Determination of acrylamide in roasted coffee by UPLC-MS/MS

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Abstract: A method for the determination of acrylamide in roasted coffee by UPLC-MS/MS was established. Acrylamide was extracted with water, and clean up on a HLB and MCX SPE cartridge, separated on a C18 column using acetonitrile-0.1% formic acid as mobile phase, and then detected under multiple reaction monitoring mode. Deuterium labelled D3-acrylamide was used as internal standard. The recoveries ranged from 87.2 % to 92.0% at three spiked concentrations with the relative standard deviations lower than 6%. The LOD and LOQ were 5 and 15μg/kg, respectively.

Introduction

Acrylamide has been classified as probably carcinogenic to humans by the International Agency for Research on Cancer[1]. Maillard reactions have been shown to be the major pathways of acrylamide formation in a wide range of foods[2]. Bakery products and potato are the most important sources of acrylamide, coffee also contribute to the total acrylamide content [3]. Acrylamide intake through coffee consumption varies widely in the world. For instance, coffee consumption accounts for about 30% of total acrylamide in the diet of adults in Norway, but coffee consumption population in china was less then Europe[4].

Numerous analytical approaches for the determination of acrylamide in foods have been published. Methods include UV[5], gas chromatography (GC)[6], liquid chromatography-mass spectrometry (LC-MS)[7], liquid chromatography-tandem mass spectrometry(LC-MS/MS)[4,8], enzyme-linked immunosorbent assay(ELISA)[9]. ELISA can give quick and sensitive result, however, ELISA often shows false positive result. And the GC-MS analysis with transformation or derivatization is more tedious and time-consuming. The ability to detect acrylamide at regulated levels has been dramatically developed by the application of LC-MS, and with the development of technology, UPLC-MS/MS become a important determination and confirmation method.The aim of this study was to develop a novel ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method with high selectivity, sensitivity, easy to operate and fast to perform to detect acrylamide in roasted coffee.

Reagents, Instruments and materials

Acetonitrile were chromatographic grade obtained from Spectrum. HLB SPE cartridge (6cc,

200mg), MCX SPE cartridge (3cc,60mg)was obtained from waters. Acrylamide, D3-acrylamide were chromatographic grade (purity≥98%) and purchased from Dr.Ehrenstorfer company. D3-acrylamide was employed as the internal standard (IS) for the quantification of acrylamide. Their stock solutions were prepared individually at a concentration of 100mg/L in methanol. All working solutions were prepared by serial dilution of the stock solutions with methanol and stored at 4 ℃.

The following instruments were used in this study: ACQMITY UPLCTM-TQ (waters),MS3 Vortex mixter and T25 homogenize(IKA), Milli-QA1(Millipore), CR22GⅢ centrifuge(HITACHI), EE120H Ultrasonoscope (Elma).

Roasted coffee were purchased from local markets of Zhanjiang, Guangdong Province.

Analytical procedures

Sample extraction

Roasted coffee powder sample 1.00 g, 20 μL of D3-acrylamide(10 mg/L), and 10 mL of water was added into a 50mL centrifuge tube in turn. The mixture was homogenized for 1 min at 8000 r/min and shaken periodically for 5 mins. Then, the mixture was extraction by ultrasonic bath for 2 mins followed by centrifuging at 8000r/min for 6 mins.The supernatant was decanted into another 50 mL centrifuge tube. 5 mL of hexane was added to the supernatant in the centrifuge tube, and shaken periodically for 5 mins. Next, the mixture was centrifuging at 8000r/min for 6 mins and the hexane was removed.

Sample purification

For sample clean-up, the HLB SPE cartridge was washed by 3 mL methanol,3 mL water and then 1.50 mL extraction solution was passed through. Then the SPE column was eluted by 4mL 80% methanol solution.The MCX SPE cartridge was washed by 2 mL methanol,2 mL water and then all 4.0mL eluted solution was passed through.All solution was collected and heating in a water bath set at 40℃, the contents were rotoevaporated just to dryness under a reduced pressure.The obtained contents was dissolved with 1.00 mL 0.1% formic acid solution followed by centrifuging at 15000 r/min for 6 mins.The solution was passed through a 0.22 μm membrane which was finally subjected to UPLC-MS/MS determination and confirmation.

Instrument condition

A gradient UPLC system (Table 1) using acetonitrile and 0.1 % formic acid solution at a flow rate of 0.30 mL/min, was used to separate acrylamide on a Waters ACQUITY UPLC BEH C_{18} column(50×2.1 mm,1.7 μm). The column temperature was 40 ℃. The injection volume was 10.0 μL. The analysis was performed using positive-ion electrospray interface (ESI+) with multiple reaction monitoring mode. Interface conditions were as follows: capillary voltage was 3.0 kV; source temperature was 110℃; desolvation temperature was 350 ℃; the flow rates of cone and desolvation gas (nitrogen) were 50 L/h and 700 L/h, respectively; collision gas was argon; MS/MS parameters were shown in Table 2.

Table 1 UPLC gradient profile for determination of acrylamide

Time(min)	Flow rate(mL/min)	Acetonitrile (%)	0.1% formic acid (%)	Curve
0	0.30	5	95	
0.5	0.30	5	95	6
2.0	0.30	90	10	6
3.0	0.30	50	50	6
5.0	0.30	5	95	1

Table 2 MS/MS parameters for determination of acrylamide

Compound	Precursor ions(m/z)	Product ions(m/z)	Cone voltage(V)	Collision(eV)
Acrylamide	72.2	43.7	25.0	10.0
		54.7*	25.0	10.0
D3-acrylamide	75.2	57.7	20.0	10.0

*Ions for quantification

Results and discussion

Optimisation of the UPLC

Due to the kinds of mobile phase have a important roles in UPLC analysis effect, the use of different mobile phase(acetonitrile-0.1% formic acid, methanol-water, acetonitrile-5mmol/L ammonium acetate solution) were tested. The results shows that when acetonitrile-0.1% formic acid used as mobile phase to seperater acrylamide in ACQUITY UPLC BEH C_{18} can get a better separation effect, also acrylamide have a higer signal/noise and a better chromatographic peak shape (Fig.1).

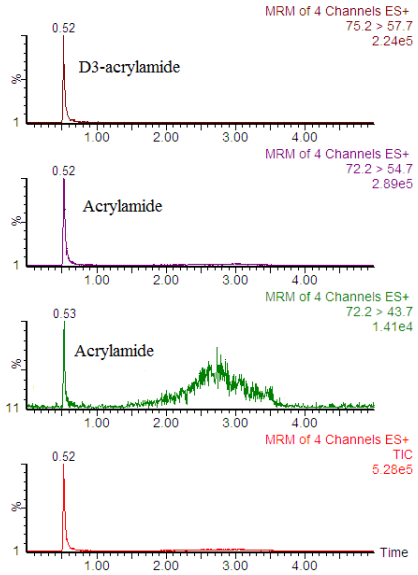

Fig.1 MRM chromatograms of standard under optimum conditions
(The injection volume was 10 μL, and the injection concentration was 50 ng/mL)

LOD and LOQ

The samples were added with the acrylamide standard solution, then pretreated and analyzed following the method described above. The limit of detection (LOD) and the limit of quantitation (LOQ) for acrylamide were extrapolated from the signal-to-noise (S/N) ratio, calculated by the instrument software, for the signal of ion at m/z 54.7 of all the samples. The LOD (S/N=3) and the

LOQ (S/N=10) obtained were 5 and 15 μg/kg, respectively.

Accuracy

For estimation of accuracy, samples were added with acrylamide standard solution. Six replicate tests, at each of the three fortification levels, were analysed. The recovery of the method was determined using roasted coffee powder fortified at 5.0μg/kg、20.0μg/kg、100.0 μg/kg. Mean recovery (n=6) of the analytes, determined in three separate assays shown in Table 3 was between 87.2 % to 92.0 % for acrylamide and the relative standard deviations(RSD) less than 6 %.

Table 3 RSD of acrylamide in samples (n=6)

Compound	Spiked concentrations (μg/kg)	Mean measured concentrations (μg/kg)	Mean recovery (%)	RSD (%)
Acrylamide	5.0	4.36	87.2	5.2
	20.0	18.4	92.0	4.1
	100.0	88.3	88.3	4.7

Conclusions

In this study, a UPLC-MS/MS method was developed for determination of acrylamide in roasted coffee. The results shows that this method has the advantages of easy to operate, fast to perform, with high sensitivity and accuracy, and it is suitable for detection of acrylamide in roasted coffee.

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