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High Sensitivity Analysis of Acrylamide in Potato Chips by LC/MS/MS with Modified QuEChERS Sample Pre-treatment Procedure

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Introduction

Acrylamide was found to form in fried foods like potato-chips via the so-called Maillard reaction of asparagine and glucose (reducing sugar) at higher temperature (120°C) in 2002 [1,2]. The health risk of acrylamide present in many processing foods became a concern immediately, because it is known that the compound is a neurotoxin and a potential carcinogen to humans [3]. Various analytical methods, mainly LC/MS/MS and GC/MS based methods, were established and used in analysis of acrylamide in foods in recent years [4]. We present a novel LC/MS/MS method for quantitative determination of acrylamide in potato chips with using a modified QuEChERS procedure for sample extraction and clean-up, achieving high sensitivity and high recovery. A small sample injection volume (1uL) was adopted purposely to reduce the potential contamination of samples to the interface of MS system, so as to enhance the operation stability in a laboratory handling food samples with high matrix contents.

Experimental

Acrylamide and isotope labelled acrylamide-d3 (as internal standard) were obtained from Sigma-Aldrich. The QuEChERS kits were obtained from RESTEK. A modified procedure of the QuEChERS was optimized and used in the sample extraction of acrylamide (Q-sep Q100 packet, original unbuffered) in potato chips and clean-up of matrix with d-SPE tube (Q-sep Q250, AOAC 2007.01). Acrylamide and acrylamide-d3 (IS) stock solutions and diluted calibrants were prepared using water as the solvent.

Method development and performance evaluation were carried out using spiked acrylamide samples in the extracted potato chip matrix. A LCMS-8040 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A polar-C18 column of 2.5µm particle size was used for fast UHPLC separation with a gradient elution method. Table 1 shows the details of analytical conditions on LCMS-8040 system,.

Table 1: LC/MS/MS analytical conditions of LCMS-8040 for acrylamide

LC condition

Column	Phenomenex Synergi 2.5u Polar-Rp 100A (100 x 2.00mm)
Flow Rate	0.2 mL/min
Mobile Phase	A: water B: 0.1% formic acid in Methanol
Elution Mode	Gradient elution, B%: 1% (0 to 1 min) → 80% (3 to 4.5 min) → 1% (5.5 to 10min)
Oven Temp.	40°C
Injection Vol.	1.0 µL

MS Interface condition

Interface	ESI
MS mode	Positive, MRM, 2 transitions each compound
Block Temp.	400°C
DL Temp.	200°C
CID Gas	Ar (230kPa)
Nebulizing Gas Flow	N2, 1.5L/min
Drying Gas Flow	N2, 10.0L/min

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Results and Discussion

QuEChERS Sample Pre-treatment

The details of a modified QuEChERS procedure for potato chips are shown in Figure 1. Hexane was used to defat potato chips, removing oils and non-polar components. In the extraction step with Q-sep Q100Packet extraction salt (contain 4g $MgSO_4$ & 0.5g NaCl), additional 4g of $MgSO_4$ was added to absorb the water completely (aqueous phase disappeared). Acrylamide is soluble in both aqueous and organic phases. With this modification, high recovery of acrylamide was obtained. It is believed that this is because complete removal of water in the mixed extract solution could promote acrylamide transferring into the organic phase. Dispersive SPE tube was used as PSA to remove organic acids which may decompose acrylamide in the process.

Method Development

As acrylamide is a more polar compound, a Polar-RP type column was selected. Isotope labeled internal standard (acrylamide-d_3) was used to compensate the variation of acrylamide peak area caused by system fluctuation and inconsistency in sample preparation of different batches.

The precursor ions of acrylamide and acrylamide-d_3 (IS) were their protonated ions (m/z72.1 and m/z75.1). The MRM optimization was carried out using an automated program of the LabSolutions workstation, which could generate a list of all MRM transitions with optimized CID voltages accurate to (+/-) 1 volt in minutes. Two MRM transitions of acrylamide and acryl-amide-d_3 were selected as quantifier and confirmation ion as shown in Table 2.

The obtained extract solution of potato chips was used as "blank" and also matrix for preparation of post-spiked calibrants for establishment of calibration curve with IS (acrylamide-d_3). To obtain reliable results, the blank and each post-spiked calibrant as shown in Table 3 were injected three times and the average peak area ratios were calculated and used.

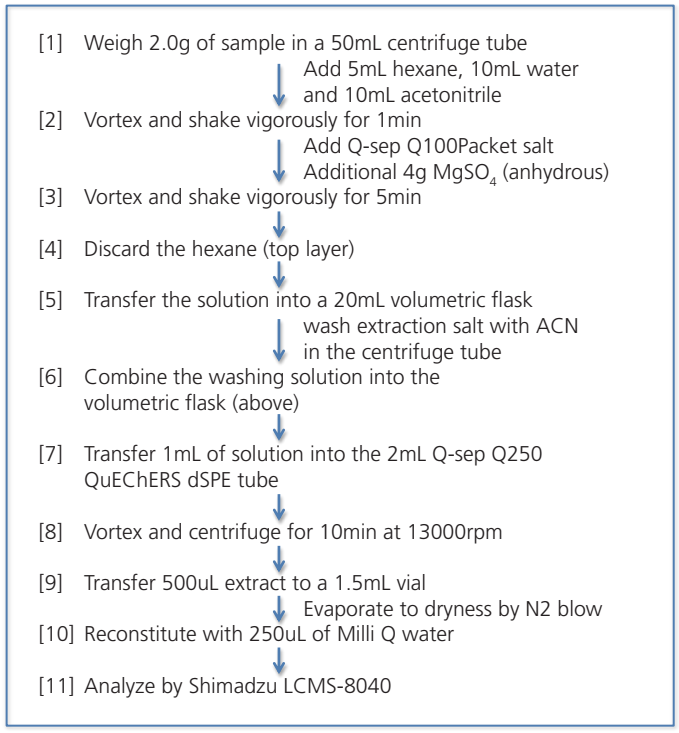

Figure 1: Flow chart of sample pre-treatment with modified QuEChERS.

Table 2: MRM transitions and CID voltages

Name	MRM (m/z)	CID Voltage (V)		
		Q1	CE	Q3
Acrylamide-d_3	75.1 > 58.0*	-29	-15	-22
	75.1 > 30.1	-29	-24	-30
Acrylamide	72.1 > 55.0*	-17	-16	-24
	72.1 > 27.1	-17	-22	-30

**MRM transition as quantifier*

Table 3: Acrylamide spiked samples and peak area ratios of measured by IS method

Acrylamide post-spiked	IS post-spiked	Conc. Ratio Calculated	Area Ratio measured*
L0, Blank	50ppb	0	0.6033
L1, 1ppb		0.02	0.6120
L2, 5ppb		0.10	0.6786
L3, 10ppb		0.20	0.8239
L4, 50ppb		1.00	1.7686
L5 100ppb		2.00	2.8196
L6, 500ppb		10.00	11.8330

**= Area (acrylamide) / Area (IS)*

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It was found that the potato chips used as "blank" in this study was not free of acrylamide. Instead, it contained 27.1 ng/mL of acrylamide in the extract solution. A linear calibration curve was established with an intercept of 0.594 at zero spiked concentration (L0) as shown in Figure 2. Good linearity with correlation coefficient (R2) greater than 0.9999 across the range of 1.0 ng/mL– 500.0 ng/mL was obtained.

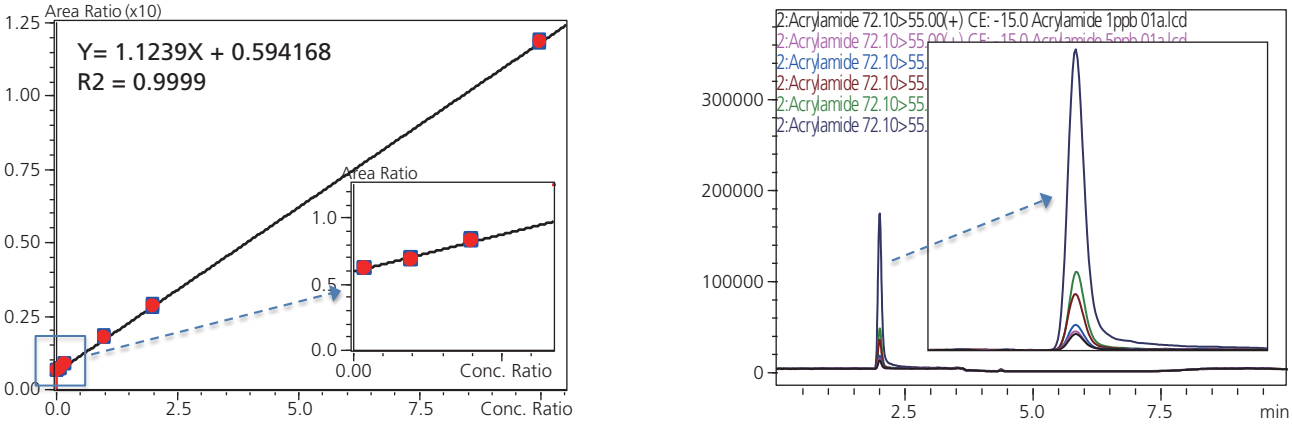

Figure 2: Calibration curve (left) and MRM peaks (right) of acrylamide spiked into potato chips matrix, 1-500 ppb with 50 ppb IS added.

Method Performance Evaluation

It was hard to estimate the LOD and LOQ of the analytical method due to the presence of acrylamide (27.1 ng/mL) in the "blank" (extract of potato chips). However, as reported also by other researchers, it is difficult to obtain potato chips free of acrylamide actually. To obtain actual concentration, it is normally subtracting the background content of acrylamide of a "blank" sample used as reference from a measurement of testing sample. The same way was used to estimate actual S/N value in this work. As a result, the LOD and LOD of acrylamide of this method with 1ul injection volume were estimated to be lower than 1ng/mL and 3ng/mL, respectively. This is consistence with the results estimated with the IS.
The repeatability of the method was evaluated with L2 and L4 spiked samples. The results are shown in Table 4 and Figure 3. The peak area %RSD of acrylamide and IS were below 4%.
The matrix effect (M.E.), recovery efficiency (R.E.) and process efficiency (P.E.) of the method were determined with a duplicate set of spiked samples of 50 ng/mL level except for the non-spiked sample. The chromatograms of "set 2", i.e., non-spiked extract, pre-spiked, post-spiked and the standard in neat solution are shown in Figure 4. Noted that, the existing acrylamide in the extract of the potato chips used as reference was accounted for 27.1 ng/mL, corresponding to 135.5 ng per gram of potato chips. The average R.E, M.E and P.E of the method for extraction and analysis of acrylamide obtained are shown in Table 6.

Table 4: Repeatability Test Results (n=6)

spiked Sample	Compound	Conc. (ng/mL)	%RSD
L2	Acrylamide	5	3.5
	Acrylamide-d_3	50	3.8
L4	Acrylamide	50	3.9
	Acrylamide-d_3	50	3.6

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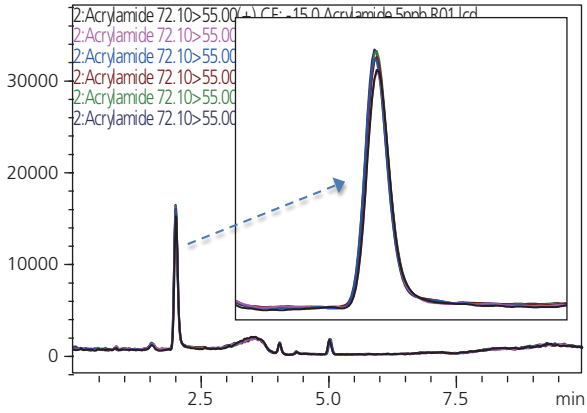

Figure 3: Overlay MRM chromatograms of 5 ng/mL acrylamide spiked in potato chips extract (total: 27.1+5 = 32.1 ng/mL)

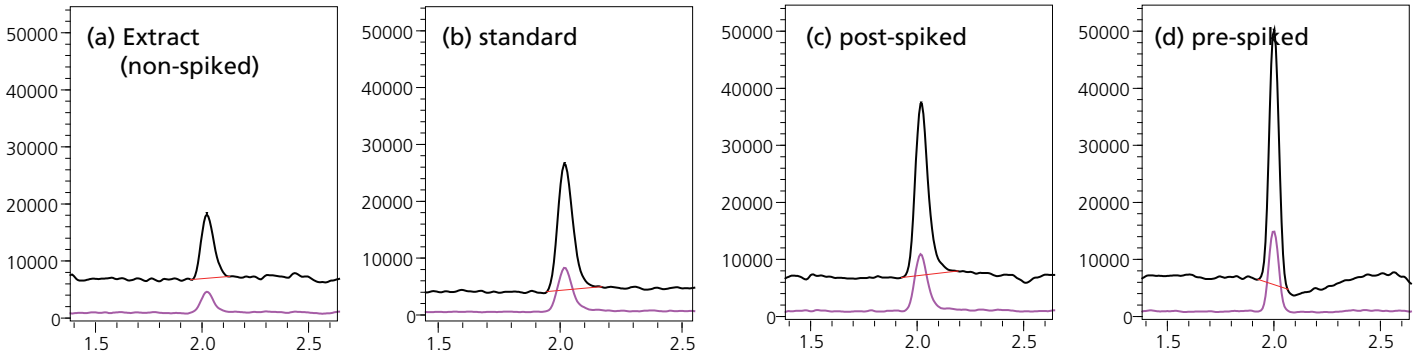

Figure 4: The MRM peaks of acrylamide detected in "blank" extract of potato chips (a), neat standard of 50ppb (b) post-spiked sample of 50ppb (c) and pre-spiked sample of 50ppb.

Table 6: Method evaluation of at 50.0ng/mL concentration in potato chips matrix

Parameter	Set 1	Set 2	Average
R.E.	104.7%	112.0%	108.4%
M.E.	96.5%	84.6%	90.5%
P.E.	100.8%	94.5%	97.6%

Conclusions

Acrylamide is formed unavoidably in starch-rich food in cooking and processing at high temperature like potato chips, French fries, cereals and roasted coffee etc. The analysis method established in this work can be used to monitor the levels of acrylamide in processing food accurately and reliably. The QuEChERS method is proven to be fast and effective in extraction of acrylamide from potato chips. The excellent performance of the method in terms of sensitivity, linearity, repeatability and recovery are related to the outstanding performance of the LC/MS/MS used which features ultra fast mass spectrometry (UFMS) technology. The high sensitivity of the method allows the analysis to be performed with a very small injection volume (1µL or below), which would be a great advantage in running heavily food samples with high matrix contents and strong matrix effects. Maintenance of the interface of a mass spectrometer could also be reduced significantly.

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