Method for Improving the Determination of Dibromoacetic Acid in Cefathiamidine

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Abstract [**Objectives**] To establish an LC-MS method for the determination of dibromoacetic acid in Cefathiamidine and Cefathiamidine for Injection. [**Methods**] Shiseido PC HILIC column (2.0 mm × 100 mm, 3 μm) was used. The mobile phase was 0.005 M ammonium formate and the mobile phase was acetonitrile with gradient elution. The flow rate was 0.3 - 0.8 mL/min. [**Results**] The limit of detection was 0.250 0 ng/mL and the limit of quantification was 0.500 ng/mL, which were 2.5% and 5.0% of the impurity limits, respectively. The recovery rate was 103.85%. [**Conclusions**] This method improved the detection of dibromoacetic acid impurities in Cefathiamidine, and has that advantage of good specificity, low limit of detection and limit of quantification, high sensitivity, high accuracy, interference resistance, can meet the detection requirements of Cefathiamidine, and is suitable for the quality control of Cefathiamidine.

Key words Cefathiamidine, Dibromoacetic acid impurities, LC-MS

1 Introduction

Cefathiamidine is a kind of cephalosporins and plays a bactericidal role by inhibiting the synthesis of bacterial cell wall[1]. It has antibacterial activity against Gram-positive bacteria and some Gram-negative bacteria, especially against Gram-positive cocci. It is mainly used to treat respiratory system, hepatobiliary system, five sense organs, urinary tract infection, endocarditis and septicemia caused by sensitive bacteria. Bromoacetyl bromide is an important raw material for the synthesis of Cefathiamidine[2]. In the preparation process, the carbonyl α position of bromoacetyl bromide may form polybrominated substitutes[3], and polybrominated acetyl bromide will also participate in the drug synthesis reaction, thus introducing impurities. Due to the presence of dibromoacetyl bromide as an impurity in bromoacetyl bromide, it will be converted to dibromoacetic acid under post-treatment conditions (alkaline, aqueous solution). Therefore, dibromoacetic acid may remain in Cefathia midine[4]. In order to study and control dibromoacetic acid in Cefathiamidine, first, its qualitative and quantitative detection is necessary. Due to the low impurity limit and weak ultraviolet absorption, it is difficult to use the conventional HPLC-UV method for detection, and the method specificity is poor[5]. Therefore, it is necessary to develop a sensitive, specific and anti-interference method for the qualitative and quantitative detection of dibromoacetic acid impurities in Cefathiamidine.

2 Materials and methods

2.1 Instruments and reagents Liquid chromatograph, tandem mass spectrometer, Sartorious, chromatographic column (Lot No.: PC HILIC, 2.0 mm × 100 mm, 3 μm) Shiseido, formic acid (MS) acetonitrile (HPLC) Merck, dibromoacetic acid [1 mg/mL in MtBE (219041608)] Accustandard, Cefathiamidine (Lot No.: LMA1911003), Reference Cefathiamidine for Injection (Lot No.: 190901, Baiyunshan Chemical Pharmaceutical Factory of Guangzhou Baiyunshan Pharmaceutical Holdings Co., Ltd.), Cefathiamidine for injection (Lot No.: SSJ200401, SSJ200404, Guangxi Kelun Pharmaceutical Co., Ltd.).

2.2 Working conditions of instrument

2.2.1 Chromatographic conditions. Shiseido PC HILIC column (2.0 mm × 100 mm, 3 μm) was used with gradient elution of 0.005 M ammonium formate and acetonitrile; injection volume: 5 μL, flow rate: 0.5 mL/min, column temperature: 30 ℃, running time: 5 min, injection temperature: 5 ℃.

2.2.2 Mass spectrum conditions. The ion source was AJS-ESI in negative ion mode and MRM mode. Gas flow: 8 L/min, gas temperature: 320 ℃, nebulizer: 50 psi, sheath gas temperature: 350 ℃, sheath gas flow: 11 L/min, capillary: -3 500 V, nozzle voltage: -500 V; scan time: 0 - 1.8 min, Delta EMV(-): -200 V, cell accelerator voltage: 4 V, dwell: 80 ms.

2.3 Solution preparation

2.3.1 Stock solution of reference substance. Measured 100 μL of dibromoacetic acid reference substance (1 mg/mL methyl tert-butyl ether solution), put it into a 10 mL volumetric flask, added methyl tert-butyl ether to dilute it to the scale, and shook it up; took 0.5 mL, put it into a 50 mL volumetric flask, diluted it to the scale with 90% acetonitrile solution, and shook it up to obtain the stock solution of reference substance.

2.3.2 Reference substance solution. Took 1 mL of reference substance stock solution, put it into a 10 mL volumetric flask, diluted it to the scale with 90% acetonitrile solution, and shook it up to obtain the reference substance solution.

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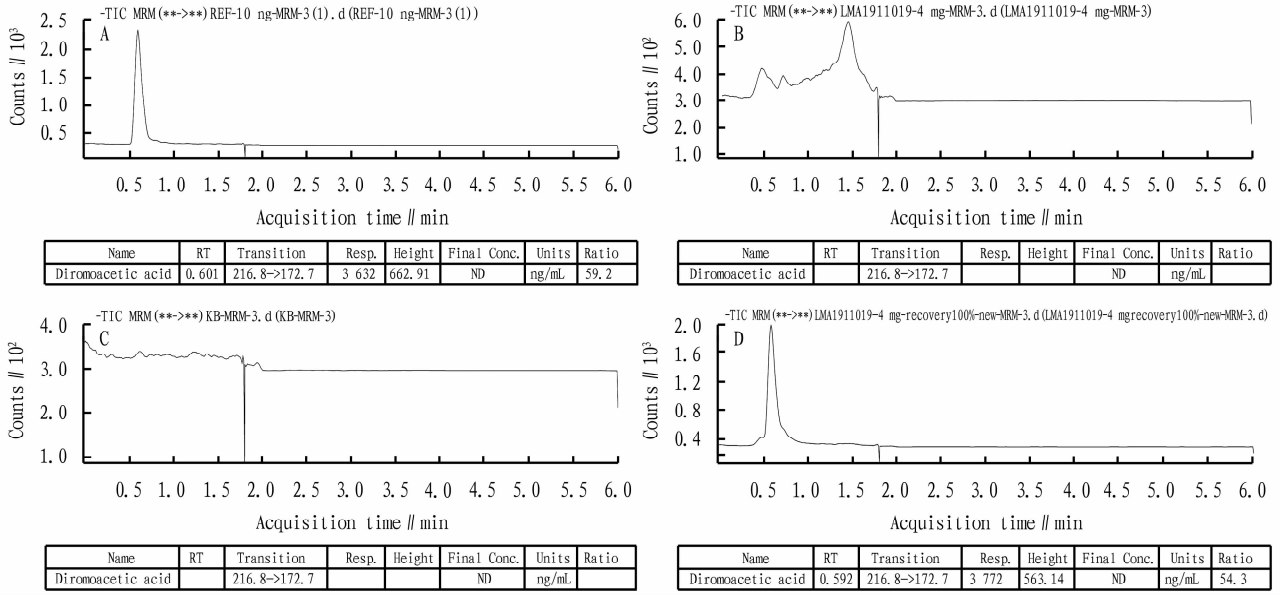
2.3.3 Test solution. Took (40 ± 0.5) mg of Cefathiamidine test sample to be tested, weighed it accurately, put it into a 10 mL volumetric flask, added 90% acetonitrile solution to dissolve and dilute it to the scale, and shook it up to obtain the test solution.

2.3.4 Recovery solution. Accurately weighed 41.38 mg of Cefathiamidine test sample, put it into a 10 mL volumetric flask, added 1 mL of reference substance stock solution, added 90% acetonitrile solution to dissolve and dilute it to the scale, and shook it up.

3 Results and analysis

3.1 Specificity investigation Took blank solution (90% acetonitrile solution), reference substance solution, test solution and recovery solution for sample injection and detection, and the results are shown in Fig. 1. The results showed that the diluent, test solution and other impurities did not interfere with the determination of impurity dibromoacetic acid, and the method had good specificity.



Name	RT	Transition	Resp.	Height	Final Conc.	Units	Ratio
Diromoacetic acid	0.601	216.8->172.7	3 632	662.91	ND	ng/mL	59.2

Name	RT	Transition	Resp.	Height	Final Conc.	Units	Ratio
Diromoacetic acid		216.8->172.7			ND	ng/mL	

Name	RT	Transition	Resp.	Height	Final Conc.	Units	Ratio
Diromoacetic acid		216.8->172.7			ND	ng/mL	

Name	RT	Transition	Resp.	Height	Final Conc.	Units	Ratio
Diromoacetic acid	0.592	216.8->172.7	3 772	563.14	ND	ng/mL	54.3

NOTE A. reference substance solution; B. test solution; C. blank solution (90% acetonitrile solution); D. recovery solution.

Fig. 1 Sample injection and detection chromatogram of each solution

3.2 Linear range Took appropriate amount of stock solution of reference substance, diluted it to different concentrations step by step with 90% acetonitrile solution, and calculated the regression curve and regression coefficient of dibromoacetic acid when the sample concentration is 4 mg/mL. The results showed that there was a good linear relationship in the concentration range of 0.50 – 20.00 ng/mL (5.0% – 200% limit), and the linear equation was $y = 499.786\ 3x - 74.367\ 1$, $R^2 = 0.998\ 0$ (Fig. 2).

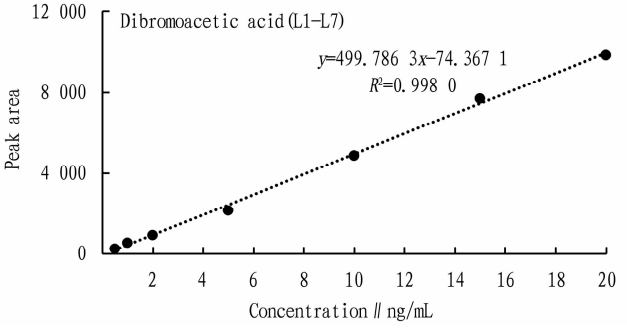


Fig. 2 Regression curve and linear equation

3.3 Limit of quantification (LOQ) and limit of detection (LOD) The LOD concentration for the impurity dibromoacetic acid was 0.250 0 ng/mL (2.5% limit) below the 20% limit based on a signal-to-noise ratio of ≥3, and the LOQ concentration was 0.500 0 ng/mL (5.0% limit) below the 30% limit based on a signal-to-noise ratio of ≥10.

3.4 Accuracy test The recovery test was performed at 50%, 100%, and 150% of the dibromoacetic acid impurity limit (2.5 ppm). Recovery solution-50%, 100%, 150%: weighed 40 mg of the test sample, put it into a 10 mL volumetric flask, added 0.5, 1.0, 1.5 mL of the reference substance stock solution separately, added 90% acetonitrile solution to dissolve and dilute to the scale, shook up. As shown in Table 1, through adding the impurity dibromoacetic acid with a limit concentration of 50% – 150% to the sample, the mean recovery was 91.14%, and the *RSD* ($n = 6$) was 5.55% < 15%.

3.5 Precision test The results showed that the repeatability *RSD* of the control solution was 3.39%, the repeatability *RSD* of the recovery solution was 3.77%, the peak area had no obvious change trend, the recovery rate of impurity dibromoacetic acid in the recovery solution was 103.85%, the experimental results showed that the method had good precision.

3.6 Stability test Took the reference substance solution and recovery solution – 100% and placed them at 5 ℃ for different time, and then performed injection and analysis. As indicated in Table 2, when the reference substance solution was placed at 5 ℃ for 6.5 h and the recovery solution was placed at 5 ℃ for 5.9 h,

Table 1 Accuracy test results

No.	Peak area	Measured amount // ng/mL	Background ng/mL	Added amount // ng/mL	Recovery rate // %	Average recovery rate // %	*RSD* // % (n = 6)
50% -1	2 248	4.631	Not detected	5.000	92.62	91.14	5.55
50% -2	2 364	4.870			97.39		
50% -3	2 330	4.800			95.99		
100% -1	4 028	8.298		10.00	82.98		
100% -2	4 687	9.655			96.55		
100% -3	4 163	8.576			85.76		
150% -1	6 632	13.66		15.00	91.08		
150% -2	6 602	13.60			90.67		
150% -3	6 354	13.09			87.26		

there was no significant difference in the peak area of the impurity dibromoacetic acid between 0 and 5.5 h (the peak area change values were all less than 20%), indicating that the reference substance solution and the test solution were stable within 5.9 h at 5 ℃.

Table 2 Stability test results

Retention time // h	Areference solution	Change // %	Retention time // h	Arecovery solution	Change // %
0	5 225	-	0	4 028	-
1.8	4 764	8.82	1.2	3 840	4.67
2.4	4 567	12.59	1.8	3 768	6.45
4.0	5 062	3.12	3.3	3 640	9.63
6.5	5 238	0.25	5.9	3 942	2.14

3.7 Durability recovery solution test The test parameters were finely adjusted, and the test results showed that the solvent blank had no interference, the average recovery rate of the recovered solution was 103.45%, and the *RSD* (n = 5) was 2.02% <15%, and the results were shown in Table 3, which met the requirements.

Table 3 Recovery rate of durability recovery solution

Method	Detection conditions						Recovery rate // %	Statistical analysis (n = 5)
	Column temp // ℃	Gas temp // ℃	Gas flow // L/min	Nebulizer // Psi	Sheath gas temp // ℃	Sheath gas flow // L/min		
Basic conditions	30	320	8	50	350	11	103.85	Average recovery rate:
Durability 1	25	320	8	50	350	11	104.90	103.45%
Durability 2	35	320	8	50	350	11	101.33	*RSD*: 2.02%
Durability 3	30	300	7	45	330	10	101.29	
Durability 4	30	340	9	55	370	12	105.91	

4 Conclusions

The accurate quantitative analysis of the content of dibromoacetic acid with low quantitation limit is very helpful for monitoring the formation of dibromoacetic acid in the production process and controlling its content within an acceptable range[6-8]. This method for detecting dibromoacetic acid impurities in Cefathiamidine and its preparation can effectively carry out qualitative and quantitative detection on the Cefathiamidine bulk drug and the dibromoacetic acid impurity possibly existing in the Cefathiamidine medicine bottle for injection. It has good specificity, the limit of detection (LOD) concentration is 0.250 0 ng/mL and the limit of quantification (LOQ) concentration is 0.500 ng/mL, which are 2.5% and 5.0% of the impurity limit respectively. It has high sensitivity, high accuracy and anti-interference, and can meet the requirements of Cefathiamidine detection[9-12]. The method for detecting Cefathiamidine and dibromoacetic acid impurities in its preparation adopts an LC-MS detection method and improves the ionization efficiency and the stability of dibromoacetic acid through the optimization of mass spectrum atomization chamber parameters and chromatographic conditions, improving the response, thereby improving the sensitivity, the precision and the recovery rate. The method is simple, the operation is simple, the applicability is good, and the guarantee is provided for the quality control of the Cefathiamidine bulk drug and the preparation thereof.

In summary, the detection method of dibromoacetic acid impurities in Cefathiamidine by LC-MS not only has high sensitivity and accuracy, but also has good specificity and anti-interference ability, which provides strong support for the quality control of Ce-

fathiamidine. This is of great significance to ensure the safety and effectiveness of drugs and the safety of patients, and provides a strong guarantee for the quality control of Cefathiamidine raw materials and their preparations.

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