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Targeted and Sensitive Detection of Food Allergens in Complex and Processed Foodstuffs Using UPLC-MS/MS

Mélanie Planque,[1] Antonietta Wallace,[2] and Nathalie Gillard[1]
[1]CER Groupe, Marloie, Belgium; [2]Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Sensitive multi-allergen method using UPLC-MS/MS.
- Allergens monitored in this method were assessed from the recommended levels provided by VITAL (Voluntary Incidental Trace Allergen Labelling) and the AOAC SMPR for food allergens (2016.002).
- This multi-allergen detection method has the lowest limits of quantification available to date (expressed in total proteins and not soluble proteins).

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY UPLC BEH130 BEH Column

Xevo® TQ-S

MassLynx® MS Software

KEYWORDS

Proteomics, allergens, LC-MS/MS, egg, peanut, milk, soybean

INTRODUCTION

Food allergy is a worldwide health problem affecting both adults and children. To avoid allergic reactions, allergens must be totally excluded from the diet. Consequently, allergic customers can only refer to mandatory labeling to try and avoid coming into contact with the food allergen. However, the undeclared presence of these allergens is still widespread.

To help food industries in the management of hidden allergens, sensitive, specific quantitative, and robust analytical methods need to be developed.

Traditionally techniques such as ELISA and PCR have been used for routine analysis, but in recent years, there has been increasing interest in the utility of LC-MS based methods. In March 2016, AOAC released the first standard method performance requirements (SMPR) specifically for the analysis of four food allergens using LC-MS/MS.[1] The detection levels tested are benchmarked against the levels stated in the AOAC SMPR 2016.002 and VITAL (Voluntary Incidental Trace Allergen Labelling)[2] reference doses.

In this application note, we describe the targeted analysis of four food allergens in a variety of matrices using Waters® ACQUITY UPLC System and Xevo TQ-S.

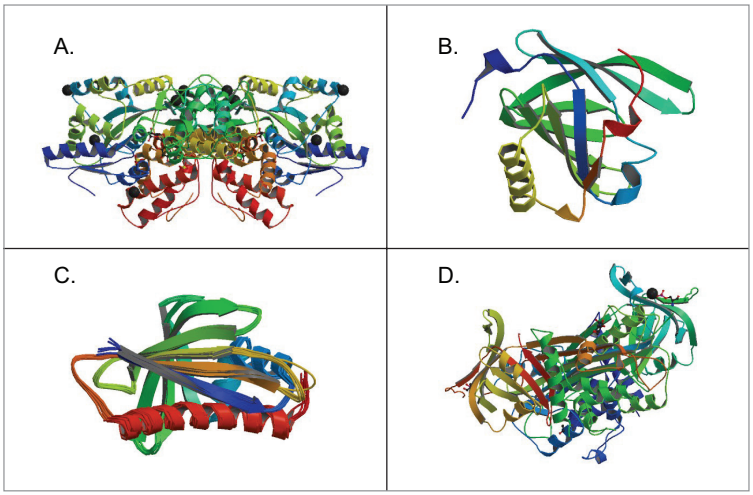

Figure 1.
1A. MBP-fusion protein of the major peanut allergen Ara h 2: DOI: 10.2210/pdb3ob4/pdb;
1B. Bovine allergen Bos d 2 in the trigonal space group P3221:DOI: 10.2210/pdb4wfu/pdb;
1C. NMR solution structure of soybean allergen Gly m 4: DOI: 10.2210/pdb2k7h/pdb;
1D. Crystal structure of uncleaved ovalbumin at 1.95 angstroms resolution: DOI: 10.2210/pdb1ova/pdb.
Images courtesy of the RSCB Protein Data Bank.

EXPERIMENTAL

This method is based on a single protocol applicable to the different tested allergens and foodstuffs. Details on the sample preparation step are described elsewhere.[3]

The four allergens investigated in this method were milk (Bos Taurus), egg (Gallus gallus chicken), peanut (Arachis hypogaea) and soybean (Glycine Max (Glycine hispida).

The protocol was tested on processed and complex food matrices including chocolate, ice cream, tomato sauce, and cookies.

LC conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH130, 2.1 x 150 mm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	0.2 mL/min
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid
Gradient:	0 to 1 min: 86% A; 1 to 16.5 min: 86% to 60% A; 16.5 to 16.6 min: 60% to 0% A; 16.6 to 21 min: 0% A; 21.0 to 21.1 min: 0% to 86% A; 21.1 to 24 min: 86% A

MS conditions

MS system:	Xevo TQ-S
Ionization mode :	ESI+ in MRM mode
Capillary voltage:	2.0 kV
Collision gas flow:	0.12 mL/min
Cone voltage:	35 V
Cone gas flow:	150 L/h
Desolvation flow:	1200 L/h
Source temp.:	150 °C
Desolvation temp.:	500 °C

Data solutions

Skyline (MacCoss Lab)

UniProt

MassLynx

Table 1. Multiple reaction monitoring (MRM) parameters for the identification of milk, egg, soybean, and peanut proteins by ACQUTIY UPLC and Xevo TQ-S.

Food	Peptide	RT* (min)	Precursor (charge state) (*m/z*)	Product ion (fragment)	Collision energy (eV)
Egg	GGLEPINFQTAADQAR	7.5	844.4 (++)	1331.7 (y12+)	26
				1121.5 (y10+)	28
				666.3 (y12+)	25
	LTEWTSSNVMEER	5.9	791.4 (++)	1052.5 (y9+)	31
				951.4 (y8+)	23
				864.4 (y7+)	23
	ISQAVHAAHAEINEAGR	2.3	887.5 (++)	1138.6 (y11+)	33
				1067.5 (y10+)	33
				996.5 (y9+)	32
	EALQPIHDLADEAISR	7.8	593.3 (+++)	761.4 (y7+)	19
				690.3 (y6+)	15
				668.8 (y12++)	15
	NIPFAEYPTYK	7.5	671.8 (++)	1115.5 (y9+)	15
				508.3 (y4+)	16
				558.3 (y9++)	29
	NIGELGVEK	4	479.8 (++)	731.4 (y7+)	12
				674.4 (y6+)	10
				545.3 (y5+)	19
	YLLDLLPAAASHR	10.4	480.6 (+++)	709.4 (y7+)	15
				582.3 (y11++)	10
				355.2 (y7++)	14
	NFLINETAR	6.2	539.3 (++)	816.5 (y7+)	14
				703.4 (y6+)	16
				590.3 (y5+)	16

(Table 1 continues on the next page.)

(Table 1 continued.)

Food	Peptide	RT* (min)	Precursor (charge state) (*m/z*)	Product ion (fragment)	Collision energy (eV)
Peanut	NTLEAAFNAEFNEIR	10.7	869.9 (++)	1139.5 (y9+)	27
				992.5 (y8+)	26
				878.4 (y7+)	26
	RPFYSNAPQEIFIQQGR	7.3	684.4 (+++)	748.4 (y6+)	20
				608.3 (y10++)	19
				836.4 (b7+)	17
	FNLAGNHEQEFLR	6.2	525.6 (+++)	692.4 (y5+)	20
				600.8 (y10++)	13
				565.3 (y9++)	14
	TANELNLLILR	11.2	635.4 (++)	983.6 (y8+)	21
				854.6 (y7+)	20
				741.5 (y6+)	22
Soybean	ISTLNSLTLPALR	10.5	699.9 (++)	984.6 (y9+)	23
				870.5 (y8+)	25
				783.5 (y7+)	25
	EAFGVNMQIVR	8.1	632.3 (++)	859.5 (y7+)	18
				760.4 (y6+)	17
				646.4 (y5+)	22
	ELINLATMCR	8.3	610.8 (++)	865.4 (y7+)	21
				751.4 (y6+)	21
				638.3 (y5+	17
	LITLAIPVNKPGR	7.9	464.6 (+++)	767.5 (y7+)	15
				583.4 (y11++)	9
				476.3 (y9++)	11
Milk	HQGLPQEVLNENLLR	8.1	587.3 (+++)	871.5 (y7+)	17
				758.4 (y6+)	16
				436.2 (b4+)	17
	FFVAPFPEVFGK	13.5	692.9 (++)	991.5 (y9+)	18
				920.5 (y8+)	18
				676.4 (y6+)	28
	YLGYLEQLLR	12.3	634.4 (++)	934.5 (y7+)	21
				771.5 (y6+)	20
				658.4 (y5+)	21
	NAVPITPTLNR	5.1	598.3 (++)	911.5 (y8+)	17
				456.3 (y8++)	14
				285.2 (b3+)	12
	VYVEELKPTPEGDLEILLQK	10.6	771.8 (+++)	912.0 (y16++)	19
				790.9 (y14++)	19
				627.9 (y11++)	20
	VLVLDTDYK	6.4	533.3 (++)	853.4 (y7+)	15
				754.4 (y6+)	14
				641.3 (y5+)	16
	LSFNPTQLEEQCHI	8.9	858.4 (++)	1254.6 (y10+)	26
	N-terminal peptide			928.4 (y7+)	27
				627.8 (y10++)	27

**Retention time (RT) is in sauce.*

RESULTS AND DISCUSSION

The software package Skyline, was used for *in silico* enzymatic digestion of food allergen proteins and to help produce potential MRMs for the experiment. From the list produced by Skyline, each MRM was analyzed using the ACQUITY UPLC System coupled to the Xevo TQ-S for sensitivity and reproducibility (in different food matrices).

In this method a total of 23 peptides and 69 MRMs were included as part of the analysis, although no regulations as yet state what determines a positive identification of an allergenic protein. (e.g. number of proteins and peptides to be monitored). For egg and milk, peptides representative of the different components of the egg: egg white (ovalbumin) and the yolk (vitellogenin), milk (casein), and whey (β-lactoglobulin), are included in the method.

METHOD SENSITIVITY

Current regulations address the analytical levels of detection for gluten, and so for the allergens monitored in this method, levels were assessed from the recommendation levels provided by VITAL and the AOAC SMPR for food allergens.

For each allergen, a single, common LOQ was determined for all targeted matrices (Figure 2). For each peptide, two MRM transitions in allergen-free matrices and incurred matrices were shown to demonstrate the specificity of the method and to confirm detection of the food allergens at the LOQ. The LOQ was defined as the minimum concentration giving a signal-to-noise ratio (S/N) of 10 for the most intense MRM transition of the targeted food allergen. The sensitivity of detection for the food allergen peptides was determined on the worst case, mainly processed cookies. The LOQs recorded are: 0.5 mg milk proteins/kg for caseins, 5 mg milk proteins/kg for whey, 3.4 mg egg proteins/kg for egg white, 30.8 mg egg proteins/kg for egg yolk, 2.5 mg/kg for peanut proteins, and 5 mg/kg for soybean proteins.

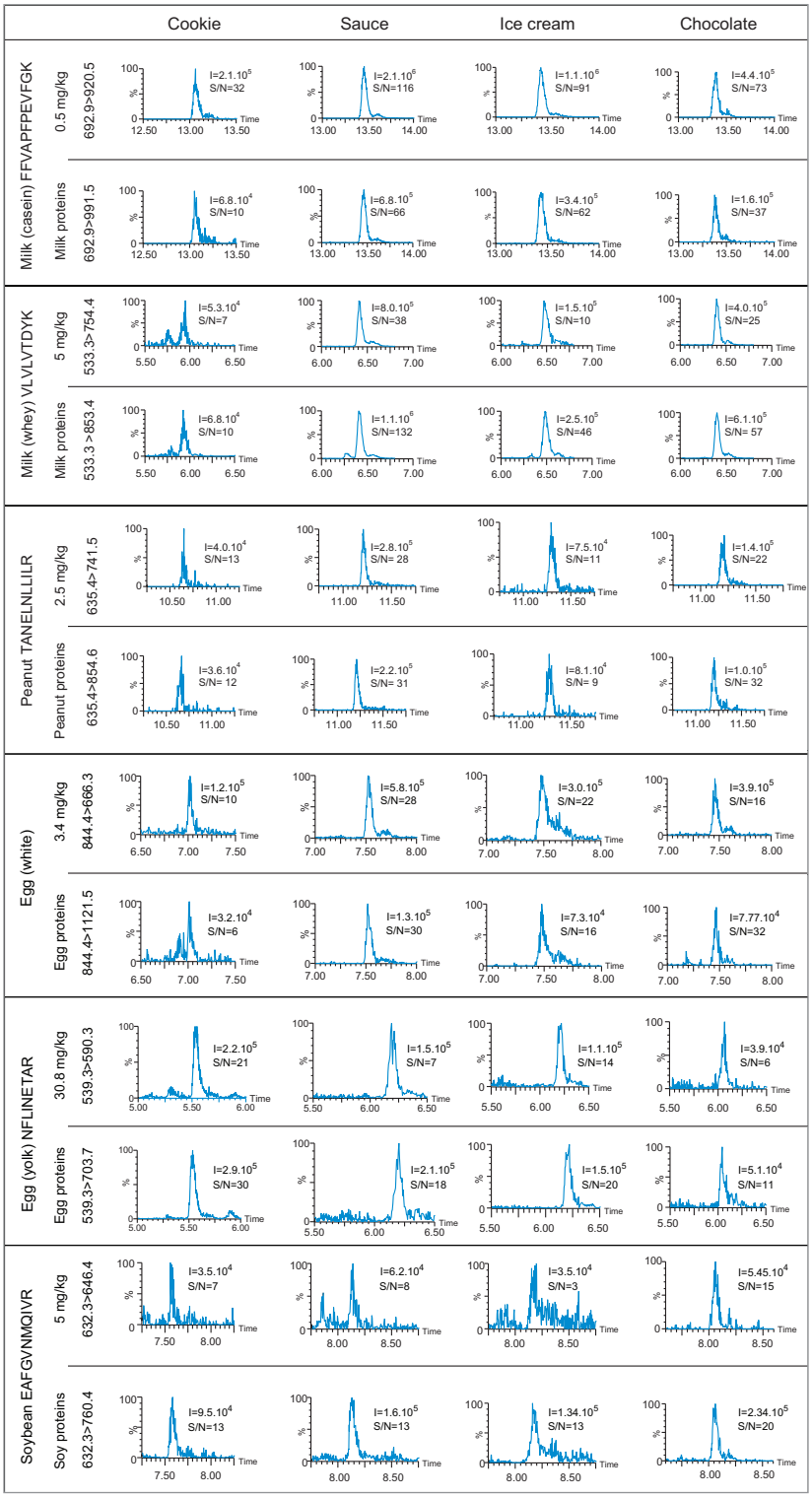

Figure 2. Chromatograms of the two higher MRM transitions of milk casein peptide FFVAPFPEVFGK, whey milk peptide VLVLDTDYK, and peanut peptide TANELNLLILR. Egg white peptide GGLEPINFQTAADQAR, egg yolk peptide NFLINETAR, and soy peptide EAFGVNMQIVR in chocolate, ice cream, tomato sauce, and cookies. Data of incurred or processed matrices at the limit of quantification are presented without any data treatment.

METHOD LINEARITY

Linearity and matrix effects were tested by analyzing three independent foodstuff preparations (incurred chocolate and ice cream and processed cookies and sauce) that contained different concentrations of milk, egg, soy, and peanut food allergen proteins (Figure 3).

Although the matrix effect and the effect of the thermal process were not the same for both targeted peptides from the same food allergen, the linear coefficient of regression supported the reliability of the method even the absence of an internal standard.

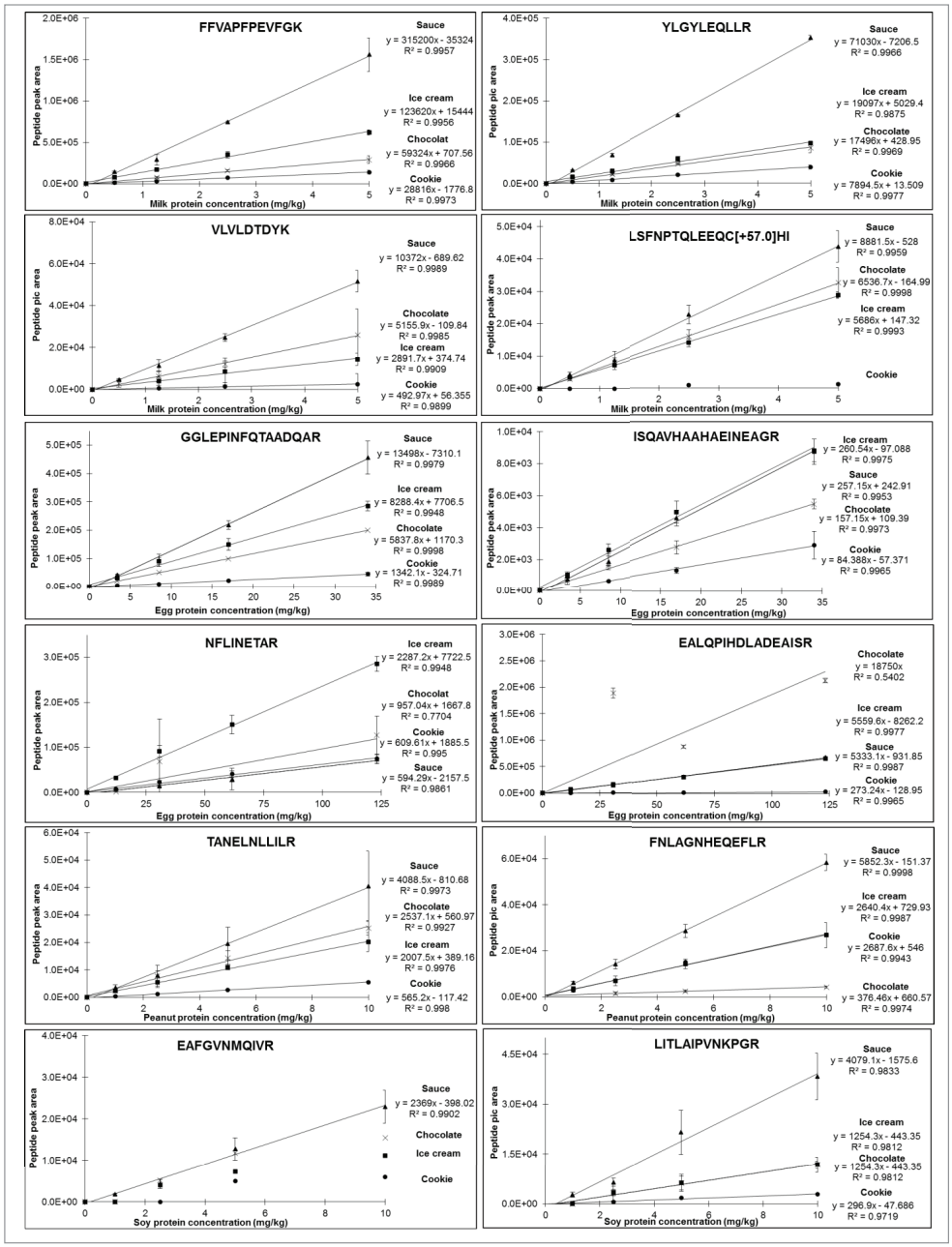

Figure 3. Linear regression of peptide peak area of the higher MRM in function of the concentration of food allergen proteins performed in three independent replicates in incurred tomato sauce, chocolate, ice cream, and processed cookies. The linearity was controlled for each food allergen: milk casein FFVAPFPEVFGK (692.9>920.5) and YLGYLEQLLR (634.4>771.5); whey milk VLVLDTDYK (533.3>853.4) and LSFNPTQLEEQC[+57] HI (858.4>928.4) (carbamidomethylation of cysteine amino acids by addition of iodoacetamide before an enzymatic digestion to block the onset of disulfur bridges); egg white GGLEPINFQTAADQAR (844.4>666.3) and ISQAVHAAHAEINEAGR (887.5>1067.5); egg yolk NFLINETAR (539.3>703.4) and EALQPIHDLADEAISR (593.3>668.8); peanut TANELNLLILR (635.4>741.5) and FNLAGNHEQEFLR (525.6>600.8; and soybean EAFGVNMQIVR (632.9>760.4) and LITAIPVNKPGR (464.6>583.4).

CONCLUSIONS

Sensitive detection of food allergens (milk casein, whey, egg white, egg yolk, peanut, and soybean) was achieved by analyzing food allergen peptides using the ACQUITY UPLC System coupled to the Xevo TQ-S.

In keeping with food production requirements, the targeted matrices were processed (tomato sauce, cookies) or incurred (chocolate, ice cream). This multi-allergen detection method has the lowest limits of quantification available to date (expressed in total proteins and not soluble proteins): 0.5 mg milk proteins/kg for caseins, 5 mg milk proteins/kg for whey, 3.4 mg egg proteins/kg for egg white, 30.8 mg egg proteins/kg for egg yolk, 2.5 mg peanut proteins/kg, and 5 mg soybean proteins/kg.

While matrix effects can be observed from the data shown, further work will involve the inclusion of internal standards in order to make the method quantitative.

References

1. AOAC SMPR 2016.002, Standard method performance requirements for Detection and Quantitation of Selected Food Allergens.
2. http://allergenbureau.net/vital/
3. 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 foodstuffs. *J Chrom A.* 1464: 115–123, 2016.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com