



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

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ABSTRACT

Complex matrices commonly affect the sensitivity and selectivity of liquid chromatography–mass spectrometry (LC–MS) analysis. Thus, selective sample enrichment strategies are useful particularly to analyze organic biomarkers present in low abundance in samples. A selective immunomagnetic extraction procedure to isolate trace peanut allergen protein Ara h3/4 from breakfast cereals combined with microwave-assisted tryptic digestion and liquid chromatography–electrospray ion-trap tandem mass spectrometry (LC–ESI–IT–MS/MS) measurement was developed. Using protein A-coated magnetic bead (MB) support, anti-Ara h3/4 monoclonal antibodies (Abs) were used as selective capture molecules. The results obtained by LC–ESI–IT–MS/MS in terms of limit of detection (3 mg peanuts/kg matrix) and a significantly reduced matrix effect demonstrated that the Ab-coated magnetic bead was very effective to selectively trap Ara h3/4 protein in breakfast cereals. The magnetic bead-based sample treatment followed by LC–IT–MS/MS method here developed can be proposed as very rapid and powerful confirmatory analytical method to verify the reliability of enzyme-linked immunosorbent assay (ELISA) screening methods, since the magnetic bead–LC–IT–MS/MS method combines good sensitivity to the identification capabilities of mass spectrometry.

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1. Introduction

Hidden allergens in foods represent a major health problem in the food safety issue, since they are recognized to induce a wide variety of hypersensitivity reactions [1]. A substance is a hidden allergen when it is unrecognized or not declared on the product label. This omission is not always intentional and there are many ways for allergens to be hidden in food, for example, through misleading labels, allergenic foods that can contaminate other safe foods, food that is listed by an uncommon term, and ingredient switching. The USA and EU legislations [2,3] on food labelling only refer to the allergenic foods added as known ingredients and do not prevent the unintentional presence of trace allergens.

The most sensitive method used to screen the presence of hidden allergens in food rely on enzyme-linked immunosorbent assay (ELISA) [4–6]. ELISA allows to obtain detection limits between 0.1 and 10 mg peanuts/kg matrix and is easy to use. However, the presence of false-positive and false-negative results demands the

availability of confirmatory methods. In addition, as well documented, the ELISA quantitative data are strongly dependent on the matrix tested leading to lack of accuracy, that still remains the main discussed analytical topic [7]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been proposed as confirmation technique to identify and also quantify proteins, but its major limitation still remains the low sensitivity achieved in complex matrices with respect to immunoassays [8–11].

In a research program dealing with the development of new analytical strategies for the determination of hidden allergens in foods, we developed an ELISA-inductively coupled plasma mass spectrometry (ICP–MS) method [12] and a LC–electrospray (ESI) MS/MS method [8] for screening and confirmation purposes, respectively. In particular, we demonstrated the potential of LC–ESI–MS/MS with triple quadrupole (QqQ) analyzer for the detection of peanut allergens (Ara h2 and Ara h3/4) in foods [8]. A proteomic bottom up-based approach based on the direct identification of Ara h2 and Ara h3/4, the biomarker peptides were used as a strategy to identify peanuts in complex food matrices. Selected reaction monitoring (SRM) analysis allowed to obtain very good selectivity in the biomarker peptide detection even with a low-resolution mass spectrometer by keeping a narrow mass window during precursor and

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product-ion transmission. This restriction allows to analyze complex digest extracts, even if it limits method sensitivity (0.5% (w/w) peanuts/matrix). In addition, besides ESI and LC column dirtying, a significant matrix effect reducing biomarker signal intensity was observed, affecting accuracy of the quantitative analysis. To achieve better sensitivity and accuracy of the LC–MS/MS method together with an improvement of column life-time, an efficient sample treatment step involving a purification and concentration of allergen proteins from complex matrices is needed.

The procedures routinely used for the extraction of compounds from liquid or solid matrices (i.e. solid-phase extraction, solid–liquid extraction, etc.), despite their attractive features of simplicity and availability of supports, lack selectivity leading to a partial co-extraction of interfering substances. In addition, a multi-step treatment can lead to analyte loss. Recently, to enhance selectivity of the extraction procedure and improve accuracy of the LC–MS results, immunosorbent (IS) supports have been proposed for the different applications [13–16]. Based on the high affinity and selectivity of antigen–antibody (Ag–Ab) interactions, the IS materials allow a selective extraction of the target compounds from complex mixtures. A strategy based on immunorecognition with materials combined with LC–ESI–MS/MS can therefore represent one of the most specific and sensitive analytical approaches to reach the analytical challenge of hidden allergen detection in foods.

Herein we propose an antibody magnetic bead (MB)-based method for the selective enrichment of Ara h3/4 protein in food extracts. Protein A-magnetic beads bound to monoclonal anti-Ara h3/4 antibodies were used as affinity probes to isolate the specific target protein. Further, we discuss a LC–ESI–MS/MS method with a linear ion-trap (IT) mass analyzer for the identification and determination of the Ara h3/4 peptide biomarkers in breakfast cereals. As a result, the combination of the use of magnetic affinity probes with LC–ESI–IT–MS/MS analysis allows us to obtain an excellent sensitivity and selectivity for the identification and quantification of peanut as contaminant in this kind of food matrix.

2. Experimental

2.1. Chemicals

Anti-Ara h3/4 (Pn-b) monoclonal antibodies were obtained by using peanuts, which were roasted in shell to prevent molding and kindly provided by the Leibniz-Center for Medicine and Biosciences at the Research Center Borstel (Borstel, Germany). Antibody solutions were diluted with deionized water (Millipore, Bedford, MA, USA). Phosphate buffer (pH 8.0), acetonitrile (HPLC purity), and formic acid (analytical reagent grade) were purchased from Carlo Erba (Milan, Italy). Trypsin from bovine pancreas, leukine–enkefalin acetate hydrate, Tris–HCl, Bradford reagent, bovine serum albumin (BSA), triethanolamine and DMP (dimethylprimelinidated·2HCl) were from Sigma–Aldrich (St. Louis, MO, USA). EDC {1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide} was from Pierce (Bonn, Germany). A commercial ELISA kit (Veratox quantitative kit) specific for peanut allergens was purchased from Neogen (Lansing, MI, USA) and used following the instructions given by the producers.

2.2. Samples

Roasted peanuts and different commercial breakfast cereals samples (cornflakes, breakfast cereals based on cereal flakes and dried fruit, cereal mix with fruit and chocolate, cereal mix with rice crispy and powder milk) were obtained at a local food store. The sample treatment and the LC–MS/MS methods were developed

and validated by using cornflakes free of peanuts. In all the other products used to test the method peanuts were not present as an ingredient and the labeling “may contain trace of nuts and soy” was reported.

2.3. Preparation of functional magnetic beads

Magnetizable polystyrene beads (diameter $2.8 \pm 0.2 \mu\text{m}$, surface area $3\text{--}9 \text{ m}^2/10^9$ beads) (Dynabeads, Invitrogen Dynal, Oslo, Norway) covalently coupled with recombinant protein A were used to capture anti-Ara h3/4 monoclonal immunoglobulins G (IgG) for the small-scale purification of Ara h3/4 protein in food extracts.

The antibody solution (100 μL of a 1 mg/mL anti-Ara h3/4-IgG in sodium phosphate solution, 100 mM) was added to an Eppendorf containing 50 μL of pre-washed protein A-magnetic beads. Incubation was performed at room temperature for 4 h to allow the binding of the Fc portion of the immunoglobulin to protein A. The protein A-anti-Ara h3/4 IgG complex was isolated using the Dynal MPC (Invitrogen Dynal) magnet and washed twice with 500 μL of 200 mM triethanolamine, pH 8.2.

To ensure an efficient binding of the IgG to protein A, a cross-linking reaction was added to the procedure. Protein A-IgG complex was then incubated with 500 μL of freshly made 20 mM DMP (dimethylprimelinidated·2HCl) in 200 mM triethanolamine solution for 30 min. Cross-linking reaction was quenched by isolating the magnetic beads and incubating with 500 μL of 50 mM Tris–HCl pH 7.5 for 15 min. The beads were thus rinsed three times with 500 μL of 100 mM phosphate buffer with 0.02% Tween 20 before use.

2.4. Sample treatment

Peanut-containing food extracts were prepared by adding 10 mL of 100 mM phosphate buffer to 1 g of ground sample. Proteins were extracted by shaking for 2 h at 37°C , then the extract was centrifuged ($3500 \times g$, 5 min) and filtered on 0.2 μm nylon filter before treatment with the magnetic beads.

Sample extract (800 μL) was incubated at different times (15 min, 30 min, 1 h, 3 h and overnight) under slight agitation at room temperature with the protein A-IgG magnetic bead complex prepared as reported above. The beads were then washed three times with 500 μL of 100 mM phosphate buffer, pH 8.0. After discharging the phosphate buffer, the beads-conjugated sample was then re-suspended in 60 μL and digested with trypsin (5 μL , 0.6 mM in 100 mM phosphate buffer) by using an accelerated microwave-assisted digestion procedure (1 min, 700 W) as reported in the literature [17]. The internal standard (5 μL of 130 mM of leukine–enkefalin) was added to the solution just before LC analysis.

2.5. Liquid chromatography–ion-trap mass spectrometry

LC separation was carried out on a Poroshell C18 300 Å (75 mm $\times$ 2.1 mm, 5 μm particles) (Agilent Technologies, Santa Clara, CA, USA) using a gradient solvent elution system [(A) 0.1% (v/v) formic acid in water/(B) 0.08% (v/v) formic acid in acetonitrile] as previously described [8]. The flow-rate was 200 $\mu\text{L}/\text{min}$.

The mobile phase was delivered by the Surveyor chromatographic system (ThermoElectron Corporation, San José, CA, USA) equipped with a 200-vial capacity sample tray. Injection volume was 50 μL .

A LTQ XL linear ion-trap instrument (ThermoElectron Corporation) equipped with a pneumatically assisted ESI interface was used. The system was controlled by the Xcalibur software.

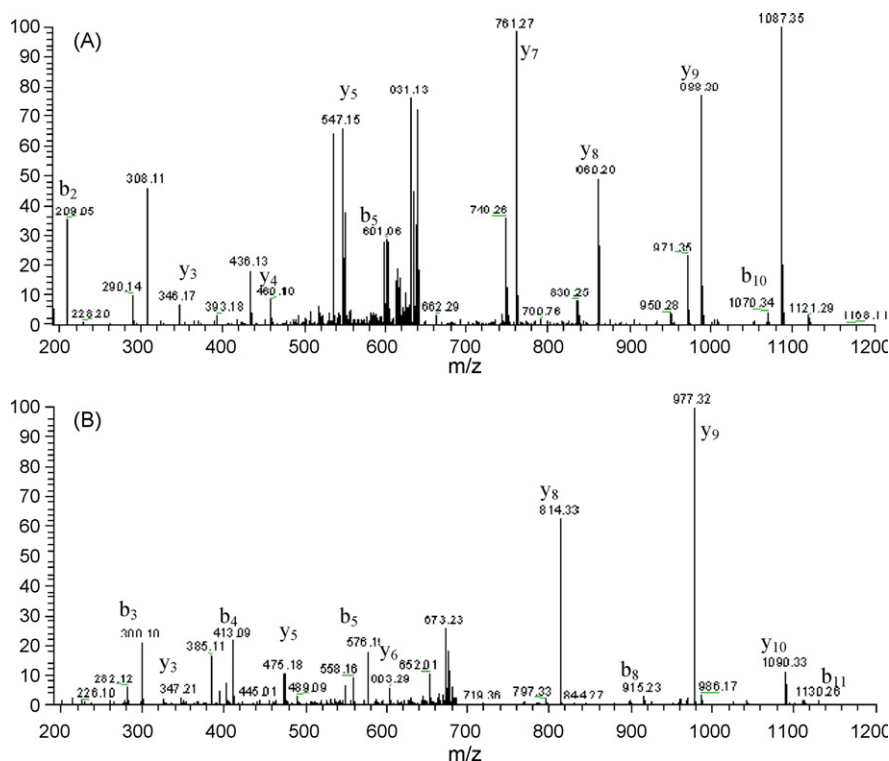


Fig. 1. LC-IT-MS/MS product-ion mass spectra of the doubly charged tryptic peptides of Ara h3/4: (A) m/z 649/761 (AHYQVVDSNGDR) and (B) m/z 695/977 (SPDIYNPQAGSLK).

The sheath gas (nitrogen, 99.999% purity) and the auxiliary gas (nitrogen, 99.998% purity) were delivered at flow-rates of 45 and 5 arbitrary units, respectively.

Optimized conditions of the interface were as follows: ESI voltage 3.5 kV, capillary voltage 20 V, capillary temperature 200 °C. MS/MS experiments were performed under both product-ion and SRM conditions with a collision gas (He) pressure of 2.3×10^{-3} mbar in the collision cell. In the product-ion scan mode the 200–1400 m/z range was monitored. The SRM transitions monitored were as follows: m/z 649/761 (AHYQVVDSNGDR, Ara h3/4), m/z 695/977 (SPDIYNPQAGSLK, Ara h3/4) both operating with collision energy (CE) 35 eV. Leukine-enkefalin (m/z 557/397; CE 20 eV) was used as the internal standard.

2.6. Magnetic bead-LC-MS/MS method validation

Validation of the whole analytical procedure was performed on the fortified samples of breakfast cereals (mix of cereal flakes). For this purpose, breakfast cereal samples were fortified with different peanut amounts and measurements were carried out by monitoring the most abundant MS/MS transition for each peptide as above reported. The detection limits (LODs) and the quantitation limits (LOQs) were calculated as the concentration of the two peptides corresponding to a signal-to-noise ratio (S/N) 3 and 10, respectively. Linearity was assessed in the 2.5–2500 mg peanuts/kg matrix range. Precision was evaluated by performing five independent extractions of corn flake samples fortified with 1 mg peanuts/kg matrix using the functionalized magnetic beads and by performing two LC-MS/MS injections for each extract. The matrix effect was assessed by comparing the slopes of an instrumental and a matrix-matched calibration curve applying a *t*-test. The matrix-matched calibration curve was obtained by analyzing the breakfast cereal sample extracts fortified with different peanut amounts and treated applying the whole analytical procedure.

3. Results and discussion

3.1. Liquid chromatography-ion-trap-tandem mass spectrometry method

This study started from the previously published results dealing with the ESI-MS behaviour of the biomarker peptides selected for Ara h2 and Ara h3/4 [8]. The biomarker peptides were previously chosen following different criteria (i.e. absence of missed cleavages, good ESI sensitivity, no post-translational modification sites and detectability in both raw and roasted peanuts, sequence specificity) [8,11]. By analyzing a peanut protein aqueous solution, the LC-IT-MS/MS profile of the peptides resulted to match that observed by LC-QqQ-MS/MS in terms of chromatographic behaviour and relative intensity. Also using the ESI-IT-MS instrument, the peptides chosen for Ara h3/4 exhibited a higher ionization efficiency with respect to those selected for Ara h2. On the basis of these results, in this work the immuno-strategy extraction method was developed targeting Ara h3/4 protein.

The chromatographic profile of the two Ara h3/4 peptides showed both good peak shape and retention time stability [peptide of m/z 649 (t_R 1.50 ± 0.03 min); peptide of m/z 695 (t_R 8.76 ± 0.17 min)]. The identity of these two peptides was confirmed by both the doubly charge state detection, obtained by tuning the mass analyzer to higher resolution (UltraZoom scan) and by the IT-MS/MS product-ion profiles. The MS/MS spectra exhibited several fragments of the y- and b-series to cover and confirm the peptide sequences (Fig. 1).

By using the IT mass analyzer, product-ion and SRM acquisition modes were performed at the same time allowing simultaneously the unambiguous identification of the two peptides together with the quantitative analysis without loss of sensitivity. By contrast, when using the QqQ analyzer, as in the previous work, the product-ion mode has lower sensitivity with respect to SRM acquisition mode due to the different duty cycles and did not allow to obtain qualitative information at low concentration levels [8].

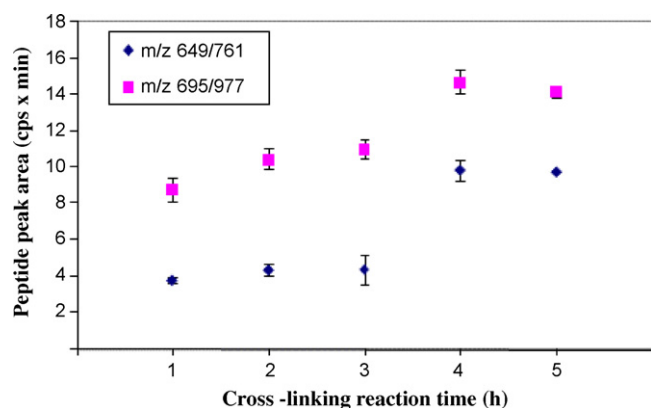


Fig. 2. Plot of the Ara h3/4 LC-MS/MS peptide peak areas as a function of the antibody incubation time in the presence of the cross-linker.

These findings highlight the great appeal of the linear IT mass spectrometer for this application.

3.2. Development of the immunoaffinity-based Ara h3/4 extraction method

Isolation of target low-abundant proteins in foods containing a number of protein rich-ingredients (i.e. milk, soy, rice, eggs, etc.) represents a great analytical challenge. The use of cut-off filters or other traditional approaches (i.e. ultracentrifugation) does not allow to obtain target-enriched purified samples and in most cases loss of analytes can occur.

The magnetic bead support functionalized with anti-Ara h3/4 monoclonal antibodies could represent an easy-to-use, fast and selective approach for extraction, purification and concentration of the target protein from complex mixtures. In this work, different Ab-protein A-magnetic bead immobilization conditions were tested. Further, the optimization of the Ara h3/4 protein extraction from breakfast cereal samples free of peanuts fortified with different peanut amounts was performed.

Selectivity of the monoclonal Abs used in this work has been previously tested in term of cross-reactivity with other food ingredients by ELISA-ICP-MS [12]. The results obtained evidenced an excellent Ag-Ab selectivity, thus proving the suitability of these monoclonal Abs for magnetic bead coating in this application.

The efficiency of the protein A-anti-Ara h3/4 IgG binding reaction was initially tested by incubating the beads with the antibody solution (100 µg Ab) at different times (1–5 h) with and without the cross-linking reaction. The antibody-coated beads thus obtained were incubated with a peanut protein aqueous solution (4 h) and the extract digested with trypsin by microwave-assisted digestion (1 min, 700 W). The LC-MS/MS analysis of the peptides at *m/z* 649 and *m/z* 695 exhibited a significant increase (>40%) of their peak area in all the cases in which the IgG was covalently bound to the magnetic beads by the cross-linker. Further, the increase of the antibody incubation time from 1 to 4 h in the presence of the cross-linker allowed to obtain magnetic beads with the maximum extraction efficiency. No significant increases were observed from 4 to 5 h binding reaction (Fig. 2).

In a further step of the work, the efficiency of the protein A-anti-Ara h3/4 IgG complex was studied by varying the amount of Abs (50, 70 and 100 µg) while keeping the incubation time constant at 4 h. The best LC-MS/MS results were obtained using 100 µg of antibody (data not shown). Confirmation of these findings was obtained by quantifying the antibody amount after the binding reaction with the beads using a Bradford assay with a standard IgG. In fact, we found the amount of anti-Ara h3/4 bound to be 15.1 ± 0.7 µg for

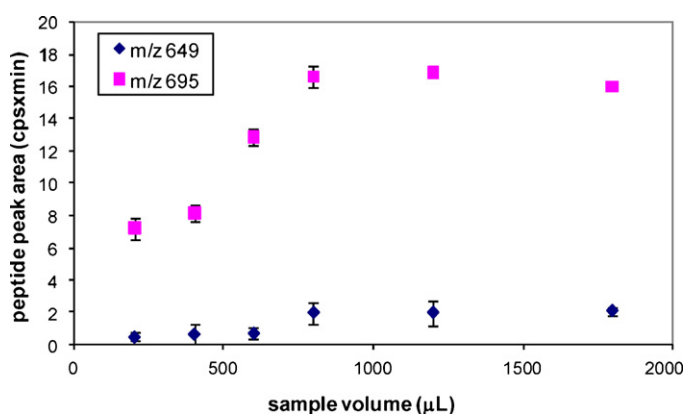


Fig. 3. Plot of the Ara h3/4 LC-MS/MS peptide peak areas as a function of the sample volume (magnetic beads prepared by incubating the antibodies and the cross-linker for 4 h, sample incubation overnight, elution volume 70 µL). Data corrected by the dilution factor.

50 µL bead solution. This result is in good agreement with the typical amount of antibody bound capacity of magnetic beads declared by the manufacturer (i.e. 50 µL Dynabeads protein A are reported to isolate approximately 12–15 µg antibodies).

In the past, the use of EDC reagent to activate terminal carboxyl groups was proposed to improve the antibody coating on protein A-magnetic beads [18]. On this basis, we tested the efficiency of EDC-activated magnetic beads following the procedure reported in the literature [16]. By analyzing a peanut protein aqueous solution (50 µg peanuts/mL solution) no significant differences were observed between the magnetic beads prepared with and without EDC. These findings could be explained taking into account the results reported above about the typical amount of Ab bound to the beads that already reach an excellent value.

By keeping the coating reaction time at 4 h, the same amount of magnetic beads (50 µL of a 1.3 g/mL product density correspond to approximately 65 mg of beads) was used for the extraction of different volumes (200, 400, 600, 800, 1200 and 1800 µL) of peanut aqueous extract in order to evaluate both the trapping capacity of the functionalized support and the effect of different concentration factors. The sample was then incubated overnight. By keeping the sample concentration constant, it was possible to observe a significant increase in the amount of trapped Ara h3/4 protein when sample volume increased from 200 to 800 µL, in particular for the peptide of *m/z* 695 (Fig. 3). Instead, by increasing the sample volume from 800 to 1800 µL, no signal increase was observed. The peanut protein extract was quantified using a Bradford assay with BSA as the standard and the total protein concentration resulted to be around 7 mg/mL. This finding suggests that the magnetic beads prepared following a 4 h-Ab and cross-linker incubation protocol reach the saturation when 800 µL of a 7 mg/mL peanut protein extract are used as incubation sample.

Further, different sample incubation times were investigated (15 min, 30 min, 1 h and 3 h and overnight) using 800 µL of aqueous peanut protein extract. The best results in terms of signal intensity and repeatability (RSD < 8%) were obtained by performing sample incubation for 3 h. No further signal increase was observed after the overnight incubation (data not shown).

As for selectivity, the overnight incubation of the magnetic beads without the Abs in a peanut aqueous extract resulted in a positive LC-MS/MS signal at the transitions monitored for the chosen peptides, suggesting the occurrence of non-specific binding. However, even if the results evidenced the capability of protein A to bind Ara h3/4, the MS signal was significantly lower (<10%) with respect to that obtained for the Ab-Ara h3/4 demonstrating that the Abs

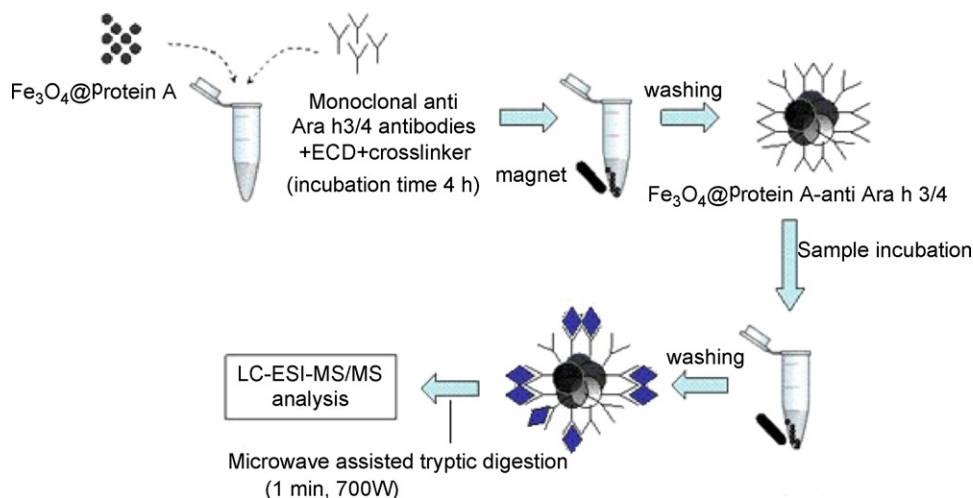


Fig. 4. Scheme of the analytical protocol: Fe_3O_4 -protein A-anti-Ara h3/4 IgG as affinity probes for selective enrichment and determination of Ara h3/4 protein from food sample using LC-ESI-MS/MS methods.

are required to reach a good enrichment factor in the real samples. By keeping the sample incubation time at 3 h, a decrease of non-specific interactions giving a signal comparable with that of the noise was observed.

No decrease in capture efficiency was observed within 3 weeks when the antibody-coated magnetic beads were kept at 4 °C. The final scheme of the extraction procedure and sample analysis is reported in Fig. 4.

3.3. Method validation and application to the analysis of Ara h3/4 in breakfast cereal fortified with peanut

A good linearity with different sensitivity was established by using an aqueous peanut extract at different dilutions (percentages from 2.5 to 2500 μg peanut/mL solution) for the two Ara h3/4 peptides ($y = 29.2(\pm 0.7)x$, r^2 0.988, $n = 12$ and $y = 1.6(\pm 0.2)x$, r^2 0.986, $n = 12$ for the peptides at m/z 695 and m/z 649, respectively). The

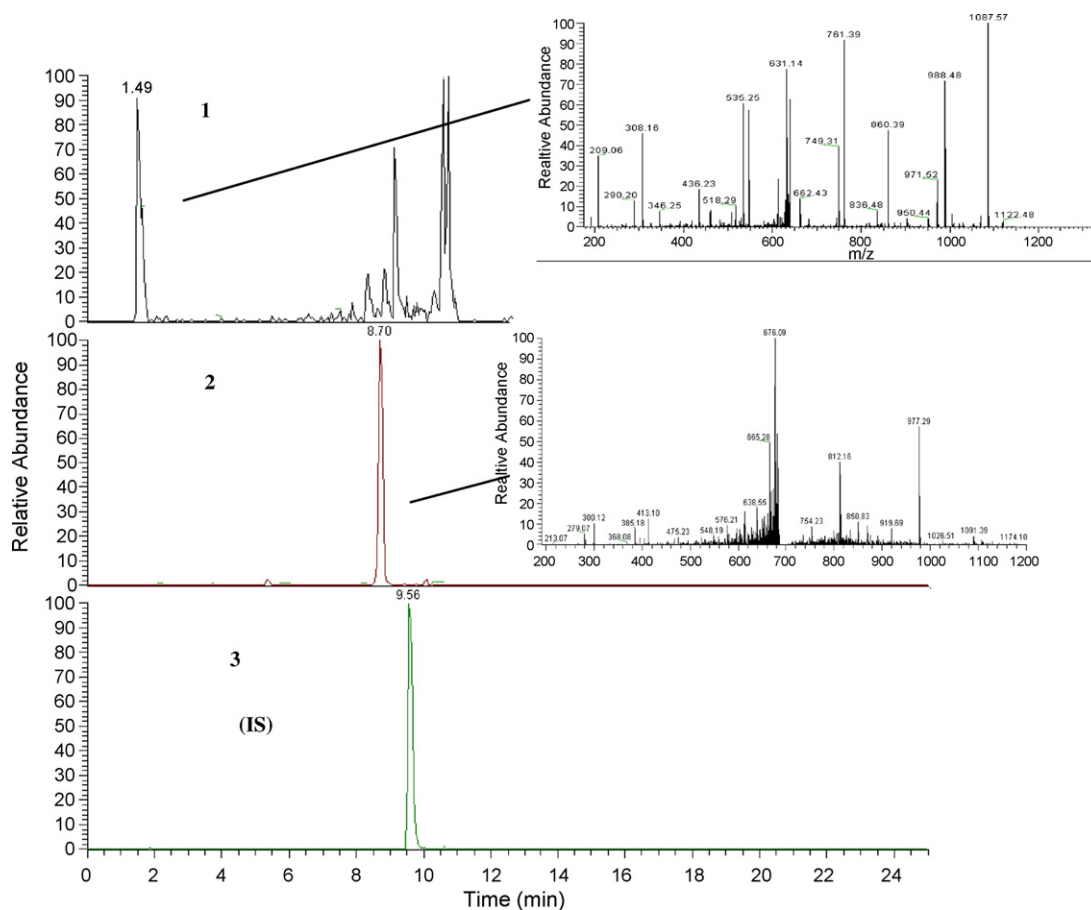


Fig. 5. LC-MS/MS profile of a breakfast cereal (mix of cereal flakes) sample containing peanuts, treated with magnetic beads: Peaks: 1, peptide at m/z 649/761 and relative MS/MS product-ion mass spectrum (inset); 2, peptide at m/z 695/977 and MS/MS product-ion mass spectrum (inset); 3, IS.

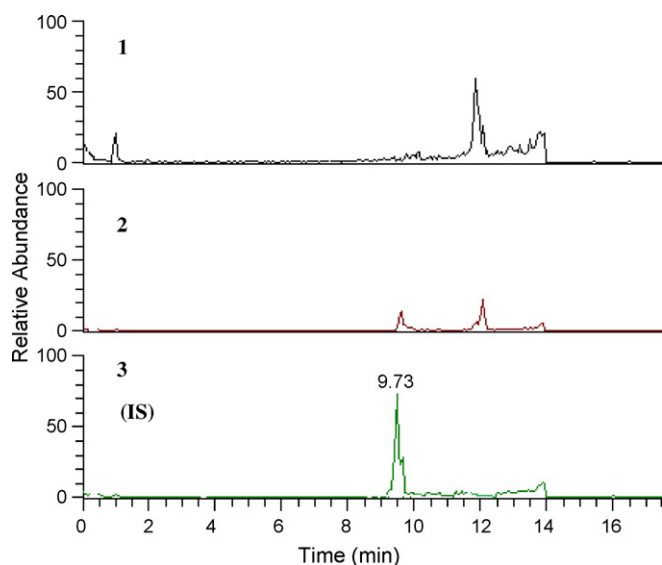


Fig. 6. LC-MS/MS profile of a breakfast cereal (mix of cereal flakes) sample containing peanuts without magnetic bead treatment. No signals corresponding to the two peptides investigated were detected. Chromatograms: 1, peptide at m/z 649/761; 2, peptide at m/z 695/977; 3, IS.

instrumental LOD and LOQ obtained for the peptide at m/z 695 exhibiting higher sensitivity resulted to be 0.8 and 2.5 μg peanut aqueous extract/mL solution, respectively.

Matrix-matched calibration curve calculated by using breakfast cereal fortified with different peanut amounts (percentages from 0.001 to 0.25% w/w peanut/matrix corresponding to 10–2500 mg peanuts/kg matrix range) and treated with the magnetic beads exhibited an excellent linearity ($y = 69.1(\pm 0.9)x$, r^2 0.998, $n = 12$ and $y = 3.4(\pm 0.3)x$, r^2 0.997, $n = 12$ for the peptides at m/z 695 and m/z 649, respectively). Trueness assessment allowed us to evidence a significant signal enhancement by comparing with a t -test the slope of the curve calculated analyzing the aqueous peanut extract and that of the matrix-matched calibration curve (peptide at m/z 695 $t_{\text{calculated}} = 188$, peptide at m/z 649 $t_{\text{calculated}} = 18$, $t_{\text{tabulated}, 0.05, 11} = 2.2$). The slope of the matrix-matched calibration curve increased more than two times for both the peptides. This result can be explained on the basis of the effects of purification and enrichment of the samples when Ab-coated beads are used. The LOD and LOQ obtained on matrix were 3 and 10 mg peanuts/kg matrix, respectively.

The method precision expressed as RSD was lower than 8% when calculated at 10 and 100 mg peanuts/kg matrix concentration levels for all the peptides investigated.

The method here developed was then applied for the detection of hidden peanut allergen Ara h3/4 in different commercially available breakfast cereal samples. In two out of seven samples (i.e. the cereal mix and the cereal mix with dried fruits, declared to “may contain traces of nuts”) the presence of Ara h3/4 was confirmed, and the other samples were found to be negative. The production mass spectra and the LC retention times were used to confirm the presence of peanut in samples (Fig. 5). In the positive samples, the peanut concentration was approximately 9.8 ± 0.4 and 12.3 ± 1.3 mg/kg, respectively.

The positive samples were analyzed by performing the same extraction procedure without the use of magnetic beads. No signals corresponding to the two peptide investigated were observed at their corresponding retention times (Fig. 6).

In a final step, the LC-MS/MS quantitative results were verified by testing the same samples with a commercially available ELISA kit to detect peanut hidden allergens. The ELISA data were in agreement with those obtained by LC-MS/MS for positive and negative samples. In the case of the two positive samples, the ELISA screening data were 14.4 ± 0.9 and 17 ± 2 mg/kg, respectively. Taking into account that the Veratox kit could over estimate samples [19], the ELISA data were consistent with those obtained by LC-MS/MS to indicate that this method was successful in confirming the presence of hidden peanut allergens in breakfast cereals.

4. Conclusions

A strategy to coat protein A-magnetic beads with monoclonal anti-Ara h3/4 antibodies was devised. To our knowledge so far this is the first time that a selective antibody magnetic support is developed for enrichment of hidden peanut allergens.

In conclusion, our methodology demonstrated numerous advantages. The application of immunomagnetic isolation of Ara h3/4 from complex matrices coupled with a microwave-assisted digestion resulted in the most fast and reliable method to purify, concentrate and digest sample extracts before LC-MS/MS analysis. The method works with a single monoclonal antibody with the linear ion-trap mass spectrometer as high selective detector. Taken together, the sensitivity and selectivity reached by the sample treatment method coupled with the LC-IT-MS/MS analysis developed (LOD 3 mg peanuts/kg matrix) allow to propose a powerful method suitable to confirm the results of the ELISA screening procedures.

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References

- [1] B. Añibarro, F.J. Seoane, M.V. Múgica, J. Invest. Allergol. Clin. Immunol. 17 (2007) 168.
- [2] Food Allergen Labelling and Consumer Protection Act of 2004, US Food and Drug Administration (FDA), Public Law 108-282, 2 August 2004.
- [3] European Parliament and Council, Off. J. Eur. Commun. L109 (2000) 29–42; European Parliament and Council, Off. J. Eur. Union L308 (2003) 15–18.
- [4] P. Koch, G.F. Schappi, R.E. Poms, B. Wuthrich, E. Anklam, R. Battaglia, Food Addit. Contam. 20 (2003) 797.
- [5] G.M. Brett, V.J. Bull, M.R. Morgan, Allergy 53 (1998) 109.
- [6] T. Holzhauser, S. Vieths, J. Agric. Food Chem. 47 (1999) 603.
- [7] P.R. Goodwin, J. AOAC Int. 87 (2004) 1383.
- [8] M. Careri, A. Costa, L. Elviri, J. Boquett Lagos, A. Mangia, M. Terenghi, A. Cereti, L. Perono Garoffo, Anal. Bioanal. Chem. 389 (2007) 1901.
- [9] K.J. Shefcheck, J.H. Callahan, S.M. Musser, J. Agric. Food Chem. 54 (2006) 7953.
- [10] K.J. Shefcheck, S.M. Musser, J. Agric. Food Chem. 52 (2004) 2785.
- [11] H. Chassaing, J.V. Nørgaard, A. van Hengel, J. Agric. Food Chem. 55 (2007) 4461.
- [12] M. Careri, L. Elviri, A. Mangia, C. Mucchino, Anal. Bioanal. Chem. 387 (2007) 1851.
- [13] F. Guan, C.E. Uboh, L.R. Soma, E. Birks, J. Chen, J. Mitchell, Y. You, J. Rudy, F. Xu, X. Li, G. Mbuy, Anal. Chem. 79 (2007) 4627.
- [14] F. Becher, E. Duriez, H. Volland, J.C. Tabet, E. Ezan, Anal. Chem. 79 (2007) 659.
- [15] J.R. Whiteaker, L. Zhao, H.Y. Zhang, L.C. Feng, B.D. Piening, L. Anderson, A.G. Paulovich, Anal. Biochem. 362 (2007) 44.
- [16] C.Y. Hong, Y.C. Chen, J. Chromatogr. 1159 (2007) 250.
- [17] W.Y. Chen, Y.C. Chen, Anal. Chem. 79 (2007) 2394.
- [18] G. Prioult, C. Turcotte, L. Labarre, C. Lacroix, I. Fliss, Int. Dairy J. 10 (2000) 627.
- [19] B. Whitaker, K.M. Williams, M.W. Trucksess, A.B. Slate, J. AOAC Int. 88 (2005) 161.