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Quantitative Analysis of Acrylamide in Peanut Butter using LC Triple Quadrupole Mass Spectrometry

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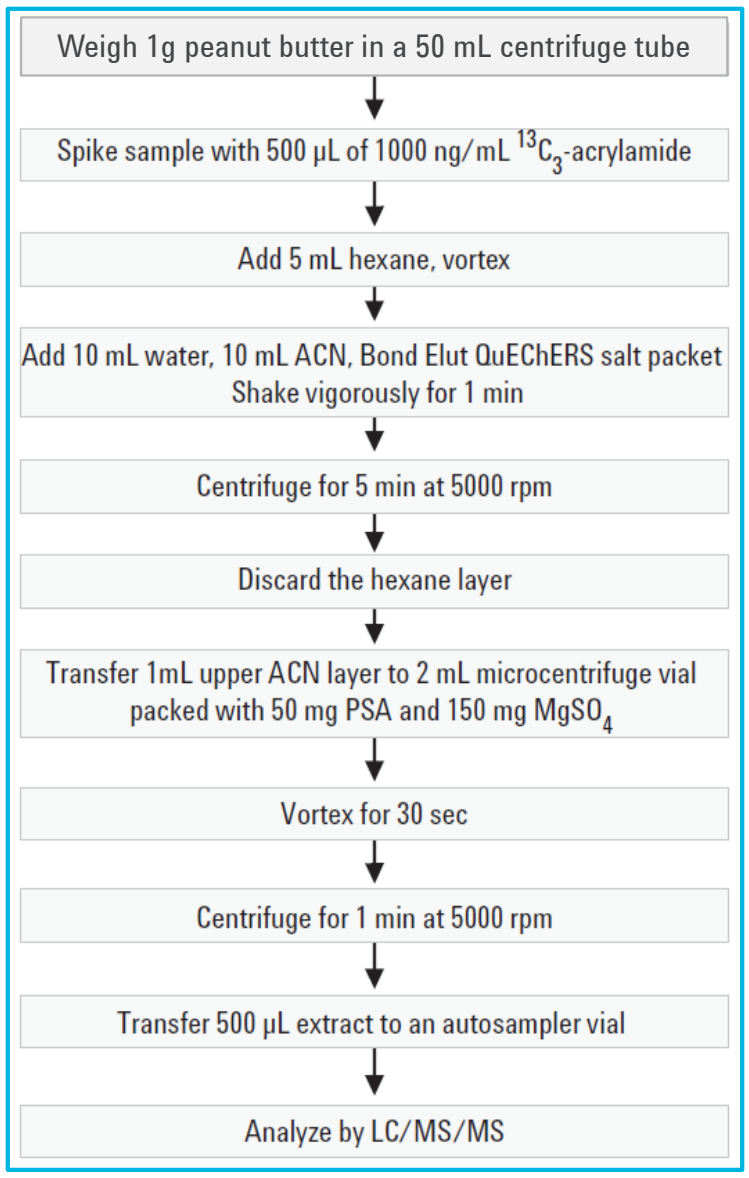
Introduction

Acrylamide forms in carbohydrate-rich foods that are subjected to high temperature such as frying, baking and extrusion. Though epidemiological studies suggest it is unlikely that dietary acrylamide consumption increases people's risk of developing cancer despite it being a probable carcinogen, high levels of acrylamide are found primarily in potato products, bakery products, etc. Due to the low molecular weight and the high solubility in water, the challenges of acrylamide analysis in food arises in both sample preparation and the mass spectrometry method. Herein, this study presents a simple and rapid sample preparation procedure for the analysis of acrylamide in peanut butter that would be directly compatible with a fast and sensitive LC/MS/MS assay.

Experimental



Chemicals and Standard Solutions

Both acrylamide and internal standard acrylamide-d3 were purchased from Sigma Aldrich. Peanut butter was purchased from local grocery store. Both acrylamide standard and internal standard stock solutions were prepared in acetonitrile.

Sample Preparation

The flow chart of the QuEChERS sample preparation procedure is shown on left[1]. One gram of peanut butter was weighed into a 50 mL centrifuge tube from the Agilent Bond Elut QuEChERS Extraction Acrylamide kit (p/n 5982-5850). The internal standard was spiked into the peanut butter sample at 50 ng/g. Hexane (5 mL) was used to defat the extract with water (10 mL) and acetonitrile (10 mL) added[2]. The extraction salt packet was added to the spiked sample and the tube was shaken for 1 min vigorously and centrifuged at 5000 rpm for 5 min. 1 mL of acetonitrile layer was transferred to a 2 mL Bond Elut QuEChERS AOAC Dispersive SPE tube (p/n 5982-5022). The tubes were vortexed for 30 sec and then centrifuged at 5000 rpm for 1 min. The supernatant was then placed in an autosampler vial for LC/MS analysis.

Calibration Curves

Due to the lack of matrix blank, both standard addition ISTD curve and reversed ISTD curve were tested in this study. The standard addition approach started from 5 to 2000 ng/g and the reversed curve was performed from 0.1 to 200 ng/g.

LCMS Method

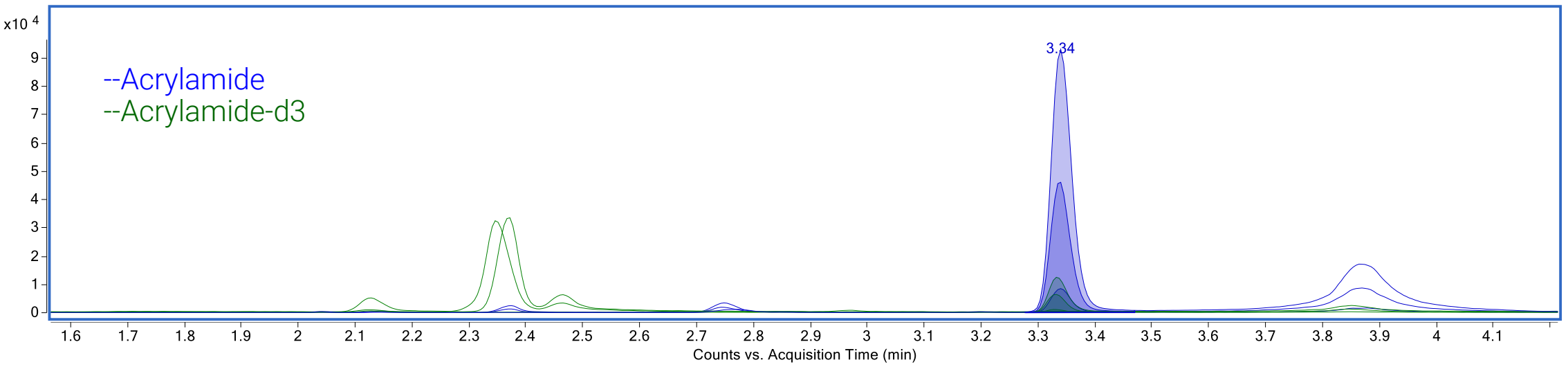
Agilent 1290 II UHPLC Conditions			
Column	Poroshell 120 EC-C18, 2.7 um, 3x150mm, p/n 693575-302		
Column temp	40 °C		
Injection volume	1 µL		
Autosampler temp	5 °C		
Needle wash	10 sec, MeOH:water 50:50		
Mobile phase	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile		
Gradient program	Time	B (%)	Flow rate (mL/min)
	0.00	2	0.250
	4.00	2	0.250
	4.01	100	0.250
	6.00	100	0.250
	6.01	2	0.250

Agilent 6470 Source Parameters		MRM Parameters	
Ion mode	AJS Positive	Resolution	Q1 / Q2 = unit
Gas temp	150 °C	Cell Accelerator	2 V
Drying gas flow	4 L/min	Total MRMs	6
Nebulizer gas	60 psi	Cycle time	312 ms
Sheath gas temp	400 °C	Dwell time	50 ms
Sheath gas flow	12 L/min	Fragmentor	50 V
Capillary voltage	2000 V		
Nozzle voltage	0 V		

MRM Transitions	Precursor	Product	CE
Acrylamide-d3	75.1	58.1/44.2/30.2	12/36/28
Acrylamide	72.1	55.1/44.2/27.2	12/36/28

Extraction, Clean Up and LC Separation

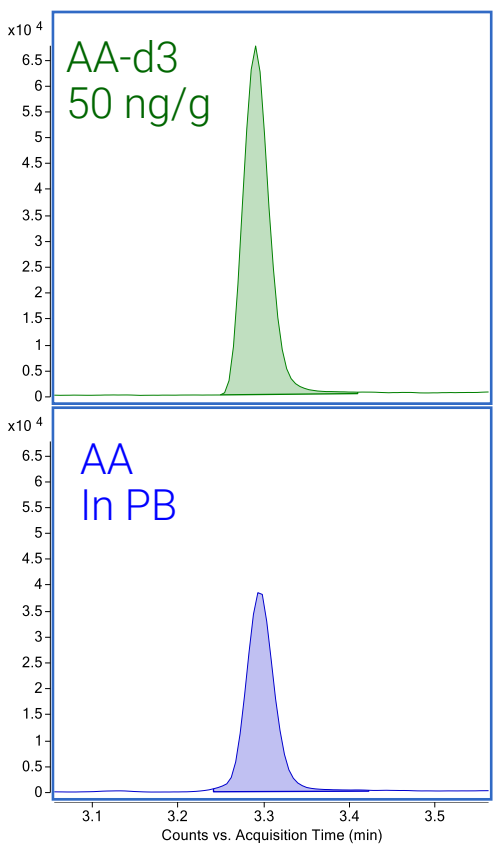
In the extraction, the hexane was used to remove fat from peanut butter. The addition of water was used to facilitate extraction of acrylamide from the matrix. Dispersive SPE was employed for direct sample clean up, without solvent evaporation, to simplify and speed the long and tedious SPE process. The 3 mm ID reversed phase column was compatible with the cleaned-up extract. Only 1 uL of extract, which is mainly in acetonitrile, was injected to avoid possible solvent effects due to acrylamide hydrophilicity. An isocratic gradient with 2% mobile phase B and a low flow rate of 0.25 mL/min was applied to separate acrylamide from the complicated matrix interference. This isocratic gradient retains acrylamide at 3.34 min. A flushing gradient with high organic solvent was applied to clean the column, followed with a longer equilibration time to assure the reproducibility of retention times from injection to injection.

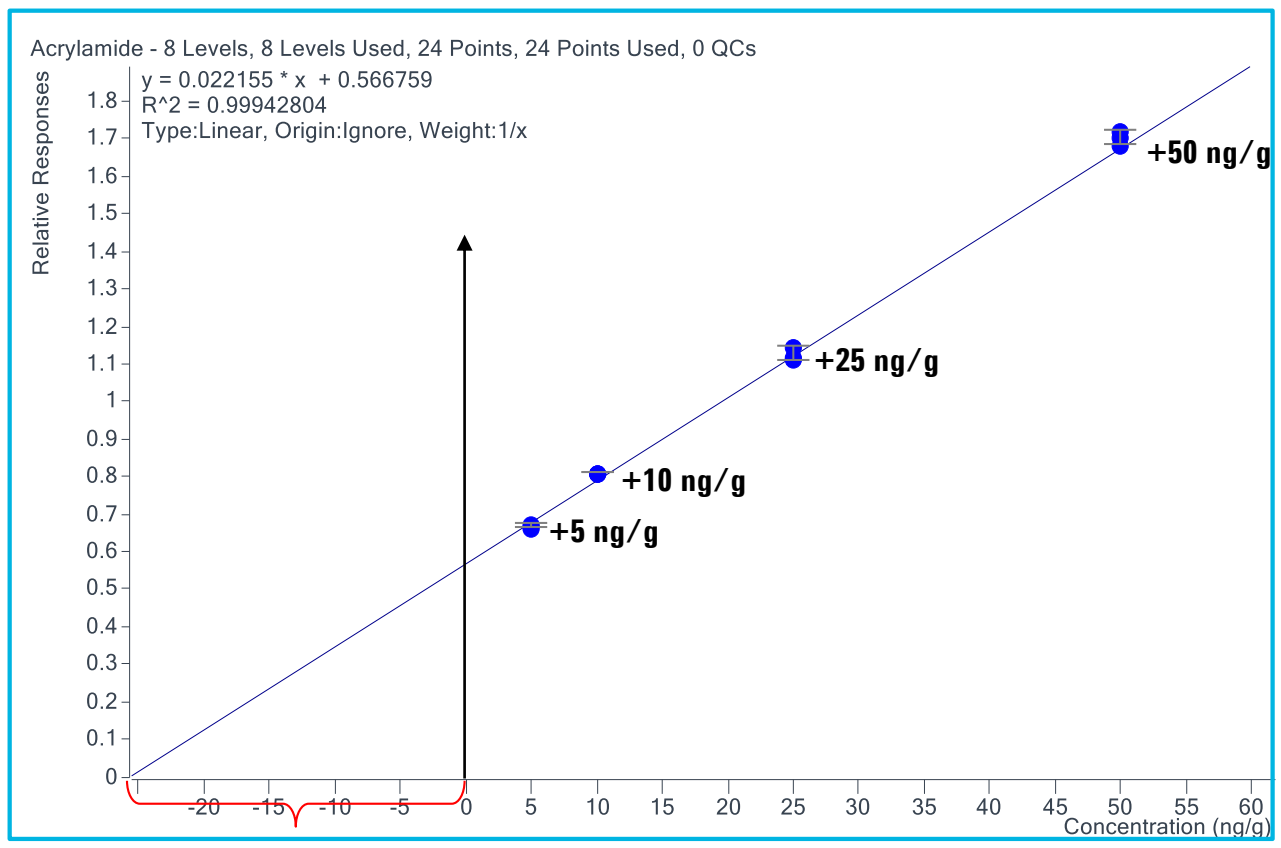


Standard Addition Calibration Curve

Before extraction, standard addition ISTD calibration curves were obtained by constantly spiking the internal standard (acylamide-d3) at a concentration of 50 ng/g, to the peanut butter sample containing the calibration standard (acrylamide) at levels from 5 to 2000 ng/g. Excellent linearity was observed (r^2>0.9994). Accuracy across the dynamic range was from 87.3 to 107.9% (n=3) with the %RSD lower than 6.30% (n=3) (table on left). Abundant response of acrylamide was observed in peanut butter without the spiked internal standard (middle). The acrylamide amount in peanut butter can be calculated by using the linear equation obtained from the standard addition curve (right), y=0.022155x+0.566759. When the addition amount is zero, i.e. x=0, the response on y axis represents the endogenous level of acrylamide in peanut butter as 25.58 ng/g (%RSD of 0.59, n=3)

Conc. (ng/g)	Accuracy (%, n=3)	%RSD (n=3)
5	87.3	6.30
10	107.9	0.67
25	100.4	3.39
50	102.3	1.53
100	101.3	2.92
500	99.4	1.13
1000	102.6	2.96
2000	98.7	0.63





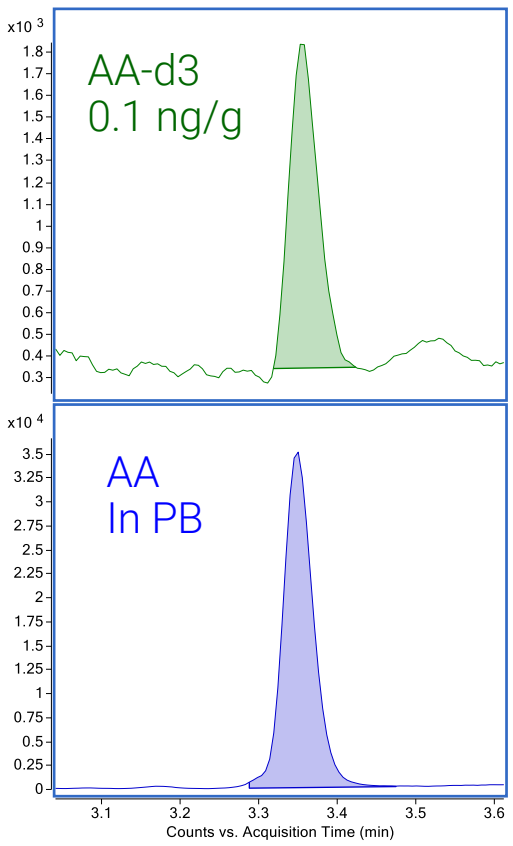
Reversed ISTD Calibration Curve

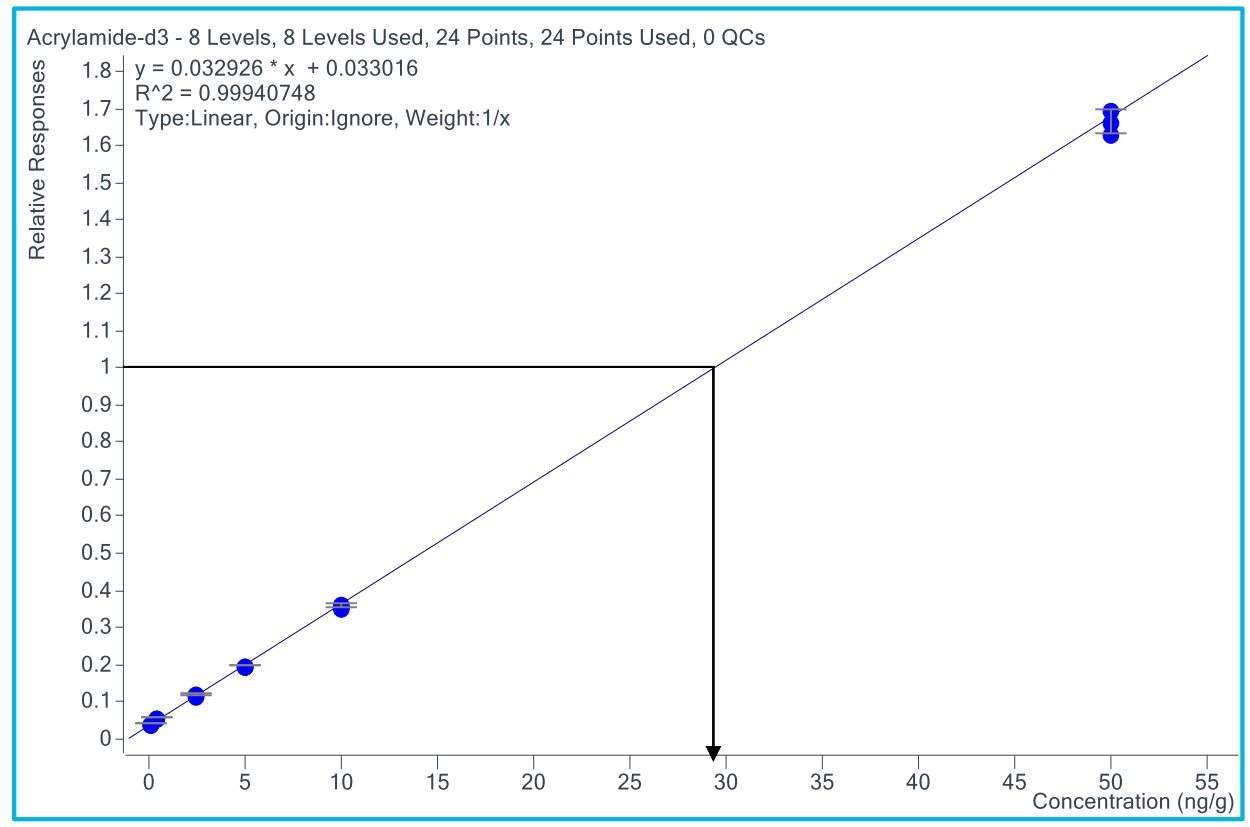
In the reversed ISTD calibration approach, only the internal standard was spiked into the peanut butter from 0.1 to 200 ng/g before the extraction. The endogenous acrylamide in the peanut butter is assigned as the internal standard while acrylamide-d3 is the targeted compound in the quantitative batch.

Results and Discussion

Excellent results were achieved in the reversed calibration approach with linearity (r^2>0.9994), accuracy from 96.9 to 103.4% (n=3) and %RSD lower than 6.15% (n=3) (table on left). The LOQ of acrylamide-d3 at 0.1 ng/g was obtained in peanut butter (middle) to demonstrate the whole assay performance, from sample prep to LCMS analysis. The acrylamide amount in peanut butter can be calculated by using the linear equation obtained from the reversed curve (right), y=0.032926x+0.033016. When the STD/ISTD ratio is 1. i.e. y=1, the calculated concentration represents the endogenous level of acrylamide in peanut butter as 29.21 ng/g.

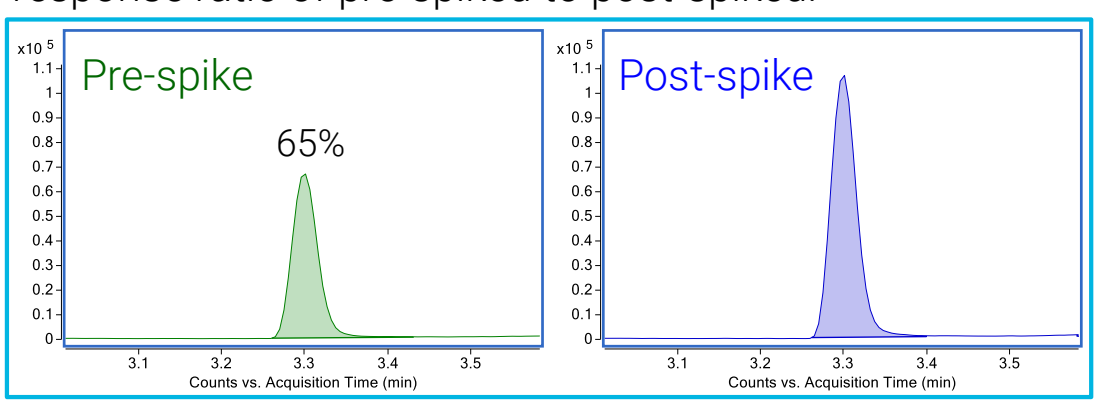
Conc. (ng/g)	Accuracy (%, n=3)	%RSD (n=3)
0.1	103.4	6.15
0.5	102.2	1.38
2.5	100.2	4.47
5	96.9	1.26
10	97.7	1.78
50	98.9	2.05
100	100.4	2.55
200	100.2	3.14





Sample Recovery

Sample recovery was evaluated by spiking 50 ng/g d3-acrylamide into the peanut butter pre- and post-sample preparation. A recovery of 65% was calculated as the response ratio of pre-spiked to post-spiked.



Sample Preparation Reproducibility

The two-step sample preparation was repeated in triplicate and followed with a triplicated LCMS analysis, which had an overall RSD less than 4%. Table below.

AA-d3 50ng/g	LCMS repeats (ng/g)	Average (ng/g)	Stdev (ng/g)	%RSD
	42.47			
Sample Prep 1	43.37	42.46	0.91	2.14
	41.55			
	43.07			
Sample Prep 2	44.08	43.32	0.67	1.54
	42.82			
	46.75			
Sample Prep 3	45.60	45.72	0.98	2.15
	44.79			
	Overall %RSD	3.85		

Conclusions

- Acrylamide was detected in peanut butter by using both the standard addition ISTD calibration and the reversed ISTD calibration with similar results.
- The %RSD of this fast and simple preparation, coupled with LCMS analysis, is less than 4%.
- Excellent reproducibility, accuracy, and linearity were achieved on both assays.
- Good LC separation on acrylamide from matrix background was performed by using low flow rate (0.25 mL/min) and larger i.d. column (3 mm).

References

[1]Fadwa Al-Taher: Agilent App Note 5990-5940EN: Analysis of Acrylamide in French Fries using Agilent Bond Elut QuEChERS AOAC kit and LC/MS/MS.

[2]KATERINA MASTOVSKA AND STEVEN J. LEHOTAY: Rapid Sample Preparation Method for LC–MS/MS or GC–MS Analysis of Acrylamide in Various Food Matrices. Journal of Agricultural and Food Chemistry 54(19):7001-8. Oct 2006

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