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Comparative study on the quality control of trihydroxybenzophenones compounds

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The quality control of three highly active anti-inflammatory and anti-oxidation structurally similar trihydroxybenzophenones, 4,5,2'-trihydroxy-2,5'-dichlorobenzophenone (LC), 4,5,2'-trihydroxy-2,5'-dibromobenzophenone (LB) and 4,5,2'-trimorpho- linyloxy-2,5'-dichlorobenzophenone (LMC) were developed in this paper. According to the common parent nucleus structure and characteristic groups of the three compounds, the quality comparison studies were illuminated from four aspects: traits, identification, inspection and content determination. The appearance, taste, solubility, melting point, melting range, absorption coefficient and acidity of the compound were examined in terms of properties. LC is a dark yellow powder crystal, LB is pale yellow needle one, and LMC is white one but a slight acid odor. They are all weakly acidic compounds.The melting points of both LC and LB are a litter bit higher than 200°C, but LMC's is only 150°C. The absorption coefficients of three compounds are order of magnitude of 10^4, they are 2.8×10^4 (for LC), 3.0×10^4 (for LB), 1.7×10^4 (for LMC), respectively. The absorption coefficient of LB is the largest because of its highest conjugatestructure. Three compounds were identified by chemical method, UV and HPLC method. Among them, the advantage of the chemical method is that it has an intuitive color reaction, so is simple in operation. The UV identification results shown that the maximum absorption wavelength of LMC is 230 nm, but the maximum absorption wavelengths of LB and LC are so similar (261 nm and 259 nm) that they are hard to separate. The HPLC method had very good specificity that it is able to identify the three compounds with completely accuracy. The contents of three compounds were determined severally by acid-base titration, UV and HPLC methods. The acid-base titration method is simple, accuracy, but its sensitivity is a little bit low. The HPLC is with high sensitivity, simple operation and universal applicability, UV had high accuracy and good repeatability. The quality of three batches of samples for three compounds were all in compliance with general Quality Control Standard of Pharmacopoeia including drying weight loss, ignition residue, heavy metal, and sulfate test. The results of these studies shown that the quality control of the three compounds are reliable, stable, accurate and controllable, and the contents of three compounds could be controlled in the range of 98.0 ~ 102.0%.

Keywords: Benzophenone, traits, identification, inspection, content determination

INTRODUCTION

Modern research had found that benzophenone halophenols have high activity in antitumor, antiinflammatory, antioxidation, antibacterial, antithrombosis, biological antifeeding, liver protection and diabetic nephropathy. The natural marine origin three patented compounds [1] including LC, LB and LMC researched by our laboratory group in this paper have good activity on the injury of vascular endothelial EA.hy 926 cells induced by H_2O_2. So the study of their quality control is extremely urgent. The comparative study on quality control of the three compounds had never been studied before. Since they not only have a common nucleus of diphenylketone, but also have characteristic groups, the comparative studies of quality control are meaningful for further predicting their biological activity and studying its *in vivo* detection, monitoring and drug metabolism. This research aims to describe a comparative study on their quality standards including traits, identification, inspction and content determination [2-4]. Quality control researches about these three compounds could not only provide guidance for the preparation of raw materials and pharmaceuticals, but also offering quality standards for preclinical pharmaceuticals and pharmaceuticals for pharmacodynamics, toxicology research and new drug research and development.

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EXPERIMENTAL

Chemicals and reagents

The standard substance (their purity were over 99.0% by HPLC analysis after three recrystallizations) and three compounds (their purity were over 98.0% by HPLC analysis) including LB, LC add LMC were all independently by our research group of Shanxi Medical University. The other chemicals used, such as silver nitrate, nitric acid, phosphoric acid, hydrochloric acid and sodium hydroxide, were obtained from Tianjin damao technology Co., Ltd (Tianjin, China). All solvents used were of HPLC grade. The

chromatographic solvents and reagents such as methanol and acetonitrile were purchased from Tianjin kemiou technology Co., Ltd (Tianjin, China). Deionized water was prepared using a Milli-Q water purifying system from Millipore Corp. (Bedford, MA).

Character check for three compounds

1. Solubility

Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a liquid solvent to form a homogeneous solution of the solute in the solvent. The solubility of a substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as on temperature, pressure and the pH of the solution. The extent of the solubility of a substance in a specific solvent is measured as the saturation concentration, where adding more solute does not increase the concentration of the solution and begin to precipitate the excess amount of solute. Excessive samples were added to 10 mL of solvent of the plugged triangular bottle and the bottle was placed into a 37℃ thermostatic water bath shaker (BS-1E, Honghua instrument factory, China) for 48 h, then was centrifuged for 15 min at 5000 r/min with a centrifuge (TGL-16gR, Anting scientific instrument factory, Shanghai, China). The supernatant was filtered with 0.45 μm microporous filter membrane and the continuous filtrate were taken to determine the absorbance (OD) at the maximum absorption wavelength with a UV spectrophotometer (MAPADA-1200, Meipuda instruments co. LTD, Shanghai, China).

2. Melting point

Melting is a physical process that results in the phase transition of a substance from a solid to a liquid. The internal energy of a substance is increased, typically by the application of heat, resulting in its temperature to the melting point, at which the ordering of ionic or molecular entities in the solid breaks down to a less ordered state and the solid liquefies. 1 mg of dried sample was placed on glass plate and the initial temperature of the automatic melting point meter (SGW X-4, INESA instruments co. LTD, Shanghai, China) was set at 130℃. The glass plate with the sample was heated at a rate of 1.0 ℃/min. The melting point of three compounds were the temperature at which it melts when heated it. The sample should be measured three times and the average value was taken as melting point.

3. Absorption coefficient

The absorption coefficient is a quantity that characterizes how easily a material or medium can be penetrated by a beam of light. 2 mg of samples were weighed precisely and 1000 mL of water was used to dissolve, diluted quantitatively to make a solution containing about 20 μg/mL, and then the solution were quantitatively half diluted to 10 μg/mL. The absorbance values were determined at the maximum absorption wavelength using five UV spectrophotometers (MAPADA-1200, MAPADA-1600 and MAPADA-1800, Shanghai, China; 1900, Shimadzu, Japan; Cary60, Agilent, USA) according to the General Rule 0401 of Part 4 in Chinese pharmacopoeia (2015 edition). The absorbance values (OD) of test solutions should be controlled between 0.3 and 0.7. The percentage absorption coefficients were calculated according to the following formula : $E_{cm}^{1\%} = A / c \times L$, where A refer to the absorbance values, c represented concentration with the unit (g / 100 mL), and L was thickness of object [5].

4. Acidity value

10 mg samples were added to 50 mL of water to prerare a solution. The solution was heated at 60 ℃ for 10 min, then cooled to 25℃, and filtered with 0.45 μm microporous membrane. The filtrate were taken to determine pH value according to general rule 0631 by pH acidity meter (pHS-2C , Shanghai lida instruments co. LTD, China).

Identification of three compounds

1. Chemical identification

One drop of $FeCl_3$ solution was added to the sample solution, and the changed color was used as the identification basis of phenol hydroxyl group. The dried samples were co-heated with an hydrous Na_2CO_3 powder until it turned to black from colorless, then 1 mL HNO_3 (0.1 mol/L) was added to adjust the solution to acidic. Finally, silver nitrate $AgNO_3$ was used to identificate the phenolic hydroxyl, bromide and chloride groups. After $AgNO_3$ solution (0.1 mol/L) was added into above acidic solution, it was used as the basis for the identification of chlorine groups if white precipitate was generated, and light yellow precipitate would be generated as the basis for the identification of bromine groups.

2. UV identification

1 mg of sample was added to methanol to make a solution containing about 10 μg/mL LC, LB and

LMC. The solution was scanned by UV spectrophotometer from 200 to 400 nm to obtain the maximum UV absorption wavelength.

3. HPLC identification

HPLC analysis was carried out using a Agilent 1200 HPLC system (Agilent technology co. LTD, USA) which consisted of a photodiode array detector, an autosampler, and a degasser according to the General rule 0512 of Part 4 in Chinese pharmacopoeia (2015 edition). Chromatographic column was a Diamonsil C18 (250 mm × 4.6 mm, 5 μm) from Dikma Technologies (Beijing, China), and it's temperature was set at 25℃. The mobile phase for HPLC analysis consisted of 80% methanol and 20% water with a rate of 1 mL/min. The detection wavelength was the maximum absorption wavelength of each of the three compounds. Concentration of three compounds were all 200 μg/mL. Sample volume of 20 μL was injected. Prior to use, the mobile phase was filtered through a 0.45 μm hydrophilic membrane filter. The retention time of there compounds under this chromatographic condition was used for identification.

Limit inspection for general impurity

1. Drying weight loss

1.0 g of Samples which were dried to constant weight were measured precisely, and then were dried at 105°C for 30 min. The reduced mass of three samples before and after weighing was recorded and the lossed weight was calculated as the drying weight loss.

2. Residue on ignition and heavy metal

1.0 g of samples were placed in a crucible which had been ablated to a constant weight. The samples were heated slowly until full carbonization, and then 0.5 mL of concentrated H_2SO_4 （98.0%） were added to it, and the sulfur vapor was removed by low-temperature heating (55°C). Then the samples were ignited at 500°C until ashing completely, placed in the dryer to make it cold, then weighed precisely. The residue which left under ignited residue were used to check the content of heave metals according to the second method of General rule 0821 of Part 4 in Chinese pharmacopoeia (2015 edition)

3. Chloride check and sulfate check

2.0 g of the samples were added to 100 mL of water to make the mixed solution. The solution was heated to a boil, then was cooled to 25℃. 25 mL of the filtrate was taken to check chloride according to General rule 0801 of Part 4 in Chinese pharmacopoeia (2015 edition). It would not be thicker than the control solution made of 5 mL of standard sodium chloride solution (0.01%). 25 mL of the filtrate which left under chloride check was taken to check sulfate according to general rule 0802 of Part 4 in Chinese pharmacopoeia (2015 edition) . It will not be thicker than the control solution prepared from 1 mL of standard potassium sulfate solution (0.02%).

Content determination for the three compounds

1. Acid-base titration

4 mg of samples were weighed precisely, and added into 20 mL methanol to prepare test solution which concentration was about 200 μg/mL. The NaOH standard solution (0.01 mol/L) was taken as titrant. 20 mL of methanol was titrated with NaOH as blank correction. Phenolphthalein solution was used as indicator. The color of titration endpoint was shown as in Fig.1. The NaOH standard solution (0.01 mol/L) was used to titrate the sample solution which concentration was about 200 μg/mL. The initial volume and final volume of titrant were recorded, and the consumed volume of titrant NaOH standard solution were measured, thus the content of the sample was calculated as the following formula: Sample content (%) = (V_{NaOH} × c_{NaOH} × M_{sample}) × 100% / (1000 × w), where V_{NaOH}, c_{NaOH}, M_{sample} and w refer to the consumed volume (mL), concentration (mol/L) of NaOH standard solution, molar mass of measured compounds (g / mol) and the mass of weighing sample (g), respectively. The sample would be measured three times and then the average content (%) and RSD (%) value were calculated [6].

Fig.1. The color of titration endpoint for acid - base titration

2. UV-VIS spectrophotometry

10 mg of standard substances and the test three compounds were weighed precisely, transfered quantitatively into a 100 mL volumetric flask after

dissolution with methanol to prepare the corresponding standard store solution (100 μg/ mL), then stored in a refrigerator at 4°C [7-12]. With methanol, the standard store solution were proportionally diluted to different gradients solution which concentration were 0, 1, 5, 25, 50, 75, 100 μg / mL, respectively. The absorbance values OD of these solutions were measured by UV spectrophotometer at the maximum absorption wavelength for making standard curves with corresponding gradient concentration. In order to verify the precision of the method, the OD values of the standard substance solution (50 μg/mL) were measured six times by UV spectrophotometer under the maximum UV absorption wavelength of three compounds, and the RSD (%) value of these six measured OD were calculated. In order to verify the repeatability of the method, five portions test solution (50 μg/mL) were determined by UV spectrophotometer under the maximum UV absorption wavelength of three compounds, and their OD values were used to calculate the RSD (%) as the repeatability. 5 mg of the test samples were weighed precisely, and was added into a 100 mL volumetric flask and diluted to scale with methanol to prepare the test sample solution. To verify the recovery rate of the method, the nine samples of known concentrations were divided into three groups, and then each group was added with a 50 ug / mL control sample solution of 0.8 mL, 1.0 mL and 1.2 mL, respectively. Nine portions solutions were diluted with methanol, and the OD values were measured by UV spectrophotometer under the maximum UV absorption wavelength of three compounds, to calculate the recovery rate according to the following formula: The recovery rate (%) = (the measured quantity after added standard substance – the known quantity of sample) × 100%/ the mass of added standard substance. Three batches of samples were taken to prepare 50 μg/mL of test solution and standard solution with methanol, respectively. The OD values of three batches samples were determined to calculate the content (%) according to standard curve.

3. HPLC chromatography

HPLC analysis as "*3.3 HPLC identification*" was carried out using a Agilent 1200 HPLC system to determinate contents of the three compounds. Chromatographic column was also Diamonsil C18 (250 mm × 4.6 mm, 5 μm), and the detect temperature was 25°C. The velocity of the mobile phase is 1 mL/min. Different chromatographic conditions [13-19] were shown in Tab.1.

Table 1. Different chromatographic conditions for three compounds

Comp.	Detection wavelength (nm)	Mobile phase
LB	261	Methanol: water (75:25)
LC	259	Methanol: water (75:25)
LMC	230	Acetonitrile:water (60:40)

10 mg of standard substances and the test three compounds were weighed precisely, transfered quantitatively into a 100 mL volumetric flask after dissolution with methanol to prepare the corresponding standard store solution (100 μg/ mL), then stored in a refrigerator at 4℃. The standard and test substance store solutions were diluted to 50 μg/mL with mobile phase for detecting the degree of separation, tailing factor and theoretical plate number by HPLC according to the chromatographic conditions shown in Tab.1. With methanol, the standard store solution were proportionally diluted to 0, 1, 5, 25, 50, 75, 100 μg / mL as "*5.2 UV-VIS spectrophotometry*". The peak area of these solutions were measured by HPLC to make a standard curve with corresponding concentration. The RSD (%) of the precision, accuracy, stability and repeatability of the experiments were also validated as "*5.2 UV-VIS spectrophotometry*". The standard substance solution were diluted step by step and were detected to find LOD and LOQ which S/N equal to 3 and 10, respectively. Three batches of samples (about 50 μg / mL) of test solution and standard solution with mobile phase were determined to calculate the content (%) according to standard curve.

RESULTS AND DISCUSSIONS

The result of character check

The appearance, taste, solubility, melting point, melting range, absorption coefficient and acidity of three compounds were examined in terms of properties. LC is a dark yellow powder crystal, but LB is pale yellow needle one, and LMC is white and a slight acid odor.

Table 2. The result of physical constants

	LB	LC	LMC
Melting point (°C)	224	203	150
Percentage absorption coefficient $E_{cm}^{1\%}$	3×10^4	2.8×10^4	1.7×10^4
Acidity value	5.5 - 7.5	5.5 - 7.5	6.4 - 8.0

All there compounds are soluble in methanol and ethanol acetonitrile, dissolve in ethyl acetate, almost insoluble in dichloromethane, petroleum ether and water. The parts of their physical constants were determined and shown in Tab.2.

The result of identification

Refer to the General rule 0301, identification of the chemical identification were shown in Fig.2. After $AgNO_3$ solution (0.1 mol/L) was added into the HNO_3 acidic solution, phenolic hydroxyl was seen as Fig.2a, and light yellow precipitate would be bromine groups as Fig.2b, conclusively white precipitate was generated as chlorine groups as Fig.2c.

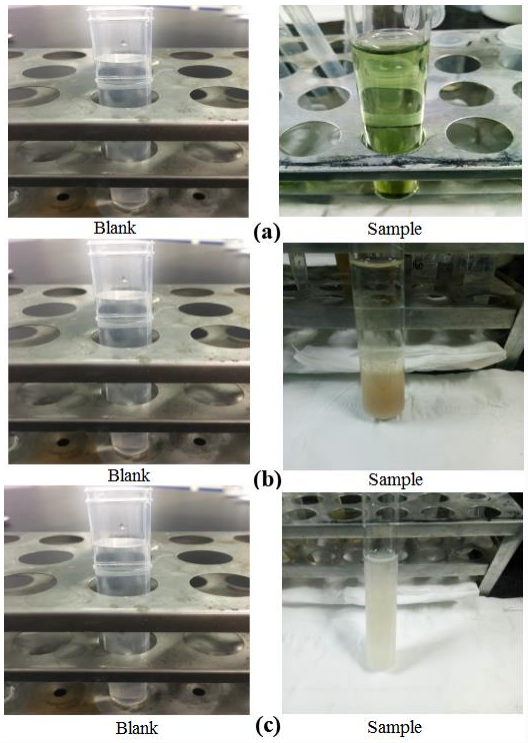

Fig.2. The phenomenon of the chemical identification (a) phenolic hydroxyl (b) bromide (c) chloride

The chemical identification results of three compounds were shown in Tab.3. The blank solution does not interfere with the identification. The chemical methods was specific for identification of three compounds's three batches samples.

Table 3. The result of chemical identification

	phenolic hydroxyl	bromide.	chloride
LB	+	+	
LC	+		+
LMC	-		+

UV wavelength scanning for there compounds were showed in Fig.3. With UV full wavelength scanning from 200 to 400 nm, the maximum absorption of LB, LC and LMC at 261, 259, and 230 nm, respectively. The UV identification results shown that the maximum absorption wavelength of LMC is 230 nm, but the maximum absorption wavelengths of LB and LC are so similar (261 nm and 259 nm) that they are hard to separate.

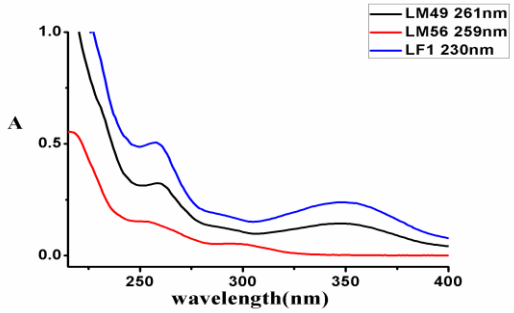

Fig .3. UV wavelength scanning for there compounds

Three compounds were identificated by HPLC. The result of HPLC identificationa for there compounds shown in Fig.4. The retention time of LB, LC and LMC at 9.35, 9.905, and 8.947 min for three batches samples, respectively. The HPLC method had a very good specificity that it is able to identify the three compounds with completely accuracy.

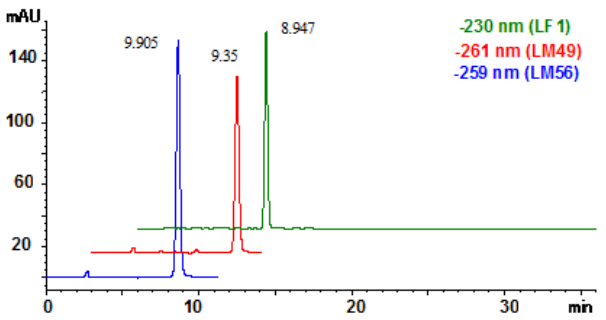

Fig .4. HPLC identificationa for there compounds

The result of limit inspection for general impurity

The weight loss would not exceed 0.5% according to the General rule 0831. According to the General rule 0841, the residue on ignition would not exceed 0.1%. The contents of heavy metals would not exceed 10 parts per million according to the General rule 0821. The solution of the examined chloride would not be thicker than the control solution made of 5 mL of standard sodium chloride solution (0.01%) according to the

General rule 0801. The solution of the examined sulfate would not be thicker than the control solution prepared from 1 mL of standard potassium sulfate solution (0.02%) according to the General rule 0802. Limit inspections for general impurity including the weight loss, residue on ignition, contents of heavy metals, solution of the examined chloride, solution of the examined sulfate, all complied with the limit of Part 4 in Chinese pharmacopoeia (2015 edition) for three batches samples of compounds.

The content determination for the three compounds

The contents of three compounds were determined by acid-base titration, UV and HPLC methods. Using UV method, the linear regression equation of LB, LC and LMC were: $Y = 0.034\ X + 0.0048$ (r = 0.9997), $Y = 0.028\ X + 0.0026$ (r = 0.9994), $Y = 0.0168\ X + 0.0036$ (r = 0.9991) had been obtained, respectively. The results of methodo-logical validation for UV content determination were shown in the Tab.4.

Table 4. The result of methodological validation about UV method

Validation	LB	LC	LMC
RSD (%) for precision	1.4	1.0	1.2
RSD (%) for repeatability	1.5	1.6	1.7
RSD (%) for stability	1.4	1.3	1.4
Recovery rate (%)	98.3	99.2	101.6

Using HPLC method, the linear regression equation of LB, LC and LMC were: $Y = 43.207\ X + 0.261$ (r = 0.9997), $Y = 50.482\ X + 34.059$ (r = 0.9993), $Y = 45.15\ X - 25.68$ (r = 0.9996) had been obtained, respectively. The results of methodological validation for HPLC content determination were shown in the Tab.5.

Table 5. The result of Methodological validation about HPLC method

Validation	LB	LC	LMC
RSD (%) for accuracy	0.8	0.8	0.9
RSD (%) for repeatability	1.1	1.0	1.1
RSD (%) for stability	1.7	0.8	1.7
Recovery rate (%)	99.8	100.2	99.8
LOD (ng/mL)	1.0	1.5	3.0
LOQ (ng/mL)	3.0	5.0	10.0

According to the results of menthod evaluation, the RSD (%) of the accuracy, stability and repeatablity of the experiments were all within 2 %. All of these methodological verification could met the requirements of quantitative analysis. The result of content determination was shown in Tab.6.

Table 6. The result of Content determination (n = 3)

Methods	LB	LC	LMC
UV- Content (%)	100.2	99.1	101.8
HPLC- Content (%)	99.9	99.5	100.2
Chemical- Content (%)	98.3	98.5	98.7

CONCLUSIONS

Through the comparative study of the quality of the three compounds, we can see that LC and LB are similar in appearance, solubility, melting point and other physical parameters due to their highly similar structures. But they can be identified easily by chemical and HPLC methods because they have their own characteristic groups. LMC is fundamentally different from LC and LB in appearance and melting point, which is due to the characteristic group of its structure. The general impurity test results of the three compounds were all in compliance with the General Quality Control Standard of Pharmacopoeia (2015). Among three methods for measuring the content of compounds, although the acid-base titration method is simple to operate, the accuracy and precision of the results were slightly lower than those of the other two methods because of the subjective error in judging the color of titration endpoint. The results of the three content determination methods shown that the quality control of the three compounds is all reliable, stable, accurate and controllable. The contents of three compounds could be controlled in the range of 98.0~102.0%. Through the research in this paper, the quality standards of the three compounds have been preliminarily established. The establishment of the quality standard can not only provide guidance for the production process of the compound, but also provide theoretical basis for later pharmacological and pharmacokinetic studies.

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NOMENCLATURE

RSD - relative standard deviation;
UV - ultraviolet&visible spectrophotpmetry;
LOQ - limit of quantitation;
LOD - limit of determination;
S/N - Signal to noise ratio;
HPLC - High Performance Liquid Chromato-graphy;

REFERENCES

[1] Zhao WY, Feng XE, Ban SR (2010) Synthesis and in vitro antioxidant activity of novel halophenols. *Chinese journal of pharmaceutical chemistry* **20**, 264-268.
[2] China Pharmacopoeia Committee. Pharmacopoeia of the People’s Republic of China (Ed). China Chemical Industry Press, Beijing, 2015.
[3] Liao B, Chen ZH (2003) Research and Formulation of Quality Standards for New Chemicals. *Chinese Journal of New Drugs* **12**, 164-168.
[4] Liu ZL, Yuan BJ. Research and Formulation of Quality Standards for New Drugs Western Medicine） **28**, 6-9, 1993.
[5] Wu MZ, Yuan BJ, Lu Ml. Establishment and application of expert system for the formulation of new drug quality standards **19**, 453-456, 1993.
[6] Zhang N, Guo DL Introductionto silver nitrate titrationfor chloride ion content determination in 93-98.
[7] Lu W, Sun XM, Sun JJ. Comparison of two methods for the determination of Astragaloside. *Clinical Journal of Chinese Medicine* **8**, 43-45, 2016.
[8] Wang YX, He JJ, Fang D. Exploration of the Methods by Quantitative Determination of VC in Common Fruits and Vegetables. *Anhui Agri. Sci. Bull* **21**, 20-22, 2015.
[9] Zhang NJ, Liu Y. Study on Determination Content of Ioversol. *Guangzhou Chemical Industry* **43**, 146-148, 2015.
[10] Xie Q, Wang W, Chen QX (2014) Comparative Study on Three Different Methods for the Determination of Total Phenolics in Chinese Olive **25**, 204-207, 2014.
[11] Tang YL, Jing K. Improvement of the determination method of the assay of midodrinehy drochloride, **38**, 59-61, 2016.
[12] Chen ZG, Shan GQ, Tan XQ. Determination of Quercetin Content in Raw Drug By Ultraviolet Spectrophotomery. *Progress in Veterinary Medicine* 37, 122-124, 2016.
[13] Li Y, Feng XE. Determination of 2,5’- Dibromo-4,5,2’-trihydroxydiphenylmethanone and Its Related Substances by HPLC. *Chin JMAP* **30**, 1104-1107, 2013.
[14] Li J, He LL, Chou LY. Determination of Contents and Related Substance in Citrate Tandospirone Tablets by Hplc. *Journal of Southwest University for Nationalities, Natural Science Edition* **42**, 183-187, 2016.
[15] Li QX, Zheng SF. Determination of the Content and Related Substances of Metoprolol SuccinateUsing HPLC Method. **31**, 632-636, 2017.
[16] Chen H, Zhu R. Content Determination of Allopurinol Sustained-release Tablets and Related Substances. *China Pharmacy* **24**, 80-82, 2013.
[17] Zhao HL, Wang C, Xie DF. RP-HPLC Determination of Eplerenone and Its Related Substances. *Chin Pharm* **51**, 663-667, 2016.
[18] Wang Y, Sun KL, Shi BY. Determination of related substances in cangrelor by HPLC. *Chinese Journal of New Drugs* **27**, 110-114, 2018.
[19] Feng XE, Zhao WY, Liang TG. Study on the determination of 3-chloro-3',4'-dihydroxy diphenylmethanone and its related sub-stances by RP-HPLC. *Chin Hosp Pharm* **31**, 185-187, 2011.

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