The Determination of Metronidazole and Chlorhexidine Gluconate in Metronidazole and Chlorhexidine Lotion by HPLC

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Abstract. Objective "To establish an HPLC method for the determination of metronidazole and chlorhexidine gluconate in metronidazole and chlorhexidine lotion. Method Using Agilent Eclipse-XDB-C18 chromatographic column, with 0.05 mol·L⁻¹ potassium dihydrogen phosphate solution 1000 ml plus 13.2 ml 10% tetrabutylammonium hydroxide aqueous solution (pH adjusted to 3.5 by phosphoric acid)-acetonitrile (77:23) as Mobile phase, detection wavelength 230 nm. Results The two components could be separated well. The linear ranges of metronidazole and chlorhexidine acetate were 36.33~59.04 μg·ml⁻¹ (r = 0.9994) and 35.45~220.11 μg·ml⁻¹ (r =1); The average recoveries were 100.6% and 100.5 %, and the RSD were 0.42% and 0.58%. Conclusion: The method is simple and specific, and the result is more accurate and reliable. Which is suitable for simultaneous determination of two components in compound preparations.

Keywords: High Performance Liquid Chromatography, Metronidazole, Chlorhexidine Acetate, Chlorhexidine Gluconate.

Metronidazole and Chlorhexidine Lotion is a compound preparation with metronidazole and chlorhexidine gluconate as the main components. It is mainly used to treat various vaginitis caused by bacteria, trichomonas and molds. In the national drug standards, UV-visible spectrophotometry is used to determine the content of chlorhexidine gluconate and metronidazole respectively [1]. This content determination method is cumbersome to operate and there are many interference factors in the experiment. In order to make the determination method of metronidazole and chlorhexidine lotion more accurate, refer to the literature [2-4] this time and improve it. The HPLC method is used to simultaneously determine metronidazole and glucose in metronidazole and chlorhexidine lotion. The content of chlorhexidine acid is suitable for retention under the same chromatographic conditions, providing a reliable analysis method to ensure the quality controllability of the drug [5-6].
1. Instruments and Reagents

1.1 Apparatus
High performance liquid chromatograph: Agilent 1260 (UV detector)
   Chromatographic column: Agilent Eclipse-XDB-C18 (4.6×250mm, 5μm)
   Ultraviolet spectrophotometer: Shimadzu UV2450

1.2 Drug Test
Metronidazole reference substance (batch number: 100191-200606), chlorhexidine acetate reference substance (batch number: 100183-201003) are all from the National Institute for the Control of Pharmaceutical and Biological Products, metronidazole and chlorhexidine lotion (200 ml: metronidazole 0.04 g of azole and chlorhexidine gluconate 0.24 g) 3 batches of commercially available raw materials of metronidazole and chlorhexidine gluconate are provided by Hainan Huanglong Pharmaceutical Co., Ltd. Acetonitrile and triethylamine are chromatographically pure (TEDIA), Other chemical reagents are of analytical grade.

2. Methods and Results

2.1 Chromatographic Conditions
Take 0.05 mol·L-1 potassium dihydrogen phosphate solution 1000 ml, add 13.2 ml 10% tetrabutylammonium hydroxide aqueous solution (phosphoric acid adjusted to pH 3.5)-acetonitrile (77:23) as mobile phase; flow rate 1 ml/min; detection The wavelength is 230 nm; the injection volume: 20 μl. see picture 1.

![Figure 1. HPLC chromatograms of metronidazole and chlorhexidine acetate reference substances (1-metronidazole, 2-chlorhexidine acetate)](image-url)
2.2 Preparation of the Solution
Accurately weigh the appropriate amounts of metronidazole and chlorhexidine acetate reference substances, and dilute them with mobile phase to each 1ml containing 0.04 mg metronidazole and 0.16 mg chlorhexidine acetate as reference solutions. Precisely measure an appropriate amount of this product and dilute it with mobile phase to contain 0.04 mg of metronidazole per 1 ml and 0.24 mg of chlorhexidine gluconate per 1 ml as the test solution.

2.3 Methodological Verification

2.3.1 Specificity test. In order to investigate whether the degradation products of metronidazole and chlorhexidine gluconate interfere with the main components under various conditions, the above-mentioned high performance liquid chromatography was used to perform oxidative destruction and acid destruction on metronidazole and chlorhexidine gluconate APIs. Alkali damage, 95 °C water bath heating damage and light damage samples are measured. The results showed that metronidazole drug substance was not degraded significantly, was relatively stable, had good resolution, and had strong specificity; while chlorhexidine gluconate drug substance had multiple degradation product peaks, but had good resolution and strong specificity.

2.3.2 Linearity test. Take 40 μg metronidazole and 160 μg chlorhexidine acetate reference solution per 1 ml, and take 16, 18, 20, 22, 24, and 26 μl injections respectively. Take the sample concentration as the abscissa (X), peak area is the ordinate (Y), and the standard curve is drawn. The results show that metronidazole has a good linear relationship with its peak area in the concentration range of 36.33~59.04 μg·ml⁻¹, y=22.817x-32.709, r=0.9994; Acetic acid chloride It has a good linear relationship with its peak area in the concentration range of 135.45~220.11 μg·ml⁻¹, y=51.41x-4.845, r=1.

2.3.3 Instrument precision test. The metronidazole and chlorhexidine acetate reference substances were accurately weighed, dissolved and diluted with mobile phase in an appropriate amount to contain 0.04 mg metronidazole and 0.16 mg chlorhexidine acetate per 1 ml, and injected 6 times continuously. The RSD of metronidazole and chlorhexidine acetate were 0.75% and 0.24%, respectively, with good precision.

2.3.4 Recovery rate test. Weigh three parts each of about 16, 20, and 24 mg of metronidazole raw materials, put them in a 50 ml volumetric flask, dissolve them with an appropriate amount of mobile phase ultrasonically, and make the volume constant. Weigh three parts each of about 51, 64, and 77 mg of chlorhexidine gluconate raw materials, put them in a 50 ml volumetric flask, and add 5 ml of the above-mentioned metronidazole raw material solution and 10 ml of the blank auxiliary material solution to them, using the mobile phase Dilute and make to volume. The average recovery rate of metronidazole was 100.6%, RSD was 0.42%, n=9; the average recovery rate of chlorhexidine gluconate was 100.5%, RSD was 0.58%, n=9.

2.3.5 Repeatability test. Prepare the solution according to 2.3.5. Metronidazole averaged 99.8%, RSD was 0.1%, n=9; Chlorhexidine gluconate averaged 99.0%, RSD was 1.6%, n=9, with good repeatability.

2.3.6 Stability test. Take the test solution at room temperature, take samples and insert them into the needles after 0, 2, 4, 6, and 8 hours. In 8 hours, the change rate of metronidazole content is 0.17~1.16%, and the change rate of chlorhexidine gluconate content is: -0.80~0.26%. The content of the two components is also relatively stable with time.

2.3.7 Assay. Three batches of commercially available metronidazole and chlorhexidine lotion were measured using the high-performance liquid method established in this experimental study and the
UV-Vis spectrophotometry stipulated in the new drug conversion standard of the Ministry of Health of the People’s Republic of China (Volume 15) Method to determine the content of metronidazole and chlorhexidine gluconate [1], the results are shown in Table 1.

**Table 1. HPLC method and UV test of Metronidazole and chlorhexidine lotion**

| Sample number | HPLC          | UV          |
|---------------|---------------|-------------|
|               | Metronidazole content% | Chlorhexidine gluconate content% | Metronidazole content% | Chlorhexidine gluconate content% |
| 1#            | 101.4         | 101.9       | 102.0        | 102.7       |
| 2#            | 102.4         | 107.9       | 103.7        | 107.6       |
| 3#            | 100.6         | 107.6       | 102.6        | 106.1       |

2.4 The Result
In the national drug standards, UV-visible spectrophotometry is used. After the color reaction of chlorhexidine acetate, the absorbance is measured at a wavelength of 475 nm. The reference substance is operated in the same way to calculate the content of chlorhexidine gluconate; The absorbance is measured at a wavelength of 318 nm, and the content of metronidazole is calculated by the E value [1]. The operation is cumbersome, the specificity is not strong, and the interference factor is high. The newly established HPLC method is used to determine the content of metronidazole and chlorhexidine lotion, which not only makes metronidazole and chlorhexidine gluconate, which have a large difference in polarity, have a suitable retention time under the same chromatographic conditions. The precision, specificity, linearity, recovery, stability, repeatability and other experimental verifications of this method are accurate, simple to operate, sensitive and specific, and suitable for the determination of metronidazole and chlorhexidine lotion.

3. Discussion

3.1 Conversion
In the ultraviolet absorption spectrum of chlorhexidine acetate and chlorhexidine gluconate, the main absorption is chlorhexidine, so the content of chlorhexidine acetate can be replaced by chlorhexidine gluconate by the conversion factor of 1.435.

3.2 Selection of Detection Wavelength
After UV scanning, it was found that metronidazole and chlorhexidine gluconate had a higher response at a wavelength of 230 nm. Therefore, 230 nm is selected as the detection wavelength of this method.

3.3 Selection of Mobile Phase
Reference [3], using methanol-water-triethylamine (56:44:0.1) (adjust the pH to 3.0 with 50% phosphoric acid) as the mobile phase, the column Waters C18, the flow rate is 0.8 mL·min⁻¹. The detection wavelength is 295 nm, and the retention time of metronidazole and chlorhexidine acetate is about 2min and 5min. Under this chromatographic condition, due to the short retention time of metronidazole, it is easily interfered by related substances, which may affect the determination of metronidazole.

The sample is two-component. In order to keep the retention time of the two components within a more appropriate range, an ion pair reagent is selected as one of the mobile phase components.
3.3.1 Selection of mobile phase component ratio. Take 1000 ml of 0.1 mol·L⁻¹ potassium dihydrogen phosphate solution, add 20 ml of 10% tetrabutylammonium hydroxide aqueous solution (adjust the pH to 4.0 with phosphoric acid)-acetonitrile as the mobile phase, according to different ratios (80:20, 75:25, 77:23, 70:30, 60:40) for testing. It is found that when the mobile phase ratio is 77:23, the resolution, column efficiency, peak shape and retention time of the two components are better.

3.3.2 Selection of mobile phase concentration. Compare through four different mobile phases: phosphate buffer (0.1 mol·L⁻¹ potassium dihydrogen phosphate solution 1000 ml), add 10% tetrabutylammonium hydroxide aqueous solution 20 ml (pH adjusted to 4.0 by phosphoric acid)-acetonitrile (77:23); Phosphate buffer (0.1 mol·L⁻¹ potassium dihydrogen phosphate solution 1000 ml), add 13.2 ml of 10% tetrabutylammonium hydroxide aqueous solution (pH adjusted to 4.0 by phosphoric acid)-Acetonitrile (77:23); Phosphate buffer (0.0 5 mol·L⁻¹ potassium dihydrogen phosphate solution 1000 ml), add 13.2 ml of 10% tetrabutylammonium hydroxide aqueous solution (pH adjusted to 4.0 by phosphoric acid)-acetonitrile (77:23), the above mobile phase metronidazole There was no significant change in the peak time. Considering the peak time of chlorhexidine acetate and the amount of reagent, the solution was selected.

3.3.3 Selection of mobile phase pH. Take 0.05 mol·L⁻¹ potassium dihydrogen phosphate solution 1000 ml, add 13.2 ml 10% tetrabutylammonium hydroxide aqueous solution (adjust the pH to 3.0, 3.5, 4.0, 4.5, 5.0 with phosphoric acid respectively)-acetonitrile (77:23) as Mobile phase. The retention times of metronidazole and chlorhexidine acetate did not change significantly, but there were different degrees of tailing. When the mobile phase pH was 3.5, the peak shapes of metronidazole and chlorhexidine acetate were the best.

3.4 Choice of Solvent
Chlorhexidine gluconate dissolves well in water, but metronidazole is not easy to dissolve in water; the test product, metronidazole, and chlorhexidine gluconate are easy to dissolve in acetonitrile and mobile phase. In order to eliminate the interference of solvents, The mobile phase is used as the solvent, and the peak shapes of metronidazole and chlorhexidine gluconate are better.

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