. F.) Merr. and Flacourtia inermis Roxb trees. Due to the high number of compounds and the complexity of the sample matrix, a gradient elution program of 80 min was employed. In another application, a fluorinated porous-shell column (Ascentis Express F5) was proposed for the determination of 15 polyphenolic compounds in Passiflora subpeltata fruit pulp with a 38 min gradient elution program [14].

Regarding the ionization of polyphenols, electrospray (ESI) in negative mode [10–13], positive mode [9], or exploring both positive and negative modes [6–8, 14] is generally employed. However, other atmospheric pressure ionization (API) sources have also been described in the literature for the determination of polyphenols in food characterization and authentication. For example, Parets et al. [12] compared the use of ESI, atmospheric pressure chemical ionization (APCI), and dopant-assisted atmospheric pressure photoionization using four organic solvents as dopants (toluene, acetone, chlorobenzene, and anisole) for
The Role of Liquid Chromatography-Mass Spectrometry in Food Integrity and Authenticity

DOI: http://dx.doi.org/10.5772/intechopen.85087

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<th>Data analysis</th>
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<tbody>
<tr>
<td>Polyphenols (pomegranate)</td>
<td>Ascentis Express C18 column (150 × 3.0 mm, 2.7 μm) Gradient elution (0.4 mL·min⁻¹): (A) water with 2% formic acid (B) methanol/water 90:10 (v/v) with 0.5% formic acid H-ESI (+) IT (full-scan 100–1500 m/z and MS² product ion scan mode 50–1500 m/z)</td>
<td>–</td>
<td>[6]</td>
</tr>
<tr>
<td>Polyphenols (Passiflora subpeltata fruit)</td>
<td>Ascentis Express F5 (150 × 2.1 mm, 2.7 μm) Gradient elution (0.2 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile H-ESI (+) QqQ (MRM acquisition mode)</td>
<td>–</td>
<td>[14]</td>
</tr>
<tr>
<td>Phenolic compounds (honey)</td>
<td>VENUSIL C18 column (100 × 2.1 mm, 3 μm) Gradient elution (0.5 mL·min⁻¹): (A) water with 0.05% formic acid (B) methanol H-ESI (+) IT (full-scan and auto-MSⁿ mode)</td>
<td>–</td>
<td>[7]</td>
</tr>
<tr>
<td>Phenolic compounds (Placacourta indica and Placacourta inermis fruit)</td>
<td>C18 amide column (250 × 3 mm, 5 μm) Gradient elution (0.2 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid H-ESI (+) Q-_TRAP (MRM acquisition mode)</td>
<td>–</td>
<td>[13]</td>
</tr>
<tr>
<td>Phenolic compounds (artichoke, garlic and spinach)</td>
<td>Zorbax Eclipse Plus C18 column (100 × 2.1 mm, 1.8 μm) Gradient elution (0.2 mL·min⁻¹): (A) methanol (B) water with 0.1% formic acid and 30 mM of ammonium acetate H-ESI (+) QqQ (MRM acquisition mode)</td>
<td>–</td>
<td>[8]</td>
</tr>
<tr>
<td>Phenolic compounds (berries)</td>
<td>Wakosil C18 column (150 × 4.6 mm, 5 μm) Gradient elution (1 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid H-ESI (+) Quadrupole MS (full-scan mode 100–800 m/z)</td>
<td>–</td>
<td>[9]</td>
</tr>
<tr>
<td>Phenolic compounds (tomato fruits)</td>
<td>BEH Shield RP18 column (150 × 1 mm, 1.7 μm) Gradient elution (0.13 mL·min⁻¹): (A) water/acetonitrile 95:5 (v/v) with 0.1% formic acid (B) water/acetonitrile 40:60 (v/v) with 0.1% formic acid H-ESI (–) QqQ (full-scan and product ion scan mode)</td>
<td>ANOVA</td>
<td>[10]</td>
</tr>
<tr>
<td>Polyphenols (fruit extracts)</td>
<td>Kinetex C18 (100 × 4.6 mm, 2.6 μm) Gradient elution (1 mL·min⁻¹): (A) water with 0.1% formic acid (B) methanol H-ESI (–) QqQ (MRM acquisition mode)</td>
<td>PCA</td>
<td>[11]</td>
</tr>
<tr>
<td>Polyphenols (cranberry-based pharmaceutical preparations and natural extracts)</td>
<td>Hypersil Gold C18 column (50 × 2.1 mm, 1.9 μm) Gradient elution (0.285 mL·min⁻¹): (A) water with 0.1% formic acid (B) methanol H-ESI (–)/APCI (–)/APPI (–) QqQ (MRM acquisition mode)</td>
<td>PCA</td>
<td>[12]</td>
</tr>
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</table>

Heated-electrospray ionization (H-ESI), multiple reaction monitoring (MRM), quadrupole-ion trap (Q-TRAP), electrospray ionization (ESI), analysis of variance (ANOVA), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI).

Table 1.
Selected targeted LC-MS/(MS) methods using polyphenols as biomarkers to address food integrity and authenticity.
the determination of 29 polyphenols in grape- and cranberry-based fruit extracts and cranberry-based pharmaceutical preparations. ESI and acetone-assisted APPI showed a good performance for the ionization step of the targeted polyphenolic compounds, providing good sensitivity for most of the analyzed polyphenols. However, when addressing the classification and authentication of the analyzed extracts, the authors described that results obtained by UHPLC-APPI-MS/MS were more satisfactory and the discrimination of the sample classes was excellent in comparison to UHPLC-ESI-MS/MS, attributing this behavior to the higher robustness of APPI source in the presence of matrix effects.

Quadrapole MS, QqQ, and IT instruments are the most employed for the LC-MS/(MS) determination of polyphenols in food integrity and authenticity. Regarding the acquisition mode, full-scan and product-ion scan modes are typically employed with IT instruments, while MRM acquisition mode is applied with QqQ instruments due to the sensitivity improvement observed in comparison with product-ion scan in this kind of instruments. Nevertheless, some authors are also proposing the use of MRM acquisition mode with Q-TRAP instruments [7], although no special improvement in sensitivity is described. Regarding the use of MS², several acquisition strategies can be found in the literature. For instance, Brighenti et al. [6] proposed the use of the SmartFrag function of the IT mass analyzer to ensure that every precursor ion receives the appropriate collision energy in order to obtain adequate product-ion scan spectra with the better fragmentation possible.

In order to address food integrity and authenticity, the comparison of data obtained from different sample matrices is required. Therefore, chemometric methodologies, such as PCA, that allow the comparison of multiple variables play an important role in this aspect. However, several works are addressing food authenticity directly by comparison of targeted bioactive substances' content, without the requirement of employing any chemometric strategy. This is the case, for example, of the work described by Ribas et al. [10] that showed significant differences in the phenolic content of three Spanish tomato varieties depending on the cultivar variety (“Caramba,” “Montserra,” and “Pera de Girona”).

Even though the concentration data of some targeted bioactive substances may allow to directly differentiate some food attributes, as previously commented, this data could also be subjected to chemometric methods to address food integrity and authenticity issues. For instance, Puigventós et al. [11] describes the use of LC-ESI-MS/MS method for the determination of 26 polyphenolic compounds in fruit-based products and fruit-based pharmaceutical preparations. The polyphenolic content was then employed as chemical descriptors to achieve sample classification and authentication by means of PCA. As an example, Figure 1 shows the PCA plot of scores (a) and plot of loadings (b) for the analyzed samples.

As shown in the plot of scores (Figure 1a), grape and cranberry products appeared in different zones so that PCA was basically able to distinguish among the two fruits or origins, allowing the authentication of fruit-based extracts. In particular, grape and related samples were located to the top-left part of the graph. In contrast, cranberry samples were mainly spread out on the bottom area. Regarding the plot of loadings (Figure 1b), it was found that gallic acid and polydatin were characteristic of grape-related samples so they were present in higher levels in this class of products. In contrast, analytes located to the right part of PC1 such as sinapic, ferulic, p-coumaric and chlorogenic acids, as well as quercitrin, were comparatively more abundant in cranberry products.

Therefore, the chemometric analysis of targeted bioactive substance contents in food products could give an idea of the more discriminant chemical descriptors of a given sample, allowing the proposal of future biomarkers to address food integrity and authenticity.
The Role of Liquid Chromatography-Mass Spectrometry in Food Integrity and Authenticity
DOI: http://dx.doi.org/10.5772/intechopen.85087

2.2 LC-HRMS methodologies

Even though LC-MS and LC-MS/MS have proved to be useful techniques in some food authenticity and integrity applications, as previously described, sometimes a more sensitive and selective technique, such as LC-HRMS, is needed mainly due to the complexity of food sample matrices and the huge variability on bioactive compounds, with different structures and physicochemical properties, that they contain [5]. HRMS and accurate mass measurements are emerging as one of the best options for the analysis of food samples in order to guarantee the unequivocal determination of the elemental composition of a target compound, which allows its distinction of other co-eluting isobaric compounds. There are mainly four types of HRMS instruments: magnetic sector, time-of-flight (TOF), Orbitrap, and Fourier transform ion cyclotron resonance (FT-ICR) instruments, being TOF and Orbitrap, as well as some of their hybrid configurations with quadrupole or IT analyzers, the most frequently employed in combination with LC techniques. In general, TOF instruments present a resolution (instrument’s ability to measure the mass of two closely related ions precisely) of approximately 10,000–40,000 FWHM (full width at half-maximum) with accuracies in the mass determination of 1–5 ppm. In contrast, the resolution of Orbitrap instruments is in the range of 10,000–140,000 FWHM (or even higher) with 1–2 ppm mass accuracy (for comparison, conventional quadrupole MS instruments show a resolution of 1000 FWHM and accuracies of 500 ppm) [5]. Recent advances in both LC-TOF-MS and LC-Orbitrap-MS methods have reduced instruments costs, make the analysis more simple, and have considerably improved accuracy, offering today bench-top instrumentation that is amenable to screening and identification of a great variety of compounds in food matrices, not only for targeted ones, but also for non-target or unknown chemicals [5].

In this section, the use of targeted LC-HRMS methodologies in order to address the food integrity and authenticity issue will be discussed. Table 2 summarizes some selected applications described in the literature employing targeted LC-HRMS methodologies in food integrity and authenticity.

As can be seen in Table 2, and in line with previously commented targeted LC-MS and LC-MS/MS methodologies, polyphenols are ubiquitously used as biomarkers in targeted LC-HRMS approaches [15–19], whether considering a
specific polyphenolic class or a wider selection. However, polyphenols are not always the best choice to solve the analytical problem even when plant-related food products are addressed, and therefore some other compounds can be employed. For instance, Megías-Pérez et al. [20] used the determination of low molecular weight

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<tbody>
<tr>
<td>Kaempferol derivatives (saffron)</td>
<td>Ascentis Express Fused-core C18 column (100 × 2.1 mm, 2.7 μm) Gradient elution (0.4 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid H-ESI (−) Q/TOF (full-scan mode 100–1700 m/z)</td>
<td>ANOVA, PCA and SLDA</td>
<td>[15]</td>
</tr>
<tr>
<td>Polyphenols (kiwifruit juice)</td>
<td>Waters XTerra MS C18 column (250 × 4.6 mm, 5 μm) Gradient elution (0.8 mL·min⁻¹): (A) water with 0.5% acetic acid (B) water:acetonitrile 1:1 (v/v) with 0.5% acetic acid H-ESI (−) Q/TOF (full-scan mode 50–1500 m/z)</td>
<td>PCA-DA and OPLS-DA</td>
<td>[16]</td>
</tr>
<tr>
<td>Polyphenols (berry fruit juice)</td>
<td>Phenomenex C18 column (100 × 2.1 mm, 2.6 μm) Gradient elution (0.3 mL·min⁻¹): (A) water with 0.1% formic acid (B) methanol with 0.1% formic acid H-ESI (+) Q/TOF (full-scan mode 50–1000 m/z)</td>
<td></td>
<td>[17]</td>
</tr>
<tr>
<td>Polyphenols (red spice paprika)</td>
<td>Syncronis C18 column (100 × 2.1 mm, 1.7 μm) Gradient elution (0.25 mL·min⁻¹): (A) water with 0.01% acetic acid (B) acetonitrile H-ESI (−) LTQ-Orbitrap (full-scan mode 100–1000 m/z)</td>
<td>PCA</td>
<td>[18]</td>
</tr>
<tr>
<td>Low molecular weight carbohydrates (cocoa beans)</td>
<td>BEH X-Bridge Amide column (150 × 4.6 mm, 3.5 μm) Gradient elution (0.4 mL·min⁻¹): (A) water with 0.1% ammonium hydroxide (B) acetonitrile with 0.1% ammonium hydroxide H-ESI (−) TOF (full-scan mode 50–1200 m/z)</td>
<td>ANOVA, PCA and PLS-DA</td>
<td>[20]</td>
</tr>
<tr>
<td>Polyphenols (cranberry-based extracts)</td>
<td>Ascentis Express C18 column (150 × 2.1 mm, 2.7 μm) Gradient elution (0.3 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid H-ESI (−) Q/Orbitrap (full-scan mode 100–1500 m/z)</td>
<td>PCA and PLS</td>
<td>[19]</td>
</tr>
<tr>
<td>Small bioactive lipids (rice)</td>
<td>Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm) Gradient elution (0.5 mL·min⁻¹): (A) water with 10 mM ammonium hydroxide (B) acetonitrile:isopropanol 90:10 (v/v) H-ESI (−) Q/TOF (full-scan mode 50–1200 m/z)</td>
<td>PCA and OPLS-DA</td>
<td>[21]</td>
</tr>
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</table>

Stepwise linear discriminant analysis (SLDA), principal component analysis-discriminant analysis (PCA-DA), orthogonal partial least-squares discriminant analysis (OPLS-DA), partial least-squares regression (PLS).

Table 2.
Recent advances of targeted LC-HRMS methodologies in food integrity and authenticity.
carbohydrates for the classification of cocoa beans from different origins and status of fermentation, whereas Zhu et al. [21] studied the presence of small bioactive lipids as markers to differentiate among diverse varieties of rice.

Regarding the chromatographic separation, almost all the works described in the literature propose the use of C18 stationary phase columns [15–19, 21]. In fact, Guijarro-Díez et al. [15] tested and compared a C18 and a cyano column, both having the same size and particle diameter, for the chromatographic separation of five kaempferol derivatives and geniposide in the analysis of saffron samples, obtaining a better resolution and peak efficiency when using the C18 column. Alternatively, the separation of polar compounds, such as low molecular weight carbohydrates, can be improved and optimized by using hydrophilic interaction liquid chromatography (HILIC) columns as it offers ample chromatographic resolution [20].

As shown in Table 2, H-ESI has been established as the most common option for the ionization step whether positive mode [20], negative mode [15, 16, 18, 19, 21] or both [17] are employed. In order to ensure a good ionization of the targeted compounds (avoiding in-source fragmentation), Guijarro-Díez et al. [15] studied both positive and negative ionization modes, as well as different mobile phase compositions, looking for the highest signal-to-noise (S/N) ratio in the determination of kaempferol derivatives in saffron samples. Negative ESI mode and the addition of 0.1% formic acid to the mobile phase showed to be the best option, being a general trend described in the literature for the determination of polyphenolic compounds [5].

Among all the range of mass analyzers, TOF and Orbitrap-based mass spectrometer technologies are usually employed in these type of targeted studies, especially hybrid instruments such as quadrupole-time-of-flight (Q-TOF) [15–17, 21], quadrupole-Orbitrap (Q-Orbitrap) [19], and linear trap quadrupole-Orbitrap (LTQ-Orbitrap) [18] configurations. The main advantage of these types of instruments in front of single HRMS analyzers is the possibility to make data dependent MS/MS experiments. These acquisition modes provide interesting spectral information that compared with online-databases and with the obtained accurate mass measurements can improve the tentative identification and confirmation of a given targeted compound. It should be mentioned that only when pure standards are available, the theoretical chromatographic retention time as well as the standard fragmentation pathway are also compared and an undoubtedly identification can be done. In contrast, authors who work with simple HRMS analyzers, such as TOF, normally resort to LRMS analyzers able to perform tandem experiments such as QqQ or IT instruments in order to obtain this fragmentation pathway data [20].

Data treatment selection is strongly related to the food analytical challenge that has to be solved. In some applications, such as some adulteration food frauds, particular biomarkers are significantly discriminant between native and adulterated food samples and their determination allows the detection of that illegal practice. As an example, characteristic and endogenous glycosylated kaempferol derivatives were used as authenticity markers able to detect and quantify the adulteration in saffron samples regardless the substance used as adulterant [15]. Depending on the compound selected as biomarker, a limit of detection for the adulteration content between 0.2 and 2.5% was achieved. However, a statistical procedure like ANOVA is usually needed for the evaluation of the significance of difference for targeted compounds among different types of samples. Guo et al. [16] used this strategy to verify the capability of targeted polyphenols to classify kiwifruit juices according to their variety and geographical origin. Even though the presence of certain polyphenols was significantly different in each case, none of them was able to cluster the samples by itself. It is in these types of situations when multivariate data analysis techniques have gained relevance, as they can
combine information regarding the content of a large number of compounds. As an example, an unsupervised PCA was applied in order to study the geographical and botanical origin of Serbian red spice paprika samples (Lemeška and Lakošnička varieties) by using the concentration of 25 polyphenols, obtained by a targeted LC-HRMS method, and 13 carbohydrates, quantified by high performance anion exchange chromatography with pulsed amperometric detection [18]. The scores plots for the first two PCs, which explain 52.75% of the total variance, show a good discrimination between samples of different origin. In fact, loadings plot revealed a strong influence of particular phenolic acids and flavonoid glycosides in the separation of Lemeška paprika samples, while flavonoid aglycones and carbohydrates mainly affected the discrimination of the Lakošnička variety. In addition, Lakošnička samples were also classified according to their harvesting year (2012 or 2013). On the other hand, Barbosa et al. [19] also used chemometric multivariate analysis tools, but with the aim of preventing the possible adulteration of cranberry-based commercial pharmaceuticals with other ineffective and less expensive fruit-based extracts. Cranberry (Vaccinium macrocarpon) and its derivatives are known to prevent urinary tract infections as they contain A-type proanthocyanidins, which exhibit bioactive activity; in contrast, less expensive fruits like blueberry, raspberry, or grapes are richer in B-type proanthocyanidins, which does not exhibit this beneficial effect in human health. The authors developed a targeted polyphenolic UHPLC-HRMS (Orbitrap) method for the classification, authentication and detection of frauds in fruit-based extracts, and a total of 106 samples including cranberry-, grape-, blueberry-, and raspberry-based natural products, as well as cranberry-based pharmaceutical preparations presented in different formats were analyzed. Then, a built user-accurate mass database of 53 polyphenols containing spectral data and several confirmation parameters (accurate mass measurements, isotopic pattern matches, product-ion scan spectra, and chromatographic retention time) was applied for screening and
confirmation purposes. The obtained polyphenolic content, which was described in a data matrix containing the peak area of each detected target compound, was employed as chemical descriptors by PCA. As can be seen in Figure 2, the obtained scores plots show a good discrimination between cranberry-based samples in front of any other fruit-based sample, showing the ability of the developed method to clearly authenticate the fruit extracts according to the type of fruit employed.

Moreover, a PLS model was developed to predict and quantify fraud levels of adulterant fruit (grape, blueberry, and raspberry) extracts in cranberry-fruit extracts, reaching calibration errors below 0.01% and prediction errors in the range of 2.71 and 5.96%, demonstrating the suitability of polyphenolic targeted LC-HRMS methods in food integrity and authenticity.

3. Non-targeted LC-HRMS (metabolomics) approaches

Modern HRMS analyzers, such as TOF and Orbitrap (and their hybrid configurations), have focused the analysis of food samples from a totally different perspective as did before, mainly due to their high capacity to generate and register a large amount of information, especially when working in full-scan or in data-dependent scan acquisition modes. In fact, in the last years, there has been a trend toward non-targeted LC-HRMS metabolomic approaches, either by studying the metabolomic profiles of food, where chemometrics plays an essential role in the data treatment, or by a retrospective analysis in order to identify unknown compounds that could become new food biomarkers. Although non-targeted metabolomic approaches are potentially much more informative than targeted approaches in practice, the annotation of the features either obtained by using databases or by matching with pure standard data is frequently required. However, in many cases, metabolomic fingerprinting is enough to classify and discriminate among food samples; therefore, further metabolite identification is not needed. Moreover, it should be taken into account that when dealing with non-targeted metabolomics, the final annotated metabolites are strongly dependent on the global experimental approach employed (including sample treatment, separation and detection, as well as the specific instrumentation used). As an example, Díaz et al. [22] studied the influence of the global approach on the final annotated metabolites in non-targeted metabolomic analysis of 42 red wine samples (from three different Spanish PDO) when comparing two LC-MS interplatforms that differed in columns, mobile phases, gradients, chromatographs, mass spectrometers (Q-Orbitrap [Platform #1] and Q-TOF [Platform #2]), data processing, and marker selection protocols. Figure 3 shows a scheme of the experimental workflow described by the authors. The authors showed that despite the ability of the platforms to distinguish the wine classes at both the spectral and the annotated metabolite level, a strong divergence among the annotated metabolites involved in the discrimination was found. For example, at the annotated features level, PDO classes were separated using both experimental setups (Figure 4a and b). When annotated metabolite level was employed, a total of 9 and 8 features were identified for Platforms #1 and #2, respectively, although none of them was common. PCA models built using only these annotated features resulted in a clear separation when the Q-TOF was employed (Figure 4d), but with the Q-Orbitrap, wines from Ribera del Duero PDO and Rioja PDO were not completely separated (Figure 4c). When results obtained using both compared platforms were considered, the resulting PCA model performed including only the annotated features common for both platforms showed a high degree of similarity between them (Figure 4e and f).
This study shows the complications that may arise on the comparison of non-targeted metabolomic platforms even when metabolite focused approaches are used in the identification.

Table 3 summarizes a wide variety on non-targeted LC-HRMS applications in food integrity and authenticity. As previously indicated, these non-targeted methodologies can be considered as blind approaches toward the unknown metabolomic composition of a particular group of samples. For that reason, the selection and optimization of the chromatographic separation or ionization technique have to be done conscientiously as they will delimit the detected compounds according to their hydrophobicity and ionization capacity. As can be seen in the table, reversed-phase stationary columns are also usually chosen to conduct the chromatographic separation in non-targeted LC-HRMS approaches. This is because this separation mode
<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromatographic separation and mass spectrometry</th>
<th>Data analysis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregano</td>
<td>Acquity HSS T3 column (100 × 2.1 mm, 1.8 μm) Gradient elution (0.4 mL·min⁻¹): (A) water with 0.1% formic acid (B) methanol with 0.1% formic acid H-ESI (+) Q-TOF (full-scan mode 50–1200 m/z)</td>
<td>PCA and OPLS-DA</td>
<td>[39]</td>
</tr>
<tr>
<td>Lamb</td>
<td>ZIC p-HILIC column (100 × 2.1 mm, 5 μm) Gradient elution (0.25 mL·min⁻¹): (A) acetonitrile with 0.1% formic acid (B) water with 16mM ammonium formate ESI (+) Orbitrap (full-scan mode 55–1100 m/z)</td>
<td>Fold change, t-test and PCA</td>
<td>[44]</td>
</tr>
<tr>
<td>Tomato juices</td>
<td>C20 column (250 × 4.6 mm, 3 μm) Gradient elution (1.3 mL·min⁻¹): (A) methanol:methyl tert-butyl ether:water with 2% ammonium acetate 60:35:5 (v/v/v) (B) methyl tert-butyl ether:methanol:water with 2% ammonium acetate (v/v/v) APCI (+) Q-TOF (full-scan mode 100–1700 m/z)</td>
<td>Fold change and t-test</td>
<td>[41]</td>
</tr>
<tr>
<td>Coffee</td>
<td>Ascentis Express C18 column (150 × 2.1 mm, 2.7 μm) Gradient elution (0.2 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid H-ESI (+) Q-TOF (full-scan mode 100–1700 m/z)</td>
<td>PCA and PLS-DA</td>
<td>[23]</td>
</tr>
<tr>
<td>Tomato</td>
<td>Phenomenex Luna C8 column (100 × 2 mm, 3 μm) Gradient elution (0.35 mL·min⁻¹): (A) water:methanol 98:2 (v/v) (B) methanol:water 98:2 (v/v) H-ESI (+) Q-Orbitrap (full-scan mode 74–1100 m/z)</td>
<td>PCA</td>
<td>[40]</td>
</tr>
<tr>
<td>Beef meat</td>
<td>Hypersil Gold C18 column (100 × 2.1 mm, 1.9 μm) Gradient elution (0.4 mL·min⁻¹): (A) water (B) methanol ESI (+) LTQ-Orbitrap (full-scan mode 50–2000 m/z)</td>
<td>PCA and PLS-DA</td>
<td>[24]</td>
</tr>
<tr>
<td>Citrus fruit/fruit juices</td>
<td>Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm) Gradient elution (0.6 mL·min⁻¹): (A) water with 10 mM ammonium acetate (B) acetonitrile H-ESI (+) Q-TOF (full-scan mode 50–1200 m/z)</td>
<td>PCA, PLS-DA and SIMCA</td>
<td>[31]</td>
</tr>
<tr>
<td>Wild strawberry</td>
<td>XSelect CSH C18 column (150 × 2.1 mm, 1.6 μm) Gradient elution (0.2 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile:water 80:20 (v/v) with 0.1% formic acid H-ESI (+) LTQ-Orbitrap (full-scan mode 200–1600 m/z)</td>
<td>PCA</td>
<td>[32]</td>
</tr>
<tr>
<td>Eggs</td>
<td>Phenomenex Luna Omega C18 column (150 × 2.1 mm, 3.5 μm) Gradient elution (0.3 mL·min⁻¹): (A) water with 0.1% formic acid and 5 mM ammonium formate (B) methanol with 0.1% formic acid and 5 mM ammonium formate H-ESI (+) Q-Orbitrap (full-scan mode 75–1000 m/z)</td>
<td>PCA</td>
<td>[33]</td>
</tr>
<tr>
<td>Garlic</td>
<td>Mediterranea Sea C18 column (150 × 4.6 mm, 5 μm) Gradient elution (0.7 mL·min⁻¹): (A) water with 0.1% formic acid (B) methanol with 0.1% formic acid H-ESI (+) Q-TOF (full-scan mode 30–1700 m/z)</td>
<td>ANOVA and PCA</td>
<td>[34]</td>
</tr>
<tr>
<td>Sample</td>
<td>Chromatographic separation and mass spectrometry</td>
<td>Data analysis</td>
<td>Ref.</td>
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</tbody>
</table>
| Parmigiano Reggiano cheese | Kinetex XB C18 column (100 × 3 mm, 2.6 μm)  
Gradient elution (0.5 mL·min⁻¹): (A) water with 0.2% formic acid (B) acetonitrile with 0.2% formic acid  
H-ESI (−)  
Orbitrap (full-scan mode 50–900 m/z) | PCA Class Method                                   | [35] |
| Cheeses             | Hypersil Gold C18 column (100 × 2.1 mm, 1.9 μm)  
Gradient elution (0.4 mL·min⁻¹): (A) water with 0.1% acetic acid (B) acetonitrile with 0.1% acetic acid  
H-ESI (±)  
Orbitrap (full-scan mode 65–1000 m/z) | PCA                                           | [36] |
| Myrtle berry        | XSelect CSH C18 column (150 × 2.1 mm, 3.5 μm)  
Gradient elution (0.2 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid  
ESI (−)  
LTQ-Orbitrap (full-scan mode 200–1600 m/z) | PCA                                           | [37] |
| Saffron             | Ascentis Express C18 column (100 × 2.1 mm, 2.7 μm)  
Gradient elution (0.4 mL·min⁻¹): (A) water with 0.1% formic acid or 10 mM ammonium formate (B) acetonitrile with 0.1% formic acid or 10 mM ammonium formate  
H-ESI (±)  
Q-TOF (full-scan mode 100–3700 m/z) | PCA, PLS-DA and OPLS-DA                        | [38] |
| Eggs                | Thermo Scientific Accucore C18 column (100 × 2.1 mm, 2.6 μm)  
Gradient elution (0.3 mL·min⁻¹): (A) water with 0.1% formic acid and 5 mM ammonium acetate (B) methanol with 0.1% formic acid and 5 mM ammonium acetate  
H-ESI (±)  
Q-TOF (full-scan mode 100–1000 m/z) | PCA                                           | [25] |
| Olive oil           | Acclaim RSLC C18 column (100 × 2.1 mm, 2.2 μm)  
Gradient elution (0.2 mL·min⁻¹): (A) water:methanol 90:10 (v/v) with 5 mM ammonium acetate (B) methanol with 5 mM ammonium acetate  
H-ESI (−)  
Q-TOF (full-scan mode 50–1000 m/z) | PCA                                           | [26] |
| Saffron             | Phenomenex Kinetex C18 column (100 × 2.1 mm, 1.7 μm)  
Gradient elution (0.4–0.6 mL·min⁻¹): (A) water with 5 mM ammonium formate or acetate (B) methanol with 5 mM ammonium formate  
H-ESI (±)  
Q-TOF (full-scan mode 100–1200 m/z) | PCA and OPLS-DA                               | [27] |
| Honey               | Hypersil Gold C18 column (100 × 2.1 mm, 1.9 μm)  
Gradient elution (0.3 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid  
H-ESI (±)  
Q-Orbitrap (full-scan mode 80–1200 m/z) | PCA and PLS-DA                               | [28] |
| Beer                | Hypersil Gold aQ column (100 × 2.1 mm, 1.9 μm)  
Gradient elution (0.6 mL·min⁻¹): (A) acetonitrile with 0.1% formic acid (B) water with 0.1% formic acid  
H-ESI (±)  
LTQ-Orbitrap (full-scan mode 50–1000 m/z) | PCA and PLS-DA                               | [43] |
| Tiger nut           | BEH C18 column (100 × 2.1 mm, 1.7 μm)  
Gradient elution (0.4–0.5 mL·min⁻¹): (A) water:methanol 95:5 (v/v) with 0.1% formic acid and 5 mM ammonium formate (B) 2-propanol:methanol:water 65:30:5 (v/v/v) with 0.1% formic acid and 5 mM ammonium formate  
H-ESI (±)  
Q-TOF (full-scan mode 100–1200 m/z) | PCA and OPLS-DA                               | [29] |
The Role of Liquid Chromatography-Mass Spectrometry in Food Integrity and Authenticity
DOI: http://dx.doi.org/10.5772/intechopen.85087

provides a great chromatographic separation of semi-polar metabolites, which comprise a wide number of compounds (phenolic acids, flavonoids, alkaloids, or glycosylated species) that have proved to be useful and interesting for the authentication of food samples. Although C18 is generally proposed [23–38], some strategies have employed other reversed-phases such as HSS T3 [39], C8 [40], and C30 [41]. For instance, Black et al. [39] used a HSS T3 column for the separation of potential biomarkers able to identify adulteration in oregano samples, while Martinez et al. [40] proposed a C8 column with the aim to separate unknown food markers for the discrimination of tomato samples obtained in organic or conventional crops.

On the other hand, the enhanced bioavailability of tangerine tomato lycopene in front of red tomato lycopene, which is attributed in part to tetra-cis lycopene geometric configuration in tangerine variety rather than all-trans configuration of red variety, took Cichon et al. [41] to study and compare the metabolomic phytochemical composition between them. A C30 column was chosen for the chromatographic separation taking advantage of its high selectivity toward the separation of hydrophobic structurally related isomeric compounds. Even though RPLC is widely exploited in these applications, there is a range of polar metabolites (amino acids, carbohydrates, sugars, amines, or organic acids) that normally elute in the solvent front. Thus, polar endcapped C18 [42, 43] or HILIC [44] columns can be employed for their study as they offer an alternative selectivity. As an example, Gallart-Ayala et al. [43] used a polar-endcapped C18 column for the separation of moderate polar compounds in order to compare beers obtained by different brewing procedures.

Accordingly to targeted approaches, H-ESI is also the most employed ionization technique in the non-targeted LC-HRMS approach. However, in this case, normally both positive and negative ionization modes are studied [23, 27–30, 32–34, 36, 38, 39, 42–44], since it is not intended to find determined compounds but rather to study which of them provides a solution to the food integrity and authentication challenge, even if they are not identified. In some applications, other API sources can offer an interesting ionization range as well as less matrix effect. For instance, instead of H-ESI, APCI operated in positive ionization mode was used to analyze lipophilic extracts of tomato juices detecting a total of 423 compounds among which 352 were significantly different between the two types of juices studied [41].

As described in Table 3, hybrid HRMS analyzers (Q-Orbitrap, LTQ-Orbitrap and Q-TOF) are also widely employed in non-targeted LC-HRMS methodologies. The possibility to study the fragmentation of unknown molecular features allows their identification and confirmation in order to establish them as future targeted compounds for particular applications. Even though single HRMS analyzers do

<table>
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<th>Data analysis</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Tea</td>
<td>Hypersil Gold C18 column (100 × 2.1 mm, 1.9 μm)</td>
<td>PCA, HCA and PLS-DA</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Gradient elution (0.2 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H-ESI (+) Q-Orbitrap (full-scan mode 100–1500 m/z)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>Hypersil Gold aQ column (100 × 2.1 mm, 1.9 μm)</td>
<td>PCA [42]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gradient elution (0.3 mL·min⁻¹): (A) water with 0.1% formic acid (B) acetonitrile with 0.1% formic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H-ESI (+) Q-TOP (full-scan mode 100–1000 m/z)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Soft independent modeling by class analogy (SIMCA).

Table 3. Recent advances of non-targeted LC-HRMS methodologies in food integrity and authenticity.
not provide fragmentation data of the detected ions, metabolomics data can solve authenticity problematics without the identification of any compound (fingerprinting strategy) as previously commented [35].

The first step of non-targeted LC-HRMS approaches data treatment is the conversion of raw data in a matrix built by retention time, m/z values and the area or signal of each peak detected. Sometimes, chemical interferences are removed from the matrix by fixing some parameters to be achieved such as mass tolerance for peak alignment, total intensity threshold, maximum peak shift, and S/N threshold. At this point, the generated matrix can be treated by univariate or multivariate data analysis. For instance, d’Urso et al. [32], who aimed to compare wild strawberry samples of different geographical origin (Sarno and Petina, Italy), growing conditions (spontaneous and cultivated populations), and germplasm (autochthonous and non-autochthonous), created a unique data matrix from raw data obtained in both positive and negative ionization mode in the performed LC-HRMS analysis following a data fusion procedure. PCA was then applied to the data matrix obtaining a scores plot that clearly discriminates between spontaneous and cultivated samples regardless the other variables. Moreover, a good classification was also observed for the five groups of samples studied, which were different combinations of the above geographical origin, growing conditions and germplasm mentioned variables.

Anyways, when the objective of the study is the identification of molecular features that could behave as a biomarker in food integrity and authenticity, the matrix needs to be reduced. Thus, measures like the elimination of those molecular features that are not detected in a minimum percentage of the samples or of those that are not observed in the quality controls, which usually consists in a mix formed by a constant volume of all the analyzed samples, are normally implemented in the non-targeted LC-HRMS workflow.

As an example, Cavanna et al. [33], whose objective was the identification and selection of biomarkers responsible of the freshness of egg products, proposed a first reduction of data matrix by establishing some critical parameters values: (i) precursor ion deviation of 5 and 10 ppm for negative and positive runs, respectively, (ii) maximum peak shift of 0.3 min, (iii) a total intensity threshold of 1,000,000 AU, and (iv) a 30% of relative intensity tolerance used for isotope search. The authors removed the molecular features that showed a coefficient of variation bigger than 40% in the quality control sample, which was prepared by mixing 10 μL of each extract sample and was injected at the beginning of the sequence as well as every 10 samples analyzed. As a clear separation between fresh and non-fresh egg samples was observed when making a PCA study on positive and negative ionization modes with the reduced matrixes, the authors then applied supervised OPLS-DA. As can be seen in Figure 5, an expected increase in the discrimination was achieved.

![Figure 5](image_url)

**Figure 5.** ESI + OPLS-DA scores plot of the fresh samples against the “1 day” samples. Left area dots (0 h), fresh samples; right area dots (1D), “1 day” samples. Reproduced from Ref. [33]. Open Access Journals.
S-plots, which correspond to OPLS-DA loading plots, and variable importance in projection (VIP) values were used to select the most significant features in the clusterization of the samples. In order to identify those molecular features, exact mass, the isotopic pattern and MS/MS fragmentation were studied. As a result, 12 compounds were completely identified (standard injection confirm their identity) and 19 were tentatively identified by the authors.

4. Summary and concluding remarks

The role of LC-MS and LC-HRMS methodologies to address food integrity and authenticity have been presented and discussed by means of some selected applications published in the last years.

Most of the methods described in the literature opt for RPLC with mainly C18 columns, with gradient elution using acidified aqueous solutions and methanol or acetonitrile as mobile phase components, probably due to the strong capacity of this separation mode when dealing with low molecular weight chemicals with a relatively wide range of polarities. The use of other stationary phases such as C18 amide or perfluorinated columns are also proposed in some specific applications.

ESI continues to be the ionization source of choice when dealing with LC-MS and LC-HRMS analysis of food products, although in some cases other API sources are also employed. APPI has shown to provide similar or slightly better sensitivity for some specific applications, such as in the case of the determination of polyphenols, but it resulted in a very feasible option when addressing the characterization and classification of natural extracts due to the higher robustness of APPI source in the presence of matrix effect. Therefore, although it has not been widely exploited in food integrity and authenticity issues up to now, it is strongly recommended because of the sample matrix complexity of foodstuffs.

Regarding the mass analyzers, QqQ and IT instruments are the chosen ones when LRMS is employed, and TOF and Orbitrap analyzers for HRMS applications. However, the selection of LC-MS or LC-HRMS methods usually depends on the targeted or non-targeted approach. When targeted strategies are proposed, some specific biochemical food components are determined as food features to address food integrity and authenticity, requiring a quantitation step using standards for each targeted component. In those cases, LC-MS((MS)) methodologies, mainly using QqQ instruments, are very appropriate due to the low sensitivity attainable with these analyzers, and their good performance for quantitative analysis. Obviously, LC-HRMS methods providing higher resolution and accurate mass measurements are also a very good option for targeted food analysis, although it is more expensive and requires a more specialized staff. In order to achieve sample characterization and authentication, the comparison of the content and distribution of the targeted chemicals is sometimes enough, but the use of chemometric methods to try to find food feature similarities between the analyzed samples is highly recommended, especially when both the number of samples and the number of targeted bioactive substances increase.

In many applications, the quantitation of some chemicals may be a difficult task due to food matrix complexity, especially due to the possibility of unknown interfering compounds. In those cases, non-targeted approaches (based on metabolomic fingerprinting) using LC-HRMS have shown to be the best option to address food integrity and authenticity. As non-targeted analysis is performed, the high resolution and accurate mass measurements attainable with TOF and Orbitrap instruments are required. In non-targeted approaches, the measurement of peak intensity values as a function of \( m/z \) and retention times is frequently enough to
achieved food integrity and authenticity. Obviously, due to the huge amount of data obtained, especially when working in full-scan mode, the use of chemometrics is mandatory. Nevertheless, it has been reported that when dealing with metabolomic HRMS methodologies, the final annotated metabolites are strongly dependent on the global experimental approach employed (sample treatment, separation and detection, instrumentation employed, etc.). This is very important when searching for possible food biomarkers, as those will depend on the methodology used.

In conclusion, targeted and non-targeted LC-MS and LC-HRMS methodologies, especially in combination with multivariate chemometric methods, are powerful tools to address a hot topic nowadays such as food integrity and authenticity, and the number of publications in this field will continue to increase in the near future.

Acknowledgements

This work was supported by the Agency for the Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the Project 2017 SGR-310.

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The Role of Liquid Chromatography-Mass Spectrometry in Food Integrity and Authenticity
DOI: http://dx.doi.org/10.5772/intechopen.85087

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The Role of Liquid Chromatography-Mass Spectrometry in Food Integrity and Authenticity

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