Determination of Related Substances of Mosapride Citrate in the Capsule Formulation by HPLC

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ABSTRACT: OBJECTIVE To establish an HPLC method for the assay of mosapride citrate related substances in the capsule that might originate from synthesis processes or degradation. **METHODS** The related substances in mosapride citrate capsules were analyzed by a chromatographic system comprising a reverse phase C_{18} analytical column, a mobile phase of methanol-0.02 mol·L^{-1} potassium dihydrogen phosphate buffer (pH adjusted to 4.0 with o-phosphoric acid) (50∶50), a flow rate of 1 mL·min^{-1} and a UV detector set at 274 nm. **RESULTS** The HPLC method had shown good chromatographic resolution for mosapride citrate and its related substances. The analyte concentration was found to be 500 μg·mL^{-1}, and RSD of peak area responses of the related substances was 1.3%. The sample and standard solutions were stable for 24 hours at room temperature. The calibration curve was linear in the range from 3.0 to 15.0 μg·mL^{-1} (r=0.999 7, n=6). The precision RSD at three different concentration levels (3, 5, 10 μg·mL^{-1}) were less than 1.5%. The recovery was between 100.5% and 101.4%. In addition, analysis of samples subjected to accelerated stability conditions showed that all degradants were resolved from the active component, resulting in a stability-indicating assay. **CONCLUSION** The HPLC method is simple, rapid, selective and suitable for quantitative determination of mosapride citrate related substances in the capsule.

KEY WORDS: mosapride citrate; HPLC; related substances; capsule

HPLC测定枸橼酸莫沙必利胶囊中的有关物质

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摘要：目的 建立反相高效液相色谱法测定枸橼酸莫沙必利胶囊中有关物质的总量。**方法** 分析柱采用 Shimadzu C_{18} (150 mm×4. 6 mm, 5 μm)，流动相为 0.02 mol·L^{-1} 磷酸二氢钾缓冲液(用磷酸调 pH 至 4.0)-甲醇(50∶50)，流速为 1 mL·min^{-1}，检测波长为 274 nm。**结果** 该法能将枸橼酸莫沙必利与其有关物质有效分离。供试溶液的浓度确定为 500 μg·mL^{-1}，室温下稳定，有关物质的峰面积重复性良好。莫沙必利浓度在 3.0~15.0 μg·mL^{-1} 与峰面积线性关系良好(r=0. 999 7，n=6)，精密度 RSD<1.5%，回收率为 100.5%~101.4%。而且，该法能用作稳定性试验的分析方法。**结论** 该法简单、快速、灵敏，适合用于枸橼酸莫沙必利胶囊中有关物质的检查。

关键词： 枸橼酸莫沙必利；高效液相色谱法；有关物质；胶囊剂

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Impurity profiling is an important issue in pharmaceutical analysis, particularly during product development and quality control. Mosapride citrate, a novel gastroprokinetic agent with a benzamide ring, behaves as a selective 5-HT_4 agonist and enhances only upper gastroprokinetic motor activity[1]. And mosapride citrate capsule is a recent listing of new drugs in China. There were several compounds, including degradants and process related intermediates originated from the synthesis that could be present as potential impurities in the capsule of mosapride citrate. Although some assay methods had been applied for the quantification of mosapride citrate and its process related impurities in bulk drugs and tablets [2-4], there were no analytical methods appeared in the literature for resolution and determination of mosapride citrate and its impurities in the capsule. Aim of this study was developing a simple, reliable determination method for mosapride citrate and its related substances. Proposed method was allowed to monitor the level of related substances in mosapride citrate capsules. Accelerated and

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long-term stability tests showed that the method was reliable and suitable for application to stability studies.

1 Experimental

1.1 Apparatus

An Agilent 1100 series liquid chromatograph system comprising vacuum degasser, quaternary pump, thermostatted column compartment, and UV detector was used. The column used was a Shimadzu C_{18} (150 mm×4.6 mm, 5 μm). The column was operated at 40 ℃. Detection wavelength was 274 nm. The mobile phase adopted for this study was methanol-0.02 mol·L^{-1} potassium dihydrogen phosphate buffer (50∶50). Buffer solution was prepared by dissolving 2.722 g potassium dihydrogen phosphate in 1000 mL water and the pH was adjusted to 4.0 with *o*-phosphoric acid. The analysis was performed at a flow rate of 1.0 mL·min^{-1}. The sample injection volume was 20 μL. Manual injections were made using a Rheodyne injectable valve (20 μL loop).

1.2 Samples and materials

The standard substance of mosapride citrate (purity 99.6%, lot 021211) was obtained from Jiangsu Hansoh Pharmaceutical Co., Ltd., China, and three lots of mosapride citrate capsules (5 mg) were obtained from Shanghai Pharmaceutical (Group) Co., Ltd., China. Potassium dihydrogen *ortho* phosphate and orthophosphoric acid were procured from Chengdu Chemical Reagent Factory (Chengdu, China). Methanol was of HPLC grade. All the other chemicals and reagents were of analytical grade. Water was deionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a membrane filter (pore size 0.45 μm).

1.3 Preparation of standard solutions and samples

Stock standard solution was prepared separately by accurately weighing 10.0 mg of mosapride citrate reference standards into 500 mL volumetric flasks, dissolving in 250 mL methanol with the aid of sonication and being made up to the volume with 0.02 mol·L^{-1} potassium dihydrogen phosphate buffer. Working standard solutions, 3.0-15.0 μg·mL^{-1}, were prepared from the stock standard solutions by diluting with the mobile phase. The content of mosapride citrate capsules, weighed approximately equal to 5 mg mosapride citrate, were dissolved in 5 mL methanol and made up to 10 mL with 0.02 mol·L^{-1} potassium dihydrogen phosphate buffer as for the test solutions (approximately 500 μg·mL^{-1}). 1.0 mL portion of the resulting solution was then transferred into 50 mL volumetric flasks. The content was diluted to the mark with the mobile phase. Diluted solutions were used as the control solutions (approximately 10 μg·mL^{-1}). Approximately 1 mL of each final solution was filtered through a 0.45 μm membrane filter prior to injection for HPLC analysis.

1.4 Assay procedure

According to the Pharmacopoeia, control solutions were injected into the HPLC, and peak height responses were obtained. Then chromatographic conditions were adjusted to elute mosapride citrate at 10% full scale response. Injections were made for the test solutions (500 μg·mL^{-1}). The sample chromatograms obtained, recording double relative retention time of mosapride citrate. A relative response factor was 1.0 because the related compounds were not unidentified. The following equation was used to calculate the content of total related substances: % of total related substances=$(A_T \times 100)/(A_C \times 50)$ where A_T and A_C represented the total peak area of the related substances detected in the test solutions and peak area of mosapride citrate detected in the control solutions.

2 Results

2.1 Concentration of test solution

The analyte concentration was determined by injecting four sample solutions of mosapride citrate (100, 200, 500, 1000 μg·mL^{-1}). Chromatograms of different analyte concentration of the drug indicated that there was the maximum number of the related impurities at concentrations of 500, 1000 μg·mL^{-1}. The results demonstrated that all of the related substances could be well resolved from mosapride citrate (Rs>1.5) and signal–noise ratios of 11 was obtained at concentration of 500 μg·mL^{-1}, while the proposed method could not well separate mosapride citrate from adjacent related substance (Rs<1.5) at concentration of 1 000 μg·mL^{-1}. Five replicate injections were made for 500 μg·mL^{-1} drug solution and the total peak area ratio response of the related substances was 101.9±1.3. The RSD of peak area responses for five replicate injections was 1.3%, which could meet the acceptance criterion established

for the determination of related substances. The analyte concentration of mosapride citrate was found to be 500 μg·mL^{-1} through these results.

2.2 Stability of analytical solutions

The stability of a standard solution of the drug substance was examined by analyzing separate portions of the solution stored at room temperature for 24 hours and at 4 ℃ in a refrigerator for 7 days against a freshly prepared standard solution. Both solutions did not show any change in the concentration of the analyte after the storage period. The solution stored at room temperature gave an assay value of 100.3% of mosapride citrate, while that stored in the refrigerator contained 100.1% mosapride citrate. These values were in excellent agreement with 100.0% of mosapride citrate detected in the initial (fresh) standard solution indicating that a standard solution of the drug substance in the mobile phase was stable for 24 hours at room temperature and at least 1 week at 4 ℃. The stability of the sample was determined by injecting the test solutions of the drug at 0, 6, 12 and 24 h of post-preparation at room temperature with protection of samples from direct sunlight. No peaks corresponding to the degradation products were observed and there was no significant change in the drug's peak area. The test solution was found to be stable in the mobile phase for at least 24 h.

2.3 Selectivity and specificity

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. According to recommended methods by the Pharmacopoeia, intentional degradation was achieved by exposing the contents of the capsules to stress conditions of direct heat (80 ℃), humidity (92.5% *RH*), light (4500 *lx*, a 125 *W* daylight lamp), acid (2 mol·L^{-1} HCl), alkali (2 mol·L^{-1} NaOH) and oxidation (3% H_2O_2) in order to test the ability of the proposed method to separate mosapride citrate from the degradation products. In each case, about 5 mg of mosapride citrate was allowed to stand under above stress conditions. After the degradation treatments were completed, all samples were analyzed following the chromatographic conditions. Chromatograms of degraded samples of mosapride citrate by direct heat, humidity and light were individually shown in Fig 1 (A–F), indicating that all of the degradation products could be well resolved from mosapride citrate (*Rs*>1.5). The proposed method was found to be specific to mosapride citrate and its related substances.

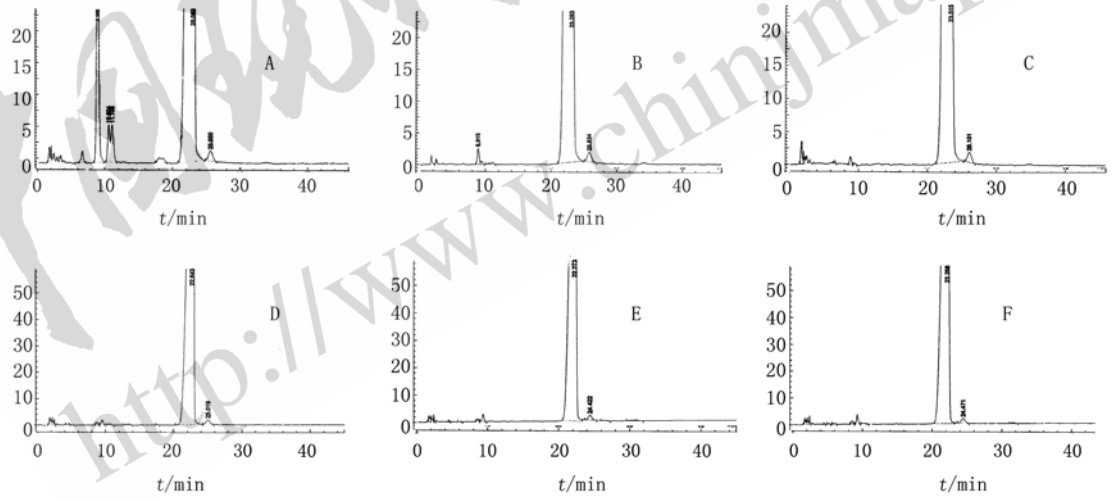

Fig 1 Chromatograms of degraded samples of mosapride citrate under the stress conditions

A–heat; B–humidity; C–light; D–acid; E–alkali; F–oxidation

图 1 枸橼酸莫沙必利破坏条件下的色谱图

A–高温；B–高湿；C–强光；D–酸；E–碱；F–氧化

2.4 Linearity

The degree of linearity was assessed by the correlation coefficient, intercept and slope. The test samples varying in concentration from 3.0 to 15.0 μg·mL^{-1} were prepared and analyzed to evaluate the linearity of the method. It could be seen that the plot of peak area responses was linear over the concentration of mosapride citrate yielding a regression equation Y=1 117X–3.16(n=6) with a correlation coefficient r=0.999 7. A P-value of

1.319 0 for the *y*-intercept indicated that the intercept was statistically equal to zero (P>0.2). These results demonstrate that a single point calibration could be used for potency assay of mosapride citrate.

2.5 Precision and recovery

System precision was determined from the RSD of five replicate injections of the standard solution of mosapride citrate at three different concentration levels. The RSD of peak area responses was respectively 1.4%, 1.3%, 1.2% at 3, 5, 10 μg·mL^{-1}, which met the acceptance criterion established for the method. The contents of mosapride citrate capsules apart from the drug were dissolved in 50 mL mobile phase. 1.0 mL portions of the resulting solution were then transferred into 10 mL volumetric flasks separately. The samples were prepared by accurately transferring 1.5, 2.5, 5.0 mL of stock standard solution (20 μg·mL^{-1}) into above flasks, and being made up to the volume with the mobile phase. The recovery of mosapride citrate was determined by the ratios of concentrations calculated by calibration curve to with those of the standard solution. The results showed the recovery was respectively 100.5%, 100.8%, 101.4% at 3, 5, 10 μg·mL^{-1}.

2.6 Robustness

Although a formal robustness assay had not been achieved, this method had been applied over two years for a stability test of the drug capsules with assays at 0, 1, 3, 6, 9, 12, 18 and 24 month. Two different equipment (Agilent 1100 LC and Shimadzu LC 10A) and alternate C_{18} silica column from different manufacture (Shimadzu and Agilent) had been employed. The method had always passed the system suitability test. Therefore, it could be considered robust.

2.7 Method application

The validated HPLC method was applied to the determination of mosapride citrate related substances in the capsule. Three lots of mosapride citrate capsules were determined. For all the lots, the total amount of the related substances was <2.00%. Furthermore, accelerated stability tests and long-term stability tests of the drug capsules were analyzed using the present HPLC method. The results were presented in Tab 1. The results clearly showed the differences in the impurity profiles of the samples, so the HPLC method was suitable for quantitative determination of mosapride citrate related substances in the capsule.

Tab 1 HPLC assay of stability tests of mosapride citrate capsules for its related substances

表 1 枸橼酸莫沙必利胶囊稳定性实验的有关物质含量

Time/month	Long-term stability tests[1)]/%			Accelerated stability tests[2)]/%		
	Lot 030301	Lot 030302	Lot 030303	Lot 030301	Lot 030302	Lot 030303
0	0.97	0.98	0.96	Same as long-term stability tests		
1	0.94	0.96	0.94	1.04	1.04	1.04
2		\		1.07	1.06	1.05
3	0.99	1.00	0.99	1.09	1.10	1.09
6	1.03	1.02	1.02	1.12	1.17	1.11
9	1.05	1.05	1.06			
12	1.11	1.15	1.12			
18	1.25	1.23	1.21		\	
24	1.35	1.28	1.32			

Note: [1)]Drug capsules stand under conditions of room temperature (25 ℃) and humidity (64% *RH*); [2)]Drug capsules stand under stress conditions of heat (40 ℃) and humidity (75% *RH*)

注：[1)]药物胶囊置于室温25 ℃和相对湿度64%条件下；[2)]药物胶囊置于温度40 ℃和相对湿度75%条件下

3 DISCUSSIONS

The chromatographic separations of mosapride citrate and its related substances were investigated using different mobile phases consisting of citrate and phosphate buffers in combination with methanol or acetonitrile. The separation of the analytes varied substantially with the chromatographic conditions examined. For instance, a composition of acetonitrile or methanol-buffer solution (0.1 mol·L^{-1}citric acid) produced no resolution between mosapride citrate and the adjacent peak. A trial using a mobile phase consisting of potassium dihydrogen phosphate (0.02 mol·L^{-1}) and acetonitrile did not produce good separation between mosapride citrate and its related compounds. Finally, a mobile phase consisting of 50 ∶ 50 of 0.02 mol·L^{-1} potassium dihydrogen phosphate buffer (pH 4.0)/methanol offered a good separation of mosapride citrate and its related substances, and resolution (Rs>1.5) could meet the acceptance criterion established for the method.

An HPLC method for the assay of mosapride citrate related substances in the commercial drug products was validated in this study. Mosapride citrate and the other related substances that may coexist with it as impurities or as degradants gave chromatograms of well resolved peaks, which indicated the specificity of the method. All the statistical values (linearity, precision, RSD, recovery) were within the acceptable limits. The validated method may be regarded as a stability indicating one, which proved to be suitable for assessing the stability of mosapride citrate capsules.

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