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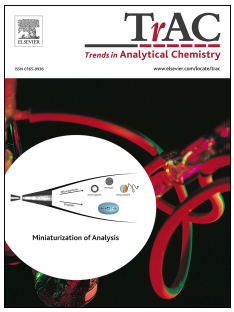

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Comprehensive overview and recent advances in proteomics MS based methods for food allergens analysis

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ABSTRACT

Food allergies are a serious health concern with increasing worldwide prevalence. Food legislation in place in several countries, requires detailed declaration of allergens in foods implying capability of methodologies to reliably trace food allergens. However, detecting and quantifying food allergens remains a challenge. Current common methods for food allergen analysis utilize antibody-based assays although some drawbacks are encountered such as matrix/processing effects and epitope masking especially when dealing with complex and processed foods. Therefore, sensitive, reliable, robust, fast, reproducible, and standardized methods are necessary for improved allergen analysis and reduce the risk of allergen contamination. In the last decade, mass spectrometry (MS) techniques have been developed and applied with success to food allergen detection. This review compares different aspects of food allergen quantification using advanced MS techniques including multiple reaction monitoring. The latter provides low limits of quantification for multiple allergens in complex food matrices, while being robust and reproducible.

Keywords: food allergen, mass spectrometry, peptide markers, quantitative allergen detection, incurred materials, analytical method validation

1. Introduction

Allergenic food proteins, also termed food allergens, represent a threat for the allergic population due to the likelihood to trigger undesired reactions in sensitive individuals whenever introduced through the diet [1]. Although these proteins are normally harmless, they can pose risk in hypersensitive individuals due to their effects ranging from mild or even fatal reactions [2]. It has been reported that the prevalence of food allergies is increasing worldwide affecting 4–8% of children and 1–5% of adults estimated on large population studies [3].

According to the available data, approximately ninety percent of all food allergies are caused by only eight food groups often referred to as “the Big 8” including egg, fish, milk, peanut, shellfish, soy, tree, nuts, and wheat [2]. In addition, since each allergenic source contains more than one allergenic protein it results that within the same allergenic food there might be several proteins capable of inducing allergic reactions.

In order to protect allergic individuals from developing health problems, legislation mandating the correct food labeling of allergenic ingredients contained into a food has been issued in different countries across the globe. In particular in the European Union Directive 2006/142 prescribe the labeling of 14 different allergenic foods [4]. More recently, disclosure of certain substances or products capable of inducing allergies or intolerances has been strengthened for prepacked foods by the EU regulation on food information to consumers and extended to non-prepacked foods since December 2014 in the EU [5]. For further legislative details, we defer to a very recent overview of the historical development of the European directives and regulations provided by Popping and Diaz-Amigo in 2018 [6]. Worldwide, the regulation of food allergens labeling shows regional differences according to local prevalence data and common dietary habits, an overview of the mandatory labeling legislation worldwide is reported in Brockmeyer 2018 [7].

Even small amounts of allergens in the ppm range can trigger a reaction in allergic consumers. In light of this, it is essential to label food products with respect to the allergen content. Currently, avoidance of foods containing their trigger allergens is the only regime that can be followed by people with food allergies. EU labelling law does not cover allergens that may be present from cross-contact or cross-contamination for which voluntary precautionary allergen labelling is applied but widely regarded as unsatisfactory [8].

It is therefore of paramount importance to have at disposal reliable and sensitive methods able to detect at, a certain confidence level, any eventual presence of allergens contaminating foods.

Current methods typically utilized by food industries for allergen monitoring plans employ an antibody-based recognition in the format of enzyme-linked immunosorbent assay (ELISA) or lateral flow devices. Although this approach offer several advantages that contributed to the wide commercialization of different kits for the detection of single allergens, they encounter also several drawbacks that decrease the confidence in the results obtained. Among them, cross-reactivity phenomena, the presence of interfering compounds in complex food matrices that could lead to false positives or could hamper the final detection due to interaction of matrix components with food allergens or antibodies [9,10,11]. In addition, food processing or sample preparation can also generate false negatives consequent to allergen modification that might mask the allergen itself from being recognized by the target antibody [12,13].

In view of the numerous drawbacks of the current established methods for allergen analysis and in order to overcome such limitations, alternative non-immunological methods have been investigated in the last decade with particular emphasis placed on mass spectrometric methods. In particular, the coupling between mass spectrometry (MS) and liquid chromatographic separation proved to be successful in food allergen identification/characterization, and more recently in allergen quantification as well.

In this paper, we illustrate the latest advancement made in MS-based methodologies tailored to the allergen detection with emphasis given to the forefront technology of mass analysers available on the market applied to the multi-allergen screening in foods. At last, gaps that still need to be addressed and future trends are tackled and discussed.

2. Advances in proteomics methods for food allergen detection

Mass spectrometry has been the technique of election in proteomic studies mainly aimed at protein characterization rather than quantification, due to the difficulty to analyze the intact protein itself and due to the limitation posed by the scarce sensitivity obtained for the ionization of large macromolecule like proteins. Nonetheless, thanks to the performance offered by the latest generation of mass analyzers, new efforts have been placed on the development of MS methods able to deliver both qualitative and quantitative information about allergenic proteins in food. In particular, the disclosure of this technology as potential high throughput screening tool to quantify allergen traces, has prompted the need for multiplex detection methods, capable of quantifying several allergenic ingredients in complex food matrices at the highest confidence level and within a single chromatographic run. Dspite the need for expensive equipment and trained

personnel, the chance to provide multiplexing and unequivocal allergen identification accounts for the overall strength of the MS based approaches compared to previously established methods. Noteworthy, current knowledge in the allergen detection field suggests that the challenge to design a unique protocol feasible for different food matrices potentially contaminated by several allergens it still far to be accomplished. A more realistic objective would be to develop tailored approaches based on matrix similarity, whether rich in carbohydrate (such as bread, cookies, etc) or rich in fat (e.g. chocolate bar, chocolate dessert, etc).

As far as MS-based allergen detection is concerningtwo methodological options are to dateavailable; i) detection of the intact protein representative of the allergenic ingredient, that is usually the most abundant in the proteomic profile, ii) detection of the target analytes namely markers that are signature peptides, properly selected, resulting from the enzymatic digestion of the whole allergenic ingredient (Figure 1). In both cases, the sampling is a curcial step to provide a proper representativeness of the analysis, and for an accurate absolute quantification, the protein/peptide content should refer to that of a suitable standard either the whole protein or a derived peptide likely isotopically labelled. The availability and eventual costs of such standards, together with their ionization efficiency are often the main drivers guiding the choice between the two aforementioned approaches. More comments about this topic will be given in the following sections.

Different coupling with with various mass analysers are to date available such as ESI-qTOF (referred as qTOF in this review), ESI-IT (referred as IT in this review), or MALDI-TOF more addressed to qualitative investigations and protein/peptide characterization [14 , 15 , 16]. Quantitative triple quadrupole and IT systems have the advantage of identification and quantification through fragmentation settings in the MS collision cell [17].

The application of MS for allergen identification is, however, not commonly applied for routine analysis, as discussed in detail later [18,19,20,21].

The main advantages offered by MS analysis are to be high-throughput and multiplexing. Nevertheless, the analysis of proteins requires a set of methodological steps that includes enzymatic digestion of proteins to generate proteotypic peptides followed by HPLC separation and MS analysis (**Figure 1**). Sometimes some chemical modifications like post-translational modifications (PTMs) occurring on a protein moiety as a consequence of the heat treatment applied, can induce a change in the secondary and tertiary structure of an allergen sometimes

compromising ELISA result that can depend on the 3-D structure of allergens [18,22]. By contrast, MS is structurally independent whereas is based on the amino acid sequence of the protein and the generated peptides. Moreover, being MS robust and stable it can be easily automated and standardized compared with other methods also offering competitive sensitivities [23,24,25].

2.1. Detection of intact food allergens

In the direct quantification of intact proteins, according to a "top-down" approach, the signal of a multi-charged analyte is compared with that of an internal or external intact standard protein. The major advantage in using a protein standard is that brought through the whole extraction procedure any variability related to recovery, digestion efficiency and extraction yield can be eliminated, assuming the process is identical for both the sample and the standard alike.

However, the quantitation of intact proteins in complex matrices, such as processed food is extremely complicated. The sensitivity of "top-down" methods is limited by the high complexity of the related spectra characterized by multiplex and often overlying charge states of the target proteins which require high resolution MS instruments to resolve isotopologue distribution. Also the accuracy of the quantitative information is strongly affected since the characteristic multi-charge cluster can be influenced by the processing the food has undergone. Moreover, the use of a specific intact protein standard is hampered by the commercial availability of "purified" proteins, even more using isotopically labeled proteins for accurate and traceable quantification; indeed, only few papers have been reported in the literature showing HPLC-MS methodology applied to the detection and quantification of allergenic proteins by using isotopically labeled equivalents [26]. Alternatively, label free methods based on HPLC-MS detection operating in selected ion monitoring mode might be implemented for the detection of intact proteins although such approach had objective sensitivity limitations when applied to highly processed foods. With this aim, a few papers reported the use of MS systems for the identification and characterization of intact proteins in food commodities especially when the investigation was tailored to the study of modifications sites in heated proteins [27,28,29,30]. Among them, lactoglobulins were the highly investigated proteins due to the good multi-protonated features shown by this class of proteins generating a reproducible multi-charged ions envelope by electrospray ionization [18,31,32,33]. In most recent studies potentials of High Resolution mass analysers might tackle some drawbacks and provide in thorough information about the modification sites and more insights in the structural elucidation [34,35].

2.2. Detection of peptides markers tracing for allergenic proteins

Due to the aforementioned limitations, the proteomics approach mostly used for allergen detection also called "bottom-up" route (mainly as "shotgun" approach, see **Figure 1**) where targets are specific peptides resulting from the enzymatic digestion of protein extracts. The use of "bottom-up" methodologies for quantitative analysis is steadily increasing and has proved to adapt well also for allergen quantitative analysis in complex food matrices.

Quantification at the peptide level can be classified in methods involving stable isotopes: tagging by light (^{12}C) and heavy (^{13}C-labelled) tags and using isotopically labelled synthetic peptide to achieve, respectively, relative or absolute quantification. Further details about the use of targeted proteomic approach coupled with isotopically labelled internal standards also known as AQUA peptides is out of the scope of the present paper.

More recently, a cost-effective label-free quantitative method based on signal intensity has also been proposed and widely used as an alternative route for relative quantification excluding the involvement of stable isotopes.

In **Figure 2**, is schematized the typical workflow underpinning the development of an analytical method for allergen detection by bottom-up strategy. Five main steps were itemized starting from the identification of candidate peptide markers (step 1), proceeding with the development of HPLC-MS instrumental set-up (step 2), the optimization of the protein extraction/purification conditions (step 3), as well as optimization of the enzymatic digestion conditions (step 4), up to the single-laboratory validation of the analytical method (step 5). Noteworthy, in the bottom-up approach the first step of marker peptides identification is one of the most important step in the method development, since the robustness and sensitivity of the overall analytical method will strictly depend on the reliability of the signature peptides (uniqueness, specificity, stability) tracing for the target proteins. Different routes have been proposed to draw the list of candidate peptide markers (A and B in **Figure 2**) both relying on the application of the selection criteria originally devised through the MoniQA (Monitoring and Quality Assurance on the Total Food Supply) [36]. Route A in **Figure 2** was based on the preliminary in-silico selection of both target proteins and peptides according to the specific criteria detailed in the scheme. The latter was accomplished by advanced bioinformatics tools such as on-line databases for fasta sequences (Uniprot), searching tools for sequences alignment (BLAST), as well as free software for target proteomic method development, such as Skyline [37]. Most important, such in-silico prediction requires a systematic

in-vitro validation of the preliminary list in order to assess the efficient release of the predicted proteotypic peptides from both the allergenic ingredients extracts and from the artificially contaminated matrix under investigation (as either spiked or incurred matrix depending on the aim of the study). This step is strongly recommended in order to assess the marker specificity, excluding the presence of interfering peaks from the matrix, as well as discover potential susceptibility of the peptides to food processing effects, both features that cannot be foreseen in-silico. Alternatively, the identification of candidate peptide markers can also follow an evidence-based approach depicted as route B in **Figure 2**. The selection grounds on a dedicated discovery proteomic experiment consisting in untargeted MS/MS analysis (often performed by high resolution MS) and software based protein/peptide identification both performed directly on enzymatic digests of allergenic ingredients and artificially contaminated matrix extracts. Noteworthy, such approach skips the preliminary in-silico prediction and invests more efforts on the peptide validation on “real complex samples”, still applying the same selection criteria [38].

Once the specific marker peptides are identified, allergen detection and quantitation at the peptide level can be achieved with specific pro and cons by several MS platforms, a comprehensive overview of various methods will be provided in the next section. Noteworthy, both chromatographic separation and MS instrumental set-up require a fine tuning of the operational parameters in order to optimize the shape, width and in-time resolution of the chromatographic peaks, as well as the ionization efficiency, the fragmentation yield and the acquisition cycle time (in particular, if quantitative multi-allergen detection is devised). The majority of published targeted methods opt for selected-reaction monitoring (SRM) acquisition mode for the high sensitivity provided by fragmentation of the analyte (peptide precursor ion) into specific ions [39]. These fragmentation events (transitions) are either in-silico predicted based on the activation mechanism or identified experimentally in the previous step of the method development.

Further crucial steps deal with the optimization of the protein extraction and purification conditions and the digestion yields, all requiring accurate evaluation based on the individual target protein properties (molecular weight, solubility, disulfide bridges, folding, and extent of glycosylation and other post-translational modifications), matrix complexity (interference from co-extracted species, processing effects) and the susceptibility to enzymatic hydrolysis [40]. Extraction conditions should be optimized based on both the highest recovery of total protein as well as relative quantification of signature peptides under the selected MS conditions. As for in-

solution enzymatic digestion, ideally, the peptide markers should be fully released from the parent protein and stable during the entire time of digestion. All additional knowledge gained at these stages should underpin further revision of the previous candidate marker list.

Finally, a single-laboratory method validation is required to assure that the method is suitable for its intended purpose and that the same method will perform equally in all laboratories. As far as validation of MS methods for food allergens detection is concerning, there has been a need over the years to harmonize analytical methods for food allergen analyses. The full validation of a multi allergen method is currently hard to achieve in absence of proper reference materials, more comments about this topic will be provided in the final paragraph.

3. Evolution of MS based methods targeting allergens in food: from single to multi-target analysis

The advent of last generation hybrid Mass Spectrometers has pushed in the direction of developing sensitive hyphenated methods, coupling separation techniques with advanced MS detectors, for absolute quantification of allergens in complex food matrices. This opened to a new concept of MS techniques henceforth also intended as rapid and high throughput analytical tool for multi-target analysis of allergens in food commodities in one run.

Typically, this peptide-based allergen quantification strategy was based on multiple SRM acquisition scheme that monitors characteristic duplets precursor/transitions of selected peptide markers generated upon proteolytic digestion on triple quadrupole instruments. The most common quantitative applications of SRM in proteomics rely on the principles of stable isotope dilution (SID) methods, which can be now considered the “golden standard” for absolute quantification [41,42].

In the very early beginning, selected ion monitoring (SIM) scheme also involving MS/MS spectra corresponding to each precursor ion, has been used for food allergen monitoring in different food commodities by using ESI-QqTOF-MS systems coupled with either UHPLC or micro-HPLC separation. The early applications date back the early 2000 where a capillary HPLC system coupled to QqTOF-MS was used for tracing peanuts or milk allergens in food products, assessing the potentials offered by such technology for qualitative and quantitative analysis [18,22-24,43,44,45,46]. The SIM method developed was based on the detection of precursor ion peptides and the respective fragmentation patterns recorded in MS/MS spectra. Notwithstanding the

feasibility assessment of this approach to food allergen detection, constrains in terms of sensitivity reached were highlighted.

With the advent of the last generation of triple quadrupole mass spectrometers providing enhanced sensitivity, the routine quantitative analysis usually accomplished on small molecules was transferred efficiently to the allergen field, by monitoring multiple transitions of the best peptide markers identified for each allergenic category. In this regards the approach described by Heick et al. in 2011 represents a milestone sincea multi-allergen SRM method capable of tracing 7 allergenic ingredients in a single run was described [20]. Once suitable peptides and transitions were properly selected, the method was applied to bread incurred with these 7 allergenic ingredients to assess the quantitative capabilities and LODs ranging from 3 to 70 $\mu g_{ingred}/g$ were obtained depending on the specific allergen. The same scheme was also used in another work for delivering an in house validated LC-MS method for the accurate quantification of milk traces in different food products based on selective determination of peptides specific for β-lactoglobulin, αS2-casein, β-casein, and κ-casein [21]. In this paper, the quantification was attained by using internal standard peptides containing isotopically labeled amino acids and LOD values as low as 0.2-0.5 μg/g, comparable to the limits obtained with ELISA kits. Despite this study represents the most complete report for measuring milk protein concentrations in food, it fails to analyse milk allergens in complex baked foods because the stable isotope-labelled milk peptides were spiked into samples only after tryptic digestion of protein extracts. In this way, the effect of processing on protein modifications were not evaluated. Later, Zhang et al. analysed traces of bovine α-lactalbumin in vegetal infant formulas, they used extended peptide precursor derived from α-lactalbumin as internal standard added into the sample prior to the tryptic digestion [25]. Similarly, the same authors achieved more recently β-casein allergen quantification also in baked foodstuffs, by using a stable isotope-labelled internal standard designed to adjust the instability of sample pre-treatment and ionisation caused by matrix effect [47].Interestingly, a feasibility study on the application of metrologically traceable MS-based reference procedures has been described by Cryar et al. [48]. The approach employed a proteolytic digestion step of wine spiked with lysozyme standard, followed by isotope dilution MS analysis. Results showed that the MS method was feasible for absolute quantitative analysis proving to be suitable for the production of allergens in food certified reference materials. Further investigations focused on single allergenic ingredient detection in food matrix by SRM based approaches were reported in the last years and some details were summarized in **Table 1** [49,50,51,52,53,54,55,56,57,58,59].

Complying with the specific need for high throughput methods, great efforts were devoted in the last decade to the development of multiplexing approaches covering a wide variety of food allergens, mainly the ingredients listed in official regulations. Several food allergens/matrices combinations were taken into consideration from easier handling liquid matrices such as wine [60,61,62], to more complex matrices such as meat [63,64], or even processed commodities [65,66,67,68]. Very interesting sensitivity were achieved like reported in **Table 2**, however, the lack of consensus in analytical performance definitions (e.g. limit of detection/quantification and recovery), in spiking procedures and in reporting units prevents full comparability of the results obtained by different laboratories and a case-by-case critical evaluation of the claimed performance should be done to appreciate any advancement.

Due to the high complexity of certain food matrixes and certain food allergens, peptide detection using lower-resolution instruments, such as triple quadrupole, can suffer from limited specificity also in SRM mode. In this case, the recourse to triple-stage MS (MS^3)-based approaches with selected secondary fragmentation monitored, was proposed by Brockmeyer and co-workers, to enhance the detection sensitivity. [7]. The Authors proposed two different investigations sharing the same multiple SRM cubed approach: the first tailored to crustaceans (shrimp and lobster) contamination monitoring, that reached sensitive detection down to levels as low as 25 µg/g (crustacean/food, 0.0025%) [69], and the second devised to detect five different nuts and peanuts in different matrix materials (bread, chocolate, ice cream), even if not accounting for the effects of food processing [70]. Other investigations were proposed mostly enhancing potentials of the linear ion trap (LIT) MS for the multi-target analysis of nuts, wheat and fish allergens in diverse food matrices [71,72,73,74,75,76]. As additional feature, some authors described also for LIT, advantages and limitations of multi-target allergen analysis by using MS^3 acquisition mode [77]. A method duly optimized for the simultaneous detection of soy, egg and milk allergens in a cookie food matrix by microHPLC–ESI-SRM, was proposed in 2014 [78]. Thanks to the innovative configuration and the versatility shown by the dual cell linear ion trap MS used, the most intense and reliable peptide markers were first identified by untargeted survey experiment, and subsequently employed to design an ad hoc multi-target SRM method for label-free quantitation. Recently, the same Authors updated such investigation by ameliorating the analytical workflow, i.e. streamlining the sample pre-treatment protocol, increasing the number of allergenic ingredients monitored simultaneously, and assessing its sensitivity also on home-made incurred cookies, the recipe for which was as close as possible to real foods [79].

An alternative to well established multiple-SRM approach provided by either triple quadrupole or LIT or their hybrids configurations, was represented by high resolution MS (HR-MS) approaches. Such route for the first time presented by Monaci et al. in 2011, proposed a single stage-Orbitrap™-mass spectrometer for the fast and high throughput screening of milk and/or egg allergens in wine samples [80,81,82] and afterwards for the detection of peanut in nuts mixture [83]. In general HR-MS offers many benefits over the classical tandem mass spectrometry [84,85,86]. Among others, the advantage of collecting full MS spectra in HR provides greater insights into the identity and chemical structure of a food component and provide a non-targeted detection method, which allows, even retrospectively, the identification of numerous allergen markers simultaneously without preliminary information required. In addition, the simultaneous acquisition of both HR-MS full scan and HCD fragmentation at the highest resolving power and mass accuracy in a single chromatographic run provides both confirmative and quantitative analyses of multiple food allergens. By following such approach, challenging LODs can be obtained thanks to the post acquisition accurate mass filtration of the selected peptide ions operated on the total ion current traces, thus representing a valid alternative to the SRM based methods (see **Tables 1-2**). A direct comparison between HR-MS and SRM based methods for a specific case study (multiplex screening of egg and milk proteins in white wines) was proposed, assessing the suitability of both approaches for quantification and screening purposes [87]. The HR-MS approach lied on the simultaneous acquisition of Full-MS and all ion fragmentation MS/MS spectra in a single chromatographic run, combining both confirmative and quantitative features. As untargeted MS approach, each chromatographic run can be reconsidered for a retrospective analysis, by applying post-run data processing without further waste of sample, solvents and time, thus providing greater flexibility than the classical SRM method where a proper decision on target peptides has to be taken in the very early beginning. However, in HR-MS based approach best sensitivity was achieved by integration of precursor markers ions, whereas the MS/MS spectra, acquired in all ion fragmentation (AIF) mode, provided only peptide structure confirmation. For simultaneous quantitative and confirmative purposes requiring specific transitions, the sensitivity shown by the SRM method was proved higher than that offered by the monostage Orbitrap™ mass spectrometer operating in AIF mode [87], thanks to precursor ion isolation. A comprehensive HR-MS approach was applied to unravel the peptide marker identification in tricky allergenic ingredients, such as peanut and most tree-nuts, where proteome profiling is challenged by high structural homology and isoforms occurrence. Allergen detection was performed in full-scan mode

at 100k resolution. The accurate mass of the marker peptides together with the expected isotopologue pattern were identified from extracted ion chromatograms and used for detection and quantification [15].

Significance of proper peptide markers selection on the resulting robustness and sensitivity of the final analytical method was discussed thoroughly in this section. An in-deep analysis of the most recent literature on the food allergen detection field, furtherly support such statement, since HR-MS has become the preferred choice for the preliminary untargeted MS/MS analysis, software based peptide identification and marker selection [88,89]. On his regards, particular attention was paid to food processing affecting the reliability of peptide targets. First systematic studies were undertaken to investigate the effects of thermal processing in terms of protein extractability and peptide thermic stability of peptide targets for peanut [90] and walnut allergenic proteins [91], the final goal being the identification of processing-stable targets, coupled with more efficient extraction procedures for successfully quantifying such allergens in processed food matrices.

4. Advances in High Resolution Mass Spectrometry applied to food allergen detection

Recent advanced in HR-MS platforms, such as the Q-Orbitrap [92] and Q-ToF [93] resulted in the development of alternative MS/MS analysis modes. Among these, the Parallel Reaction Monitoring (PRM) option available on a Q-Orbitrap equipment acquires a Full MS/MS fragmentation spectrum for a specific parent ion, providing the simultaneous monitoring of all the product ions at high accuracy and resolving power [94]. As main benefits, the PRM option boasts a potentially higher specificity accounted by the high accuracy and resolving power in the fragmentation pattern acquisition, a simplified assay development with no need of preliminary transitions selection, a multiplexing capacity comparable to SRM approaches available on QqQ platforms [95]. On the other hand, the sensitivity and duty cycle efficiency were reported not to be comparable with triple quadrupole instruments [94]. An alternative targeted option for quantitative proteomics available on the Q-Orbitrap is the targeted-selected ion monitoring coupled with data dependent MS/MS acquisition (t-SIM/dd2). The t-SIM/dd2 mode provides reliable peptide identification based on the accurate detection of the peptide precursor ion and the relevant confirmative fragmentation pattern. As general statement, both the aforementioned options require preliminary selection of markers and no retrospective analysis can be carried out.

The choice between them should be guided by the signal background, which is directly related to the system complexity. PRM is fragmentation based so has inherently higher specificity, however the overall "base" sensitivity is potentially lower as the intensity of the available precursor ions is distributed across multiple transitions; still with highly complex matrix PRM works properly in improving signal-to-noise ratio.

PRM and t-SIM/dd2 modes, together with the untargeted Full HR-MS approach were recently applied to the multi-allergen detection in cookie matrices, and relevant performances compared [96]. The three modes provided a slight difference in the sensitivities achieved and in all cases, the t-SIM/dd2 turn out the best compromise among sensitivity, specificity and reliability, given the dual information available about both the precursor ion and the MS/MS spectrum. Noteworthy, the t-SIM/dd2 acquisitions resulted in best overall sensitivity by monitoring the precursor peptide ions, whereas fragmentation spectra were considered only for confirmative purposes.

Finally, an alternative HR-MS/MS analysis mode is the data independent acquisition (DIA) available on both Q-Orbitrap and Q-ToF platforms. Originally devised for comprehensive proteomic characterization, DIA combines the sequential isolation of a large precursor window with full MS/MS spectrum acquisition [97]. By post-acquisition processing (accurate mass extraction) the data can be used for targeted analysis after proper selection of specific transitions for each marker peptide. Differently from SRM, the instrumental set up is completely independent from specific target ions; therefore, various precursors can be activated simultaneously in the same m/z isolation window, resulting in a composite fragmentation spectrum which needs software based deconvolution. The MacCoss lab, developer of the Skyline software proposed a variant of DIA for improved spectral deconvolution that randomly combines five 4 m/z isolation windows and cover 400 m/z range per duty cycle [98]. The randomly permuted isolation windows result in a unique combination of peptide ions analyzed during each duty cycle, which reduces signal interferences and facilitates unambiguous spectra deconvolution. Peptides sequences can be either identified by database search or detected by targeted extraction. The so-called SWATH (Sequential Windowed Acquisition of all Theoretical fragmentation spectra) approach represents a natural evolution of the same basic idea underneath the DIA acquisition, noteworthy relying on a wider isolation windows (typically 2-25 m/z) [99]. The latter increase the complexity of the product ions spectra and relevant deconvolution, too challenging for traditional sequence based search engines. However, again extraction of product ion chromatograms similar to SRM or computational deconvolution by specific developed approaches are both feasible options [99].

The concept of DIA presents appealing advantages for quantitative proteomics mainly for its untargeted MS/MS nature. Indeed, the resulting data set would be always available for retrospective analysis, maintaining, at least partially, the specific correlation about the parent peptide ion. This feature overcomes the objective limitation that constrained the sensitivity and specificity achievable with the aforementioned AIF approach. Nevertheless, in DIA the wider is the isolation window set, the higher will be the loss of information about the precursor and consequent complexity in the reassignment of product ions. The high mass resolution and accuracy of MS/MS spectrum together with LC elution profiles result in specificity comparable to SRM [99].

To the best of our knowledge, DIA was not applied so far to the quantitative detection of allergen contamination in complex food matrixes, however two investigations were reported which benefit from the untargeted quantitative information provided by this analysis mode for the comprehensive profiling of allergen composition in relevant food ingredient, namely peanut [89] and wheat gluten [100].

5. Towards validation of multi-allergen MS methods

After the proper development of the MS-based method, a final validation of the workflow in order to assess the robustness of the method among different laboratories is strongly recommended. The validation process includes a number of steps to demonstrate that the developed method complies with the established performance criteria set in the guidelines issued by different international standardization bodies (like IUPAC, ISO, AOAC International).

As far as validation of MS methods for food allergens detection is concerning, there has been a need over the years to harmonize analytical methods for food allergen analyses. This was an objective of international Associations like MoniQA and the objective of other European funded Projects (e.g. iFAAM) [40] and represents the main objective of the recently funded EFSA project at European level.

Lately, in 2016 the AOAC STAKEHOLDER PANEL on Food Analytical methods (SPSFAM) drew guidelines on setting Standard Method Performance Requirements for detection and quantitation of selected food allergens to guide method developers in the validation of MS based methodologies for food allergen analysis [101]. Although some criteria have been established setting performance requirements based on key parameters such as the analytical range, limit of

438 detection, limit of quantification, recovery and precision, other aspects are still open issues and
439 need to be better tackled. As already mentioned the full validation of a multi allergen method is
440 currently hard to achieve in absence of proper reference materials. Only few options are available
441 on the market for specific allergenic ingredients and incurred matrices, supplied with proper
442 characterization of total protein content, allergen profiling, homogeneity and stability tests.
443 Among these, it deserves to be mentioned the MoniQA milk allergen reference materials (positive
444 and negative controls and two incurred cookie matrices), NIST spray-dried whole egg reference
445 material, FAPAS cake mix reference materials including egg, gluten and milk, just to cite a few. In
446 absence of these, a preliminary characterization of the raw materials at least in terms of total
447 protein content and homogeneity should be carried out, unless such information were made
448 already available from the provider, for confident inclusion in validation studies. Noteworthy,
449 great efforts have been devoted in the last years to the development and commercialization of
450 more reference allergenic ingredients, reference-incurred matrices, and quality control materials
451 for allergen detection to be included in the development MS methods.

452 According to the published papers, a factor that needs to be taken into account is the reporting
453 units used along the study (either whole ingredient or its protein content), because the non-
454 uniformity of such information does not allow comparability among the different methods
455 developed. Regulatory bodies define the food allergens as whole food commodity, excluding the
456 direct association to a specific protein and referring to the total allergenic commodity in the total
457 food matrix. However, risk assessment methods focus on protein components, requiring input
458 data on allergen levels expressed in milligrams of total protein. The direct correlation to the
459 concentration levels in foods would facilitate in addition to assess if the sensitivities achieved by
460 methods under development could detect and quantify allergens down to clinically relevant
461 reference doses for the established serving size.

462 Among the major parameters to be accurately discussed in any new method evaluation is the food
463 matrix under investigation and the protocols used for the preparation of artificially contaminated
464 food matrices. Despite the widespread employment of foods spiked or fortified with the allergenic
465 ingredient, only a few report the performance characteristics assessed in “incurred”. This last
466 approach, if on one-hand leads to a lower sensitivity, on the other hand appears to be more
467 realistic in the perspective application to a food accidentally contaminated with the allergenic
468 ingredient before entering the production line.

In **Table 3** a selection of the most recent papers on the field was provided sharing the common effort to develop and validate (at least partially) multi-allergen analytical methods onto incurred food matrices. Interestingly, some of these also included in the recipe, reference materials for some of the allergenic ingredients (e.g. whole egg (NIST RM 8445), non-fat dry milk (NIST SRM 1549), etc) [54,68,102,103]. For absolute quantification, the common approach requires resorting to SID for the construction matrix-matched calibration curves. These labelled peptides are identical in physicochemical structure, chromatographic performance, and ionization efficiency to the corresponding light peptide mitigating the effect of instrumental fluctuation and signal suppression caused by matrix components. However, the approach poses new challenges, once applied to incurred food matrices because demands the elaboration of proper conversion factors, which translate the absolute peptide amount, extrapolated from stable isotope dilution curves, into milligrams of total protein of the allergenic ingredient under investigation. The work proposed by Parker et al. in 2015 [66] represented the first attempt to elaborate such conversion factor, afterwards recalled in 2017 by Boo et al. [103] and in 2018 by Sayers et al. [104], all based on the main assumption that the selected marker peptide is totally released by the parent protein (molar equivalence between peptide and protein concentration).

Noteworthy, the paper authored by Sayers et al in 2018 [104] proposed a microfluidic-SRM approach based on novel ceramic based microflow devices, as valid alternative to conventional chromatographic separation. This promising technology provided enhanced sensitivity for the specific application grounding on the increased ionization efficiency at low levels, reduced susceptibility to matrix interference and increased peptide stability over a wider dynamic range.

In their last work, Planque et al. extended the previous investigation [68] with inclusion of up to 10 allergens into incurred matrices [102]. The final goal was to devise a sample preparation protocol feasible in one working day, with the prospective to make it suitable for routine analysis. Interesting sensitivity were achieved with 90.7% of the coefficients of regression higher than 0.97 and only 11.6% of the relative standard deviations higher than 20%. The Authors suggested that the method precision could be improved by inclusion of labeled internal standard.

More recently, we accomplished the first in-house validation of an HR Orbitrap™-based MS method for the multiple detection of five allergenic ingredients in incurred cookies [96]. Different acquisition schemes were independently optimized and compared in terms of sensitivity. Targeted-selected ion monitoring with data-dependent fragmentation turned out the best choice

as good compromise between sensitivity and accuracy, accomplishing the detection of 17 peptides, belonging to five allergens in the same run with relevant HR-MS/MS spectra acquisition. Systematic evaluation of matrix and processing effects was featured as well as a preliminary in-house validation of the HR-MS method assessing trueness (recovery), precision, and sensitivity.

Notwithstanding, the undeniable advances provided by the selected investigations in Table 3, compared to the previous existing literature, some criticisms still need to be tackled, mainly concerning the results comparability about different methods. Firstly, the different ingredients selected for the incurred matrix preparation will inevitably affect the detection sensitivity (e.g. defatted peanut vs peanut butter vs peanut flour), but more importantly a consensus about the definition of LOD and LOQ values is highly desirable to advance in results comparability towards the full validation of multi-allergen MS methods. In order to have more trustful estimation of LODs and LOQs that can be comparable among the different laboratories and unaffected by the instrumental noise, the authors do encourage the analytical community to calculate LODs and LOQs by considering the variability over the all calibration range investigated or the variability of ten independent samples at the lowest concentration level, to avoid over-optimistic conclusions. Thereby is authors opinion that the calculation made from the following approach would be the most preferred and reliable for such determination: LOD = 3SD/slope, LOQ =10SD/slope, where the SD should be either (i) the standard deviation of the intercept, or (ii) the residual standard deviation of the linear regression, or (iii) the standard deviation of 10 independent samples fortified at the lowest acceptable concentration.

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CONFLICT OF INTERESTS

The authors declare that have no conflict of interest.

ABBREVIATION LIST

AIF, all ion fragmentation

AOAC, Association of Analytical Communities

BLAST, basic local alignment search tool

DIA, data independent acquisition

EFSA, European Food Safety Agency

ELISA, enzyme-linked immunosorbent assay

ESI, electrospray ionization

HCD, higher energy collisional dissociation

HPLC, high performance liquid chromatography

HR, high resolution

IT, ion trap

ISO, International Organization for Standardization

IUPAC, International Union of Pure and Applied Chemistry

LIT, lineat ion trap

LOD, limit of detection

LOQ, limit of quantification

MALDI, matrix assisted laser desorption ionization

MS, mass spectrometry

PRM, parallel reaction monitorins

PTM, post-translational modification

qTOF, quadrupole time of flight

QqQ, triple quadrupole

SID, stable isotope dilution

SIM, selected ion monitoring

SRM, selected-reaction monitoring

556 SWATH, sequential windowed acquisition of all theoretical fragmentation spectra

557 t-SIM/dd2, targeted selected ion monitoring with data dependent MS/MS acquisition

558 UHPLC, ultra-high performance liquid cromatography

559

560

561 ## FIGURES AND TABLES CAPTIONS

562 **Figure 1.** Summary of the different MS-based analytical approaches available for food allergen
563 identification, characterization, and quantitative detection.

564 **Figure 2.** Scheme of the typical workflow underpinning the development of an analytical method for
565 allergen detection by bottom-up strategy.

566 **Table 1.** Overview of the LC-MS methods devised for single allergen detection in food products.

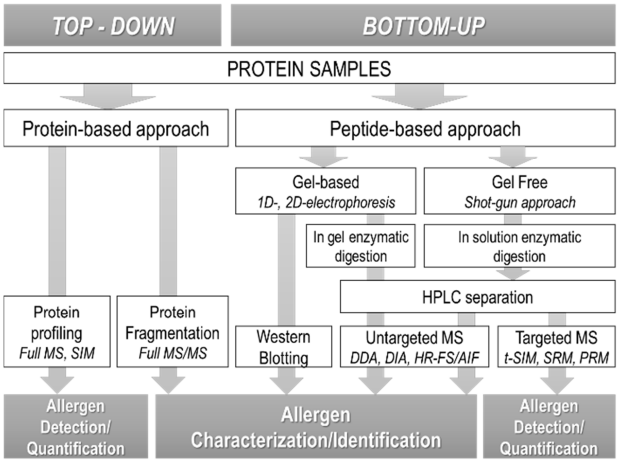
567 **Table 2.** Overview of the LC-MS methods devised for multiple allergen detection in food products.

568 **Table 3**. Detailed description of the most recent analytical methods for food allergen detection developed
569 and validated with incurred food matrices (abbreviations: RT, room temperature; TBS, Tris-buffered saline;
570 DTT, dithiothreitol; ON, overnight; SDS, sodium dodecyl sulfate).

571

572 # FIGURES AND TABLES

573 **Figure 1**



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577 **Figure 2**

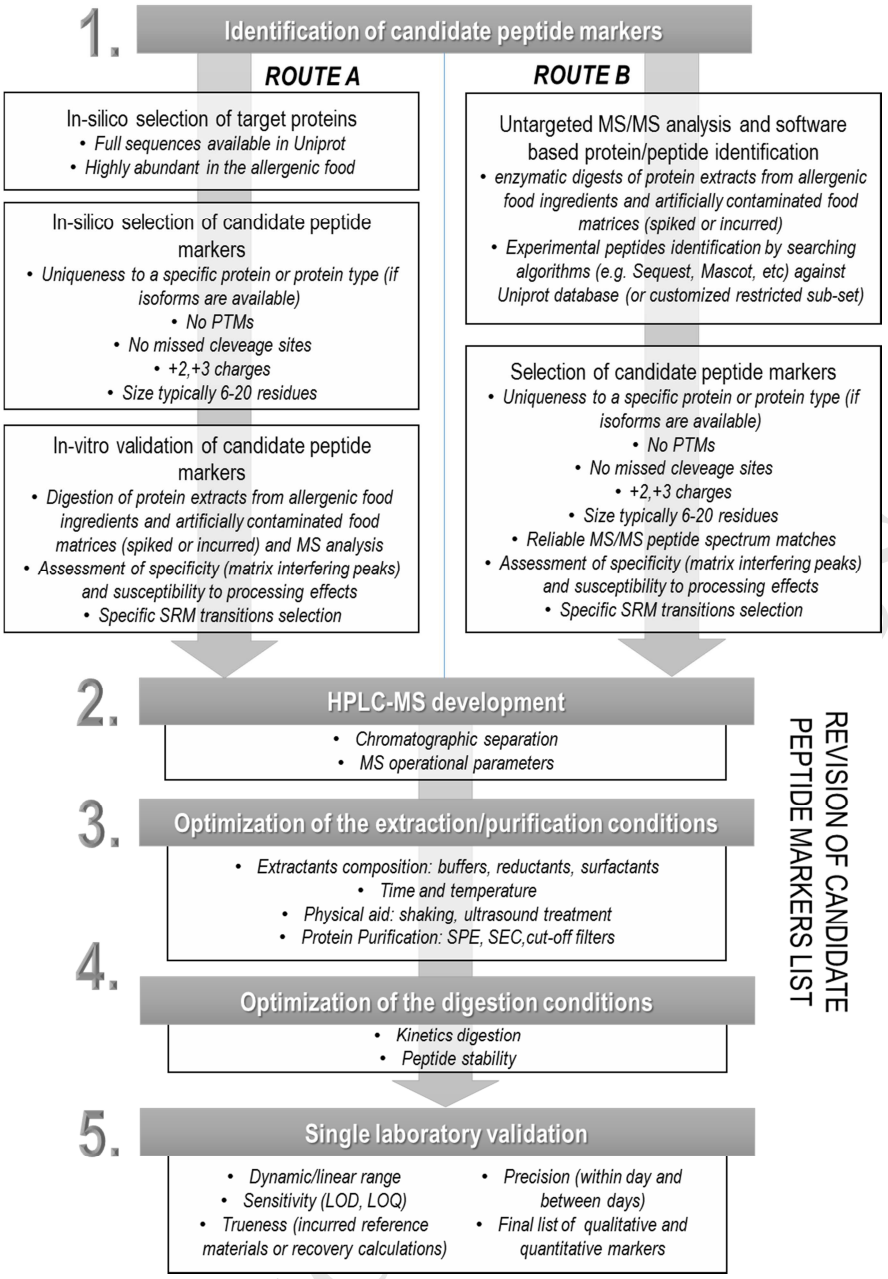

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Table 1.

Reference	Allergenic Ingredient	Target Protein	Foodstuff	Analytical method	LOD/LOQ
Shefcheck et al. 2004 [44]	Ara h 1	Ara h 1	Vanilla ice cream	HPLC-quadrupole ToF (MS^2)	LOD: 10 $\mu g_{ara\ h\ 1}/g$
Shefcheck et al. 2006 [23]	Peanut	Ara h 1	Dark chocolate	HPLC-triple quadrupole (SRM)	LOD: 2 $\mu g_{tot\ prot}/g$
Weber et al. 2006 [43]	Milk powder	αS1-casein	Cookie	Capillary HPLC-quadrupole ToF-(MS^2)	LOD: 1.25 $\mu g_{milk\ powder}/g$
Careri et al. 2007 [72]	Peanut	Ara h 2, Ara h 3/4	Chocolate/rice crispy based snacks	HPLC-triple quadrupole (SRM)	LOD: 1-5 $\mu g_{tot\ prot}/g$ LOQ: 3.7$_{tot\ prot}/g$
Careri et al. 2008 [73]	Peanut	Ara h 3/4	Breakfast cereals	HPLC-linear ion trap (SRM)	LOD: 3 $\mu g_{peanut}/g$ LOQ: 10 $\mu g_{peanut}/g$
Monaci et al. 2010 [19]	Caseinate	αS1-casein, β-casein	White wine	Capillary HPLC-quadrupole/ToF-(MS^2)	LOD: 50 $\mu g_{caseinate}$/mL
Monaci et al. 2010 [45]	Milk powder	αS1-casein, αS2-casein, BSA	Incurred cookie	Capillary HPLC-quadrupole/ToF-(MS^2)	LOD: 100 $\mu g_{milk\ powder}/g$
Monaci et al. 2011 [81]	Caseinate	αS1-casein, β-casein	White wine	HPLC-Orbitrap™ (Full MS/AIF)	LOD: 39-51 $\mu g_{caseinate}$/mL
Lutter et al. 2011 [21]	Milk	β-casein, β-lactoglobulin, αS2-casein, k-casein	Soy-based infant formula, Breakfast cereals, Infant cereals, Baby food	HPLC-triple quadrupole (SRM)	LOD: 1-20 $\mu g_{\beta\text{-casein}}/g$, 1-5 $\mu g_{\beta\text{-lactoglobulin}}/g$, 1-5 $\mu g_{\alpha S2\text{-casein}}/g$, 1-20 $\mu g_{k\text{-casein}}/g$ LOQ: 1-50 $\mu g_{\beta\text{-casein}}/g$, 2-10 $\mu g_{\beta\text{-lactoglobulin}}/g$, 2-20 $\mu g_{\alpha S2\text{-casein}}/g$, 5-50 $\mu g_{k\text{-casein}}/g$
Pedreschi et al. 2012 [50]	Peanut	Ara h 1, Ara h 2, Ara h 3/4	Incurred cookie	HPLC-triple quadrupole (SRM)	LOD: 10 $\mu g_{peanut}/g$
Tolin et al. 2012 [60]	Egg white (Albuclar®)	Ovalbumin, Ovotransferrin, Lysozyme, Ovomucin, Serum albumin	Red wine	HPLC-quadrupole/ToF (MS^2)	LOD: 50 $\mu g_{albuclar}$/mL
Newsome et al. 2012 [26]	Skimmed Milk powder	αS1-casein	Incurred cookie	HPLC-triple quadrupole/linear ion trap (SRM)	LOQ: 3 $\mu g_{skim\ milk}/g$
Mattarozzi et al. 2012 [71]	Lupin	β-conglutinin, α-conglutinin, δ–conglutinin, γ-conglutinin	Pasta, Biscuits	HPLC-linear ion trap (SRM)	LOD: 1-13 $\mu g_{lupin}/g_{pasta}$, 1-24 $\mu g_{lupin}/g_{biscuit}$ LOQ: 4-42 $\mu g_{lupin}/g_{pasta}$, 4-80 $\mu g_{lupin}/g_{biscuit}$
Losito et al. 2013 [51]	Caseinate	αS1-casein, αS2-casein, β-casein	White wines	HPLC-3D ion trap (MS^2)	LOD: 0.09 - 0.29 $\mu g_{caseinate}$/mL
Costa et al. 2014 [52]	Hazelnut	Cor a 8, Cor a 9, Cor a 11	Chocolate	HPLC-triple quadrupole/linear ion trap (SRM)	LOD: 1 $ng_{peptide}$/mL LOQ: 2-10 $ng_{peptide}$/mL
De Ceglie et al. 2014 [105]	Hazelnut oil	Cor a 9, Cor a 11, Cor a 14	Extra virgin olive oil	MALDI-ToF (Full MS)	LOD: 4-20 $\mu g_{TOT\ protein}$/mL
Chen et al. 2015 [47]	β-casein	β-casein	Cookie	HPLC-triple quadrupole (SRM)	LOQ: 500 $ng_{\beta\text{-casein}}$/mg
Poseda-Ayala et al. 2015 [53]	Sin a 1	Sin a 1	Commercial sauces (mustard sauce, garlic mayonnaise, barbecue sauce, honey-mustard sauce, ketchup, and mayonnaise) and salty biscuit	nano-HPLC-triple quadrupole (SRM)	LOD: 0.25 $\mu g_{Sin\ a\ 1}/g$ LOQ: 0.75 $\mu g_{Sin\ a\ 1}/g$
Monaci et al. 2015 [83]	Defatted Peanut	Ara h 1, Ara h 3	Tree nut mixture	HPLC-Orbitrap™ (Full MS/AIF)	LOD: 4 $\mu g_{TOT\ prot}/g$, 26 $\mu g_{defatted\ peanut}/g$, LOQ: 14 $\mu g_{TOT\ prot}/g$, 88 $\mu g_{defatted\ peanut}/g$,
Pilolli et al. 2017 [57]	Ovalbumin	Ovalbumin	White and Rosé Wine	HPLC-linear ion trap (SRM)	LOD: 0.01 $\mu g_{ovalbumin}$/mL, LOQ: 0.03 $\mu g_{ovalbumin}$/mL
Lamberti et al. 2016 [54]	Skimmed milk powder	αS1-casein	Incurred cookie	HPLC-3D ion trap (SRM)	LOD: 1.3 $\mu g_{skim\ milk\ powder}/g$ LOQ: 4 $\mu g_{skim\ milk\ powder}/g$
Sayers et al. 2016 [90]	Defatted peanut flour	Ara h 1, Ara h 3, Ara h 2, Ara h 6, Ara h 7	Raw peanuts with skins intact, mechanically blanched and oil-fried peanuts, lightly roasted mechanically defatted peanut flour, boiled (for different time) or roasted (@ different temperature) peanuts	HPLC-triple quadrupole (SRM)	ND
Ji et al. 2017 [56]	β-lactalbumin, β-lactoglobulin, αS1-casein	β-lactalbumin, β-lactoglobulin, αS1-casein	cookie, pies, waffles	HPLC-triple quadrupole/ToF (SRM)	LOD: 0.2 $\mu g_{\beta\text{-lactoglobulin}}$/mL, 0.39 $\mu g_{\beta\text{-lactalbumin}}$/mL, 0.2 $\mu g_{\alpha S1\text{-casein}}$/mL, LOQ: 0.48$\mu g_{\beta\text{-lactoglobulin}}$/mL, 0.97 $\mu g_{\beta\text{-lactalbumin}}$/mL, 0.48 $\mu g_{\alpha S1\text{-casein}}$/mL
Inman et al. 2018 [58]	Almond	prunin	Cumin, paprika	HPLC-triple quadrupole/linear ion trap (SRM)	ND
Vandekerckhove et al. 2017 [59]	Peanut	Ara h 3, conglutin	Chili pepper powder	HPLC-triple quadrupole (SRM)	LOD: 24 $\mu g_{peanut}/g$ LOQ: 84 $\mu g_{peanut}/g$
Sayers et al. 2018 [104]	Defatted peanut flour	Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 7	chocolate dessert, chocolate bar	HPLC-triple quadrupole (SRM)	LOQ: 10 $mg_{TOT\ PROT}/kg$ (only in chocolate dessert)

Table 2.

Reference	Allergenic Ingredient	Target Protein	Foodstuff	MS method	LOD/LOQ
Bignardi et al., 2010 [77]	Hazelnut	Cor a 9	Biscuits	HPLC-linear ion trap (SRM/SRM^3)	LOD: SRM 30 μg_{NUT}/g, SRM^3 35 μg_{NUT}/g LOQ: SRM 90 μg_{NUT}/g, SRM^3 110 μg_{NUT}/g
	Cashewnut	Ana o 2			LOD: SRM 14 μg_{NUT}/g, SRM^3 30 μg_{NUT}/g LOQ: SRM 46 μg_{NUT}/g, SRM^3 98 μg_{NUT}/g
	Almond	Pru 1			LOD: SRM 17 μg_{NUT}/g, SRM^3 25 μg_{NUT}/g LOQ: SRM 58 μg_{NUT}/g, SRM^3 80 μg_{NUT}/g
	Walnut	Jug r 4			LOD: SRM 55 μg_{NUT}/g, SRM^3 50 μg_{NUT}/g LOQ: SRM 180 μg_{NUT}/g, SRM^3 1600 μg_{NUT}/g
	Peanut	Ara h 3/4			LOD: SRM 10 μg_{NUT}/g, SRM^3 27 μg_{NUT}/g LOQ: SRM 37 μg_{NUT}/g, SRM^3 90 μg_{NUT}/g
Heick et al., 2011 [20]	Egg white	Ovalbumin	Incurred Bread	HPLC-triple quadrupole/linear ion trap (SRM)	LOD: 42 $\mu g_{egg\ white}$/g
	Skimmed milk powder	αS1-casein			LOD: 5 $\mu g_{skim\ milk\ powder}$/g
	Defatted hazelnut	11S globulin			LOD: 5 $\mu g_{defatted\ hazelnut}$/g
	Defatted almond	Prunin			LOD: 3 $\mu g_{defatted\ almond}$/g
	Defatted peanut	Ara h 1			LOD: 11 $\mu g_{defatted\ peanut}$/g
	Defatted walnut	Jug r 1			LOD: 70 $\mu g_{defatted\ walnut}$/g
	Defatted soy	Glycinin			LOD: 24 $\mu g_{defatted\ soy}$/g
Monaci et al., 2013 [82]	Egg white	Ovalbumin, Lysozyme	White wine	HPLC-Orbitrap^{TM} (Full MS/AIF)	LOD: 0.4-1.1 $\mu g_{egg\ white}$/mL
	Caseinate	αS1-casein, αS2-casein, β-casein			LOD: 0.4-0.9 $\mu g_{caseinate}$/mL
Bignardi et al., 2013 [75]	Hazelnut	Cor a 9	Biscuits, Dark chocolate	HPLC-linear ion trap (SRM)	LOD: 1.3 $\mu g_{NUT}/g_{biscuit}$ - 14 $\mu g_{NUT}/g_{chocolate}$ LOQ: 4.5 $\mu g_{NUT}/g_{biscuit}$ - 49 $\mu g_{NUT}/g_{chocolate}$
	Cashewnut	Ana o 2			LOD: 0.5 $\mu g_{NUT}/g_{biscuit}$ - 15 $\mu g_{NUT}/g_{chocolate}$ LOQ: 1.6 $\mu g_{NUT}/g_{biscuit}$ - 50 $\mu g_{NUT}/g_{chocolate}$
	Almond	Pru 1			LOD: 0.9 $\mu g_{NUT}/g_{biscuit}$ - 9 $\mu g_{NUT}/g_{chocolate}$ LOQ: 3.1 $\mu g_{NUT}/g_{biscuit}$ - 30 $\mu g_{NUT}/g_{chocolate}$
	Peanut	Ara h 3/4			LOD: 0.1 $\mu g_{NUT}/g_{biscuit}$ - 7 $\mu g_{NUT}/g_{chocolate}$ LOQ: 0.3 $\mu g_{NUT}/g_{biscuit}$ - 25 $\mu g_{NUT}/g_{chocolate}$
	Walnut	Jug r 4			LOD: 0.8 $\mu g_{NUT}/g_{biscuit}$ - 5 $\mu g_{NUT}/g_{chocolate}$ LOQ: 2.6 $\mu g_{NUT}/g_{biscuit}$ - 18 $\mu g_{NUT}/g_{chocolate}$
Pilolli et al 2014 [87]	Ovalbumin, lysozyme	Ovalbumin, lysozyme	White wine	HPLC-Orbitrap^{TM} (Full-MS/AIF) HPLC-linear ion trap (SRM)	LOD: Full MS 0.3 $\mu g_{Ovalbumin}$/mL, 0.18 $\mu g_{Lysozyme}$/mL, SRM: 0.19 $\mu g_{Ovalbumin}$/mL, 0.19 $\mu g_{lysozyme}$/mL
	Caseinate	αS1-Casein			LOD: HR-MS: 0.3 $\mu g_{caseinate}$/mL, SRM: 0.2 $\mu g_{caseinate}$/mL
Monaci et al 2014 [78]	Whole egg stock solution	Ovalbumin, β-Lactoglobulin	Cookie	HPLC-linear ion trap (SRM)	LOD: 0.3 $\mu g_{TOT\ PROT}$/g; LOQ: 1 $\mu g_{TOT\ PROT}$/g
	Skimmed milk stock solution	αS1-Casein			LOD: 0.12 $\mu g_{TOT\ PROT}$/g; LOQ: 0.4 $\mu g_{TOT\ PROT}$/g
	Pre-cooked soy flour protein extract	Glycinin G2, β-Conglycinin, Glycinin G4			LOD: 2 $\mu g_{TOT\ PROT}$/g; LOQ: 7 $\mu g_{TOT\ PROT}$/g
Mattarozzi et al 2014 [61]	Caseinate (Protoclar®)	αS1-, β-caseins	Red wine	HPLC-linear ion trap-(SRM)	LOD: 0.5 $\mu g_{\alpha S1\text{-}Casein}$/mL, 0.01 $\mu g_{\beta\text{-}casein}$/mL; 0.2 $\mu g_{caseinate}$/mL LOQ: 1 $\mu g_{\alpha S1\text{-}Casein}$/mL, 0.03 $\mu g_{\beta\text{-}casein}$/mL
	Egg-white powder	ovalbumin			LOD: 0.8 $\mu g_{ovalbumin}$/mL, 1.6 $\mu g_{egg\ white}$/mL LOQ: 2 $\mu g_{ovalbumin}$/mL
Korte et al. 2016 [69]	Shrimp, Lobster	myosin light chain, myosin heavy chain, arginine kinase, slow muscle myosin S1 heavy chain, fast myosin heavy chain	Salmon lasagna	HPLC-triple quadrupole/linear ion trap (SRM/SRM^3)	LOD: SRM 1000 $\mu g_{crustacean}$/g, SRM^3: 25 $\mu g_{crustacean}$/g
Korte & Brockmeyer 2016 [70]	peanut	Ara h 3	Multigrain bread, vanilla ice cream, dairy chocolate	HPLC-triple quadrupole/linear ion trap (SRM^3)	LOD: ≤ 1 μg_{NUT}/g in bread and ice cream matrix; ≤ 3 μg_{NUT}/g in milk chocolate LOQ: ≤ 3 μg_{NUT}/g in bread and ice cream matrix; ≤ 10 μg_{NUT}/g in milk chocolate
	almond	Pru du 6.0101, Pru du 6.0201			
	cashew	Ana o 2			
	hazelnut	Cor a 9			
	pistachio	Pis v 5			
	walnut	Jug r 2, Jug r 4			
Hoffmann et al. 2017 [63]	Lupine	Conglutin β2	Meat	HPLC-triple quadrupole/linear ion trap (SRM)	LOD: 2 $\mu g_{TOT\ protein}$/g
	Pea protein isolate	Convicilin			LOD: 5 $\mu g_{TOT\ protein}$/g
	Soy protein isolate	Glycinin G2			LOD: 4 $\mu g_{TOT\ protein}$/g
Huschek et al. 2016 [67]	Soybeans	Gly m6	Raw wheat flour, cookie, soft bread	HPLC-triple quadrupole/linear ion trap-(SRM)	LOQ: 10-50 $\mu g_{whole\ ingredient}$/g
	Sesame seeds	Ses i6			
	white lupine	β-conglutin			
Planque et al. 2016 [68]	milk powder	αS1-Casein, αS2-Casein, β-lactoglobulin	Chocolate, ice cream, tomato sauce, and processed cookies	HPLC-triple quadrupole-(SRM)	LOQ: 0.5 $mg_{proteins}$/kg (detection of caseins), 5 $mg_{proteins}$/kg (detection of whey)
	eggs (from which egg yolk and egg white proteins were isolated)	Ovalbumin, Vitellogenin-2, Vitellogenin-1, Apovitellenin			LOQ: 3.4 $mg_{egg\ white\ protein}$/kg, 30.8 $mg_{egg\ yolk\ protein}$/kg
	soy flour	Glycinin, 2S-albumin, β-conglycinin			LOQ: 5 $mg_{TOT\ protein}$/kg
	peanut butter	Cupin (Ara h 1, Ara h 3/4, Ahy-1)			LOQ: 2.5 $mg_{TOT\ protein}$/kg

Parker et al 2015 [66]	egg powder, non fat dry milk, defatted peanut flour	Lysozyme, ovalbumin, αS1-casein, β-lactoglobulin, Ara h 1, Ara h 2 and Ara h 3	Incurred cereal bar and muffins	UPLC- triple quadrupole/linear ion trap (SRM)	ND
Korte, et al. 2016 [15]	peanut protein extract	ND	Milk chocolate, vanilla ice cream, commercial bread, and breakfast cereals	HPLC-Orbitrap™ (Full MS)	LOD: 0.26-2.08 $\mu g_{TOT\ prot}$/g, 1.0-8.1 μg_{NUT}/g
	almond protein extract	ND			LOD: 0.34-1.92 $\mu g_{TOT\ prot}$/g, 1.8-10.1 μg_{NUT}/g
	cashew protein extract	ND			LOD: 0,78-2,02 $\mu g_{TOT\ prot}$/g, 4,3-11,2 μg_{NUT}/g
	hazelnut protein extract	ND			LOD: 0,49-1,01 $\mu g_{TOT\ prot}$/g, 4,0-8,4 μg_{NUT}/g
	pistachio protein extract	ND			LOD: 0,91-1,90 $\mu g_{TOT\ prot}$/g, 5,1-10,6 μg_{NUT}/g
	walnut protein extract	ND			LOD: 0,8-5 $\mu g_{TOT\ prot}$/g, 5,7-35,7 μg_{NUT}/g
Sealey-Voyksner et al. 2016 [88]	peanut, almond, pecan, cashew, walnut, hazelnut, pine nut, Brazil nut, macadamia nut, pistachio nut, chestnut and coconut	ND	Raw and roasted nuts, Muffin, Cookie, Cake, Pretziel, Crackers, Crisps, Bars, cereal flours, candies, pasta, instant meals, soups, puree	HPLC-quadrupole-ToF (MS, MS^2)	LOD: 0.1 $\mu g_{peptide}$/g
Gomaa and Boye 2015 [106]	Caseinate	αS2-,β-, κ-caseins	Cookie	UPLC-quadrupole-ToF (MS^2)	LOD: 10 $\mu g_{caseinate}$/g
	Soy protein concentrate	Glycinin G1, β-conglycinin			LOD: 10 $\mu g_{TOT\ protein}$/g
	Gluten	α-amylase trypsin inhibitor			LOD: 100 μg_{gluten}/g
De Angelis et al. 2017 [62]	Caseinate	αS1-, β-casein	White wine	On line-SPE-HPLC-linear ion trap (SRM)	LOD: 50 $ng_{whole\ ingredient}$/mL; LOQ: 166 $ng_{whole\ ingredient}$/mL
	Egg white	Ovalbumin, Lysozyme			LOD: 36 $ng_{whole\ ingredient}$/mL; LOQ: 121 $ng_{whole\ ingredient}$/mL
Pilolli et al 2017 [79]	Skimmed milk powder	αS1-caseins	Spiked and Incurred cookie	On line-SPE-HPLC-linear ion trap (SRM)	LOD: 7 $\mu g_{whole\ ingredient}$/g; LOQ: 20 $\mu g_{whole\ ingredient}$/g
	egg powder	Ovalbumin			LOD: 9 $\mu g_{whole\ ingredient}$/g; LOQ: 30 $\mu g_{whole\ ingredient}$/g
	Pre-cooked soy flour	Glycinin G1-G2			LOD: 6 $\mu g_{whole\ ingredient}$/g; LOQ: 19 $\mu g_{whole\ ingredient}$/g
	peanut	Conarachin			LOD: 13 $\mu g_{whole\ ingredient}$/g; LOQ: 40 $\mu g_{whole\ ingredient}$/g
	hazelnut	11S globulin-like protein			LOD: 7 $\mu g_{whole\ ingredient}$/g; LOQ: 20 $\mu g_{whole\ ingredient}$/g
Gu et al. 2018 [65]	Milk	αS1-, αS2-, β-, κ-caseins	Chocolate	HPLC-hybrid triple quadrupole/linear ion trap (SRM)	LOD: 0.05-0.13 $\mu g_{TOT\ protein}$/g; LOQ: 0.2-0.4 $\mu g_{TOT\ protein}$/g
	Soybean	Glycinin G1, β-conglycinin (α' and β chains)			LOD: 0.4-1.2 $\mu g_{TOT\ protein}$/g; LOQ: 1.0-4 $\mu g_{TOT\ protein}$/g
	Peanut	Ara h 1, Ara h 3/4			LOD: 0.8-1.3 $\mu g_{TOT\ protein}$/g; LOQ: 2.5-4 $\mu g_{TOT\ protein}$/g
	Almond	Pru 1, Pru 2			LOD: 0.4-0.8 $\mu g_{TOT\ protein}$/g; LOQ: 1.3-2.6 $\mu g_{TOT\ protein}$/g
	Walnut	Jug r 2			LOD: 0.6 $\mu g_{TOT\ protein}$/g; LOQ: 2.0 $\mu g_{TOT\ protein}$/g
	Hazelnut	Cor a 9			LOD: 0.5 $\mu g_{TOT\ protein}$/g; LOQ: 1.7 $\mu g_{TOT\ protein}$/g
	Cashew	Ana o 2			LOD: 0.7 $\mu g_{TOT\ protein}$/g; LOQ: 2.3 $\mu g_{TOT\ protein}$/g
	Pistachio	Pis v 2			LOD: 0.4 $\mu g_{TOT\ protein}$/g; LOQ: 1.3 $\mu g_{TOT\ protein}$/g
Montowska & Fornal 2018 [64]	Soy	αS1-caseins, β-lactoglobulin	poultry meat products (sausages, frankfurters, patés)	nano-LC-quadrupole/ToF equipped with an HPLC-Chip Cube (SRM)	ND
	Milk	Glycinin, β-conglycinin			
	Egg white	ovotransferrin, lysozme C			
Boo et al. 2017 [103]	Egg powder	Ovalbumin, lysozyme C	Incurred sugar cookies	Nano-LC-triple quadrupole/linear ion trap (SRM)	LOQ 5 $\mu g_{whole\ ingredient}$/g
	nonfat dry milk	β-lactoglobulin, αS1-casein			
	Peanut	Ara h 1, Ara h 2, Ara h 3			
Planque et al. 2017 [102]	Egg powder	Ovalbumin, Vitellogenin-2, Vitellogenin-1	chocolate, ice cream, cookie, tomato sauce	HPLC-triple quadrupole (SRM)	LOQ: 3 $mg_{egg\ white\ protein}$/kg, 60 $mg_{egg\ yolk\ protein}$/kg
	Milk powder	β-lactoglobulin, αS1-Casein, αS2-Casein			LOQ: 0.5 $mg_{milk\ protein}$/kg (detection of caseins), 5 $mg_{milk\ protein}$/kg (detection of whey protein)
	Soy flour	Glycinin, 2S albumin, β-conglycinin			LOQ: 5 $mg_{TOT\ prot}$/kg
	Peanut butter	Cupin, Conglutin 7			LOQ: 2.5 $mg_{TOT\ prot}$/kg
	Almond	Prunin			LOQ: 5 $mg_{TOT\ prot}$/kg
	Pecan	7S vicilin			LOQ: 5 $mg_{TOT\ prot}$/kg
	Cashew	Ana o 2, Ana o 3			LOQ: 2.5 $mg_{TOT\ prot}$/kg
	Hazelnut	11S globulin- like protein			LOQ: 2.5 $mg_{TOT\ prot}$/kg
	Walnut	Vicilin-like protein, Albumin seed storage			LOQ: 5 $mg_{TOT\ prot}$/kg
	Pistachios	2S albumin Pis v 1, 11S globulin (Pis v 5 and Pis v 2)			LOQ: 2.5 $mg_{TOT\ prot}$/kg
Pilolli, et al. 2018 [96]	Egg powder	Gal d 2	Incurred cookie	Micro-HPLC-hybrid quadrupole/Orbitrap (t-SIM/dd2)	LOD: 14 $\mu g_{TOT\ prot}$/g, LOQ: 50 $\mu g_{TOT\ prot}$/g
	Skimmed milk powder	Bos d 5, Bos d 9			LOD: 6 $\mu g_{TOT\ prot}$/g, LOQ: 20 $\mu g_{TOT\ prot}$/g
	Pre-cooked soy flour	Gly m 5, Gly m 6			LOD:10 $\mu g_{TOT\ prot}$/g, LOQ: 30 $\mu g_{TOT\ prot}$/g
	Peanut	Ara h 1			LOD:7 $\mu g_{TOT\ prot}$/g, LOQ: 24 $\mu g_{TOT\ prot}$/g
	Hazelnut	Cor a9			LOD:4 $\mu g_{TOT\ prot}$/g, LOQ: 12 $\mu g_{TOT\ prot}$/g

Table 3

Reference/Method	Allergenic ingredient	Incurred Food Matrix	Sample preparation	Quantification method and standards inclusion	Method Validation
Parker et al. 2015 [66] UPLC- triple quadrupole/linear ion trap (SRM)	– Whole egg – Nonfat dry milk – Partially defatted dark roasted peanut flour (12% fat)	– Cereal bar – Muffins	– 3x15 min solvent-based defatting steps; – buffer extraction (2 M urea, 50 mM TBS, pH 8.0, 25 mM DTT): 5 min vortex+10 min water bath sonication at 4°C + 15min end-over-end rotation at RT; – New pellet pressure-assisted extraction chamber; – Protein reduction, alkylation and filter aided tryptic digestion (ON) with inclusion of Rapigest^{TM} surfactant.	SID matrix-matched calibration curves. Standards: yeast alcohol dehydrogenase, plant protease inhibitor cocktail, yeast enolase, rabbit phosphorylase B digest standard, angiotensin I	– Recovery; – Conversion factor (synthetic peptides → total protein of allergenic ingredient); – RT variability; – Transitions ratio (within ±20%).
Boo et al. 2017 [103] Nano-LC-triple quadrupole/linear ion trap (SRM)	– Whole egg (NIST RM 8445) – Nonfat dry milk (NIST SRM 1549) – Partially defatted lightly roasted peanut flour	– Incurred sugar cookies	– 3x15 min solvent-based defatting steps; – 2x buffer extraction steps (2M Urea, 50 mM TBS, 25 mM DTT): 5min shaking + 10min water bath sonication at RT + 15 min end-over-end rotation; – Protein reduction, alkylation and filter aided tryptic digestion (ON) with inclusion of Rapigest^{TM} surfactant.	SID matrix-matched calibration curves (matrix lysate dilution1:2). Standards: yeast enolase, rabbit phosphorylase B digest standard, angiotensin I.	– Recovery; – Conversion factor synthetic peptides → total protein of allergenic ingredient; – Sensitivity (LOD/LOQ evaluation from chromatogram S/N ratio); – RT variability (within ±3%); – Transitions ratio (within ±20%); – Precision (only technical replicates RSD< 5%).
Lamberti et al. 2016 [54] HPLC-3D ion trap-(SRM)	– Nonfat dry milk (NIST SRM 1549)	– Cookie	– 20min buffer extraction at 60°C (NH_4HCO_3/$(NH_4)_2CO_3$ + 1% SDS buffer, pH 8.2); – Protein precipitation ON at RT with methanol/chloroform; – Tryptic digestion for 90min at 37°C	Label free	– Recovery; – Dynamic range; – Sensitivity (LOD/LOQ from the calibration curve 3s/slope and 10s/slope, s= standard deviation of 10 independent measurements of blank signal -not-spiked cookies); – Precision (intra- and inter-day repeatability); – Accuracy.
Huschek et al. 2016 [67] HPLC- triple quadrupole/linear ion trap-(SRM)	– Soybeans – Sesame seeds – White lupine	– Cookie – Soft bread	– 30 min buffer extraction at RT (100 mM NH_4HCO_3, 4 M urea and 5 mM DTT at pH 8.2); – Protein alkylation and ON tryptic digestion; – SPE peptide purification	SID matrix-matched calibration curves.	– Recovery; – Sensitivity (LOD/LOQ evaluation from chromatogram S/N ratio); – Precision (independent replicates).
Planque et al. 2016 [68] HPLC-triple quadrupole (SRM)	– Lyophilized milk powder – Whole Eggs (isolated egg yolk and egg white) – Soy flour – Peanut butter (NIST SRM 2387)	– Chocolate – Ice cream – Tomato sauce – Cookies	– buffer extraction (2M urea+200mM Tris HCl pH 9.2): 30min shaking at 20°C, 15 min ultrasonic treatment at 4°C; under; – extract dilution 1:1 – Protein reduction, alkylation and ON tryptic digestion. – SPE peptide purification and concentration (evaporation at 40°C under nitrogen flow)	Label free	– Sensitivity (LOQ evaluation from chromatogram S/N ratio); – Matrix effects.
Planque et al. 2017 [102] HPLC-triple quadrupole (SRM)	– Whole egg (NIST RM 8445) – Whole milk (NIST SRM 1549a), – Soybean flour (NIST SRM 3234), – Peanut butter (NIST SRM 2387), – almond, – pecan, – cashew, – hazelnut, – walnut, – pistachios	– Chocolate – Ice cream – Tomato sauce – Cookies	– buffer extraction (2M urea+200mM Tris HCl pH 9.2): 30min shaking at 20°C, 15 min ultrasonic treatment at 4°C; under; – extract dilution 1:1 – Protein reduction, alkylation and 1h tryptic digestion. – SPE peptide purification and concentration (evaporation at 40°C under nitrogen flow)	Method developed without internal standard. Only some conclusive experiments on real samples included isotope-labelled standards	– Linearity; – Sensitivity (LOQ evaluation from chromatogram S/N ratio); – Precision (RSD of six aliquots of the same foodstuff preparation); – Specificity (detection of four allergen-free matrices); – Matrix effects.
Sayers et al. 2018 [104] microfluidic-triple quadrupole (SRM)	– Lightly roasted mechanically defatted peanut flour	– Chocolate dessert – Chocolate bars	– Buffer extraction (50 mM Tris-HCl+50 mM DTT+0.04% (w/v) RapiGest): 15 min ultrasound water bath at 60 °C (vortexed every 5 min). – Protein reduction, alkylation and tryptic digestion(two incubations with fresh trypsin addition after 3h and final ON incubation).	SID matrix-matched calibration curves (matrix dilution 1:5). Standards: yeast enolase, rabbit phosphorylase B digest standard, angiotensin I.	– Recovery; – Conversion factor synthetic peptides → total protein of allergenic ingredient; – Linearity; – Sensitivity (LOD from the calibration curve 3s/slope s= standard error of the regression line, LOQ=3xLOD); – Matrix effects.
Pilolli et al. 2017 [79] Micro-HPLC-dual pression linear ion traps (SRM)	– Skimmed milk powder, – Egg powder, – Pre-cooked soy flour, – Hazelnut, – Peanut	– Cookies	– 30 min buffer extraction (20 mM Tris-HCl pH8.2): ultrasound assisted alternate (probe-sonicator), vortexed every 10 min; – SEC purification – Protein reduction, alkylation and tryptic digestion ON – On-line automatized SPE	Label-free	– Linearity; – Sensitivity (LOD/LOQ from the calibration curve 3s/slope, 10s/slope, s= standard error of the intercept); – RT variability; – Processing effects.

Pilolli et al. 2018 [96] Micro-HPLC-hybrid quadrupole/Orbitrap™ (t-SIM/dd2)	– Skimmed milk powder, – Egg powder, – Pre-cooked soy flour Hazelnut, – Peanut	– Cookies	– 30 min buffer extraction (20 mM Tris-HCl pH8.2): ultrasound assisted alternate (probe-sonicator), vortexed every 10 min; – SEC purification – Protein reduction, alkylation and tryptic digestion ON	Label-free	– Recovery; – Linearity; – Sensitivity (LOD/LOQ from the calibration curve 3s/slope, 10s/slope respectively, s= standard error of the intercept); – RT variability; – Precursor ion accuracy <3ppm; – Precision (intra-day and inter-day); – Matrix/processing effects.

REFERENCES

[1] H.A. Sampson, Food allergy: Past, present and future, Allergol. Int. 65 (2016) 363–369.

[2] MH-Ho, W.H. Wong, C. Chang, Clinical spectrum of food allergies: A comprehensive review, Clin. Rev. Allergy Immunol. 46 (2014) 225-240.

[3] S.H: Sicherer, H.A. Sampson, Food Allergy: epidemiology, pathogenesis, diagnosis and treatment, J. Allergy Clin. Immunol. 133 (2014) 291-307.

[4] European Commission, Commission Directive No. 2006/142/EC (December 22, 2006) Amending Annex IIIa of Directive No. 2000/13/EC of the European Parliament and of the Council listing the ingredients which must under all circumstances appear on the labelling of foodstuffs, OJ. L368 (2006) 110–111.

[5] European Commission, Regulation (EU) No. 1169/2011 of the European Parliament and of the Council (October 25, 2011) On the provision of food information to consumers, amending Regulations (EC) No. 1924/2006 and (EC) No. 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive No. 87/250/EEC, Council Directive No. 90/496/EEC, Commission Directive No. 1999/10/EC, Directive No. 2000/13/EC of the European Parliament and of the Council, Commission Directive Nos. 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No. 608/2004 Text with EEA relevance, OJ. L304 (2011) 18–63.

[6] B. Popping, C. Diaz-Amigo, European Regulations for Labeling Requirements for Food Allergens and Substances Causing Intolerances: History and Future, J. AOAC Int. 101 (2018) 2-7.

[7] J. Brockmeyer, Novel Approaches for the MS-based detection of food allergens: high resolution, MS3, and beyond, J. AOAC Int. 101 (2018) 124-131.

[8] L.S. Ford, S.L. Taylor, R. Pacenza, L.M. Niemann, D.M. Lambrecht, S.H. Sicherer Food allergen advisory labeling and product contamination with egg, milk, and peanut J. Allergy Clin. Immun, 126 (2010), 384-385.

[9] A.J. van Hengel, Food allergen detection methods and the challenge to protect food-allergic consumers, Anal. Bioanal. Chem. 389 (2007) 111-118.

[10] L. Monaci, A. Visconti, Immunochemical and DNA-based methods in food allergen analysis and quality assurance perspectives, Trends Food Sci. Tech. 21 (2010) 272-283.

[11] K. Török, L. Hajas, V. Horváth, E. Schall, Z. Bugyi, S. Kemény, S. Tömösközi, Identification of the factors affecting the analytical results of food allergen ELISA methods, Eur. Food Res. Technol. 241 (2015) 127-136.

[12] L. Monaci, M. Brohée, V. Tregoat, A. van Hengel, Influence of baking time and matrix effects on the detection of milk allergens in cookie model food system by ELISA, Food Chem. 15 (2011) 669-675.

[13] T.J. Fu, N. Maks, Impact of thermal processing on ELISA detection of peanut allergens, J. Agric. Food Chem. 61 (2013) 5649-5658.

[14] L. Monaci, A. Visconti, Mass spectrometry-based proteomics methods for analysis of food allergens, TrAC-Trend Anal. Chem. 28 (2009) 581-591.

[15] R. Korte, S. Lepski, J. Brockmeyer, Comprehensive peptide marker identification for the detection of multiple nut allergens using a non-targeted LC–HRMS multi-method, Anal. Bioanal. Chem. 408 (2016) 3059–3069.

[16] U. Andjelković, M. Gavrović-Jankulović, T. Martinović, D. Josić, Omics methods as a tool for investigation of food allergies, TrAC- Trend Anal. Chem. 96 (2017) 107-115.

[17] L. Monaci, R. Pilolli, E. De Angelis, G. Mamone, Mass Spectrometry in Food Allergen Research, in: Y. Picó, (Ed.), Comprehensive Analytical Chemistry, Elsevier Science and Technology, 2015, pp. 359-393.

[18] I. Losito, T. Carbonara, L. Monaci, F. Palmisano, Evaluation of the thermal history of bovine milk from the lactosylation of whey proteins: an investigation by liquid chromatography–electrospray ionization mass spectrometry, Anal. Bioanal. Chem. 389 (2007) 2065-2074.

[19] L. Monaci, I. Losito, F. Palmisano, A. Visconti, Identification of allergenic milk proteins markers in fined white wines by capillary liquid chromatography–electrospray ionization-tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 4300–4305.

[20] J. Heick, M. Fischer, B. Popping, First screening method for the simultaneous detection of seven allergens by liquid chromatography mass spectrometry, J. Chromatogr. A 1218 (2011) 938-943.

[21] P. Lutter, V. Parisod, H. Weymuth, Development and validation of a method for the quantification of milk proteins in food products based on liquid chromatography with mass spectrometric detection, J. AOAC Int. 94 (2011) 1043-1059.

[22] B. Simonato, F. Mainente, S. Tolin, G. Pasini, Immunochemical and mass spectrometry detection of residual proteins in gluten fined red wine, J. Agr. Food Chem. 59 (2011) 3101-3110.

[23] K. J., Shefcheck, J. H. Callahan, S. M. Musser, Confirmation of peanut protein using peptide markers in dark chocolate using liquid chromatography-tandem mass spectrometry (LC-MS/MS), J. Agr. Food Chem. 54 (2006) 7953-7959.

[24] H. Chassaigne, J. V. Nørgaard, A. J. van Hengel, Proteomics-based approach to detect and identify major allergens in processed peanuts by capillary LC-Q-TOF (MS/MS), J. Agr. Food Chem. 55 (2007) 4461-4473.

[25] J. Zhang, S. Lai, Y. Zhang, B. Huang, D. Li, Y. Ren, Multiple reaction monitoring-based determination of bovine -lactalbumin in infant formulas and whey protein concentrates by ultra-high performance liquid chromatography–tandem mass spectrometry using tryptic signature peptides and synthetic peptide standards, Anal. Chim. Acta. 727 (2012) 47– 53.

[26] G.A. Newsome, P.F. Scholl, Quantification of Allergenic Bovine Milk αS1-Casein in Baked Goods Using an Intact ^{15}N-Labeled Protein Internal Standard, J. Agr. Food Chem. 6 (2012) 5659-5668.

[27] R. Grandori, Origin of the conformation dependence of protein charge-state distributions in electrospray ionization mass spectrometry, J. Mass Spectrom. 38 (2003) 11-15.

[28] J. Leonil, D. Molle, J. Fauquant, J. L. Maubois, R. J. Pearce, S. Bouhallab, Characterization by Ionization Mass Spectrometry of Lactosyl β-Lactoglobulin Conjugates Formed During Heat Treatment of Milk and Whey and Identification of One Lactose-Binding Site, J. Dairy Sci. 80 (1997) 2270-2281.

[29] F. K. Yeboah, V. A. Yaylayan, Analysis of glycated proteins by mass spectrometric techniques: qualitative and quantitative aspects, Nahrung 45 (2001) 164-171.

[30] R. Siciliano, B. Rega, A. Amoresano, P. Pucci, Modern mass spectrometric methodologies in monitoring milk quality, Anal. Chem. 72 (2000) 408-415.

[31] F. Fenaille, F. Morgan, V. Parisod, J. C. Tabet, P. A. Guy, Solid-state glycation of β-lactoglobulin monitored by electrospray ionisation mass spectrometry and gel electrophoresis techniques, Rapid Commun. Mass. Spectrom. 17 (2003) 1483-1492.

[32] L. Monaci, A.J. Van Hengel, Effect of heat treatment on the detection of intact bovine β-lactoglobulins by LC mass spectrometry, J. Agr. Food Chem. 55 (2007) 2985-2992.

[33] L. Monaci, A. J. van Hengel, Development of a method for the quantification of whey allergen traces in mixed-fruit juices based on liquid chromatography with mass spectrometric detection, J. Chromatogr. A 1192 (2008) 113-120.

[34] S.E. Abbatiello et al., New Guidelines for Publication of Manuscripts Describing Development and Application of Targeted Mass Spectrometry Measurements of Peptides and Proteins, Mol Cell Proteomics 16 (2017), 327-328.

[35] A. Kaufmann, P. Teale, Capabilities and Limitations of High Resolution Spectrometry (HRMS): Time-of-flight and Orbitrap, in: J.F. Kay, J.D. MacNeil, J. Wang (Eds.) Chemical Analysis of Non-antimicrobial Veterinary Drug Residues in Food, Wiley, 2016, pp.93-140.

[36] P. Johnson, S. Baumgartner, T. Aldick, C. Bessant, V. Giosafatto, J. Heick, G. Mamone, G. O'Connor, R. Poms, B. Popping, A. Reuter, F. Ulberth, A. Watson, L. Monaci, E. N. C. Mills Current perspectives and recommendations for the development of mass spectrometry methods for the determination of allergens in foods J. AOAC Int, 94 (2011), 1026-1033.

[37] B. MacLean, D.M. Tomazela, N. Shulman, M. Chambers, G.L. Finney, B. Frewen, R. Kern, D.L. Tabb, D.C. Liebler, M.L. MacCoss, Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics, 26 (2010) 966–968.

[38] M. Planque, T. Arnould, N. Gillard, Food Allergen Analysis: Detection, Quantification and Validation by Mass Spectrometry, in: S:S. Athari (Ed.), Allergens, Intech, 2017 pp. 7-41.

[39] S. Gallien, E. Duriez, B. Domon, Selected reaction monitoring applied to proteomics, J. Mass Spectrom. 46 (2011) 298–312.

[40] C. Nitride, V. Lee, I. Baricevic-Jones, K. Adel-Patient, S. Baumgartner, E.N.C. Mills, Integrating Allergen Analysis Within a Risk Assessment Framework: Approaches to Development of Targeted Mass Spectrometry Methods for Allergen Detection and Quantification in the iFAAM Project, J. AOAC Int. 101 (2018) 83-90.

[41] V. Mayya, D.K. Han, Proteomic applications of protein quantification by isotope-dilution mass spectrometry, Expert Rev. Proteomics 3 (2006) 597-610.

[42] P. Picotti, O. Rinner, R. Stallmach, F. Dautel, T. Farrah, B. Domon, H. Wenschuh, R. Aebersold, High-throughput generation of selected reaction-monitoring assays for proteins and proteomes, Nat. Methods 7 (2010) 43-46.

[43] D. Weber, P. Raymond, S. Ben-Rejeb, B. Lau, Development of a Liquid Chromatography−Tandem Mass Spectrometry Method Using Capillary Liquid Chromatography and Nanoelectrospray Ionization−Quadrupole Time-of-Flight Hybrid Mass Spectrometer for the Detection of Milk Allergens, J. Agr. Food Chem. 54 (2006) 1604-1610.

[44] K. J. Shefcheck, S. M. Musser, Confirmation of the allergenic peanut protein, Ara h 1, in a model food matrix using liquid chromatography/ tandem mass spectrometry (LC/MS/MS), J. Agr. Food Chem. 52 (2004) 2785-2790.

[45] L. Monaci, J. V., Nørgaard, A. J. van Hengel, Feasibility of a capillary LC/ESI-Q-TOF MS method for the detection of milk allergens in an incurred model food matrix, Anal. Methods 2 (2010) 967-972.

[46] S. Azarnia, J. I. Boye, V. Mongeon, H. Sabik, Detection of ovalbumin in egg white, whole egg and incurred pasta using LC-ESI-MS/MS and ELISA, Food Res. Int. 52 (2013) 526-534.

[47] Q. Chen, J. Zhang, X. Ke, S. Lai, B. Tao, J. Yang, W. Mob, Y. Ren, Quantification of bovine β-casein allergen in baked foodstuffs based on ultra-performance liquid chromatography with tandem mass spectrometry, Food Additiv. Contam. A 32 (2015) 25–34.

[48] A. Cryar, C. Pritchard, W. Burkitt, M. Walker, G. O'Connor, D. T. Burns, M. Quaglia, Towards absolute quantification of allergenic proteins in food, J. AOAC Int. 96 (2013) 1350-1361.

[49] J.A. Sealey-Voyksner, C. Khosla, R. D. Voyksner, J. W. Jorgenson, Novel aspects of quantitation of immunogenic wheat gluten peptides by liquid chromatography-mass spectrometry/mass spectrometry, J. Chromatogr. A 1217 (2010) 4167-4183.

[50] R. Pedreschi, J. Nørgaard, A. Maquet, Current Challenges in Detecting Food Allergens by Shotgun and Targeted Proteomic Approaches: A Case Study on Traces of Peanut Allergens in Baked Cookies, Nutrients 4 (2012) 132-150.

[51] I. Losito, B. Introna, L. Monaci, S. Minella, F. Palmisano, Development of a method for the quantification of caseinate traces in Italian commercial white wines based on a liquid chromatography–electrospray ionization-ion trap-mass spectrometry, J. Agric Food Chem. 61 (2013) 12436–12444.

[52] J. Costa, P. Ansari, I. Mafra, M. Beatriz, P.P. Oliveira, S. Baumgartner, Assessing hazelnut allergens by protein- and DNA-based approaches: LC-MS/MS, ELISA and real-time PCR, Anal. Bioanal. Chem. 406 (2014) 2581–2590.

[53] M. Posada-Ayala, G. Alvarez-Llamas, A.S. Maroto, X. Maes, E. Muñoz-Garcia, M. Villalba, R. Rodríguez, M. Perez-Gordo, F. Vivanco, C. Pastor-Vargas, J. Cuesta-Herranz, Novel liquid chromatography–mass spectrometry method for sensitive determination of the mustard allergen Sin a 1 in food, Food Chem. 183 (2015) 58–63.

[54] C. Lamberti, E. Acquadro, D. Corpillo, M. Giribaldi, L. Decastelli, C. Garino, M. Arlorio, C. Ricciardi, L. Cavallarin, M.G. Giuffrida, Validation of a mass spectrometry-based method for milk traces detection in baked food, Food Chem. 199 (2016) 119–127.

[55] K.L. Fiedler, S.C. McGrath, J.H. Callahan, M.M. Ross, Characterization of Grain-Specific Peptide Markers for the Detection of Gluten by Mass Spectrometry, J. Agric. Food Chem. 62 (2014) 5835−5844.

[56] J. Ji, P. Zhu, F. Pi, C. Sun, J. Sun, M. Jia, C. Ying, Y. Zhang, X. Sun, Development of a liquid chromatography-tandem mass spectrometry method for simultaneous detection of the main milk allergens, Food Control 74 (2017) 79-88.

[57] R. Pilolli, R. Chaudhari, F. Palmisano, L. Monaci, Development of a mass spectrometry immunoassay for unambiguous detection of egg allergen traces in wines, Anal. Bioanal. Chem. 409 (2017) 1581-1589.

[58] S.E. Inman, K. Groves, B. McCullough, M. Quaglia, C. Hopley, Development of a LC-MS method for the discrimination between trace level Prunus contaminants of spices, Food Chem. 245 (2018) 289–296.

[59] M. Vandekerckhove, B. Van Droogenbroeck, M. De Loose, I. Taverniers, E. Daeseleire, P. Gevaert, H. Lapeere, C. Van Poucke, Development of an LC-MS/MS method for the detection of traces of peanut allergens in chili pepper, Anal. Bioanal. Chem. 409 (2017) 5201–5207.

[60] S. Tolin, G. Pasini, A. Curioni, G. Arrigoni, A. Masi, F. Mainente, B. Simonato, Mass spectrometry detection of egg proteins in red wines treated with egg white, Food Control 23 (2012) 87-94.

[61] M. Mattarozzi, M. Milioli, C. Bignardi, L. Elviri, C. Corradini, M. Careri, Investigation of different sample pre-treatment routes for liquid chromatography–tandem mass spectrometry detection of caseins and ovalbumin in fortified red wine, Food Control 38 (2014) 82-87.

[62] E. De Angelis, R. Pilolli, L. Monaci, Coupling SPE on-line pre-enrichment with HPLC separation and MS/MS detection for the sensitive detection of allergens in wine, Food Control 73 (2017) 814-820.

[63] B. Hoffmann, S. Münch, F. Schwägele, C. Neusüß, W. Jira, A sensitive HPLC-MS/MS screening method for the simultaneous detection of lupine, pea, and soy proteins in meat products, Food Control 71 (2017) 200-209.

[64] M. Montowska, E. Fornal, Detection of peptide markers of soy, milk and egg white allergenic proteins in poultry products by LC-Q-TOF-MS/MS, LWT - Food Sci. Tech. 87 (2018) 310-317.

[65] S. Gu , N. Chen, Y. Zhou, C. Zhao, L. Zhan, L. Qu, C. Cao, L. Han, X. Deng, T. Ding, C. Song, Y. Ding, A rapid solid-phase extraction combined with liquid chromatography-tandem mass spectrometry for simultaneous screening of multiple allergens in chocolates, Food Control 84 (2018) 89-96.

[66] C.H. Parker, S.E. Khuda, M. Pereira, M.M. Ross, T.-J. Fu, X. Fan, Y. Wu, K.M. Williams, J. De Vries, B. Pulvermacher, B. Bedford, X. Zhang, L.S. Jackson, Multi-allergen Quantitation and the Impact of Thermal Treatment in Industry-Processed Baked Goods by ELISA and Liquid Chromatography-Tandem Mass Spectrometry, J. Agric. Food Chem. 63 (2015) 10669−10680.

[67] G. Huschek, J. Bönick, Y. Löwenstein, S. Sievers, H. Rawel, Quantification of allergenic plant traces in baked products by targeted proteomics using isotope marked peptides, LWT-Food Sci. Techn. 74 (2016) 286-293.

[68] M. Planque, T. Arnould, M. Dieu, P. Delahaut, P. Renard, N. Gillard, Advances in ultra-high performance liquid chromatography coupled to tandem mass spectrometry for sensitive detection of several food allergens in complex and processed foodstuff, J. Chromatogr. A 1464 (2016) 115–123.

[69] R. Korte, J.-M. Monneuse, E. Gemrot, I. Metton, H.-U. Humpf, J. Brockmeyer, New High-Performance Liquid Chromatography Coupled Mass Spectrometry Method for the Detection of Lobster and Shrimp Allergens in Food Samples via Multiple Reaction Monitoring and Multiple Reaction Monitoring Cubed, J. Agric. Food Chem. 64 (2016) 6219−6227.

[70] R. Korte, J. Brockmeyer, MRM3-based LC-MS multi-method for the detection and quantification of nut allergens, Anal. Bioanal. Chem. 408 (2016) 7845-7855.

[71] M. Mattarozzi, C. Bignardi, L. Elviri, M. Careri, Rapid Shotgun Proteomic Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry-Based Method for the Lupin (Lupinus albus L.) Multi-allergen Determination in Foods, J. Agr. Food Chem. 60 (2012) 5841-5846.

[72] M. Careri, A. Costa, L. Elviri, J. B. Lagos, A. Mangia, M. Terenghi, A. Cereti, L.P. Garoffo, Use of specific peptide biomarkers for quantitative confirmation of hidden allergenic peanut proteins Ara h2 and Ara h3/4 for food control by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 389 (2007) 1901-1907.

[73] M. Careri, L. Elviri, J. B. Lagos, A. Mangia, F. Speroni, M. Terenghi, Selective and rapid immunomagnetic bead-based sample treatment for the liquid chromatography-electrospray ion-trap mass spectrometry detection of Ara h3/4 peanut protein in foods, J. Chromatogr. A 1206 (2008) 89-94.

[74] M. Carrera, B. Cañas, J. M. Gallardo, Rapid direct detection of the major fish allergen, parvalbumin, by selected MS/MS ion monitoring mass spectrometry, J. Proteomics 75 (2012) 3211-3220.

[75] C. Bignardi, M. Mattarozzi, A. Penna, S. Sidoli, L. Elviri, M. Careri, A. Mangia, A rapid size-exclusion solid-phase extraction step for enhanced sensitivity in multi-allergen determination in dark chocolate and biscuits by liquid chromatography-tandem mass spectrometry, Food Anal. Method 6 (2013) 1144-1152.

[76] A. Manfredi, M. Mattarozzi , M. Giannetto, M. Careri, Multiplex liquid chromatography-tandem mass spectrometry for the detection of wheat, oat, barley and rye prolamins towards the assessment of gluten-free product safety, Anal. Chim. Acta, 895 (2015) 62-70.

[77] C. Bignardi, L. Elviri, A. Penna, M. Careri, A. Mangia, Particle-packed column versus silica-based monolithic column for liquid chromatography–electrospray-linear ion trap-tandem mass spectrometry multiallergen trace analysis in foods, J. Chromatogr. A 1217 (2010) 7579-7585.

[78] L. Monaci, R. Pilolli, E. De Angelis, M. Godula, A. Visconti, Multi-allergen detection in food by micro high-performance liquid chromatography coupled to a dual cell linear ion trap mass spectrometry, J. Chromatogr. A 1358 (2014) 136-144.

[79] R. Pilolli, E. De Angelis, L. Monaci, Streamlining the analytical workflow for multiplex MS/MS allergen detection in processed foods, Food Chem. 221 (2017) 1747-1753.

[80] L. Monaci, I. Losito, F. Palmisano, A. Visconti, Reliable detection of milk allergens in food using a high-resolution, stand-alone mass spectrometer, J. AOAC Int. 94 (2011) 1034-1042.

[81] L. Monaci, I. Losito, F. Palmisano, M. Godula, A.Visconti, Towards the quantification of residual milk allergens in caseinate-fined white wines using HPLC coupled with single-stage Orbitrap mass spectrometry, Food Addit. Contam. A, 28 (2011) 1304-1314.

[82] L. Monaci, I. Losito, E. De Angelis, R. Pilolli, A. Visconti, Multi-allergen quantification of fining-related egg and milk proteins in white wines by high-resolution mass spectrometry, Rapid Commun. Mass Sp. 27 (2013) 2009-2018.

[83] L. Monaci, E. De Angelis, S.L. Bavaro, R. Pilolli, High-resolution Orbitrap™-based mass spectrometry for rapid detection of peanuts in nuts, Food Addit. Contam A 32 (2015) 1607-1616.

[84] A. Kaufmann, The current role of high-resolution mass spectrometry in food analysis, Anal. Bioanal. Chem. 403 (2012) 1233-1249.

[85] A. Kaufmann, M. Widmer, K. Maden, Post-interface signal suppression, a phenomenon observed in a single-stage Orbitrap mass spectrometer coupled to an electrospray interfaced liquid chromatography, Rapid Commun. Mass Sp. 24 (2010) 2162-2170.

[86] A. Makarov, M. Scigelova, Coupling liquid chromatography to Orbitrap mass spectrometry, J. Chromatogr. A 1217 (2010) 3938-3945.

[87] R. Pilolli, E. De Angelis, M. Godula, A. Visconti, L. Monaci, Orbitrap™ monostage MS versus hybrid linear ion trap MS: application to multi-allergen screening in wine, J. Mass Spectrom. 49 (2014) 1254–1263.

[88] J. Sealey-Voyksner, J. Zweigenbaum, R. Voyksner, Discovery of highly conserved unique peanut and tree nut peptides by LC–MS/MS for multi-allergen detection, Food Chem. 194 (2016) 201–211.

[89] P.E. Johnson, R.L. Sayers, L.A. Gethings, A. Balasundaram, J.T. Marsh, J.I. Langridge, E.N.C. Mills, Quantitative Proteomic Profiling of Peanut Allergens in Food Ingredients Used for Oral Food Challenges, Anal. Chem. 88 (2016) 5689−5695.

[90] R.L. Sayers, P.E. Johnson, J.T. Marsh, P. Barran, H. Brown, E.N.C. Mills, The effect of thermal processing on the behaviour of peanut allergen peptide targets used in multiple reaction monitoring mass spectrometry experiments, Analyst 141 (2016) 4130–4141.

[91] M.L. Downs, J.L. Baumert, S.L. Taylor, E.N.C. Mills, Mass spectrometric analysis of allergens in roasted walnuts, J. Proteomics 142 (2016) 62–69.

[92] S. Gallien, E. Duriez, C. Crone, M. Kellmann, T. Moehring, B. Domon, Targeted proteomic quantification on quadrupole-orbitrap mass spectrometer, Mol. Cell. Proteom. 11 (2012) 1709-1723.

[93] K.P. Law, Y.P. Lim, Recent advances in mass spectrometry: data independent analysis and hyper reaction monitoring, Expert Rev. Proteom. 10 (2013) 551-566.

[94] A.C. Peterson, J.D. Russell, D.J. Bailey, M.S. Westphall, J.J. Coon, Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics, Mol. Cell. Proteom. 11 (2012) 1475-1488.

[95] V. Vidova, Z. Spacil, A review on mass spectrometry-based quantitative proteomics: Targeted and data independent acquisition, Anal. Chim. Acta 964 (2017) 7-23.

[96] R. Pilolli, E. De Angelis, L. Monaci, In house validation of a high resolution mass spectrometry Orbitrap-based method for multiple allergen detection in a processed model food, Anal. Bioanal. Chem. 2018, in press, doi 10.1007/s00216-018-0927-8.

[97] J.D. Venable, M.-Q. Dong, J. Wohlschlegel, A. Dillin, J.R. Yates, Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra, Nat. Methods 1 (2004) 39-45.

[98] J.D. Egertson, A. Kuehn, G.E. Merrihew, N.W. Bateman, B.X. MacLean, Y.S. Ting, J.D Canterbury, D.M Marsh, M. Kellmann, V. Zabrouskov, C.C Wu, M.J. MacCoss, Multiplexed MS/MS for improved data-independent acquisition, Nat. Methods 10 (2013) 744-746.

[99] L.C. Gillet, P. Navarro, S. Tate, H. Rost, N. Selevsek, L. Reiter, R. Bonner, R. Aebersold, Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis, Mol. Cell. Proteom. 11 (2012), O111.016717.

[100] S. Bromilow, L.A.Gethings, J.I. Langridge, P.R. Shewry, M. Buckley, M.J. Bromley, E.N.C. Mills, A curated gluten protein sequence database to support development of proteomics methods for determination of gluten in gluten-free foods. J. Proteom. 163 (2017) 67–75.

[101] V. Paez, et al., AOAC SMPR(®) 2016.002, J. AOAC Int. 99 (2016) 1122–1124.

[102] M. Planque, T. Arnould, M. Dieu, P. Delahaut, P. Renard, N. Gillard, Liquid chromatography coupled to tandem mass spectrometry for detecting ten allergens in complex and incurred foodstuffs, J. Chromatogr. A 1530 (2017) 138–151.

[103] C.C. Boo, C.H. Parker, L.S. Jackson, A Targeted LC-MS/MS Method for the Simultaneous Detection and Quantitation of Egg, Milk, and Peanut Allergens in Sugar Cookies, J. AOAC Int. 101 (2017) 1-10.

[104] R.L. Sayers, L.A. Gethings, V. Lee, A. Balasundaram, P.E. Johnson, J.A. Marsh, A. Wallace, H. Brown, A. Rogers, J.I. Langridge, E.N.C. Mills, Microfluidic Separation Coupled to Mass Spectrometry for Quantification of Peanut Allergens in a Complex Food Matrix, J. Proteome Res. 17 (2018) 647−655.

[105] C. De Ceglie, C.D. Calvano, C.G. Zambonin, Determination of Hidden Hazelnut Oil Proteins in Extra Virgin Olive Oil by Cold Acetone Precipitation Followed by In-Solution Tryptic Digestion and MALDI-TOF-MS Analysis, J. Agric. Food Chem. 62 (2014) 9401−9409.

[106] A. Gomaa, J. Boye, Simultaneous detection of multi-allergens in an incurred food matrix using ELISA, multiplex flow cytometry and liquid chromatography mass spectrometry (LC–MS), Food Chem. 175 (2015) 585–592.

HIGHLIGHTS

- The review compares different aspects of food allergen quantification by MS methods
- Analytical performances of MS methods for allergen detection are detailed
- Advances in High Resolution MS methods for allergen analysis are provided
- The review illustrates efforts towards validation of a multi allergen MS method