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The Detection of Allergens in Food Products with LC-MS

Something for the future?

Jacqueline van der Wielen

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Scope of Organisation

Dutch Food and Consumer Product Safety Authority:

- Law enforcement
    - Control of labelling
- Point out problems / evils
- Development of new methods
- New regulations

Scope of Organisation

CEN:

- Normalisation of methods
- CEN-TC/275-WG/12
- Providing standards/methods for (food) industries as well as control agencies.

What to measure

Allergens/Markers of allergens on the “EU-List”:

- Cereals containing gluten
- Crustaceans
- Eggs
- Fish
- Peanuts
- Soybean
- Milk
- Nuts
- Celery
- Mustard
- Sesame seeds

(Food labeling Directive 2000/13/EC)

How to measure allergens

Commonly used methods:

- ELISA
- PCR
- LC-MS

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Comparison between techniques

	ELISA	PCR	LC-MS/MS
Detect:	Intact protein	DNA	intact protein or peptides (markers)
Sensitivity	1-5 ppm	lower	1-10 ppm
Quantification	Yes	RT-PCR (semi)	Confirmatory
False Positives	Yes	use of marker (DNA)	No
SpecificProcedure	Yes (antibodies)	Yes	No
Time consuming	Yes	Yes	No

LC-MS Methods developed for Allergens

- Allergen identification: Over 230 articles
- Allergen identification in Food: Over 140 articles
- Method Development for controll of absence/presence of allergens in (several) food matrices: < 10 articles

LC-MS Methods for detection of Allergens in Food Products

3 approaches:

- Direct identification of extracted proteins with LC-MS system
- Digest of proteins are identified with LC-MS system
- Proteins interact with a bait molecule on a chip and are identified with LC-MS system

Determination of Gluten Gliadins in Food

Detection of gluten gliadins directly in food samples by characteristic gliadin mass pattern (25 – 40 kDa) using MALDI/TOF-MS:

– Extraction of gliadins from food sample in 60% (v/v) ethanol

– Extract was mixed with solution of sinapinic acid in 30% acetonitrile and 0.1% TFA and then concentrated

– Concentrate was measured with a MALDI-TOF MS

– Equipment was externally calibrated with mixtures of BSA en CC

Determination of Gluten Gliadins in Food

Results:

– method lineair on α-gliadin at mass signal around 30 kDa: 4-100 mg/kg food

– characteristic gliadin profile is revealed in unprocessed as wel as processed gluten containing samples

– LC-MS confirms ELISA results

– In processed food (bread) the gluten content has decreased compared to unprocessed flour, LC-MS provides additional information on changes in gliadin composition ratios (stability of ω-gliadin).

Determination of Gluten Gliadins in Food

– However, method gives problems with samples with high content of maize or rice (prolamines are co-extracted with gliadins)
– Resuspend dried ethanol fraction in 1M acetic acid: prolamines remain in pellet (90%) and gliadins are soluble (supernatant)
– MALDI-TOF MS is now less sensitive (>50 mg/kg)
– still there remain matrix interferences

Conclusion: This MALDI-TOF MS method is good non-immunological technique to verify ELISA results.

Detection of Milk Allergens

Trypric digests from cleaned-up food matrices were analysed using LC-QTOF-MS for allergen milk proteins (α-casein).

– food samples were extracted and desalted

– extracts were incubated for 3 h. with trypsin

– digest mixture was analysed using LC-MS/MS

Detection of Milk Allergens

Results:

– Presence of milk is detected using reconstructed ion chromatograms of *m/z* 634.2 and *m/z* 692.8

– 1.25 mg/kg milk can be detected in a cookie (spiked)

– Digestion of αS1-casein with trypsin theoretically produces about 15 peptides with masses above 500 Da

– Signals of these peptides vary in intensity according to sample concentration

Detection of Milk Allergens

– the presence of both markers (*m/z* 634.3 and 692.8) correlate only with presence of αS1 casein

–the digest by trypsin can be performed directly or in a gel: in a gel the chromatograms are cleaner, however sample process time is doubled!

–MS/MS database search of directly digested extract provides usefull data about other (milk) proteins such as whey or gliadin

– sensitivity of ELISA and LC-MS/MS were comparable

Detection of Milk Allergens

Conclusion:

This method can be used to confirm other methods such as ELISA

Conformation of Peanut Protein Ara h1

Biomarkers (peptides) are found to identify the presence of Ara h1 in food matrices

– proteins were extracted from matrix

– cleaned extracts were incubated overnight with trypsin (37°C)

– digest was analysed/characterised using LC-QTOF-MS/MS

Conformation of Peanut Protein Ara h1

Results:

– mixture of peptides identified as Ara h1 specific

– 4 most abundant peptides (*m/z* 571.3, 629.8, 869.9 and 606.6) were found to be unique for Ara h1 (unique sequences)

– to confirm 10 mg/kg Ara h1 in an Ice Cream, an extra clean up with a molecular mass cut-off filter of 50 kDa is necessary:

- concentration of Ara h1 protein
- removes other small proteins (from ice cream)

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Conformation of Peanut Protein Ara h1

Conclusion:

This method has broad applicability as a confirmatory test for ELISA

Multi-dimensional separation/identification of proteins using SELDI-TOF-MS

Presently used for identification of disease biomarkers and study of biomolecular interactions

– sample or crude extract can be applied directly to chip surface to promote interactions with bait molecule

– a serie of washes is applied to elute unbound proteins/interfering substances

– array is inserted into proteinchip reader: SELDI-TOF-MS

Multi-dimensional separation/identification of proteins using SELDI-TOF-MS

– Different varieties of proteinchip arrays:

- Chemical: an-/cationic, hydrophobic, metal etc.
- biochemically: antibody, receptor, DNA, enzyme

– Using a QTOF-MS/MS: proteins can be digested on the chip and fragments identified by tandem MS

Conclusion: Promising tool for allergens at low concentrations

Conclusions

– LC-MS Methods for detection of allergens in different food matrices are confirmatory to other techniques
– Quantification seems to be difficult
– Detection limits: 1-10 mg/kg
– More method development has to be done
– Methods need to be validated (in house as well as collaborative trial)

Conclusion

In the future we might be able to use LC-MS routinely for the detection of allergens in different kind of foods

Literature

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