Analysis of gatifloxacin injection by HPLC with fluorescence detection

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ABSTRACT: OBJECTIVE To develop an HPLC method with fluorescence detection for analysis of gatifloxacin(GTX) injection. **METHOD** The HPLC system consisted of CLC-ODS column(15cm × 6.0mm, 5μm) and a RF-10AXL fluorescence detector. The mobile phase used composed of 0.05 mol·L^{-1} citric acid-acetonitrile (80∶20, v/v), adjusted pH to 3.0 with triethylamine, and the flow rate was set at 1 mL·min^{-1}. The fluorescence detector was set at λex = 360nm and λem = 465 nm. GTX injection was diluted 1000 times with water, then 10μL was injected into HPLC System. **RESULTS** The method was linear at the GTX range from 4.0 ng to 32.0ng, the recovery of the method was (99.98 ±0.06) %(n=5), and the RSD was <2 % for precision of intra-day and between-day. **CONCLUSIONS** The method is simple, rapid and accurate, can be used for determination of GTX.

KEY WORDS: gatifloxacin, HPLC, fluorescence detection

高效液相色谱荧光法测定加替沙星注射液的含量

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摘要:目的 建立高效液相色谱荧光法测定加替沙星注射液的含量方法。方法 CLC-ODS 柱(15mm × 6.0mm, 5μm),流动相:0.05mol/L 枸橼酸-乙腈(80∶20),三乙胺调 pH3.0,流速 1 mL·min^{-1},Ex = 360nm, Em = 465nm,样品稀释 1000 倍,进样 10μL。结果 加替沙星在 4~32ng 范围内线性关系良好,回收率(99.98 ±0.06) %,日内、日间 RSD<2 %。结论 该法简便、快速、准确,可用于测定加替沙星注射液的含量。

关键词:加替沙星;高效液相色谱法;荧光检测
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Gatifloxacin(GTX) is a new 8-methoxy-fluoroquinolone with antibiotic expanded antibacteria spectrum than ciprofloxacin and ofloxacin. It has a potent activity against gram-positive, gram-negative bacteria, including penicillin-resistant streptococcus pneumonia, community-acquired pneumonia. However, there no crystalluria and skin photosensitivity were observed in healthy volunteers[1]. It was approved for use in the United States in December 1999[2]. It was reported that column-switching reversed phase HPLC with fluorescence detection was used to determination of the unchange GTX in serum, ultrafiltrate, saliva, urine, and feces[2]. In domestic, microbiology assay was used to detect the drug in serum[3]. Two methods mentioned above were complicated and time consuming. Therefore, a more simple faster method was required in manufacture. And the standards for the quality of GTX injection need to be established. In this communication, a simple, accurate method for assay of GTX injection was introduced.

1 Instruments and agents

1.1 Instruments: SHIMADZU HPLC system, including RF-10A$_{XL}$ fluorescence dectetor, LC-10AT liquid chromatograph pump and CTO-10AS column oven(Japan). Shim-pack CLC-ODS column(15cm × 6.0mm, 5μm). WEIMA General Multi-medium Chromatograph Data Workstation (shenruan electric lim.Corp. Shenzhen, China).

1.2 Agents and solutions: All used in this test were analytical purity.

1.3 Experimental drugs: GTX Injection (250mL∶400mg, Affiliated Cancer Hospital of Guangxi Medical University), GTX standard (Jiangyin Yongda Chemical Factory, NO: 20010303, Content:99.6 %)

2 Experimental Methods

2.1 The mobile phase: 0.05mol·L^{-1} citric acid-acetonitrile (80∶20, vol/vol), adjusted pH 3.0 with triethylamine, and the flow rate: 1 mL·min^{-1}. The fluorescence wavelength: λ_{ex} = 360nm and λ_{em} = 465nm. The Column temperature: 50 ℃.

2.2 Established the standard curve: Prepared a serial solutions of the GTX standard sample at 0.4, 0.8, 1.6, 2.4, 3.2ng·μL^{-1}, then injected 10μL to measure the peak height under the

above-mentioned conditions. Analyzed linear regression with concentration(C) as abscissa and peak height(H) as ordinate, obtained the regression equation: $H = 32.5732 + 15176.6921C$, The linear range of GTX was 4 ~ 32ng, and the correlation coefficient: $r = 0.999\ 95$ ($n = 5$).

2.3 Test of minimal detectable concentration: Diluted the standard sample solutions of 0.4 $ng \cdot \mu L^{-1}$ to 0.04 $ng \cdot \mu L^{-1}$, carried out as procedure in "2.2", results presented as fig.1. The results showed the minimal detectable concentration was 0.04 $ng \cdot \mu L^{-1}$, and retention time was 7.70 min. Peak of the mobile phase did not appear under the conditions.

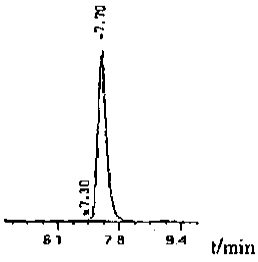

fig.1 HPLC chromatogram of gatifloxacin

2.4 Recovery and precision test: Prepared solutions of the GTX standard samples that concentration were 0.8, 1.0, 1.2 $ng \cdot \mu L^{-1}$, mixed respectively with GTX injection known concentrations. Then carried out as procedure in "2.2", at 1, 2, 4, 6, 8h each day for following 5 days. According to the regression equation, results that were calculated and obtained are presented in table 1 and table 2.

Table 1 Recovery for Assay of GTX ($\bar{x} \pm s$, $n = 5$)

added ($ng \cdot \mu L^{-1}$)	recovery ($ng \cdot \mu L^{-1}$)	mean (%)	RSD (%)
0.8000	0.7997 ± 0.0048		0.36
1.0000	0.9999 ± 0.0040	99.98 ± 0.06	0.85
1.2000	1.2000 ± 0.0096		0.55

Table 2 Precision for Assay of GTX ($\bar{x} \pm s$, $n = 5$)

added amount ($ng \cdot \mu L^{-1}$)	intra-day determinated amount ($ng \cdot \mu L^{-1}$)	RSD (%)	between-Day determinated amount ($ng \cdot \mu L^{-1}$)	RSD (%)
0.8000	0.7997 ± 0.00031	0.48	0.7995 ± 0.00014	1.12
1.0000	0.9999 ± 0.00074	1.02	0.9997 ± 0.00094	1.78
1.2000	1.2011 ± 0.00016	0.84	1.2009 ± 0.00108	1.32

2.5 Determination of GTX injection: 0.2 mL of sample was diluted to 1000 times with water. Carried out as procedure in "2.2", results presented in Table 3. There was 5%(w/v) of glucose in GTX injection used to adjust osmotic pressure. However, the glucose did not affect determination of GTX as there was not any peak belonged to glucose appeared under the conditions.

Table 3 Determination of GTX in GTX injection($n = 3$)

sample	concentration($mg \cdot mL^{-1}$)	RSD(%)
1	1.6021	1.74
2	1.6048	1.05
3	1.6005	1.32

3 Discussion and conclusion

The test for the optimal chromatographic conditions indicated that the retention time of GTX was affected with the column temperature notably. When the column temperature was set at 20 ~ 25 ℃, the retention time of GTX was about 10 min. But under this conditions, the retention time of ofloxacin was about 10 min, too. Their peaks overlapped partly. In order to separate them, the higher column temperature was tried. At 50 ℃, the retention time of ofloxacin was about 9 min and the GTX was 7.7 min. So the column temperature was set at 50 ℃ in this test.

The mobile phase of the test was similar to ofloxacin in Chinese Pharmacopoeia (2000), but changed pH 4.0 to 3.0. At pH 3.0, symmetric peak and better peak shape was obtained. In test, used peak area as ordinate, correlativity was not satisfactory. When peak area was instead of peak height, it was fine. The accuracy and precision of this method were very good that was showed by the results of recovery and precision test. Although there was glucose in GTX injection, it did not affected analysis as well.

In conclusion, the method was established simple, rapid and accurate for determination of GTX in GTX injection.

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