Application Note

Food Testing and Agriculture

LC/Q-TOF Marker Identification to TQ LC/MS Targeted Quantitation

Development and evaluation of sensitive and robust workflow for detecting peanut allergens in wheat flour

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Abstract

Consumption of peanut is one of the common causes of food allergies in humans worldwide.[1] Therefore, accurate package labeling is an imperative for the food industry and regulatory agencies. This application note describes a liquid chromatography/quadrupole time-of-flight (LC/Q-TOF) marker identification to triple quadrupole liquid chromatography/mass spectrometry (TQ LC/MS) workflow that was used to develop and optimize a reliable and accurate method to quantify peanut in raw and cooked wheat-flour-based matrices. Method development used the Agilent Auto MS/MS tool and Agilent MassHunter Qualitative Analysis software to screen tryptically-digested peanut LC/Q-TOF data for peanut peptides. The peptide markers identified with the LC/Q-TOF were then quantified on a TQ LC/MS using multiple reaction monitoring (MRM). The linearity, sensitivity, recovery, accuracy, repeatability, and reproducibility of the developed TQ LC/MS targeted method were evaluated and determined to exceed typical enzyme-linked immunosorbent assay (ELISA) performance for peanut quantification.

Introduction

Of the various food allergies experienced by humans worldwide, peanut allergy is one of the most common.[1] Accidental consumption of peanut by sensitive individuals can result in life-threatening anaphylactic reactions.[2] Regularly testing foods and their raw materials, as well as accurate package labeling, is important to protect peanut-allergic consumers, particularly from accidental contamination due to shared processing facilities and insufficient cleaning. Therefore, both the food industry and regulatory agencies need reliable and accurate methods to quantify peanut in food matrices.

Because of its ease of use and adequate sensitivity, enzyme-linked immunosorbent assay (ELISA) is frequently used for peanut allergen detection. However, quantification results among ELISA kits from different manufacturers can be inconsistent due to differences in methodology deployed to use the kits, and the allergenic proteins and antibodies chosen for quantification. Previous studies have found that ELISA methods could underestimate peanut allergen concentrations in roasted samples due to denaturing, elimination of epitopes, and changes in solubility of extraction buffers.[2-5]

Liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) offers improved sensitivity, linearity, robustness, and accuracy compared to ELISA. Two approaches have been described for LC/MS/MS-based peanut quantification. In the first, the calibration curve is built without internal standards and is based on known peanut concentrations in a food matrix and the peak areas of peptide markers.[6-11] Different concentrations of peanut are incorporated into the food matrix either before (incurred sample) or after baking (spiked sample). Preparing calibration samples is labor-intensive and time-consuming, and the results obtained depend on the variety of peanut used. The second approach builds the calibration curve using isotopically labelled peptides as internal standards.[12-16] This approach compensates for matrix effects, but assumes that the peanut proteins are fully converted into peanut peptides.

This application note describes a workflow that was used to develop a sensitive, robust, and accurate triple quadrupole liquid chromatography/mass spectrometry (TQ LC/MS) method to quantify peanut protein in both raw and cooked wheat-flour-based dry matrices (Figure 1).[17]

First, data generated from an Agilent 6545 LC quadrupole time-of-flight (Q-TOF) Auto MS/MS analysis of tryptically-digested peanut were screened for known peanut peptides using Agilent MassHunter Qualitative Analysis software to identify candidate peptide markers. The peptides with the highest response, but not presenting in blanks, were selected for TQ LC/MS quantitative method development using the Agilent 6470 Triple Quadrupole (TQ) LC/MS system and the Agilent MassHunter Optimizer for Peptides software. Raw and cooked samples of three dry wheat-flour-based matrices were analyzed to evaluate the recovery, accuracy, repeatability, and reproducibility of the optimized 6470 TQ LC/MS quantitative method using the peanut protein Ara h 1 as a standard. Linearity and sensitivity were evaluated using raw wheat flour matrix.

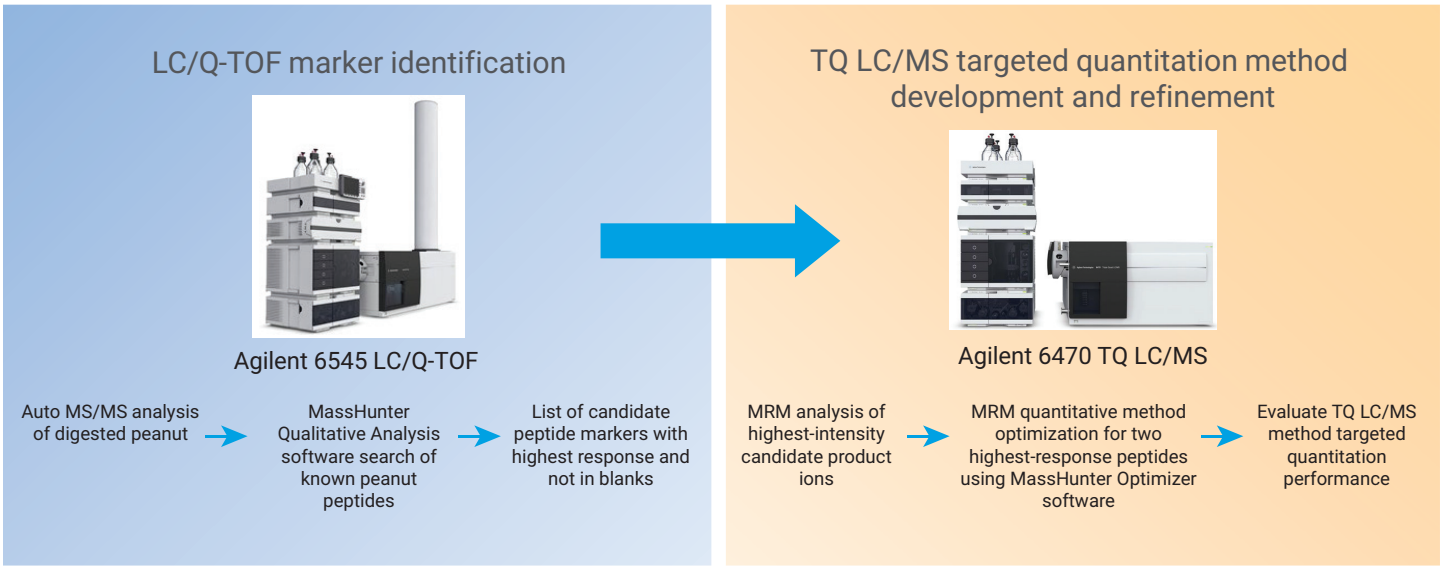

Figure 1. Workflow overview: LC/Q-TOF marker identification to TQ LC/MS targeted quantitation.

Experimental

Reagents and standards

Tris(hydroxymethyl)aminomethane, trichloroacetic acid, dithiothreitol, iodoacetamide, trypsin, and salt (NaCl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid was purchased from Fisher Scientific (Shanghai, China). Hydrochloric acid was purchased from Sinopharm (Beijing, China). Acetonitrile was purchased from Merck (Darmstadt, Germany). Ultrapure water was generated from a MilliQ system (Millipore, Bedford, MA, USA).

Raw shelled peanut, wheat flour, and hydrogenated soybean oil (HSO) were purchased from a local market. Peanut protein standard Ara h 1 (1.4 mg/mL) for calibration curve development was purchased from Indoor Biotechnologies (Charlottesville, VA, USA). Synthesized natural and isotopically labelled peptides (1 mg each) were purchased from GenScript (Piscataway, NJ, USA).

Wheat flour sample matrices

Three food matrices with peanut added at different concentrations were made for the method evaluation:

- Wheat flour (WF)
- A mixture of wheat flour and HSO (WF-HSO) (80/20, w/w)
- A mixture of wheat flour, HSO, and NaCl (WF-HSO-NaCl) (79/20/1, w/w/w).

The raw shelled peanut was ground into powder and then mixed into the matrices using a pulverizer. To obtain homogeneous samples, mixing was repeated ten times for ten seconds each. Raw samples with total peanut at a concentration of 80 g/kg were prepared and serially diluted with the food matrices to prepare samples with total peanut concentrations of 20 g/kg, 4 g/kg, 400 mg/kg, 40 mg/kg, and 10 mg/kg. Samples without peanut were prepared as negative controls.

Cooked samples were made from the corresponding raw samples. Raw sample (4 g) was weighed into a 100 g crucible and cooked at 180 °C for 15 minutes. The cooked samples were allowed to cool to room temperature and ground into powder using a mortar. Samples from individual crucibles of the same matrix were pooled and labeled: CWF for wheat flour matrix, CWF-HSO for wheat flour-HSO matrix, and CWF-HSO-NaCl for wheat flour-HSO-NaCl matrix. Cooked samples were stored at –20 °C until use.

Sample preparation: LC/Q-TOF peanut peptide marker identification

A sample (0.5 g) was weighed into a 15 mL tube and extracted with 5 mL Tris buffer (50 mM, pH 8.5) at 75 °C for 2 hours using a shaking water bath at 180 rpm. The solution was then centrifuged at 4,000 rpm for 10 minutes and the supernatant filtered. One mL of the filtrate was digested at 37 °C overnight using trypsin at a ratio of 1:40 (enzyme: total protein). Digestion was stopped by addition of 511 µL 30% trichloroacetic acid (v/v). After 10 minutes, the solution was centrifuged at 14,000 rpm for 10 minutes.

The supernatant was purified using an Agilent Bond Elute Plexa column (part number 12109603). The column was conditioned using 1 mL of acetonitrile and equilibrated using 1 mL of 1% formic acid (v/v). Next, 1.4 mL of the supernatant was loaded onto the column where interferences were washed away using 1 mL of 1% formic acid (v/v), and the analyte was eluted using 1 mL of 50% acetonitrile (v/v). The eluate was evaporated to dryness at room temperature under a nitrogen stream, and the residue was redissolved in 100 µL of 5% acetonitrile (v/v).

Instrumentation and analysis: LC/Q-TOF peanut peptide marker identification

LC/Q-TOF peptide marker identification experiments were carried out using an Agilent 1290 Infinity LC system coupled to an Agilent 6545 Q-TOF mass spectrometer. The LC system was equipped with a binary pump and the Agilent AdvanceBio Peptide Mapping column. The LC parameters are provided in Table 1.

Table 1. LC parameters for Q-TOF analysis.

Agilent 1290 Infinity LC with Binary Pump	
Analytical Column	Agilent AdvanceBio Peptide Mapping column, 2.1 × 100 mm, 2.7 µm (p/n 655750-902)
Injection Volume	10 µL
Autosampler Temperature	10 °C
Column Temperature	50 °C
Mobile Phase	A) 5% acetonitrile (v/v) with 0.05% formic acid (v/v) B) 95% acetonitrile (v/v) with 0.05% formic acid (v/v)
Flow Rate	0.3 mL/min
Gradient	Time (min) %B 2.0 hold 3 80.0 linear gradient 40 82.0 90 85.0 hold 90 86.0 3 90.0 re-equilibration 3
Run Time	90 min

The 6545 LC/Q-TOF mass spectrometer was operated in positive mode using the Agilent Auto MS/MS capability. Auto MS/MS performs real-time automated MS/MS analysis to capture the best fragmentation information from a selection of prominent ions. The top ten precursors with thresholds over 3,000 were chosen for Auto MS/MS analysis using ramped collision energy. For precursor ions with two charges, slope and offset were 3.1 and 1. For precursor ions with three or more charges, slope and offset were 3.6 and −4.8. The Q-TOF mass spectrometer parameters are summarized in Table 2.

Samples of all food matrices with 20 g/kg total peanut and blank samples were analyzed. After data acquisition, the accurate *m/z* values were extracted, and the peak areas were integrated manually. The data were screened using Agilent MassHunter Qualitative Analysis software to search a database of previously identified peanut peptides (Table 3).

The LC/Q-TOF screening results were evaluated to determine which of the peptides identified were the best candidates for TQ LC/MS quantitative method development. Candidate peptide markers were selected if a) they were detected with high and constant responses in paired raw and cooked samples containing 20 g/kg total peanut, and b) if they were not detected in blank samples. The eleven peptides shown in blue in Table 3 were selected.

Table 2. Q-TOF mass spectrometer parameters.

Agilent 6545 LC/Q-TOF	
Ionization Mode	Positive
Drying Gas Temperature	325 °C
Drying Gas Flow Rate	9 L/min
Nebulizer	45 psi
Sheath Gas Temperature	275 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	4,000 V
Fragmentor Voltage	175 V
Skimmer 1 Voltage	65 V
Octupole RF Peak Voltage	750 V
MS Scan Range	100 to 1,700 *m/z*
MS Scan Rate	8 spectra/sec
MS/MS Isolation	Narrow

Table 3. Peanut peptides used for qualitative analysis of LC/Q-TOF Auto MS/MS data. The eleven candidates selected for subsequent TQ LC/MS experiments are highlighted in blue.

Peptide	*m/z* (+1)	*m/z* (+2)	*m/z* (+3)	Allergen	Reference
VLLEENAGGEQEER	1572.7500	786.8786	524.9215	Ara h 1	18
DLAFPGSGEQVEK	1376.6692	688.8383	459.5613	Ara h 1	8
WLGLSAEYGNLYR	1541.7747	771.3910	514.5964	Ara h 3	19
QQPEENACQFQR	1477.6489	739.3281	493.2211	Ara h 3	20
CCNELNEFENNQR	1612.6479	806.8276	538.2208	Ara h 2	20
NLPQQCGLR	1028.5306	514.7689	343.5150	Ara h 2	20
CDLDVSGGR	921.4095	461.2084	307.8080	Ara h 6	20
NLPQNCGFR	1049.4993	524.7533	350.1713	Ara h 7	20
GTGNLELVAVR	1128.6371	564.8222	376.8839	Ara h 1	21
CMCEALQQIMENQSDR	1898.7864	949.8968	633.6003	Ara h 2	22
AHYQVVDSNGDR	1360.6240	680.8156	454.2129	Ara h 3	22
LNAQRPDNR	1083.5654	542.2863	361.8600	Ara h 3	23
RPFYSNAPQEIFIQQGR	2051.0457	1026.027	684.3534	Ara h 3	23
NNPFYFPSR	1141.5425	571.2749	381.1857	Ara h 1	24
SFNLDEGHALR	1258.6175	629.8124	420.2107	Ara h 1	24
NTLEAAFNAEFNEIR	1738.8395	869.9234	580.2847	Ara h 1	24
IFLAGDKDNVIDQIEK	1817.9644	909.4858	606.6596	Ara h 1	24
TANDLNLLILR	1255.7369	628.3721	419.2505	Ara h 3	21
AHVQVVDSNGDR	1296.6291	648.8182	432.8812	Ara h 3	21
AQSENYEYLAFK	1462.6849	731.8461	488.2331	Ara h 3	25
QFQNLQNHR	1184.5919	592.7996	395.5355	Ara h 1	23
LFEVKPDDK	1090.5779	545.7926	364.1975	Ara h 1	25
ANLRPCEQ	930.4462	465.7267	310.8202	Ara h 2	25
NEFENNQR	1050.4599	525.7336	350.8248	Ara h 2	25
VYDEELQEGHVLVVPQNFAVAGK	2541.2984	1271.153	847.771	Ara h3	9

Sample preparation: TQ LC/MS peanut quantification method

Sample preparation for the TQ LC/MS experiments used the optimized procedure developed by Chang *et al*.[17] Sample purification was required because the relatively high abundance of wheat flour proteins can suppress ionization in the mass spectrometer ion source, making it difficult to detect the target peptides digested from trace-level peanut proteins. Therefore, samples were heated to selectively precipitate the wheat-flour proteins, improving the detectability of peanut peptides.

For the procedure, a sample (0.5 g) was weighed into a 15 mL tube and extracted with 5 mL of Tris buffer (50 mM, pH 8.5) for 30 minutes using a multiple mixer (Heidolph, Schwabach, Germany). The solution was then heated to 75 °C for 30 minutes in a shaking water bath at 180 rpm, followed by centrifugation at 4,000 rpm for 30 minutes. The supernatant was filtered using filter paper.

Synthetic isotopically labelled peptides DLAFPGSGEQVE {Lys($^{13}C_6$,$^{15}N_2$)} and IFLAGDKDNVIDQIE {Lys($^{13}C_6$,$^{15}N_2$)}, were reconstituted and diluted to 25 ng/mL using 20% acetonitrile (v/v). Twenty microliters of the diluted synthetic isotopically labelled peptides were added as internal standards and 1 mL of the filtrate was digested at 37 °C overnight using trypsin at a ratio of 1:100 (enzyme: total protein). The ratio of trypsin to protein was chosen as the best combination of sensitivity and repeatability.[17] Digestion was stopped by addition of 511 µL of 30% trichloroacetic acid (v/v). After 10 minutes, the digested solution was centrifuged at 14,000 rpm for 10 minutes. Supernatant purification with the Agilent Bond Elute Plexa column, evaporation to dryness, and redissolution in acetonitrile used the same steps as the sample preparation for the LC/Q-TOF analysis.

Instrumentation and analysis: TQ LC/MS peanut quantification

TQ LC/MS experiments were carried out using an Agilent 1290 Infinity LC system coupled to an Agilent 6470 TQ mass spectrometer. The LC system configuration used for the 6470 TQ LC/MS analyses was the same as that used for the LC/Q-TOF analyses, along with the same chromatographic parameters (Table 1), except for the gradient (Table 4). The total run time was 36 minutes.

Table 4. LC gradient used for 6470 TQ LC/MS peanut quantitation.

Gradient	Time (min) 0 28.0 28.1 32.0 32.1 36.0	%B 5 25 100 100 5 5
Run Time	36 min	

Table 5. TQ mass spectrometer parameters.

Agilent 6470 TQ LC/MS	
Ionization Mode	Positive
Drying Gas Temperature	350 °C
Drying Gas Flow Rate	9 L/min
Nebulizer	45 psi
Sheath Gas Temperature	380 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	4,000 V
Nozzle Voltage	500 V

The 6470 TQ LC/MS was operated in positive ion mode with MRM data acquisition. The mass spectrometer parameters are provided in Table 5.

To develop the 6470 TQ LC/MS MRM method, two product ions from each of the eleven candidate peptides that had the highest intensity in the MS/MS spectrum acquired by LC/Q-TOF Auto MS/MS analysis were selected as quantifier and qualifier ions (Table 6). The raw and cooked samples containing 10 mg/kg total peanut were analyzed and the absolute peak areas obtained were compared. The peptides DLAFPGSGEQVEK and IFLAGDKDNVIDQIEK of Ara h 1 had the highest responses and were therefore selected for further TQ LC/MS quantitative method refinement.

To optimize the MRM parameters, synthesized natural and isotopically labelled target peptides, diluted to 1 µg/mL in 20% acetonitrile (v/v), were analyzed using the Agilent MassHunter Optimizer for Peptides tool. Though most TQ mass spectrometer parameters can be set using the Autotune functions in MassHunter software, the MassHunter Optimizer automatically optimizes MRM parameters for each individual compound specified, including selection of the best precursor ions and product

Table 6. Candidate peptides and their precursor, quantifier, and qualifier ions used for 6470 TQ LC/MS method development.

Peptide	Precursor	Quantifier	Qualifier
VLLEENAGGEQEER	786.9	804.4	680.8
DLAFPGSGEQVEK	688.8	930.5	465.7
GTGNLELVAVR	564.8	686.4	557.5
VYDEELQEGHVLVVPQNFAVAGK	847.8	931.5	466.3
NNPFYFPSR	571.3	821.4	1,141.5
SFNLDEGHALR	629.8	797.4	682.4
NTLEAAFNAEFNEIR	869.9	1,139.5	992.5
IFLAGDKDNVIDQIEK	606.7	779.4	722.9
TANDLNLLILR	628.4	1,083.6	741.5
AQSENYEYLAFK	731.8	1,263.6	722.8
QFQNLQNHR	592.8	583.8	554.3

ions, fragmentor voltage for each precursor ion, and collision energy for each transition. The settings used for the MassHunter optimization are provided in Table 7. The resulting optimized transitions and parameters are provided in Table 8. For each target peptide, three MRM transitions are monitored, two for the natural target peptide and one for the corresponding isotopically labeled peptide.

TQ LC/MS peanut quantification method evaluation

The linearity, sensitivity, recovery, accuracy, repeatability, and reproducibility of the optimized 6470 TQ LC/MS peanut quantification method were evaluated.

Linearity was determined using matrix-matched standards. Peanut protein was used as the standard for accurate and simple quantification. Nine concentrations of Ara h 1 were prepared by serial dilution of the Ara h 1 (1.4 mg/mL) standard solution. The concentrations of Ara h 1 ranged from 0.55 to 140 ng/mL, corresponding to 0.15 to 40 mg/kg total peanut. Fifty microliters of each of the nine solutions and 20 µL of internal standard peptides were mixed with 0.98 mL raw flour extract. After TQ LC/MS analysis, the calibration curve was created by plotting relative peak areas of target peptide (normalized with internal standards) against concentrations of Ara h 1.

The sensitivity was defined as the limit of quantitation (LOQ) at the lowest concentrations. These values were compared to results previously obtained from commercial ELISA kits.

Recovery was calculated for all the sample matrices at three concentrations of Ara h 1 in four replicate analyses. Ara h 1 was spiked into raw flour extract to prepare solutions of 3.5, 0.7, and 0.175 µg/mL, corresponding to 10, 2, and 0.5 mg/kg total peanut. Then, 50 µL of each of the three solutions were spiked into 0.5 g of each blank sample and thoroughly mixed. After TQ LC/MS analysis, recovery was calculated based on the detected and spiked concentrations.

To evaluate 6470 TQ LC/MS method accuracy, repeatability, and reproducibility, samples of all matrices spiked with 10 mg/kg total peanut were analyzed. Three batches of samples were analyzed over three days. For each batch, there were four samples (two independent samples with two replicates for each matrix). Repeatability was calculated as RSDs for four samples in each batch. Reproducibility was calculated as RSDs for three average values from all batches.

Table 7. Agilent MassHunter Optimizer software parameters used for automated MRM method development.

Parameter	Setting
Precursor Ion Selection	Positive ions with +H, charge state of 2 or 3
Product Ion Selection	Up to four with low mass cut-off value of 80% precursor mass
Fragmentor Voltage	From 100 to 150 V in steps of 5 V
Collision Energy	From 5 to 40 V
Cell Accelerator Voltage	3 eV

Table 8. MassHunter Optimizer software-generated MRM transitions and parameters for TQ LC/MS targeted quantitation of peanut.

Peptide sequence	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor Voltage (V)	Collision Energy (V)	Quantifier or Qualifier	Ion Ratio %[a]
DLAFPGSGEQVEK	688.8	930.4	120	22	Quantifier	39.8
		465.7	120	25	Qualifier	
DLAFPGSGEQVE{Lys($^{13}C_6$,$^{15}N_2$)}	692.8	938.5	120	21	Quantifier	
IFLAGDKDNVIDQIEK	606.6	779.4	115	17	Quantifier	43.8
		722.9	120	15	Qualifier	
IFLAGDKDNVIDQIE{Lys($^{13}C_6$,$^{15}N_2$)}	609.6	783.6	110	17	Quantifier	

[a] Measured using 35 ng/mL Ara h 1 standard solution (corresponding to 10 mg/kg total peanut).

Results and discussion

TQ LC/MS method performance

The LOQ of the 6470 TQ LC/MS method was lower than most previously reported results for commercial ELISA kits (Table 9).[17] The method also provided good linearity, with R^2 values above 0.99 from 0.31 to 40 mg/kg total peanut, which is wider than previously reported for the commercial ELISA kits (Table 9).

Recovery was evaluated at three concentrations of Ara h 1 representing 10, 2, and 0.5 mg/kg total peanut. Recoveries were satisfactory in all matrices, demonstrating that the method is applicable for quantification of peanut in all the matrices studied. Figure 2 shows the recovery values for the target peptides DLAFPGSGEQVEK and IFLAGDKDNVIDQIEK at the three spike levels.

Table 9. Comparison of linearity and sensitivity: 6470 TQ LC/MS method and ELISA.[17]

Method	Linear Range (mg/kg)	LOQ (mg/kg)	Kit
Agilent 6470 TQ LC/MS (Raw and Cooked Flour)	0.31 to 40	0.31	n/a
ELISA	2.5 to 25	2.5	Neogen Veratox
	1 to 20	1	Neogen BioKits
	1 to 40	1	Romer AgraQuant
	1 to 15	1	ELISA Systems
	0.3 to 20	0.3	Morinaga

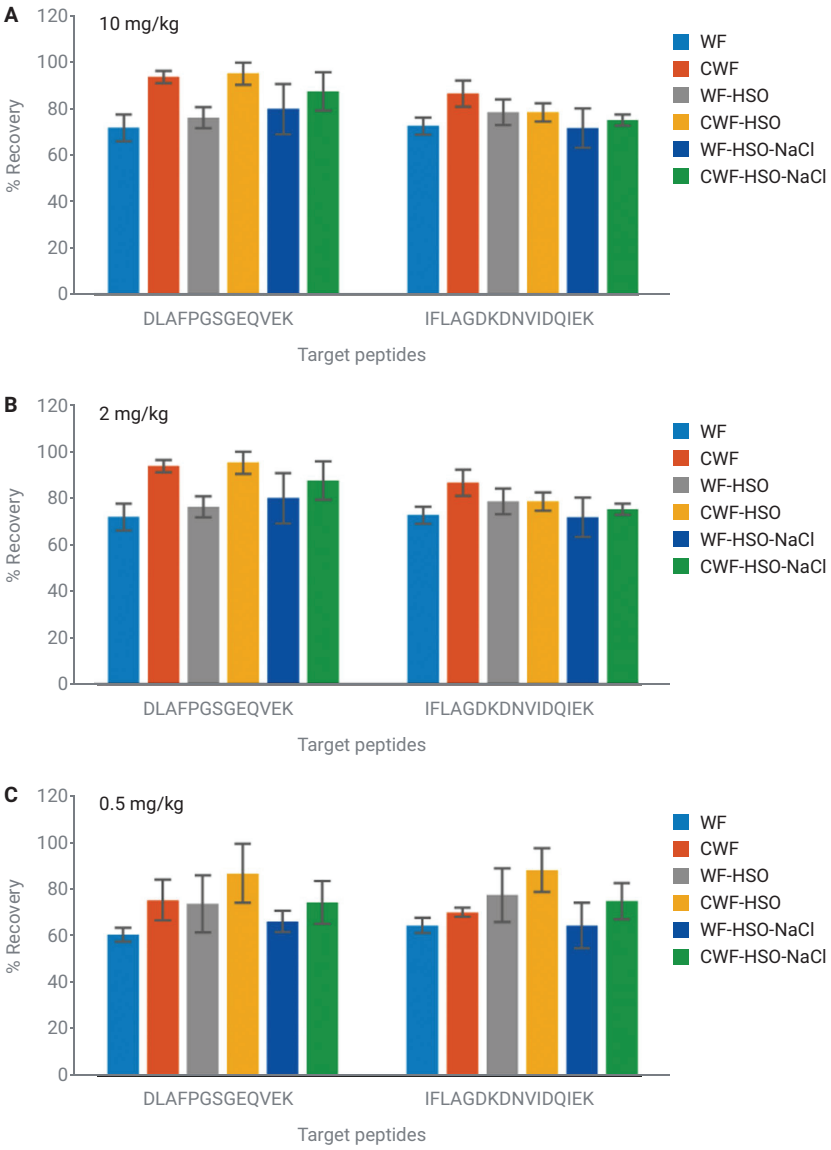

Figure 2. Percent recovery with RSD (n = 4) for target peptides digested from Ara h 1 in spiked samples containing total peanut concentrations of 10, 2, and 0.5 mg/kg.

For the quantification accuracy experiments, the results were acceptable according to EU guidance (2002/657/EC) as the ratio of qualifier ion to quantifier ion of the target peptide was within 25% of those of the standard as listed in Table 8.[26] The quantification results for each of the matrices analyzed are presented in Figure 3 and Table 10.

There was a difference of approximately 10% between the measured and actual values of peanut in the WF and WF-HSO matrices, and about a 20% difference between the measured and actual values of peanut in the CWF and CWF-HSO matrices. The reason for the difference may be that the quantification method assumes that Ara h 1 accounts for 14% of total protein in peanut. However, the percentage of Ara h 1 in total protein ranges between 12% and 16% depending on the peanut variety. In addition, the quantification used the calibration curve built with raw flour extract, which may not fully compensate for matrix effects in cooked samples. There was a difference of approximately 30% between measured and actual values of peanut in WF-HSO-NaCl and CWF-HSO-NaCl matrices, indicating that the TQ LC/MS method would need to be optimized to provide accurate quantitation in other food matrices.

As shown in Table 11, the optimized 6470 TQ LC/MS quantification method provided good repeatability and reproducibility. Repeatability was calculated as RSDs for four samples in each batch.

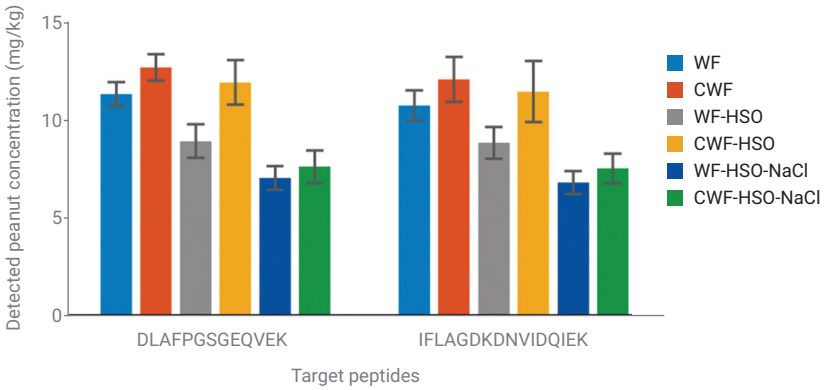

Figure 3. Total peanut concentration measured using the target peptides DLAFPGSGEQVEK and IFLAGDKDNVIDQIEK for TQ LC/MS quantification in each of the study matrices.

Table 10. Agilent 6470 TQ LC/MS quantitation accuracy with RSD% for each peptide, in each of the matrices studied.

Matrix	DLAFPGSGEQVEK		IFLAGDKDNVIDQIEK	
	Peanut concentration (mg/kg)	RSD%	Peanut Concentration (mg/kg)	RSD%
WF	11.4	5.48	10.8	7.18
CWF	12.8	5.34	12.2	9.60
WF-HSO	9.00	9.71	8.90	9.18
CWF-HSO	12.0	9.59	11.5	13.7
WF-HSO NaCl	7.00	8.77	6.80	8.75
CWF-HSO NaCl	7.60	10.9	7.60	10.2

Table 11. Agilent 6470 TQ LC/MS method repeatability and reproducibility (two replicates of two independent samples for three batches).

Peptide	Metric	WF	CWF	WF-HSO	CWF-HSO	WF-HSO-NaCl	CWF-HSO-NaCl
DLAFPGSGEQVEK	Repeatability (%)						
	Batch 1	6.26	7.40	14.7	2.66	2.49	15.0
	Batch 2	5.71	2.50	9.07	14.6	12.7	7.03
	Batch 3	2.83	5.16	4.10	8.26	7.21	2.61
	Reproducibility (%)	3.46	2.70	2.73	3.51	4.29	6.87
IFLAGDKDNVIDQIEK	Repeatability (%)						
	Batch 1	4.65	5.40	14.0	2.72	4.11	15.4
	Batch 2	11.8	5.00	5.32	19.5	9.44	4.53
	Batch 3	4.28	4.03	8.01	12.2	8.00	6.41
	Reproducibility (%)	2.35	10.0	2.22	7.07	6.16	4.70

Workflow development

The LC/Q-TOF marker identification to TQ LC/MS targeted quantitation workflow enabled the development of a sensitive, accurate, and reproducible TQ LC/MS method for peanut quantification in both raw and cooked wheat flour matrices. The workflow is applicable to the development of a range of TQ LC/MS methods for quantifying target compounds in complex matrices using the potential markers identified.

Ideally suited to untargeted sample analysis, the 6545 Q-TOF mass spectrometer offers broad screening and comprehensive profiling. Due to its resolving power, mass accuracy, and the capability of full-spectrum measurement, almost every compound that ionizes can be detected. However, the amount of data gathered this way can appear overwhelming. Auto MS/MS addresses this concern with real-time automated MS/MS analysis that captures the best fragmentation information from a selection of prominent ions for subsequent data analysis. Following Q-TOF Auto MS/MS analysis of tryptically-digested peanut, MassHunter Qualitative Analysis software enables automated screening of the acquired data for known peanut peptides.

Similarly, 6470 TQ LC/MS quantitative method development was substantially streamlined using Agilent MassHunter Optimizer for Peptides. Development of MRMs can be a challenging and time-consuming multistep process that is complicated by analyte coelution and matrix interferences. With minimal user setup, MassHunter Optimizer automatically optimizes MRM parameters for each individual compound specified, including selection of the best precursor ions and product ions, fragmentor voltage for each precursor ion, and collision energy for each transition.

Conclusion

Peanut allergy is of worldwide concern, making regular testing of foods and their raw materials and accurate package labeling an imperative for the food industry and regulatory agencies. Because quantification results among ELISA kits from different manufacturers can be inconsistent and could under- or overestimate allergen concentrations, a more accurate approach is desired. This application note presented an LC/Q-TOF marker identification to TQ LC/MS targeted quantitation workflow that was used to develop and optimize a reliable and accurate method to quantify peanut in food matrices. The linearity, sensitivity, recovery, accuracy, repeatability, and reproducibility of the 6470 TQ LC/MS peanut quantification method were evaluated and determined to exceed overall ELISA performance.

Method development used the Auto MS/MS tool and MassHunter Qualitative Analysis software to screen tryptically-digested peanut Q-TOF data for known peanut peptides. MRM method optimization was substantially streamlined using the Agilent MassHunter Optimizer. Overall, the workflow is applicable to the development of a range of TQ LC/MS MRM methods for accurately quantifying target compounds in complex matrices using the potential markers identified.

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