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Determination and Analysis of Promazine Hydrochloride in Chicken by High Performance Liquid

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Abstract: *To establish a method for the determination of promazine hydrochloride residue in pork by HPLC. The extraction of promazine hydrochloride from pork tissues by ultrasonic extraction and centrifugal extraction was studied. The peak area of promazine hydrochloride was determined by high performance liquid chromatography. The content of promazine hydrochloride in pork was calculated by standard curve equation. The mobile phase was acetonitrile-ammonium acetate (0.02mol/mL) with a ratio of 60:40, the detection wavelength was set at 258 nm, the flow rate was 1.0mL/min, and the sample size was 10μL. The column temperature was 20℃. Acetonitrile was selected as the extraction liquid, extracted by ultrasonic shock method and centrifugal method, purified by solid phase extraction method, and dried and concentrated by vacuum as the pre-treatment method of pork. The results showed that promazine hydrochloride showed a good linear relationship with the peak area in the concentration range of 1ug/mL~50ug/mL (r2=0.9983). The intra-day and intra-day precision were all less than 3%. The recoveries of three different concentrations were 79.31%~84.38%. The method used in this experiment is simple, easy to operate, high accuracy and good reproducibility, which is suitable for the analysis and detection of the residual amount of promazine hydrochloride in pork.*

Keywords: *Pork, Promazine hydrochloride, High pressure liquid chromatography, A sedative*

1. Introduction

Promazine hydrochloride is a common sedative with the molecular formula C17H20N2S.HCl and the molecular weight 320.88.As shown in Figure 1.

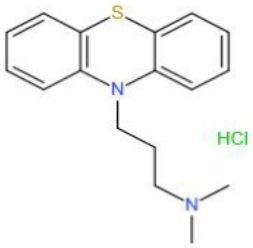

Figure 1: Molecu Molecular structure of promazine hydrochloridela

Sedative drugs as drugs inhibiting animal central nervous systems can cause mild inhibition of central nervous systems, weakening functional activities, eliminating incompetence and restoring quiet, commonly used in veterinarians [1]. In recent years, drove by economic interests, some livestock breeders add tranquilizers in the process of livestock and poultry rearing without authorization, so as to hypnotize animals, increase the weight of animals and shorten the time of breeding. In addition, in order to reduce weight loss and death and reduce meat quality during the transportation of animals. Sedatives are often used to reduce the loss of stress effects[2].If people eat these foods for a long time, they will also produce a variety of accumulated toxicity or chronic poisoning, which will bring a variety of problems to people's health, such as vomiting, nausea, numbness of the mouth and tongue, etc. In 2002, the Ministry of Agriculture of China issued Announcement No.176 and Announcement No.235, which clearly stipulated that the use of sedative drugs in animal feed and drinking water was

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strictly prohibited, and that drugs such as methaqualone should not be detected in food of animal origin [3].

Detecting the drug residues of sedatives is a necessary way to accurately know the drug residues in food of animal origin. Current detection methods include high performance liquid chromatography-tandem mass spectrometry [4], high performance liquid chromatography [5], thin layer chromatography [6], immunological detection method [7], gas chromatography/mass spectrometry [6] and other detection methods.

HPLC-MS/MS and GC-MS have the advantages of good accuracy and high sensitivity, but the instrument is expensive and the popularization is limited.

Most tranquilizers are hydrophobic and volatile, so HPLC is more suitable for the determination of tranquilizers. At present, diode array detector, as a high performance liquid chromatography detector, is still the main method for detecting most tricyclic and phenothiazine sedatives, especially in the analysis and detection of body fluid tissue [8].

In this study, ultrasonic extraction method and centrifugal extraction method were used as extraction methods, solid phase extraction technology was used as purification means, and high performance liquid chromatography combined with diode array detector was used to detect and analyze promazine hydrochloride in pork. This method is simple, repeatable and accurate, which provides a new method for the detection of sedatives in pork.

2. Materials and Methods

2.1. Materials and Reagents

3mL, 60mg SPE column (Jiangsu Jiedao High-tech Material Technology Co., Ltd.);0.22 μm organic filter membrane (Green Alliance Technology Co., Ltd.). Promazine hydrochloride (98.0% purity, Shanghai Yuanye Biotechnology Co., Ltd.);Methanol, acetonitrile, (Chromatographic Grade, Tianjin Komil Chemical Reagent Co., Ltd.); Ammonium acetate, n-hexane (analytical pure, Tianjin Komeo Chemical Reagent Co., Ltd.); The water is the first grade ultrapure water.

2.2. Instruments and Equipment

1260 Infinity Ⅱ High Performance Liquid Chromatograph (Agilent, USA); Extrapid Column - Manual Solid Phase Extraction Instrument (Beijing Leibtec Instrument Co., Ltd.); HLB solid phase extraction column (Beijing Leibtec Instrument Co., Ltd.); SB25-2DTD ultrasonic cleaning machine (Ningbo Xinzhi Biotechnology Co., Ltd.); FC5718R Centrifuge (CHAUS, Germany); SCIENTZ-48 High Throughput Tissue Grinder (Ningbo Xinzhi Biotechnology Co., Ltd.).

2.3. Preparation of Solution

2.3.1. Preparation of Standard Reserve Solution

10.0mg promazine hydrochloride drug standard was accurately weighed, dissolved in methanol (chromatographic pure) and put into a 50mL volumetric flask to prepare a standard reserve solution with the content of 200.0μg/mL, which was stored in the cold room of the refrigerator away from light.

2.3.2. Preparation of Standard Working Fluid

0.05mL of standard reserve solution was accurately measured and diluted with methanol to 50mL to prepare 1.0μg/mL of standard working solution. The concentrations of series of standard working solutions prepared by the same method were 1.0, 5.0, 10.0, 20.0, 50.0μg/mL respectively. Store in the freezer away from light.

2.3.3. Preparation of 0.02mol/l Ammonium Acetate Solution

Accurate weighed 0.774g ammonium acetate, dissolved it in ultrapure water in a 100mL beaker, transferred it to a 500mL volumetric flask, constant volume to 500mL, and shaken well. After filtering through the filter, it is used as the mobile phase for standby. Mobile phase use as you go.

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2.4. Pretreatment of Samples

Extraction of samples: After the tissue was melted in the refrigerator at room temperature, the connective tissue was removed, ground with a high-throughput tissue grinder, and homogenized with a homogenizer. Weighed a 2.0g sample into a 10mL centrifuge tube and added 8mL acetonitrile. A glass rod was used to stir and mix, and the centrifuge tube was put into the ultrasonic cleaning machine for ultrasonic extraction for 15min. The extracted liquid was centrifuged at 9400r/min for 10min. The supernatant was transferred to another 10mL centrifuge tube, 2mL n-hexane was added, and the swirling mixer was used for swirling mixing for 1min.Suction out the n-hexane layer. Add 8mL acetonitrile to the residue, cover tightly, swirl and mix for 1min, centrifuge in the same way as before, and repeat once. The extracted solution for two times was put into a vacuum dryer, dried and evaporated at 45℃ to the remaining 2mL of the solution in the centrifuge tube, and the concentrated solution was purified [8].

Purification of samples: Install the SPE column on a SPE device with vacuum extraction, activate it with 3mL water and 5mL methanol, add the above concentrated solution into the HBL column, control the flow rate to be about 1 drop per second, wash the HBL column with 5mL water after the concentrate is dried, wait for the HBL column to dry naturally, and elute it into a 10mL centrifuge tube with 5mL eluent. After filtration through 0.45μm organic membrane, samples were injected into HPLC for analysis.

2.5. Instrument Conditions

The chromatographic column: Supelce Discovery -C_{18},150nm

Mobile phase:acetonitrile: 0.02mol/LAmmonium acetate(60:40)

Detection wavelength: 258nm

Flow rate: 1.0mL/min

Sample quantity: 10μL

Column temperature: 20℃

3. Results and Analysis

3.1. Selection of Detection Wavelength

10.0μg/mL promazine hydrochloride standard solution was scanned on the UV-visible spectrophotometer with the wavelength range controlled between 200nm-400nm. As shown in Figure 2, it was found that there was a large absorption in the wavelength range of 250-260nm. Therefore, 258nm was selected as the detection wavelength.

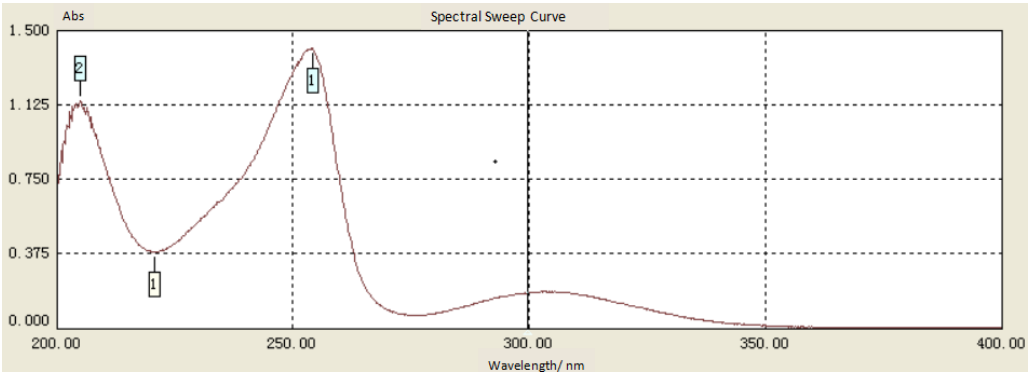

Figure 2: 10.0μg/mL promazine hydrochloride spectrogram

3.2. Determination of Mobile Phase

Cai Yugang et al. [9] used acetonitrile-ammonium acetate solution as liquid chromatography mobile phase to detect benzodiazepines. Most of them use acetonitrile as the mobile phase. Therefore, in this

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experiment, acetonitrile-ammonium acetate is considered as the mobile phase and the ratio of acetonitrile-ammonium acetate is changed to seek the optimal conditions.In this experiment, the ratio of acetonitrile-ammonium acetate was(90:10), (75:25), (60:40)and the ammonium acetate concentration is 0.02mol/L.The results show that when the ratio of acetonitrile - (0.02mol/L) ammonium acetate is (60:40), the retention time of promazine hydrochloride on the chromatographic chart is more suitable, the peak type is symmetrical, the sensitivity is high, and there is no interference of the mixed peak before and after.

3.3. Qualitative Analysis of Samples

Under the conditions of detection wavelength of 258nm and flow rate of 1mL/min, 1.0μg/mL and 5.0μg/mL standard working solution were injected once, and the chromatogram with retention time of 9.7min of promethazine hydrochloride was obtained as shown in Figure 3 and Figure 4.

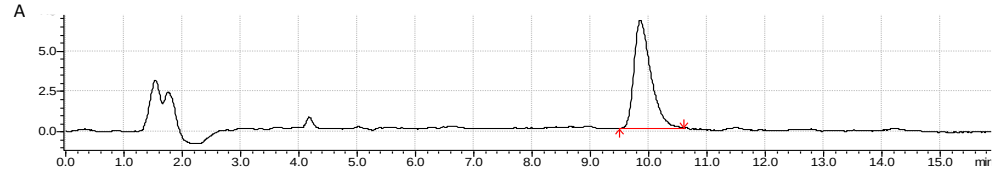

Figure 3: 1.0ug/mL promazine hydrochloride chromatogram

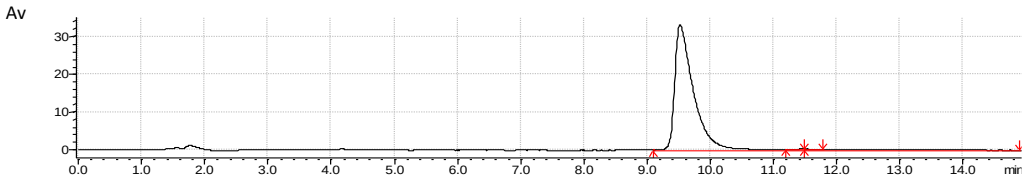

Figure 4: 5.0ug/mL promazine hydrochloride chromatogram

3.4. Standard Curve Drawing

A series of gradient standard solutions with the prepared concentrations of 1.0, 5.0, 10.0, 20.0, 50.0μg/mL were detected. The standard solution of each concentration was injected twice, and the average of the two peak areas was taken. Taking the standard solution concentration (μg/mL) as the abscissate and the mean peak area as the ordinate, the standard curve of promazine hydrochloride was plotted, and the regression equation and correlation coefficient were calculated.

When the concentration of promazine hydrochloride was in the range of 1.0-50.0 μg/mL, the chromatogram peak area was linearly correlated with the concentration. The regression equation was y=11.230x+17.325, and the correlation coefficient R=0.9991. The retention time and peak area of promazine hydrochloride in the standard solution with a series of gradient concentrations are shown in Table 1

Table 1: Peak area and retention time of promazine hydrochloride

Density of reference substance (μg/mL)	Retention time of promazine hydrochloride (min)	Peak area of promazine hydrochloride (mAU)	Mean peak area (mAU)
1.0	9.856 9.849	170513 170899	170706
5.0	9.518 9.782	711369 716655	717012
10.0	9.546 9.436	1386536 1376308	1381422
20.0	9.369 9.472	2519863 2519867	2519777
50.0	9.287 9.329	5734638 5735250	5734944

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3.5. Limit of detection (LOD) and Limit of Quantification (LOQ) Analysis

The baseline noise values of the tissue of the five standard samples and five blank samples were averaged. The detection limit was calculated according to S/N=3 and the limit of quantification was calculated according to S/N=10. The detection limit of promazine hydrochloride was 0.110 μg/mL and the limit of quantification was 0.367 μg/mL. The representative noise chromatogram is shown in Figure 5.

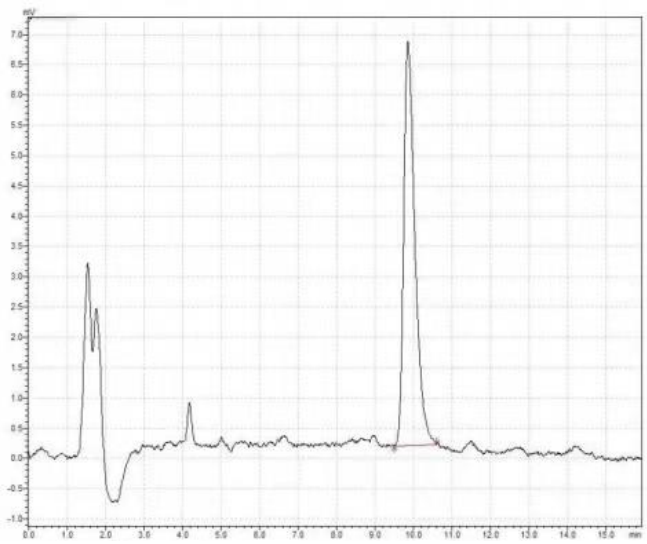

Figure 5: Chromatogram of instrument noise

3.6. Precision Analysis

The standard deviation and relative standard deviation (RSD) of 10.0μg/mL standard compound were calculated for 5 times a day and once a day for 5 days. The results were shown in Table 2 and Table 3, representing intra-day and inter-day precision, respectively.

As can be seen from Table 2 and Table 3, both S value and RSD% value are relatively small, indicating that the fluctuation between the measured data is small, indicating that the precision of the instrument is good, the determination method is stable, and the measured data can be reproduced more accurately [10].

Table 2: S value and RSD% value of 10.0μg/mL standard substance were repeated within one day

Test ripetitions	Peak area	S	RSD/%
1	1391422	15532.20	1.12%
2	1386203		
3	1388598		
4	1368982		
5	1360429		
Average value	1381127		

Table 3: S value and RSD% value of 10.0μg/mL standard substance were repeated within five days

Test ripetitions	Peak area	S	RSD/%
1	1381422	14945.56	1.08%
2	1398067		
3	1388598		
4	1368982		
5	1360429		
Average value	1381127		

3.7. Recovery Analysis

The spiked recovery test was carried out at three supplemental levels of 1.0 , 3.0 , and 5.0μg/mL,

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and the spiked recovery was shown in Table 4.

Table 4: Recycling test data with different concentrations

Fortified Concentration (μg/mL)	The determination of the concentration (μg/mL)	Recovery rate /%	Average recovery rate /%	$\bar{x} \pm SD$	CV%
	0.813	81.30			
1.0	0.792	79.20	81.03	81.03±1.72	2.11
	0.826	82.60			
	2.482	82.73			
3.0	2.375	79.16	81.03	81.03±1.80	2.21
	2.436	81.20			
	4.214	84.28			
5.0	3.996	79.92	82.19	82.19±2.19	2.65
	4.119	82.38			

The standard recovery test of promazine hydrochloride in pork was carried out through three concentration addition levels, and the average recovery rate was calculated to be above 80%. It can be seen that the method established in this paper has a good accuracy in determining the residual amount of promazine hydrochloride in pork, and the method is reliable.

3.8. Sample Determination

Pork from four stores was purchased in Baoding Market. Four kinds of pork samples were treated respectively according to the extraction and purification steps of the tested substance in the samples described in High-pressure liquid chromatography analysis was carried out according to the chromatographic conditions established in The residual amount of promazine hydrochloride in the pork samples was calculated according to the standard curve. The chromatogram of the samples was shown in Figure 6 ~ 9. As can be seen from the chromatogram, there was no peak around 9.7 min, so it was judged that no promazine hydrochloride was detected in the four pig samples.

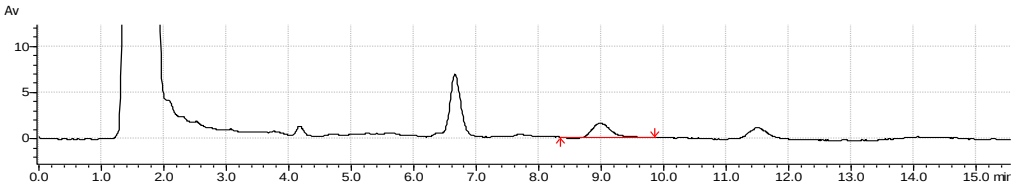

Figure 6: Chromatogram of sample 1

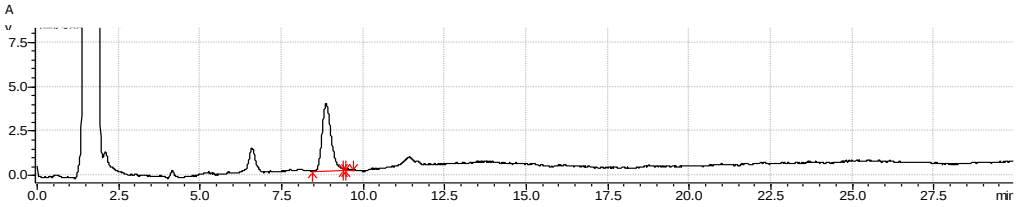

Figure 7: Chromatogram of sample 2

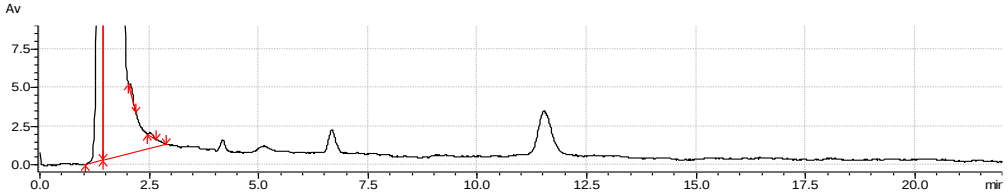

Figure 8: Chromatogram of sample 3

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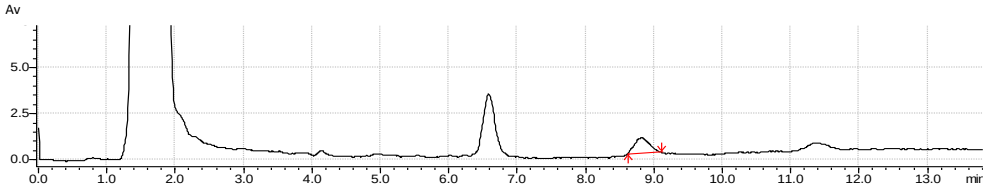

Figure 9: Chromatogram of sample 4

4. Discussion

There are three main problems to be solved in the determination of promazine hydrochloride residue in pork: the first is the determination parameters and conditions; the second is the sample pretreatment method; the third is the reliability of the method[11]. In this paper, the determination of promazine hydrochloride in pork by high pressure liquid chromatography was mainly carried out from these three aspects [12].

The recovery, precision and detection limit of this method can meet the requirements of most daily detection, and it is easy to operate. At the same time, this experiment established two kinds of extraction liquid for chicken promazine hydrochloride extraction comparative experiment, and took the solid phase extraction method to purify the extraction liquid, remove most of the impurities, reduce the interference on the chromatogram, but also effectively extended the use time of the chromatographic column. The method established in this paper has good feasibility and provides reference and basis for the analysis and determination of the residual amount of promazine hydrochloride in pork.

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