ISSN: 2688-8645

Determination of Phenylethanolamine A Residues in Milk by Solid Phase Extraction-Liquid Chromatography Tandem Mass Spectrometry

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Abstract

A method was established for the determination of phenethanolamine A residue in milk by solid phase extraction, liquid chromatography-tandem mass spectrometry. The samples were extracted with ammonium acetate buffer solution under the condition of enzymatic hydrolysis and purified by MCX solid phase extraction column. The eluent was blown dry with nitrogen at 45℃ and dissolved with 1.0 mL 0.1% formic acid solution. The samples were isolated by Poroshell 120EC-C18, 100 mm ×3.0 mm (i.d.), 2.7 μm column, and then determined by mass spectrometry in multiple reaction monitoring mode, and quantified by internal standard method. The results showed that phenethanolamine A had good linear relationship in the concentration range of 0.5-50.0 μg/L, the correlation coefficient r=0.9984, the limit of detection was 0.17 μg/kg, and the limit of quantitation was 0.57 μg/kg. The average recovery was 98.3-108.7% and the precision was 2.4-3.7% at different supplemental levels. The established method can purify the samples effectively, improve the detection efficiency, and is suitable for the detection of phenethanolamine A residue in milk.

Keywords

Phenylethanolamine A; Residues; Milk; Solid Phase Extraction; Liquid Chromatography Tandem Mass Spectrometry.

1. Introduction

The structure of phenethanolamine A is similar to ractopamine. It is used in pig, cattle and other breeding, which can stimulate growth, accelerate metabolism, promote fat decomposition in animals and improve lean meat rate. After eating the foods containing the residue of this drug, people will appear nausea, dizziness, limb weakness, hand tremor and other poisoning symptoms, long-term consumption will even induce malignant tumors, so it is prohibited to use in feed and animal drinking water. At present, the methods for the detection of phenethanolamine A reported in the literature mainly include liquid chromatoc-series mass spectrometry [1,2], enzyme-linked immunoassay [3,4], biosensor method [5], colloidal gold strip method [6], etc. The substrates for the detection of phenethanolamine A residue reported in the literature mainly include hair of Swine and Sheep [7], pork [8,9], pig urine [10] and feed

ISSN: 2688-8645

[11]. Liquid chromatography-tandem mass spectrometry is widely used because of its strong specificity and high clarity. The existing detection methods involve pre-treatment purification methods including solid phase extraction method and QuEChERS method. As a classical purification method, solid phase extraction method has advantages that can not be replaced by other purification methods, and can obtain good purification effect. In this paper, solid phase extraction combined with liquid chromatography-tandem mass spectrometry technology was used to establish the detection method of phenylethanolamine A residue in milk, so as to detect and monitor phenylethanolamine A residue in related products.

2. Experimental Part

2.1. Instruments and Materials

Agilent 1290-6460 Liquid chromatography-Tandem Mass Spectrometer, USA; Hitachi CR22G III high speed refrigeratedcentrifuge, Japan;IKACompanyGM200grindinginstrument, Germany; Organomation Assosiates nitrogen blowing concentrator,USA; IKA Vortex Mixer, Germany; Waters solid phase extraction device, USA. Methanol, n-hexane, ethyl acetate, formic acid, all chromatographically pure; Ammonium acetate, glacial acetic acid, ammonia water, all analytically pure. β-glucuronidase/arylsulfatase. MCX Solid phase extraction column: 60 mg, 3 mL. Phenethanolamine A, CAS: 1346746-81-3; Phenylethanolamine A-D3, CAS: 2507994-61-6, all purchased from Shanghai Anpu Experimental Technology Co., LTD., purity greater than98%.

2.2. Preparation of Standard Solution

Accurately weighed 100.0 mg of phenethanolamine A standard, dissolve it with methanol at a constant volume to 100 mL, mix well, and prepare 1000 mg/L standard reserve solution. Usedthe liquid to dilute step by step, prepared during use. Accurately weighed 50.0 mg phenylethanolamine A isotope into a 50 mL volumetric bottle, dissolvedit with methanol and adjust the volume to the scale, mix well, and prepare it into 1000 mg/L internal standard reserve solution. Usedthe liquid to dilute step by step, and prepared during use.

2.3. Instrument Conditions

Chromatographic column: Poroshell 120 EC-C18, 100 mm ×3.0 mm (i.d.), 2.7 μm; Column temperature: 30℃; Injection size: 5μL; Mobile phase: A: methanol, B: 0.1% formic acid water;Gradient elution conditions: 0-1.5 min,10%A; 1.5-5 min, 10-80%A; 5-8min,80%A; 8-9min, 80-10%A; 9-10min, 10%A. Flow rate: 0.3 mL/min.

Ion source: electrospray ion source; Scanning mode: positive ion scanning; Detection method: multi-reaction monitoring; Capillary voltage: 4000V; Dry temperature: 325℃; Drying gas flow rate: 10L/min; Atomizing gas pressure: 276 KPa; Sheath temperature: 350℃; Sheath gas flow rate: 11 L/min. The reference values of parent ions, daughter ions, fragmentation voltage and collision energy of phenethanolamine A and phenethanolamine A-D3 are shown in Table 1.

Table1. Parent ion, product ion, fragmentation voltage, collision energy of PhenylethanolamineA, Phenylethanolamine A-D3

compound	Ion pair/(m/z)	fragmentation voltage /V	Impact energy /eV
Phenethanolamine A	345.2/150.1*	100	20
	345.2/327.1		10
Phenethanolamine A-D3	348.0/330.0	100	10

Note: *quantitative ion pairs.

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2.4. Sample Handling

The 5g sample (accurate to 0.01g) was weighed and placed in a 50mL centrifuge tube, then added 10μg/L internal standard working liquid 200μL, followed by 20mL ammonium acetate buffer solution, homogenized for 1 min, then added 40μL β-glucuronidase/arylsulfatase, and fully oscillatedandmixed. Incubateat37℃ for16h. After thereaction was completed, centrifugeat4℃ at 9000r/min for 5min, collect the supernatant in the screw cap polypropylene centrifuge tube, add 20mL n-hexane, shake and mix well. Centrifuge at 4℃ at 9000r/min for 5min, then discard the n-hexane layer and wait for purification. The MCX column was pretreated with 3 mL methanol and 3 mL water successively, the supernatant was loaded on the MCX column, and then the column was washed with 3 mL0.1mol/L hydrochloric acid, 3 mL water, 3 mL methanol-water (50+50) solution successively, and the eluent was discarded; The MCX column was blown dry with nitrogen, eluted with 5 mL ammonia-methanol (1+19), and the eluent was collected. The eluent was blown dry with nitrogenat45℃,and 1.0 mL0.1% formicacidsolution was addedtodissolvetheresidue accurately. The eluent was mixed by ultrasound and then passed through 0.22μm organic phase filter for liquid chromatoc-tandem mass spectrometrydetermination.

3. Results and Discussion

3.1. Determination of Extraction Conditions

In order to completely change phenylethanolamine A from the bound state to the free state, it wasnecessary to add β-glucuronidase/arylsulfatase for enzymatic hydrolysis, and the samples after enzymatic hydrolysis were extracted with ammonium acetate buffer solution. According to the conditionsofenzymatichydrolysisandpurificationintheexistingstandards,it was determined that 40μL β-glucuronidase/arylsulfatase was enzymolized at 37℃for 16h, and 20 mL ammonium acetate was used to extract phenethanolamineA.

3.2. Selection of Purification Method

PhenethanolamineAhasasimilarstructuretoractopamine,whichcanberetainedontheMCX solid phase extraction column, and the purpose of removing impurities can be achieved by leaching and adjusting the pH value. In this paper, the purification method of solid phase extraction was selected, and the activation, leaching, elution and other conditions were optimized. The experimental results showed that the recovery rate of MCX solid phase extraction column without acid activation was higher. The above conditions could meet the requirements of enzymatic hydrolysis, extraction and purification of phenethanolamine A, and the recovery rate was over 95%.

3.3. Optimization of Chromatographic Conditions

Methanol, acetonitrile, ammonium acetate and water were selected for testing in different proportions. It was found that only methanol and water were needed for mobile phase. Under the condition of gradient elution, phenylethanolamine A and impurities could be separated well by adjusting the ratio of the two. In order to increase the response value of phenylethanolamine A, formic acid was added to the water, so that its content was 0.1%. In order to determine the appropriate column, Hypersil GOLD (50 mm×2.1 mm, 1.9 μm) and Poroshell 120 EC-C18 (100mm×3.0 mm, 2.7μm) columns were compared. The experiment found that,under the condition of 30 ℃ column temperature, both chromatographic columns can satisfy the chromatographic separation of phenethanolamine A. In order to meet the versatility of laboratory detection and avoid changing chromatographic columns back and forth, the Poroshell 120 EC-C18 column was selected in this paper, and other conditions were optimized on this basis.

ISSN: 2688-8645

3.4. Optimization of Mass Spectrum Conditions

Without chromatographiccolumn,100μg/LphenylethanolamineAstandardsolution was used for mass spectrometry analysis. The results showed that the response value of phenylethanolamine Ainpositiveionmode was higherthanthatinnegativeionmode, so thepositiveionmode was used for optimization. First, primary mass spectrometry was performed to obtain the parent ion and the optimal fragmentation voltage of the compound. Then select ion monitoring Scan and daughterion scan for the selected parent ions to obtain the corresponding disintegration energy and collision energy of the compound, determined the quantitative and qualitative ions used in the multi-reaction monitoring, and establishedthe multi-reaction monitoring data parameters of phenethanolamine A and its internal target through the optimization oftheobtained mass spectrum parameters. The multireaction monitoring chromatogram of phenylethanolamine A and its internal standard is shown in Figure 1.

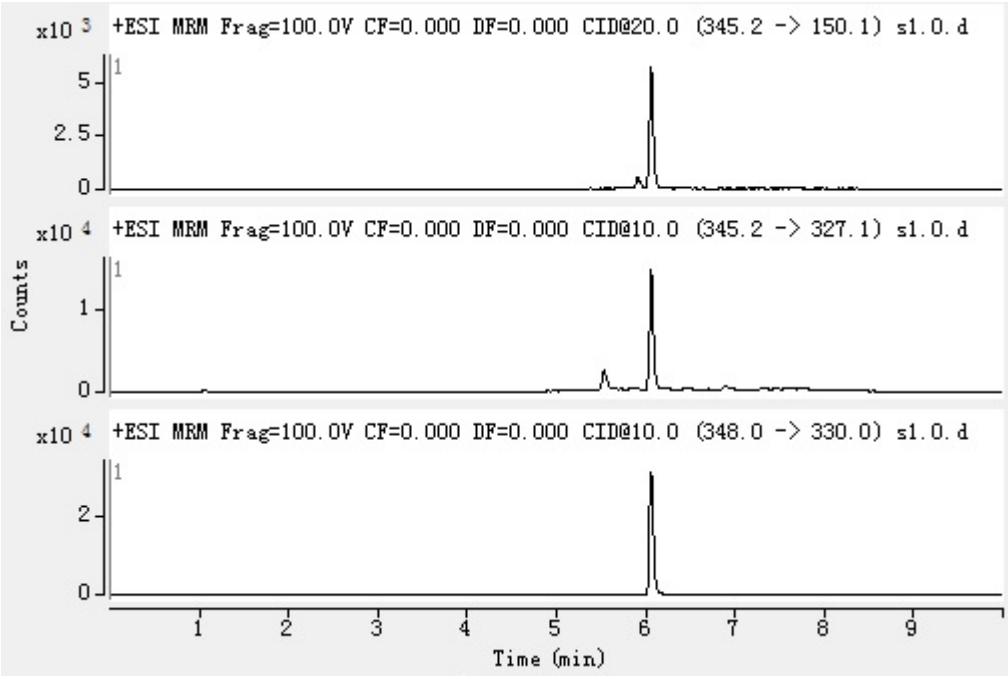

Figure 1. Multireaction monitoring chromatogram of phenylethanolamine A and its internal standard

3.5. Linear Range, Detection Limit and Quantitation Limit of the Method

Phenylethanolamine A standard solution with concentration series of 0.5, 1.0, 2.5, 5.0, 10.0 and 50.0 μg/L was prepared, which was equivalent to the test sample containing 0.1, 0.2, 0.5, 1.0,2.0 and 10.0 μg/kg target compound, respectively. 200 μL10 μg/L phenylethanolamine A-D3 standard solution was added to each concentration point. The linear regression equation of phenethanolamine A was *Y*=0.665×x-0.086, and the correlation coefficient r=0.9984, indicating a good linear relationship in the concentration range of 0.5-50.0μg/L. 5g blank milk sample was weighed, 0.5μg/kgphenethanolamineAstandardsolution was added,pre-treatment was carriedout according to the optimized conditions and mass spectrometry was performed. The obtained chromatogramsignal-to-noiseisshownin Figure 2. The detectionlimitofthemethod was 0.17 μg/kg according to 3 timessignal-to-noise ratio, and the quantitative limit of the method was0.57 μg/kg according to 10 times signal-to-noise ratio.

ISSN: 2688-8645

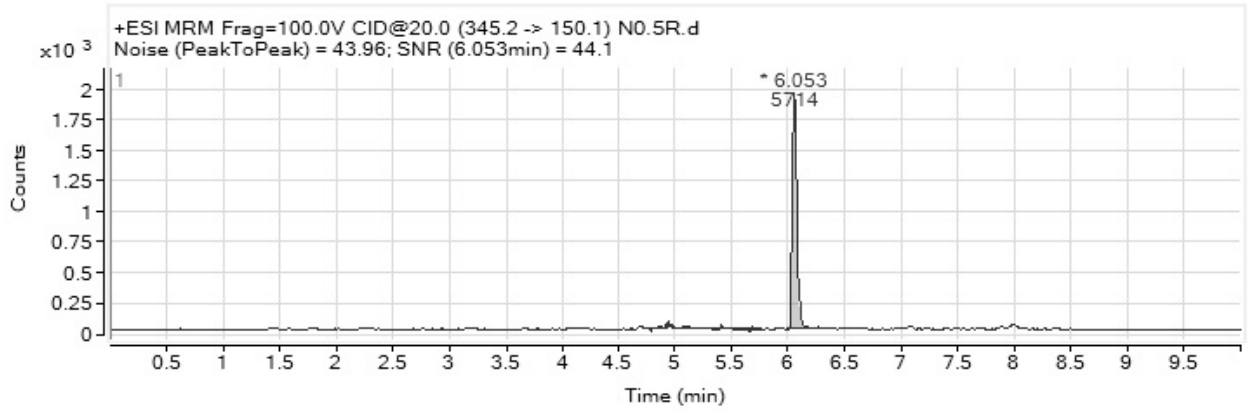

Figure 2. Chromatogram and signal-to-noise ratio of blank milk sample supplemented with phenethanolamine A

3.6. Precision and Recovery of the Method

Blank milk samples were selected for three levels of 0.6, 1.0 and 2.0μg/kg, and six parallel measurements were performed for each level. The results showed that the recovery rate andprecision of the method were good, with the recovery rate of 98.3-108.7% and the precision of 2.4-3.7%, which fully met the determination requirements of phenethanolamine A residue in milk. The precision and recovery data of the method are shown in Table 2.

Table 2. Recovery rate and precision of phenethanolamine A (n=6)

Compound	Amount to add (μg/kg)					
	0.6		1.0		2.0	
	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
Phenethanolamine A	101.3	2.4	106.3	2.6	98.3	3.7
	103.9		106.5		100.9	
	102.0		107.5		101.3	
	107.4		103.7		103.7	
	104.5		106.2		108.7	
	100.8		100.2		99.3	

4. Conclusion

Inthispaper, AmethodwasestablishedforthedeterminationofphenylethanolamineAresidue in milk by solid phase extraction, liquid chromatography and tandem mass spectrometry. By optimizing extraction conditions, purification methods, mass spectrometry conditions and liquid chromatography conditions, phenylethanolamine A residue in milk can be extracted and separated on the chromatographic column, with short analysis period, good purification effect and high recovery rate. The established method is fast and practical, and is suitable for the batch detection of phenylethanolamine A residues in milk.

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