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Determination of Residual Solvents in Marbofloxacin by Capillary Gas Chromatography

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The residual solvents in marbofloxacin were determined in this study. Under the optimized conditions, ethyl acetate, dichloro methane, methyl benzene and *N,N*-dimethyl formamide were detected by capillary gas chromatograph with flame ionization detector. The results showed that the four organic solvents could get a good separation and the internal standard acetonitrile had no effect on the determination. In the test concentration range, the linear relationship was good (r > 99 %) and the RSD values were less than 2 %. The established method is simple, efficient and can be used for the determination of residual solvents in marbofloxacin.

Key Words: Marbofloxacin, Residual solvents, Capillary gas chromatograph, FID detector.

INTRODUCTION

Marbofloxacin is a new animal-specific third-generation drug of fluoroquinolone, which was first developed by the Swiss Roche Group and came into market in 1995[1,2]. Marbofloxacin is currently a more widely-used broad-spectrum antibiotics, which has a high degree of antibacterial activity against gram-positive and gram-negative bacteria and can inhibit the growth of some mycoplasma, chlamydia and certain anaerobic bacteria[3]. Marbofloxacin has a strong antibacterial activity and low toxicity, rarely causing nausea, vomiting, diarrhea, cramps and other side effects[4,5]. The chemical structure of marbofloxacin (Fig. 1) is similar to ofloxacin[6] having molecular formula $C_{17}H_{19}N_4O_4F$ and molecular weight 362. This drug has an important role in the treatment of animal's digestive tract, respiratory tract, urogenital tract and skin infections[7]. Therefore, the detection and security control of marbofloxacin is essential in the process of production and usage.

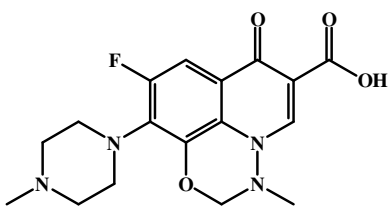

Fig.1. Structure of marbofloxacin

Residual solvents in pharmaceuticals are uncleared volatile organic chemicals that are produced or used in the manufacture process. Residual solvents not only are ineffective, but also increase the side effects and reduce the stability of drugs, so all residual solvents should be removed as much as possible[8-10]. In order to protect drug users from the damage of residual organic solvents, they need to be determined[11]. Some organic solvents such as ethyl acetate, dichloro methane, methyl benzene and *N,N*-dimethyl formamide may be introduced in the process of marbofloxacin production. The determination methods of residual solvents in marbofloxacin have not been reported. In this paper, the foregoing residual solvents were researched by gas chromatograph. Experimental results show that the method is fast, easy, accurate, and has good reproducibility.

EXPERIMENTAL

A GC8100 gas chromatograph (Shandong Jingwei Analysis Co. Ltd., China) equipped with a flame ionization detector was used for all analyses. The GC was fitted with an INNOWAX capillary column (Agilent Technologies Co. Ltd.). The detection data was acquisited by TOP-2007 workstation (Hangzhou Yangcheng Technology Co. Ltd., China).

Ethyl acetate (Tianjin Yongda Chemical Reagent development Centre), dichloro methane (Tianjin Kaitong Chemical Reagent Co. Ltd.), methyl benzene (Tianjin Kaitong Chemical Reagent Co. Ltd.), *N,N*-dimethyl formamide (Tianjin Fuyu Fine Chemical Co. Ltd.), acetonitrile (Tianjin Guangfu Fine Chemical Research Institute) were of analytical grade. Marbofloxacin was provided by Hebei Yuanzheng Pharmaceutical Co. Ltd.

Preparation of internal standard solution and test solution: Accurately transferred 64 µL of acetonitrile into a 50 mL volumetric flask by pipette, diluted it with triple-distilled water to the mark line and then acquired the internal standard solution. Precisely weighed 0.1 g marbofloxacin and transferred it into a 10 mL volumetric flask, dissolved it in the internal standard solution which should be added to the mark line. The gained solution was used as test solution.

Preparation of reference solution: Took an appropriate amount of chemical reagent, then made solutions of 50 mg/mL ethyl acetate, 8.9 mg/mL methyl benzene, 6.0 mg/mL dichloro methane and 8.8 mg/mL *N,N*-dimethyl formamide. All these solutions were used as standard stock solutions. Respectively took 10 µL of stock solution in 10 mL volumetric flask, diluted each with the internal standard solution. The concentrations of these prepared solutions were 50 µg/mL, 8.9 µg/mL, 6.0 µg/mL, 8.8 µg/mL in order. Because *N,N*-dimethyl formamide is highly unstable solvent, it should be used immediately after preparation.

Chromatographic conditions: The gas chromatography conditions were as follows: (1) injector temperature 160 ºC; (2) detector temperature 160 ºC; (3) initial oven temperature 40 ºC for 3min and increased to 150 ºC at 60 ºC/min, then maintained for 2 min; (4) usage of high-purity nitrogen as a carrier gas (1.0 mL/min) and of hydrogen and air as detector gases at 30 and 30 mL/min, respectively; and (5) capillary column 30 m × 0.53 mm × 1 µm.

RESULTS AND DISCUSSION

Choice of chromatographic conditions: The four solvents *i.e.*, ethyl acetate, methyl benzene, dichloro methane, marbofloxacin and the internal standard acetonitrile need to be separated and analyzed in this research. Most of the analysis of residual solvents requires higher resolution and sensitivity. The resolution and sensitivity of capillary column is better than the packed column. Moreover, the capillary column has some advantages, such as high phase ratio, large permeability, good thermal stability, *etc.*, so we used the INNOWAX polar capillary column. The internal standard method was applied in this study. Acetonitrile and water had no interference with the peaks of the solvents, so we selected 1000 µg/mL acetonitrile solution as the sample diluent. As the boiling points of solvents were different, in order to get better separation and analysis in shorter time, we used the method of temperature-programmed. The initial temperature was determined generally based on the lowest boiling point and could also be set at room temperature. The terminal temperature was determined according to the highest boiling point and could also be set at the maximum temperature of stationary liquid. The experimental results showed that the retention time of the solvents were 0.9 min (ethyl acetate), 1.2 min (dichloro methane), 2.3 min (methyl benzene) and 5.0 min (*N,N*-dimethyl formamide). The retention time of internal standard Acetonitrile was 2.0 min and didn't interfere with other solvent peaks. Fig. 2 is a chromatogram in which five kinds of solvents were injected at the same time.

Precision experiments: Took the standard reference solution, accurately injected 1 µL into the gas chromatograph, and determined it according to the above chromatographic conditions. Six parallel determinations were carried out and the relative standard deviation values were calculated based on the ratio of solvent peak area and internal standard peak area (Table-1). Each solvent RSD was less than 2 %, indicating good precision instruments.

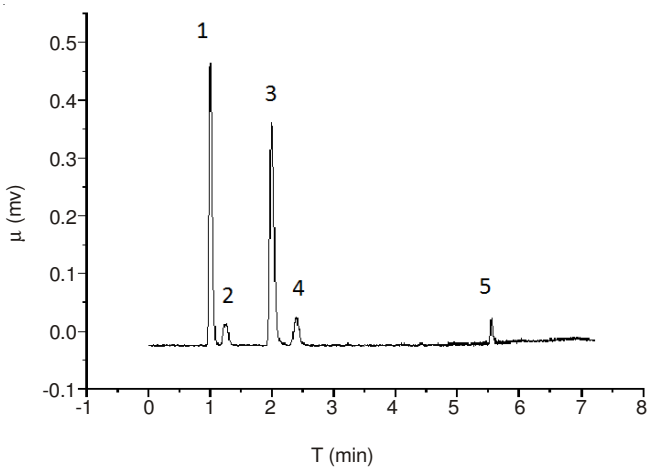

Fig. 2. Chromatogram of the standard reference solution. 1 ethyl acetate, 2 dichloro methane, 3 acetonitrile, 4 methyl benzene, 5 *N,N*-dimethyl formamide

TABLE-1
THE RESULTS OF PRECISION EXPERIMENTS

No.	Ethyl acetate	Dichloro methane	Methyl benzene	*N,N*-dimethyl formamide
1	0.850	0.093	0.103	0.00753
2	0.852	0.094	0.099	0.00756
3	0.852	0.098	0.099	0.00752
4	0.857	0.095	0.102	0.00758
5	0.851	0.095	0.099	0.00752
6	0.853	0.093	0.099	0.00763
RSD %	0.35	1.88	1.9	1.01

Linear relationship experiments: Accurately took a certain amount of standard stock solution, diluted it with internal standard solution, and gained a series of solutions of which concentration was continuously incremental. Used the solution as the linear test solution and determined it in accordance with the above chromatographic conditions and recorded the experimental data. Based on the experimental data, the linear regression was processed and the linear regression equation was gained. In addition, the detection limit of the solvent was calculated according to the ratio (S/N = 3). The results were listed in Table-2 from which it is clear that the solvents have a good linear relationship in the appropriate concentration range.

Sample recovery experiment: With the reference solution as 100 %, prepared three different solutions of low (80 %), medium (100 %) and high (120 %) concentrations. Accurately weighed the test sample, dissolved it by adding the above solution and made a solution of 0.1 mg/mL. Determined it in the above chromatographic conditions and calculated the recovery through standard addition method. Measured several times and then calculated the average recovery, with the results as ethyl acetate 100.8 %, dichloro methane 101.6 %, methyl benzene 99.6 %, *N,N*-dimethyl formamide 96.74 %.

TABLE-2
LINEAR REGRESSION EQUATION AND CORRELATION COEFFICIENT OF LINEAR RELATIONSHIP EXPERIMENTS

Solvent	Regression equation	Correlation coefficient	Linear range (mg/mL)	Detection limit (μg/mL)
Ethyl acetate	y = 2.06518x +1.23533	0.99925	8.3-50.0	0.5
Dichloro methane	y = 0.11331x +1.29067	0.99966	1.0-6.0	1.1
Methyl benzene	y = 1.55403x +0.68441	0.99916	1.3-8.0	4.8
N,N-dimethyl formamide	y = 0.05682x +1.91853	0.99960	1.5-8.8	7.8

Determination of samples: Accurately weighed a certain amount of marbofloxacin, prepared a test solution of 10 mg/mL by adding the internal standard solution, and injected the test solution 1 μL into gas chromatograph and got the chromatogram (Fig. 3). As can be seen from the figure, there was only the ethyl acetate residue in the sample, while the remaining residue solvents were not detected. The content of ethyl acetate in the sample was calculated and the results showed its content was lower than the detection limit.

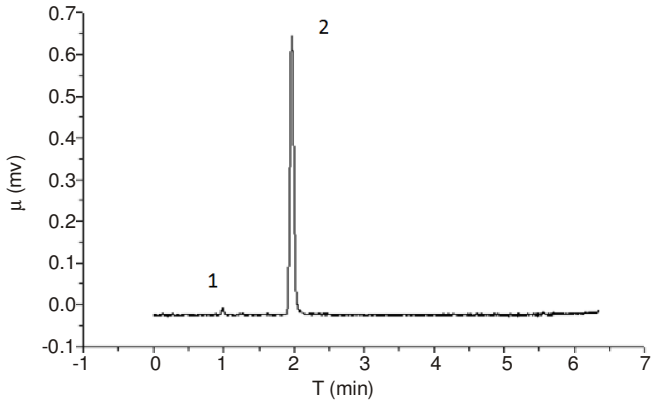

Fig. 3. Chromatogram of sample determination, 1 ethyl acetate, 2 acetonitrile

Conclusion

The organic residual solvents in marbofloxacin were analyzed in this study using internal standard method. The experimental results showed that in addition to ethyl acetate, there weren't other residual solvents detected in marbofloxacin. The detected ethyl acetate content was less than the instrument detection limit, indicating the content of this residual solvent was very low. This method has a high sensitivity, good reproducibility and fine linear relationship and can be used to determine the organic residual solvents in marbofloxacin.

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