Determination of related substances in lisinopril and amlodipine tablets by HPLC

Dao-Rui Yu¹, Gui-Fang Yang², Wen-Li Xiao², Jun Wang², Qi-Bing Liu³*  
¹Functional Laboratory, Hainan Medical College, Haikou 571199, China  
²Hainan Zhongji Pharmaceutical Technology CO., LTD, Haikou 570208, China  
³College of Pharmacy, Hainan Medical College, Haikou 571199, China

Objective: To establish an HPLC method for determining the related substances in lisinopril and amlodipine tablets. Methods: An Inertsil Thermo BDS HYPERSIL C18 (4.6 mm x 250 mm, 5 μm) column was used with the Acetonitrile-water-phosphoric acid (10:90:0.1) as mobile phase A and Acetonitrile-water-phosphoric acid (90:10:0.1) as mobile phase B by gradient elution at the detection wavelength of 215 nm. The flow rate was 1.0 mL/min and the column temperature was 30 °C. Results: The separation of the impurity peak and peak was good. Besides, all the impurities could be detected effectively. Conclusions: The method is sensitive, accurate and selective. It is suitable for control the related substances in lisinopril and Amlodipine tablets.

1. Introduction

Lisinopril amlodipine by ACEI drugs lisinopril with calcium antagonists fixed amount of ammonia acid compound preparation consisting of amlodipine, by the Hungarian Jerry large pharmaceutical research and development, for the treatment of primary hypertension[1,2]. Lisinopril amlodipine listed in 2004 in Hungary, the European Medicines Agency approval in the Czech Republic, Estonia, Lithuania, Latvia, Poland, Romania and Slovakia and other EU member states Snow listed[3] in 2008. According to the chemical structure of lisinopril and amlodipine besylate and possible degradation modes of the major degradation impurities include lisinopril lisinopril impurity A, lisinopril impurity B and impurity C lisinopril , amlodipine besylate of the major degradation impurities amlodipine impurity D.

No current domestic and international pharmacopoeia collection contains lisinopril amlodipine, and no reports of the species related substances analysis. This article was established HPLC method for the determination of lisinopril amlodipine related substances, lisinopril impurity A, lisinopril impurity B, impurity C lisinopril and amlodipine impurity D major degradation impurities individually controlled method is sensitive, accurate and specific good.

2. Materials and methods

2.1. Instruments

Shimadzu HPLC (Shimadzu of Suzhou, SPD-10A vp Plus UV - visible detector, LC-10AT vp Plus Solvent Delivery Pump, CBM-10A vp Plus System Controller, LCsolution Lite Workstation), Agilent 1100 HPLC (Agilent of USA, G1322A Degassing device, G1312A Solvent Delivery Pump, G1313A Autosampler, G1316A Column Oven, G1315B DAD).

2.2. Reagents

Lisinopril amlodipine (Hainan Ji Pharmaceutical Co., Ltd, Batch number: 120301, 120302, 120303, Specification: 10/5 mg); Lisinopril (China Institute of Food and Drug Test, Batch number: 100814-200701, Contents: 91.2%); Amlodipine (China Institute of Food and Drug Test, Batch number: 100374-200903, Contents:...
99.4%); Lisinopril impurity A (Shanghai Ye Source Biotechnology Co., Ltd, Batch number: 20111201; Purity: 98%); Lisinopril impurity B (Shanghai Ye Source Biotechnology Co., Ltd, Batch number: 20111107; Purity: 98%); Lisinopril Impurity C (self-control, Batch number: DKP111101S; Purity: 99%); Amlodipine impurity D (USP, Batch number: F01143; Purity: 100.0%); Acetonitrile (Chromatography, Company of Merck); Distilled water, Other reagents were of analytical grade.

2.3. Chromatographic conditions

Column: Thermo BDS HYPERSIL C18 (4.6 mm x 250 mm, 5 μm); The mobile phase: Acetonitrile - water - phosphoric acid (10:90: 0.1) as the mobile phase A, Acetonitrile - water - phosphoric acid (10:90: 0.1) as the mobile phase B, Gradient elution (Gradient program shown in Table 1); Velocity of flow: 1.0 mL/min; Detection wavelength: 215 nm; Column temperature: 30 °C; Injection volume: 20 μL (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>The mobile phase A (%)</th>
<th>The mobile phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>35</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>35.1</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>45</td>
<td>90</td>
<td>10</td>
</tr>
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</table>

Gradient elution program.

2.4. Preparation of the solution

2.4.1. Test solution

This product powder amount was taken (Lisinopril 10 mg, amlodipine 5 mg), accurately weighed, and set in 10 mL volumetric flask. Appropriate amount of solubilizers [acetonitrile - water (1:1)] was performed with 10 min sonication, cooled, diluted with a solvent to the mark. Test solution was shaken, centrifuged to get the supernatant.

2.4.2. Control solution

The precise amount of the test solution 1 mL was set in 100 mL volumetric flask, diluted with a solvent to the mark, and shaken.

2.5. Specificity test

2.5.1. Accessories interference test

A blank prescription matching accessories was taken, added appropriate amount of solvent, underwent ultrasonic for 10 min, then was cooled, diluted to the mark with the solvent, shaken, centrifuged to get supernatant. Chromatographic conditions were determined. Accessories for this product related substances were measured without interference.

2.5.2. Impurity separation test

A mixed solution of lisinopril, amlodipine besylate, lisinopril impurity A, lisinopril impurity B, impurity C lisinopril and amlodipine containing impurities D were taken. Press chromatographic conditions was the same as "2.1". Its chromatogram was shown in Figure 1. The separation of the impurity peak lisinopril and amlodipine between the two main component peak and between the impurity peaks were eligible.

![Figure 1. HPLC chromatograms of impurities separation test.](image)

1: lisinopril; 2-5: amlodipine-lisinopril impurities B, A, C; 6: amlodipine impurity D

2.5.3. Forced degradation testing

This product powder (lisinopril 10 mg, amlodipine 5 mg) was taken, and set in 10 mL flask. It was added with appropriate amount of solvent, and underwent ultrasonic for 10 min at 100 °C water bath for about 2 h. It was then allowed to cool, diluted with a solvent to the mark, shaken, centrifuged. Press chromatographic conditions was as "2.1", and chromatogram was shown in Figure 2B.

This product powder amount (lisinopril 10 mg, amlodipine 5 mg) was taken, and set in 10 mL flask. It was added with appropriate amount of solvent, and underwent ultrasonic for 10 min. It was then allowed to cool, then exposed for about 12 h to light (4 500 lx) next, followed by centrifugation. Press chromatographic conditions were as "2.1", chromatogram was shown in Figure 2C.

This product powder amount (lisinopril 10 mg, amlodipine 5 mg) was taken, and set in 10 mL flask. It was added with appropriate amount of solvent, and underwent ultrasonic for 10 min. It was added with 1 mol/L sodium hydroxide solution 1 mL, left at room temperature for about 2 h, added with 1 mol/L hydrochloric acid solution 1 mL, diluted with a solvent to the mark, shaken, centrifuged. Press chromatographic conditions were as "2.1", chromatogram was shown in Figure 2D.

This product powder amount (lisinopril 10 mg, amlodipine 5 mg) was taken, and set in 10 mL flask. It was added with 5 mL solvent, and underwent ultrasonic for 10 min. It was added with 30% hydrogen peroxide solution 1 mL, set about 40 °C water bath for about 2 h after cooling, diluted with a solvent to the mark, shaken, centrifuged. Press chromatographic conditions were as "2.1", chromatogram was shown in Figure 2E.

This product powder amount (lisinopril 10 mg, amlodipine 5 mg) was taken, and set in 10 mL flask. It was added with 5 mL solvent, and underwent ultrasonic for 10 min. It was added with 1 mol/L hydrochloric acid solution 1 mL, set 60 °C water bath for about 1 h, then was cooled, added with 1 mol/L sodium hydroxide solution 1 mL, diluted with a solvent to the mark, shaken, centrifuged. Press chromatographic conditions were as "2.1", chromatogram was shown in Figure 2F.

3. Results

The test results showed that the drug at high temperature, light,
strong alkalis, strong acids and oxidative damage, each degradation product peak could separate the peaks and lisinopril and amlodipine peak (Figure 2).

3.2. Linear relationship and correction factor calculation

Lisinopril, amlodipine besylate, lisinopril impurity A, lisinopril impurity B, impurity C lisinopril and amlodipine impurities D was taken with a solvent to dissolve and quantitative dilution made a series of concentrations solution, the chromatograms was recorded. In concentration as abscissa and peak area of the vertical axis, the standard curve was used to calculate the principal component regression equation with impurity correction factor linear range and various impurities corresponding main component. The results are shown in Table 2.

3.3. Accuracy tests

Lisinopril impurity A, lisinopril impurity B, impurity C and the average recovery of lisinopril amlodipine impurity D were 93.4%, 102.9%, 107.6%, 94.1%, RSD were 4.5%, 2.8%, 4.1%, 6.3% (n=9).

3.4. Calculation methods and determination of impurities in the sample

Lisinopril impurity A, lisinopril lisinopril impurity B and impurity C correction factors were within the range of 0.9 to 1.1. The correction factor was 0.78; unknown impurities were attributable to amlodipine, without a correction factor calculated using principal component self-control method.

Three batches of samples were taken, the test solution and control solution was prepared by the method of “2.2”, then chromatographic conditions were as “2.1”, the measurement results shown in Table 3.

4. Discussion

Lisinopril amlodipine piece compound, comprising lisinopril and amlodipine besylate two active ingredients, USP, BP, JP and Chinese pharmacopoeia were not for lisinopril amlodipine resumption load. USP and JP reproduces lisinopril and amlodipine besylate tablets two tablets prescribed preparations, its related substances were measured

<table>
<thead>
<tr>
<th>Name</th>
<th>Linear range (μg/mL)</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Correction factor</th>
</tr>
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<tbody>
<tr>
<td>Lisinopril</td>
<td>2.00-15.00</td>
<td>Y=293028X+3614</td>
<td>0.999</td>
<td>4</td>
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<tr>
<td>Amlodipine</td>
<td>1.00-7.50</td>
<td>Y=253048X+3371.1</td>
<td>0.999</td>
<td>4</td>
</tr>
<tr>
<td>Lisinopril impurity A</td>
<td>0.60-4.50</td>
<td>Y=273340X+467.79</td>
<td>0.999</td>
<td>0</td>
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<tr>
<td>Lisinopril impurity B</td>
<td>0.60-4.50</td>
<td>Y=318754X-3057.1</td>
<td>0.999</td>
<td>6</td>
</tr>
<tr>
<td>Lisinopril impurity C</td>
<td>2.00-15.00</td>
<td>Y=307591X-5627.1</td>
<td>0.997</td>
<td>6</td>
</tr>
<tr>
<td>Amlodipine impurity D</td>
<td>0.50-3.75</td>
<td>Y=323590X+1082.5</td>
<td>0.998</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2

Results of Linear relationship and the correction factor calculation.
by high performance liquid chromatography[4,5] use. Because of lisinopril and amlodipine besylate polarity difference between the larger, or the use of lisinopril tablets of amlodipine besylate tablets prescribed preparations related to material conditions, each known impurity and the main component is difficult to effectively separate, Therefore, we have developed acetonitrile - water - phosphoric acid system, gradient elution method, the analysis time is moderate, and the degree of separation between the impurity and the main component of the impurity between the meet the requirements.

Related Substances wavelength USP and CP were contained in the lisinopril tablets is 215 nm, the wavelength of the Related Substances of amlodipine besylate tablets to 237 nm[6]. Since lisinopril lisinopril and impurity absorption at 237 nm wavelength is very small, while the acid amlodipine amlodipine impurities at 215 nm wavelength have good absorption[7,8], so Selective related substances lisinopril amlodipine compound preparation at 215 nm wavelength.

By heat, light, acids, alkalis and strong oxidation, forced degradation tests, the preliminary understanding of the degradation pathways of drugs, combined with stress testing and long-term, accelerated stability test, the source compound preparation of impurities were assigned, the main degradation product clear of drugs and controlled to ensure product quality. In addition, the selected method of method validation test, the results of the validation tests are in line with the provisions show that the method is sensitive, accurate, specific, and can effectively control the product impurities.

<table>
<thead>
<tr>
<th>Batch number</th>
<th>Lisinopril impurityA</th>
<th>Lisinopril impurityB</th>
<th>Lisinopril impurityC</th>
<th>Amlodipine impurityD</th>
<th>Other largest single impurity</th>
<th>Total impurities</th>
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<tbody>
<tr>
<td>120301</td>
<td>0.011</td>
<td>not detected</td>
<td>0.026</td>
<td>0.037</td>
<td>0.088</td>
<td>0.343</td>
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<tr>
<td>120302</td>
<td>0.015</td>
<td>not detected</td>
<td>0.027</td>
<td>0.043</td>
<td>0.089</td>
<td>0.358</td>
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<tr>
<td>120303</td>
<td>0.010</td>
<td>not detected</td>
<td>0.025</td>
<td>0.042</td>
<td>0.086</td>
<td>0.340</td>
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References