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ORIGINAL ARTICLE

A rapid method for the determination of dopamine in porcine muscle by pre-column derivatization and HPLC with fluorescence detection

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Abstract A rapid method has been developed based on the sample preparation procedure named as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), combined with reversed-phase high performance liquid chromatography with fluorescence detector and C_{18} column after pre-column derivatization using *o*-phthalaldehyde and 2-mercaptoethanol to determine dopamine in porcine muscle. Methanol and deionized water (0.1% acetic acid, v/v) with a ratio of 60:40 was used as mobile phase. The flow rate was 0.8 mL/min and dopamine was eluted within 15 min. The linearity range was 0.003–8 μg/mL with $r=0.9992$. The detection limit for dopamine was 4 μg/kg and the quantification limit was 9 μg/kg. Recovery studies were carried out at 0.1, 0.5 and 1.0 mg/kg fortification levels and the average recoveries obtained ranged from 90.4% to 98.2% with relative standard deviations between 3.5% and 8.1%. The method was found to be suitable for detection of dopamine in animal product tissues at the maximum residue level.

1. Introduction

Dopamine, one of the naturally occurring catecholamine, plays an important role as a neurotransmitter in the central nervous system [1], and affects the regulation of blood pressure and heart rate as well when it is used for the treatment of human diseases and various veterinary diseases [2]. Sometimes dopamine is used to reduce the fat content when it is added to livestock feed of many animal species. Thus the residues of dopamine are accumulated in animal tissue after administration to livestock, and might be dangerous for an individual exposed to even very low concentrations. China has banned the usage of dopamine in livestock feed and water. However, it is still used illegally and it is necessary to develop efficient methods to detect dopamine residue in animal products.

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In recent years, several methodologies such as capillary electrophoresis [3], spectrofluorimetry [4] and high performance liquid chromatography (HPLC) have been developed for detection of dopamine. The commonly used detectors for chromatography include MS [5,6], UV [7], optical fiber detector [2,8], electrochemical detector [9,10] and fluorescence detector(FLD) [11,12]. Nevertheless, most of these methods have several disadvantages such as being expensive.

As for sample preparation, a modified method named as QuEChERS was used, which stands for quick, easy, cheap, effective, rugged and safe [13]. The approach is very flexible, and permits the direct analysis of the drugs after extraction and purification. Since its development, it has been modified several times and used to analyze hundreds of drugs in a variety of foods successfully [13,14].

This work developed a simple, reliable and fast method based on a modified QuEChERS method for sample preparation, using reversed-phase high performance liquid chromatography with fluorescence detection after pre-column derivatization using *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (2-ME) for the determination of dopamine. The results indicated that the proposed method displays enough sensibility, precision and accuracy to be used to detect dopamine in porcine muscle at the maximum residue level.

2. Experimental

2.1. Chemicals and solutions

Dopamine hydrochloride (DA, 100070-200405) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile of HPLC-grade were purchased from Burdick & Jackson (Muskegon, MI, USA). Silica C_{18} (50 μm) was obtained from Beijing Greenherbs Science and Technology Development Co., Ltd. (Beijing, China). Other reagents were of the chemical pure grade. All aqueous solutions were prepared in distilled water, which was further purified with a Molelement water purification system (Molecular, Shanghai, China). All samples were filtered through a 0.45 μm filter before chromatographic analysis. Porcine muscle purchased from a local food market was kept deep-frozen at −18 °C prior to the analysis.

Stock solution of dopamine (10 μg/mL) was prepared in methanol and stored in dark at 4 °C. The solution was stable for at least 2 months and diluted further with methanol to obtain working standard solutions, with the concentrations of 0.001, 0.003, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, and 8 μg/mL. Working standard solutions were used for the preparation of standard calibration curves. All of them were freshly made. The pre-column derivatization solution was prepared by dissolving 0.0150 g OPA in 2 mL methanol, then adding 100 μL of 2-ME and finally adding 50 mM sodium borate to 25 mL. The pre-column derivatization solution was used within 24 h.

2.2. Derivatization mechanism

In this work, we chose *o*-phthalaldehyde and 2-mercaptoethanol for the derivatization of dopamine. After derivatization, dopamine was transformed into substituted isoindole, which allows fluorescence detection, the reaction mechanism is shown in Fig. 1 [15].

2.3. HPLC-FLD and derivatization conditions

For chromatographic separation, an LC-10Avp (Shimadzu, Japan) chromatography system was used. The system comprised an LC-10ATvp secondary pump system, a DGU-12A on-line degasser, a CTO-10ASvp thermostatted column compartment, and a RF-10AXL spectrofluorimetric detector. CLASS-VP software was used to control the LC components and process fluorescence data. Chromatographic separation was achieved on a Kromasil C_{18} chromatography column (4.6 mm × 150 mm, 5 μm). The column temperature was maintained at 30 °C and for all the experiments the flow rate was 0.8 mL/min. Methanol and deionized water (0.1% acetic acid, v/v) with a ratio of 60:40 was used as mobile phase. Under the chromatographic conditions described above, the analytes were eluted within 15 min.

The derivatization of dopamine was achieved by adding derivatization reagent to dopamine solutions with a volume

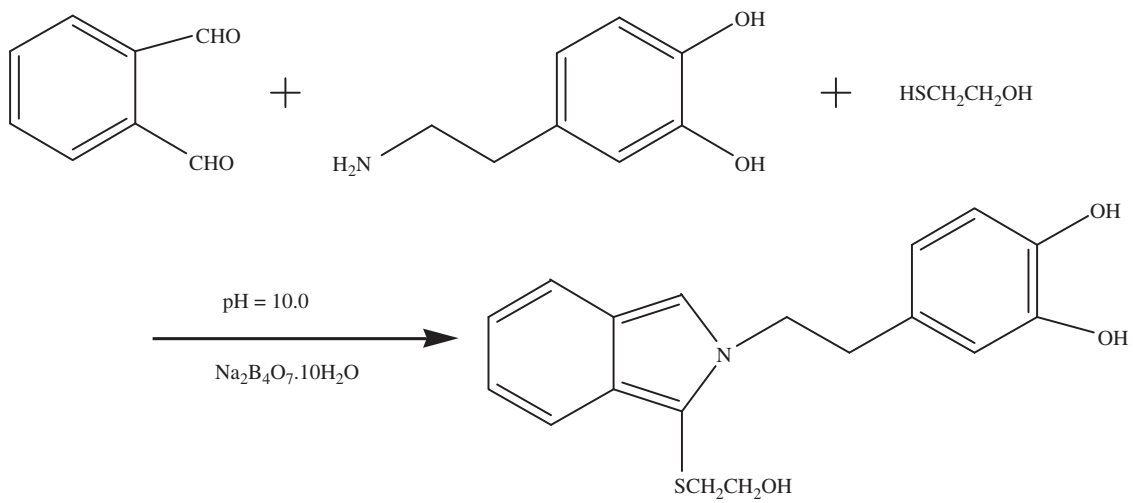

Figure 1 Mechanism of the derivatization reaction.

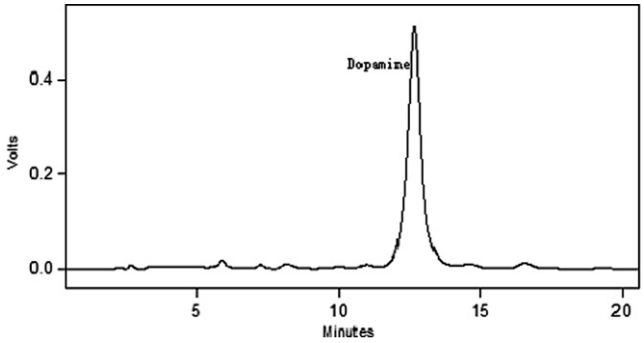

Figure 2 HPLC-FLD chromatogram of dopamine. E_x=350 nm; E_m=450 nm.

ratio of 1:5. After vigorously shaken by hand for 1 min, the resulting fluorescence was monitored at excitation and emission wavelengths of 350 and 450 nm (Fig. 2). The retention time for dopamine was 12.702 min.

2.4. *The procedure of QuEChERS*

A sample of 0.5 g blended pork was weighed into centrifuge tubes in an analytical scale, fully immersed in the standard mixture solution, and let to stand for 20 min in dark. 12 mL of acetonitrile–acetic acid (99:1, v/v) was added to the sample, followed by 2.0 g of $MgSO_4$ and 0.5 g of NaCl. The samples were hand-shaken vigorously for 1 min to ensure that the powders did not agglomerate, and then centrifuged at 3000*g* for 10 min. The supernatant was transferred to another centrifuge tube and cleaned up with C_{18} (1.5 g), and then dried with $MgSO_4$ (1.5 g). After mixing (1 min) and centrifugation (3000*g*, 10 min), the eluate was evaporated to dryness with a gentle stream of air, and then the residue was dissolved to 0.5 mL with methanol. The final solution of 20 µl was filtered through a 0.45 µm disposable syringe filter unit before injection into the chromatographic system.

3. Results and discussion

3.1. *Optimum conditions for HPLC-FLD*

Several eluents, such as acetonitrile, methanol, H_3PO_4, acetic acid, phosphate buffer, and acetic acid buffer, were tested to study if they could separate the analyte. In this study, solvent A methanol and solvent B deionized water (0.1% acetic acid, v/v) were chosen as the optimal chromatographic mobile phase. Chromatographic analysis of the samples was performed within 15 min. The resulting fluorescence of the derived product was monitored at excitation and emission wavelengths of 350 and 450 nm.

3.2. *Optimum conditions for the QuEChERS*

A modified QuEChERS approach was adopted to isolate dopamine from porcine muscle. Firstly, several solvents, such as *N*-hexane, acetoacetate, acetonitrile–acetic acid (99:1, v/v), acetonitrile–H_2O (2:1, v/v) and acetonitrile, were tested to study the recoveries of the analytes. The recoveries of the dopamine under different eluting conditions are shown in Table 1. As indicated, the proper eluting solvent was acetonitrile–acetic acid (99:1, v/v). The effect of the eluting solvent volume was also tested. The samples were eluted with 6, 8, 10, 12 and 14 mL of acetonitrile–acetic acid (99:1, v/v), and the recoveries increased rapidly to about 66% after elution with 8 mL of eluent, finally reaching an equilibrium value when using 12 mL of eluent. Therefore, the solvent volume of 12 mL was used in the subsequent studies.

Table 1 Recoveries of dopamine in different eluting conditions.

Eluting solvent	Volume (mL)	Recovery (%)
C_6H_{14}	12	ND
CH_3CN/CH_3COOH (v/v=99:1)	12	93.8
CH_3CN	12	87.9
$CH_3COOCH_2CH_3$	12	41.2
CH_3CN/H_2O (v/v=2:1)	12	62.9

ND, not detected.

In QuEChERS, the step of cleanup is very important, and C_{18} is found to be one of the most suitable sorbents for the method, so it is very crucial to investigate the relations between the dose of C_{18} and the recoveries of the analytes. 0.5 g of tissue sample (the spiking level was 0.5 µg/g) was used for each test, and 0.5, 1.0, 1.5, 2.0 and 2.5 g of C_{18} were used in the cleanup procedure to give different ratios of sample to C_{18}. It turned out that when the C_{18} weight was 1.5 g, the average recovery was better than other ratios. So 0.50 g sample and 1.5 g C_{18} were chosen for the extraction and purification.

3.3. *Optimum conditions for OPA derivatization*

The concentrations of OPA and 2-ME had effects on fluorescent derivatization. Weights of OPA at the levels of 0.0050, 0.0100, 0.0150, 0.0200, 0.0250 g and volumes of 2-ME at the levels of 10, 50, 100, 150 µL were tested, considering the maximum and constant peak areas, 0.0150 g OPA and 100 µL 2-ME were adopted for the derivatization reagents. The effect of the pH of 50 mM sodium borate was also tested at the levels of 7.0, 8.0, 9.0, 10.0, 11.0, and the pH of 10.0 was chosen.

Besides, reaction time and temperature were also investigated. Due to the rapid reaction speed of dopamine and OPA, the reaction was conducted at room temperature and because of the bad stability of the derived product, it must be monitored within two minutes.

3.4. *Method validation*

3.4.1. *Linearity*

The linearity was demonstrated for dopamine by preparing a ten-point calibration curve at different concentrations of 0.001, 0.003, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 8 µg/mL, and each concentration was measured by three replicate injections. The relation between the amount of dopamine and its peak area was linear over the concentration range of 0.003–8 µg/mL. The linear equation was: $y=2.7\times10^3\ x+4.5\times10^3$ with a correlation coefficient of 0.9992.

Table 2 Intra-day and inter-day repeatability.

Added (μg/g)	Intra-day repeatability	Inter-day repeatability
	RSD (%) (n=3)	RSD (%) (n=9)
1.0	5.1	5.6
0.5	4.7	6.0
0.1	7.8	9.4

Table 3 Average recoveries and RSD of dopamine at three levels of spiking.

Added (μg/g)	Recovery (%, n=3)			Average recovery (%)	RSD (%)
	1	2	3		
1.0	100.6	94.2	99.8	98.2	3.5
0.5	98.3	101.2	87.6	95.7	7.2
0.1	87.1	99.6	84.5	90.4	8.1

3.4.2. Precision

Two parameters were investigated at three levels of spiked pork samples (0.1, 0.5 and 1.0 μg/g) for precision, namely, intra-day (three repetitions of each concentration, within one day) and inter-day repeatability (three repetitions of each concentration, on three consecutive days), and expressed in terms of relative standard deviations (RSDs), which are shown in Table 2. The RSDs were lower than 7.8% for intra-day and lower than 9.4% for inter-day. These results indicated that the developed method had acceptable precision.

3.4.3. Accuracy

The accuracy was expressed as recovery and tested by fortification of pork samples at three known levels of 0.1, 0.5 and 1.0 μg/g. Table 3 summarizes the recoveries and the RSD obtained. The results indicated that the average recoveries were between 90.4% and 98.2%, and RSDs of the peak areas changed from 3.5% to 8.1%. The limits of detection (LOD) and quantification (LOQ) were determined with the three times and ten times criteria, respectively, using six injections of a pork blank sample. The method gave the LOD at 4 μg/kg and the LOQ at 9 μg/kg. It demonstrated that the pre-treatment and derivatization of the developed HPLC-FLD method were satisfactory for the quantification of dopamine in real animal samples.

4. Conclusion

The present study develops a new chromatographic method with fluorescence detection for the determination of dopamine residue in porcine muscle. In fact, the developed method is sufficiently selective and sensitive to allow the simple assay of animal tissues. The proposed QuEChERS methodology is relatively simple, efficient and economical compared with SPE and MSPD, and is suitable for analysis of drug residues in pork. Therefore, we believe that this method will be useful for detection of dopamine in animal product tissues at the maximum residue level.

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