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Targeted Peptide Quantitation of Seven Food Allergens in Dark Chocolate Using Triple Quadrupole LC/MS

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Introduction

Food allergy presents a significant public health issue. Hence, mandatory allergen labelling laws have been enacted to protect the allergic consumers, but allergens may be unintentionally present in food products due to cross-contact. To address this uncertainty, food manufacturers use precautionary allergen labelling (PAL). However, the lack of global consistency in PAL confuses many consumers. Thus, scientific-based allergen risk assessment has been increasingly used by the food authorities and industry to improve management of food allergens via PAL.

Allergen analysis plays an important role in the application of action levels for either voluntary or legislative labeling. A quick sample preparation procedure together with a sensitive and reliable analysis method was developed for the simultaneous targeted quantitation of egg, milk, soy, peanut, almond, hazelnut and walnut in dark chocolate.

Experimental

Materials and sample preparation

Reference materials for milk, egg, soy and peanut were obtained from NIST (Gaithersburg, MD, USA). Baked almond, hazelnut and walnut were purchased from a local supermarket and homogenized into fine pastes. For each allergen, 50 mg/mL stock solution was prepared following the second and third steps in Figure 1. They were combined and serially diluted into allergen working solutions used for spiking dark chocolate to prepare calibration standard and QC samples (Figure 1).

Targeted peptide analysis

Peptides (10 µL) were separated by a Poroshell 120 EC-C18 column using an Agilent 1290 Infinity II UHPLC system coupled to a 6495 triple quadrupole mass LC/MS (LC/TQ) system. The detailed LC and MS parameters are shown in Table 1 and 2, respectively.

Table 1. Agilent 1290 Infinity II UHPLC parameters.

LC Parameter	Value
Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (P/N 695775-902)
Mobile phase A	0.1% formic acid in water
Mobile phase B	0.1% formic acid in acetonitrile
Flow rate	0.4 mL/min
Column temp	40°C
Gradient	0.0 min → 2%B; 1.0 min → 2%B; 11.0 min → 40%B; 12.5 min → 98%B; 14.5 min → 98%B; 14.6 min → 98%B
Post time	2.4 min

Experimental

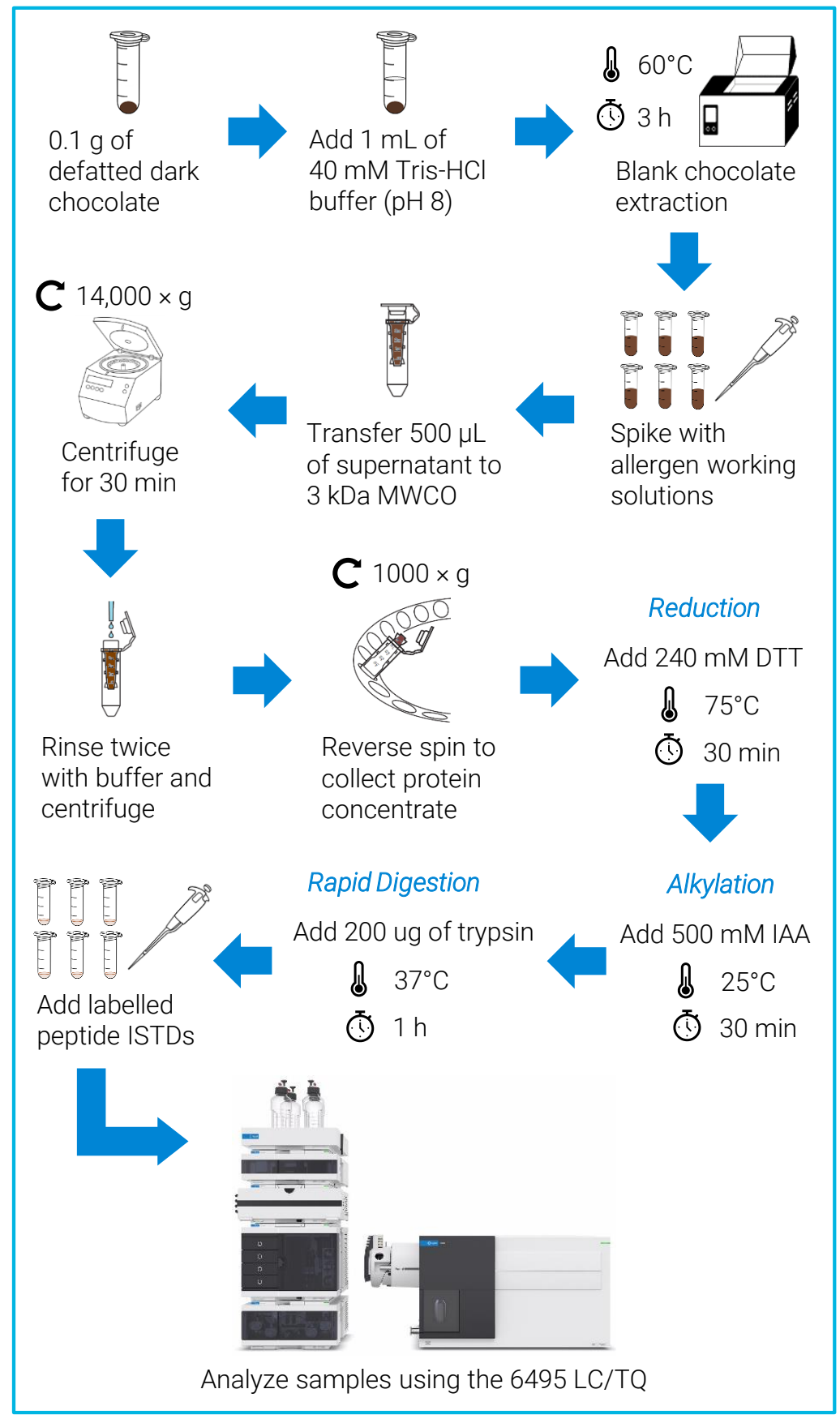

Figure 1. Sample preparation workflow.

Table 2. Agilent 6495 LC/TQ MS parameters.

MS Parameter	Value
Ionization mode	Positive AJS ESI
Gas temperature	150°C
Drying gas flow	16 L/min
Nebulizer gas	30 psi
Sheath gas temperature	350°C
Sheath gas flow	11 L/min
Capillary voltage	3500 V
Nozzle voltage	300 V
High/Low RF pressure voltage	145/65 V
Delta EMV	200 V
Scan type	Dynamic MRM
Cycle time	500 ms

Selection of peptide markers

The peptide markers were selected from peptide mapping experiments and rigorously checked to make sure that they are unique to each food allergen and have no interference with the food matrix, other food allergens or commonly used ingredients of plant or mammal origins. To ensure optimal MS sensitivity, two peptides and two MRM transitions per peptide, were used as positive identification for each allergen (Table 3). Optimal separation of the peptides was achieved using a short 10 min LC gradient (Figure 2).

Table 3. MRM transitions of the peptide markers.

Allergen	Peptide ID	Precursor m/z	Product m/z	CE (V)	Function
Milk casein	MC1	692.9	920.5	17	Quantitation
		692.9	991.5	23	
	MC2	390.8	568.3	7	Confirmation
		390.8	372.2	16	
Milk whey	MW1	533.3	853.4	15	Quantitation
		533.3	754.4	15	
	MW2	858.4	1254.6	31	Confirmation
		858.4	627.8	31	
Egg white	EW1	844.4	666.3	27	Quantitation
		844.4	1331.7	30	
	EW2	298.5	397.7	3	Confirmation
		298.5	326.7	6	
Soy	SY1	347.5	407.2	5	Quantitation
		347.5	464.3	5	
	SY2	478.3	643.4	19	Confirmation
		478.3	434.8	16	
Peanut	PN1	543.3	429.7	15	Quantitation
		543.3	858.4	18	
	PN2	628.4	741.5	21	Confirmation
		628.4	1083.7	21	
Almond	AM1	571.8	369.2	16	Quantitation
		571.8	858.4	19	
	AM2	686.9	594.8	19	Confirmation
		686.9	748.4	31	
Hazelnut	HN1	514.3	616.3	17	Quantitation
		514.3	729.4	17	
	HN2	576.3	689.4	22	Confirmation
		576.3	852.4	22	
Walnut	WN1	636.4	875.4	18	Quantitation
		636.4	397.3	15	
	WN2	479.6	662.4	13	Confirmation
		479.6	618.9	13	

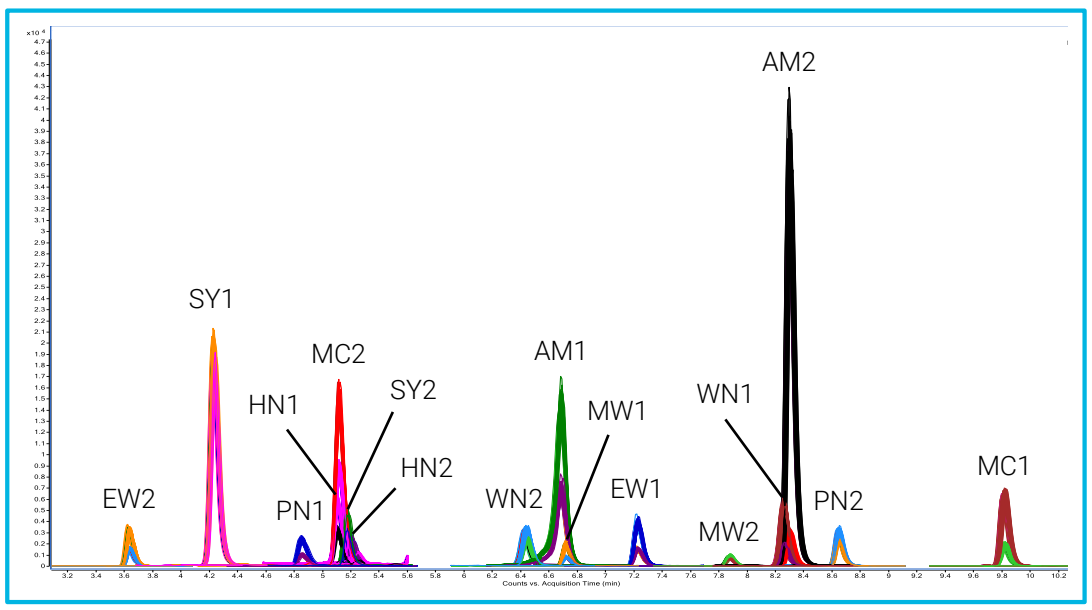

Figure 2. MRM chromatograms of the 14 peptides representing the 7 food allergens in 100 mg/kg spiked dark chocolate (overlay of 104 replicate injections).

Method sensitivity

The method limit of quantitation (LOQ) was defined as the concentration where S/N is greater than 10 and benchmarked against the recommended sensitivity levels from VITAL 3.0 and AOAC SMPR 2016.002. All peptide markers in this method demonstrated excellent sensitivity and were able to meet the minimum sensitivity levels described in VITAL 3.0 and AOAC SMPR (Table 4). The method also demonstrated good specificity and was able to accurately detect the peptides at LOQ in the dark chocolate matrix (Figure 3).

Table 4. Comparison of the method LOQ to the recommended sensitivity levels from VITAL 3.0 (reference amount of 40 g) and AOAC SMPR 2016.002.

Allergen	mg allergen per kg food		
	Method LOQ	VITAL 3.0 Action Level[1]	AOAC SMPR MQL[2]
Milk casein	10	20	10
Milk whey	10	20	10
Egg white	5	10	5
Soy	5	23	Not defined
Peanut	10	23	10
Almond	2.5	12	Not defined
Hazelnut	5	17	10
Walnut	5	5	Not defined

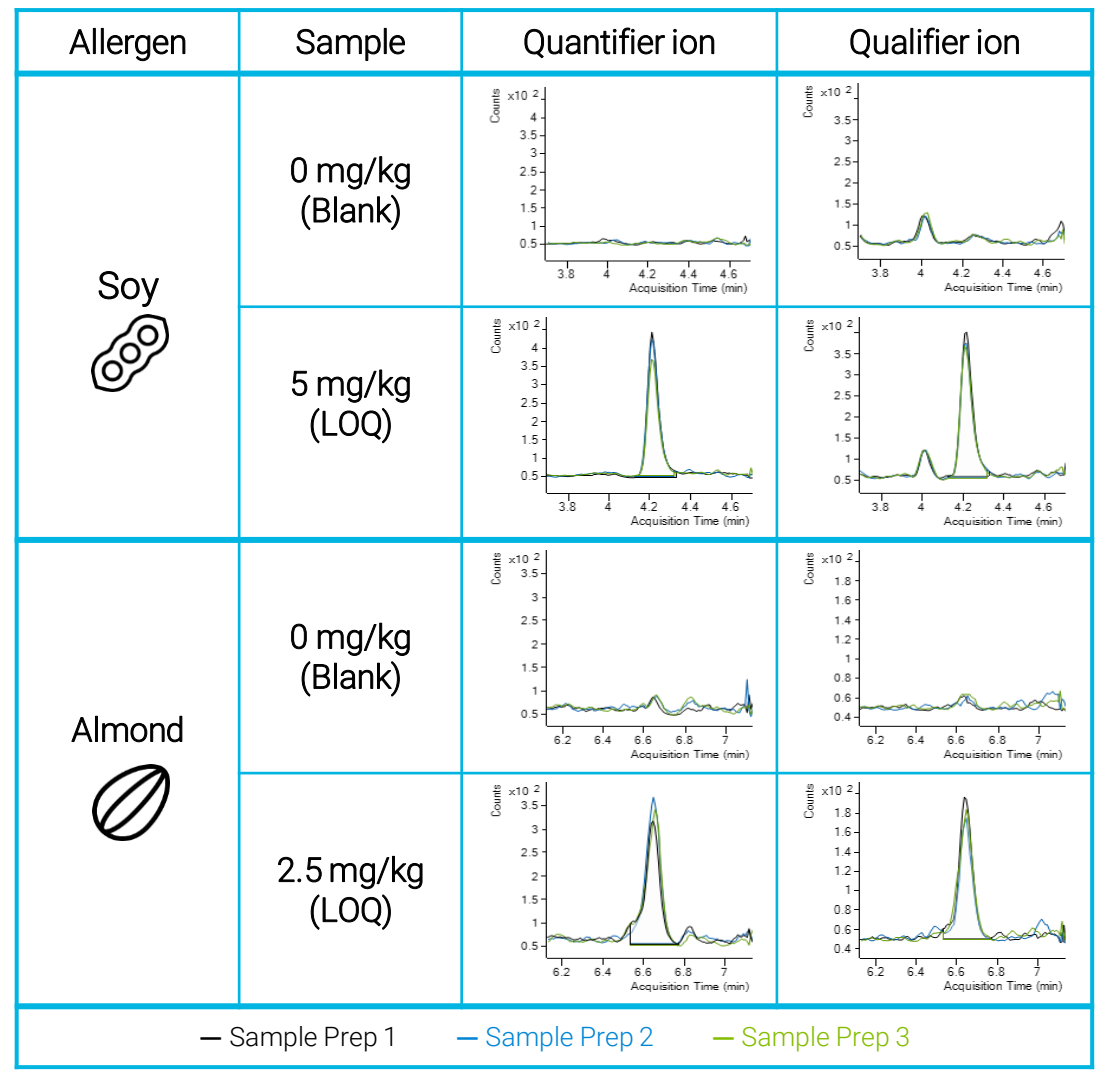

Figure 3. Overlay of MRM chromatograms for soy and almond in blank (0 mg/kg) and LOQ chocolate samples.

Results and Discussion

Analytical range and accuracy

As shown in Figure 4, all peptides demonstrated a wide analytical range of 3 to 4 orders of magnitude across 2.5 – 1000 mg/kg for almond; 5 – 1000 mg/kg for egg white, soy and walnut; 10 – 1000 mg/kg for milk and peanut; and 5 – 500 mg/kg for hazelnut. All calibration curves demonstrated good linearity with R^2 values greater than 0.99.

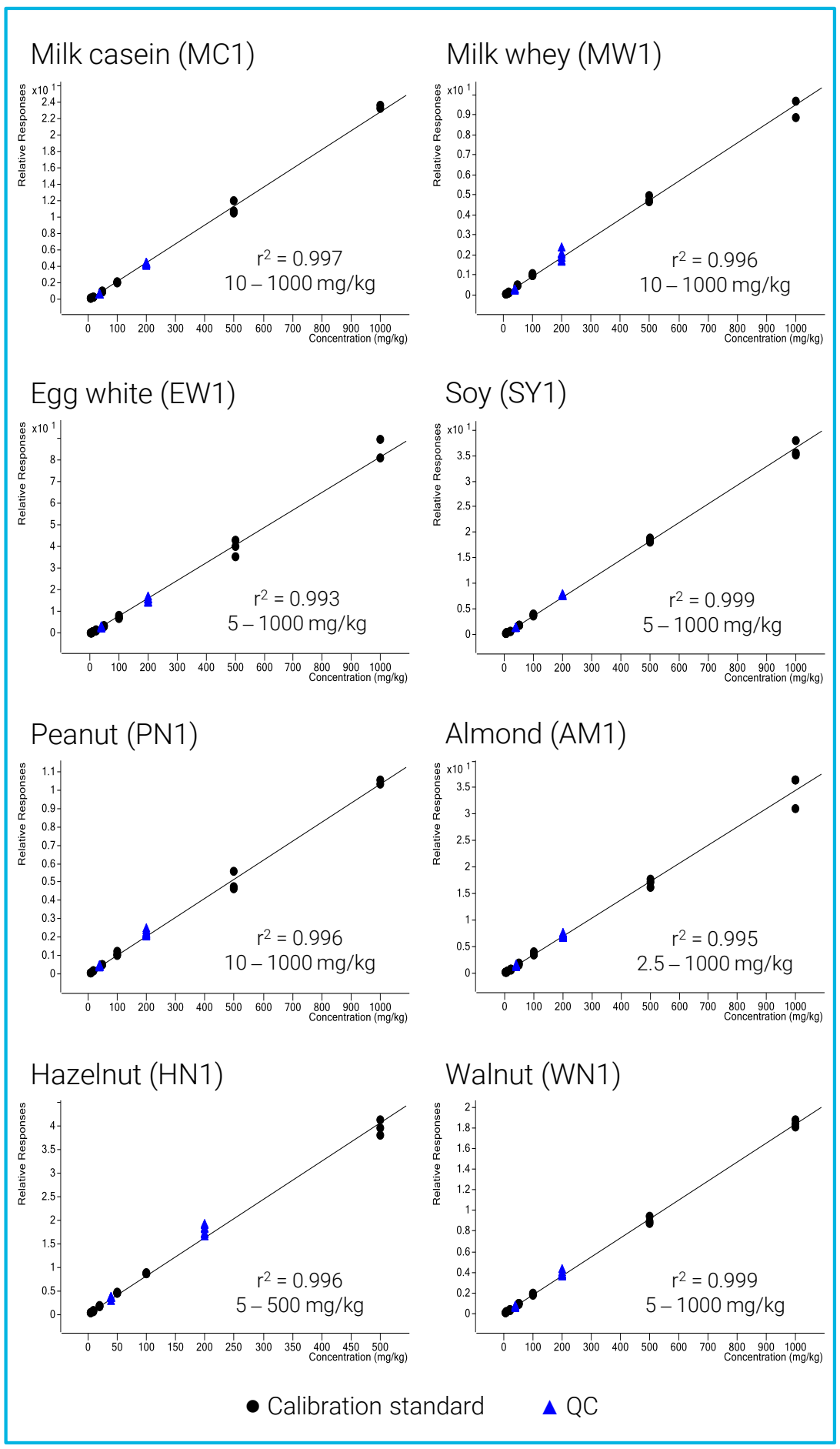

Figure 4. Calibration curves of the quantitation peptides in dark chocolate spiked with the 7 allergens (n = 3 per calibration concentration).

Method recovery and precision

The recovery and precision of the peptide markers were evaluated at two QC levels at 40 and 200 mg/kg. Nine replicate analyses of each QC level were evaluated. As shown in Figure 5, method recoveries were 75 – 102% at both QC levels for most quantitation peptides and are well within the AOAC recommended recovery of 60 – 120%. The method also demonstrated excellent precision (RSD) of 1.5 – 8.6% and 1.7 – 10.4% for 40 and 200 mg/kg, respectively.

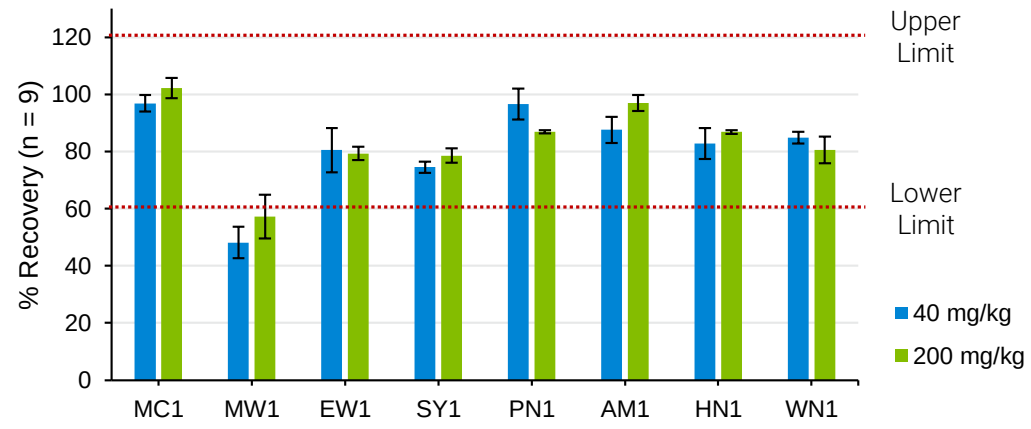

Figure 5. Recovery of allergens in QC samples at 40 and 200 mg/kg spiking levels.

Conclusions

- A rapid and simple sample preparation method was successfully developed for extracting milk (casein and whey), egg white, soy, peanut, almond, hazelnut and walnut from dark chocolate and analyzed using the Agilent 6495 Triple Quadrupole LC/MS system.
- The method was sensitive enough to meet the minimum sensitivity level recommendations in VITAL 3.0 and AOAC SMPR 2016.002, and demonstrated good analytical range, recovery and precision.
- Preliminary data showed that this method is applicable for cookies and further studies will be performed.

References

[1] The Allergen Bureau Limited. Food Industry Guide to the Voluntary Incidental Trace Allergen Labelling (VITAL) Program Version 3.0, 2019

[2] Paez V, et al. AOAC SMPR 2016.002 Standard Method Performance Requirements (SMPRs) for Detection and Quantitation of Selected Food Allergens. J AOAC Int. 2016, 99(4), 1122–1124

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