



Development and Validation of Residual Amount of Potential Genotoxic Substance 1-Bromo-2-Methylpropane in Febuxostat by GC

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Abstract

In the present work, an effective and sensitive gas chromatography (GC) method for determination of the residual solvent 1-Bromo-2-Methylpropane in febuxostat was developed using an Agilent DB-624 column (30 m × 0.53 mm × 3.0 μm) and an optimized oven program. Its working range, linearity, the limit of detection (LOD) and the limit of quantification (LOQ), accuracy, precision, robustness, and stability were measured to verify the method. The LOD and the LOQ was 0.4725 μg/mL and 0.9450 μg/mL, respectively. The method has a wide working range of 0.9450 μg/mL (1.89ppm) ~ 21.6216 μg/mL (43.24ppm) for 1-Bromo-2-Methylpropane. And the method has a good linearity with the regression coefficient of the calibration graphs was 0.9998. The accuracy was determined by the recovery rate (80%, 100%, 120%) of three concentration levels, which were in the range of 96.04 to 102.93 %. Precision was expressed as a percentage of relative standard deviation (%RSD), which was 1.34% for determined 1-Bromo-2-Methylpropane. The results showed that the developed GC method had the great specificity, linearity, accuracy and precision, and applicable for the low-level detection of 1-Bromo-2-Methylpropane in febuxostat. This method can provide a new choice for GC determination of potential genotoxic impurities in febuxostat.

Keywords: Febuxostat; 1-Bromo-2-Methylpropane; Residual solvent; Genotoxic impurity; Method verification

1. Introduction

Gout is a metabolic disorder disease caused by the deposition of monosodium urate (MSU) crystals, which can lead to recurrent attacks of chronic inflammatory arthritis, urolithiasis, nephropathy, and acute arthritis and bursitis [1-5]. A variety of complications of gout, including cardiovascular disease, diabetes, obesity, and chronic kidney disease, have added significant challenges for people with gout [6, 7].

The model drug, febuxostat (Fig. 1) [chemical name: 2-(3-cyano-4-isobutyloxy)-phenyl-4-methyl-5-thiazolecarboxylic acid], which

is the first non-purine non-competitive xanthine oxidase inhibitor (XOI) approved by the European Union in 2008 [8, 9]. It is the first drug approved by the United States Food and Drug Administration (FDA) in 2009 for the treatment of chronic gout caused by hyperuricemia [10, 11]. Additionally, allopurinol is known to be associated with severe skin reactions and mortality [12]. Unlike allopurinol, febuxostat can significantly improve renal function, oxidative stress, and cardiac function without affecting other activities of purine metabolism [13-17]. Additionally, febuxostat has also been found to ameliorates testosterone-induced benign prostatic hyperplasia [18], and has antioxidant and anti-inflammatory effects [19, 20], prevent acute lung injury triggered by uric acid-induced lung inflammation [21], and so on.

Residual solvents in pharmaceuticals refer to volatile organic compounds that are used in the production of active pharmaceutical ingredients (APIs), excipients and preparations, but cannot be completely removed during the process [22]. The residual solvents may affect the physical and chemical properties of APIs, and may pose a risk to human health due to its own potential toxicity or other adverse effects. Therefore, in order to meet the requirements of regulations and ensure the safety of patients, the determination of residual solvents has become an essential procedure for the quality control of APIs and drug products.

Toxicity is the main reason for the control of residual solvent contents, especially genotoxicity. In recent years, genotoxic impurities have attracted the attention of various regulatory agencies, such as the European Medicines Agency (EMA) and the United States Food and Drug Administration (US-FDA) [23, 24]. International Council for Harmonization (ICH) also issued a separate guideline M7(R1), involving the limits of genotoxic impurities in newly marketed drugs in 2013 [25]. In the production process of febuxostat, the starting material used a genotoxic impurity with a halogenated aliphatic alkane warning structure: 1-Bromo-2-Methylpropane (Fig. 2). According to ICH M7, it can be classified as three types of genotoxic impurities.

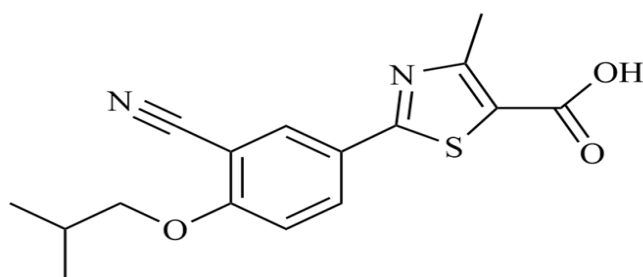


Fig. 1. Chemical structures of febuxostat.

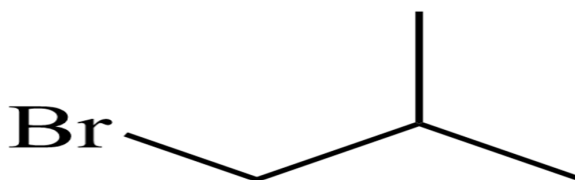


Fig. 2. Chemical structures of 1-Bromo-2-Methylpropane.

Consequently, a highly sensitive GC method was established to identify and quantify the potential genotoxic impurity viz. 1-bromo-2-methylpropane in febuxostat (Drug substance).

2. Experimental

2.1. Chemicals and reagents

The reference substance and samples of febuxostat used for method qualification were acquired from Taiipu Pharmaceutical Co., Ltd. 1-Bromo-2-Methylpropane was purchased from Beijing Bailingwei Technology Co., Ltd. *N, N*-Dimethylformamide (DMF, for HPLC) was purchased from Merck.

2.2. Instrumentation and data acquisition

The system of GC was Agilent GC 7890 (SN: CN15343046) equipped with a flame ionization detector (FID) detector and Agilent 7697 (SN: CN15420001) headspace automatic sampler. Data acquisition and processing were performed using OpenLAB CDS® software (version C.01.07 SR3[465]).

2.3. Chromatographic conditions

The chromatographic parameters of the GC method were as follows: High purity nitrogen was used as the carrier gas and the flow rate was 1.8 mL/min. The inlet temperature was 200°C and the split ratio was 10:1. The initial temperature of column oven was 50°C hold for 2 min and the temperature was raised to 90°C at a rate of 20°C/min hold for 2 min, then the temperature was raised to 170°C at a rate of 40°C/min hold for 1.5 min. Run for tens of minutes until no chromatographic peak appeared after 240°C high column flow. The FID temperature was 250°C. The equilibrium temperature of the headspace bottle, temperature of the quantitative ring and the

transmission line were 100°C, 120°C, 130°C, respectively. The equilibrium time was 30 min and the headspace injection volume was 1 mL. In this work, DMF was used as the exclusive diluent for all the experiments. The concentration of sample was 500 mg/mL.

2.4. Control strategy

In the previous study, we referred to ICH M7 to determine the treatment cycle of febuxostat for 1-10 years, with a maximum acceptable intake (10 µg/day) and the maximum daily dose of 80 mg. The limit of 1-Bromo-2-Methylpropane was determined to be 125 ppm by calculating the concentration. Nevertheless, we changed the control strategy because of we identified febuxostat as a long-term drug in subsequent studies. Based on the duration of treatment and the maximum clinical dose, the limit level of 1-Bromo-2-Methylpropane needs to be controlled below 18 ppm to meet the threshold of toxicological concern (TTC) of 1.5 µg/day.

2.5. Preparation of sample and standard

About 1000 mg of febuxostat was dissolved with 2 mL DMF to prepare febuxostat sample solution. The solution of standard was prepared by dissolving 1-Bromo-2-Methylpropane in DMF (the concentration was about 9.0 µg/mL); 2.0 mL of this solution is equal to 18 ppm (w/w) of 1-Bromo-2-Methylpropane in 1000 mg febuxostat. For neat sample analysis, all solutions were prepared directly in a 10 mL GC headspace vial. After the solution was prepared, all the vials were capped and crimped immediately.

According to gas chromatography ('Chinese Pharmacopoeia' 2015 Edition IV Part 0521) determination.

2.6. Method validation

The method verification was carried out according to the guiding

principles of methodological verification of 'Chinese Pharmacopoeia' 2015 Edition IV Part 9101.

The validation of our GC method was evaluated based on specificity, system suitability, LOD and LOQ, linearity, accuracy, precision, robustness and solution stability. The specificity of the method was assessed by injecting a blank dilute (DMF), standard solutions of 1-Bromo-2-Methylpropane, standard solutions of febuxostat and a specificity mixture solution (1-Bromo-2-Methylpropane was added to febuxostat sample solution at a concentration of 0.0018 % of its working concentration). The separation degree of 1-Bromo-2-Methylpropane and adjacent chromatographic peaks in the system suitability solution is required to meet the requirements (≥ 1.5), and the asymmetry factor of 1-Bromo-2-Methylpropane is between 0.95 and 1.05. The LOD was determined at a concentration of signal to noise (S/N) ratio ≥ 3 and the LOQ was determined at a concentration of S/N ≥ 10 . The LOQ solution of six standard substances were continuously injected and the %RSD of peak area response was not more than 10 %. The standard curve of 1-bromo-2-methylpropane was drawn with concentration as abscissa and peak area as ordinate. The accuracy of the method was evaluated by calculating the spiked solutions at three concentration levels (80 %, 100 % and 120 %). The repeatability was evaluated by repeated injection of 100 % spiked solution for 6 times. The intermediate precision of the method was evaluated by the second analyst using different equipment, chromatographic columns, and solutions. The requirements for personal repeatability were the same except for the %RSD ≤ 11 % of the two analysts. Take 1mL of the solutions of standard and sample, inject into the GC by headspace injection, and analyze at 0h, 5h, 15h, 20h, 25h, 30h and 35h respectively. The changes of the peak area of 1-Bromo-2-Methylpropane in the standard solution and the sample solution within 35h were investigated. The acceptance standard for the stability solution was the %RSD (n=6) of the peak area of 1-Bromo-2-Methylpropane in the standard solution and the sample solution should not more than 6.0% within a certain time, and no impurities interfering with 1-Bromo-2-Methylpropane should be produced. Under the initial chromatographic conditions, the column flow rate was ± 0.1 mL/min, the initial column temperature was $\pm 2^\circ\text{C}$, and the headspace heating temperature was $\pm 2^\circ\text{C}$ for robustness test.

3. Results and Discussion

3.1. Method development

The purpose of this study is to establish a gas chromatography method with good selectivity, high tolerance and applicability for the

potential genotoxic impurity 1-Bromo-2-Methylpropane. The details and key points of the method development process are discussed in turn below.

3.1.1. Chromatographic conditions

There are two programmed temperatures were set in the development process of GC method. We chose the second heating procedure for subsequent method development: the solution does not interfere with the determination of 1-Bromo-2-Methylpropane under this method. The chromatographic peak of the diluent did not appear at the end of the oven programming and were all blowout during post run. The oven program was optimized and determined with the total running time was about 9.5 min because of the need to remove the diluent (DMF) after the elution of the residual solvent, but the post-running time is increased.

3.1.2. Diluent

The diluent is a key factor affecting the headspace equilibration temperature, the sample peak response and sensitivity of GC method. DMF and *N*-Methyl pyrrolidone (NMP) were studied as potential diluents. The results showed that DMF provided the best baseline and did not interfere with the target peak. Therefore, DMF was selected as the diluent of the GC method.

3.1.3. Volume of diluent

3 mL and 2 mL of NMP interfered with the determination of 1-Bromo-2-Methylpropane, while 2 mL and 3 mL of DMF did not interfere with the determination of 1-Bromo-2-Methylpropane during the development process. Comprehensive consideration, the volume of the diluent was selected to be 2 mL.

3.1.4. Concentration of sample

Appropriate amount of 1-Bromo-2-Methylpropane was precisely measured, dissolved with DMF and quantitatively diluted to a concentration of S/N ratio close to 10.0. The LOQ of 1-Bromo-2-Methylpropane was 0.9450 $\mu\text{g/mL}$, which it can meet the sensitivity requirements of the method. The concentration of febuxostat sample was 500 mg/mL was selected in the present study because of the limit of 1-Bromo-2-Methylpropane is 18 ppm.

Using the above strategy, a new GC method for the determination of the residual amount of the potential genotoxic substance 1-Bromo-2-Methylpropane in febuxostat was successfully developed. GC chromatographic conditions and headspace parameters of the final method are summarized in Table 1.

GC Conditions	
Column	Agilent DB-624, 30 m \times 0.53 mm, 3.0 m film thickness
Injector Temperature	200 $^\circ\text{C}$
Injector Mode	Split
Split Ratio	10:1
Carrier Gas	Nitrogen
Mode	Constant flow, 1.8 mL/min
Oven Program	Initially set at 50 $^\circ\text{C}$ hold for 2 min, then 20 $^\circ\text{C}/\text{min}$ –90 $^\circ\text{C}$ hold for 2 min, 40 $^\circ\text{C}/\text{min}$ –170 $^\circ\text{C}$ hold for 1.5 min
Detector	FID
Detector Temperature	250 $^\circ\text{C}$
Headspace Parameters	
Oven temperature ($^\circ\text{C}$)	100 $^\circ\text{C}$
Loop temperature ($^\circ\text{C}$)	120 $^\circ\text{C}$
Transfer line temperature ($^\circ\text{C}$)	130 $^\circ\text{C}$
Equilibration time (min)	30 min
Injection volume (mL)	1 mL

Table 1. GC Conditions and Headspace Parameters of the Final Method

3.2. Method validation

3.2.1. Specificity

Specificity refers to the ability of the analytical method to accurately determine the analyte in the presence of some impurities, degradation products, excipients and other components. DMF did not interfere with the determination of 1-Bromo-2-Methylpropane and the resolution of 1-Bromo-2-Methylpropane in the specificity mixture solution was 16.21 (≥ 1.5) under the selected chromatographic conditions.

3.2.2. System Suitability

The system suitability test in the methodology validation is to examine the instrument and equipment, test operation and the analyzed sample as a whole. The main purpose is to ensure that the chromatographic system used in the analysis is effective and applicable. Under the selected chromatographic conditions, the resolution of 1-Bromo-2-Methylpropane in the solution of system suitability was 16.44 (≥ 1.5), and the asymmetry factor of 1-Bromo-2-Methylpropane in the solution of system suitability was 0.99, between 0.95 and 1.05. All the parameters were indicated that the established chromatographic system was applicable for the analysis of 1-Bromo-2-Methylpropane in febuxostat.

3.2.3. Limits of detection and quantitation

Under the selected chromatographic conditions, the concentration of LOD of 1-Bromo-2-Methylpropane was 0.4725 $\mu\text{g/mL}$. The RSD ($n = 2$) of the retention time of 1-Bromo-2-Methylpropane in 2 copies solutions of LOD was 0% ($< 1.0\%$), and the two S/N were not less than 3.0. The RSD ($n = 6$) of the retention time of 1-Bromo-2-Methylpropane in 6 parts of the solution of LOQ was 0.01 % ($< 1.0\%$), and the RSD ($n = 6$) of the peak area of 1-Bromo-2-Methylpropane was 2.0% ($< 8.0\%$). The six S/N were not less than 10.0.

3.2.4. Linearity

The linear equation of 1-Bromo-2-Methylpropane was: $Y = 2.7917X + 0.2122$, the correlation coefficient (R) was 0.9998, the absolute value of Y axis intercept was less than 25 % of 100 % response, and the % RSD of response factor is 1.49 % ($< 6.0\%$).

3.2.5. Accuracy

The accuracy of the method was expressed by spiked recovery. The spiked recovery was calculated according to the following formula:

$$R = \frac{c_2 - c_1}{c_3} \times 100\%$$

Among them, R was the spike recovery; c_1 was the initial concentration of the sample; c_2 was the measured concentration of the spiked sample; c_3 was the spiked amount.

The recovery rate of 80 % ~ 120 % limit concentration of 1-Bromo-2-Methylpropane in the solution of 9 recovery rates were in the range of 80 % ~ 115 %, 9 recovery data RSD ($n = 9$) was 2.03% ($< 6.0\%$).

3.2.5. Precision

The precision of the method was evaluated by repeatability and intermediate precision. The recoveries of 1-Bromo-2-Methylpropane in 6 repeated solutions were in the range of 80 % ~ 115 %, and the %RSD ($n = 6$) of 6 recoveries was 0.91 % ($< 6.0\%$). The recovery rates of 1-Bromo-2-Methylpropane in 12 precision solutions were in the range of 80 % ~ 115 %, and the %RSD ($n = 12$) of 12 recovery rates was 1.34 % ($< 11.0\%$).

3.2.6. Robustness

The robustness of the method was appraised by slightly changing the chromatographic conditions of GC and headspace parameters such as the column flow rate was $\pm 0.1\text{mL/min}$, the initial column temperature was $\pm 2^\circ\text{C}$, and the headspace heating temperature was $\pm 2^\circ\text{C}$. The robustness of the analysis method was compared with the results under conventional method conditions under each variable

condition. Under each slightly changed method conditions, the %RSD ($n = 6$) of the six 1-Bromo-2-Methylpropane contents in the sample solutions were less than 6.0%. These study results established that the test method is robust, the key instrument parameters fluctuate slightly, and the performance of the method will not be affected.

3.2.7. Solution stability

The standard solution and sample solution were stable within 35 h under conventional laboratory storage conditions. Within 35 hours, the RSD ($n = 6$) of the peak area of 1-Bromo-2-Methylpropane in the standard solution was 1.68 % ($< 6.0\%$), and no impurities interfering with 1-Bromo-2-Methylpropane were produced. 1-Bromo-2-Methylpropane was not detected in the sample solution, and no impurities interfering with 1-Bromo-2-Methylpropane were produced.

4. Discussion

The method was fully validated, indicating that it can be successfully applied to the determination of the residual solvent 1-Bromo-2-Methylpropane in febuxostat. The method has the advantages of simple operation, high sensitivity and low cost, which provides convenience for further determination of the residual solvent 1-Bromo-2-Methylpropane in febuxostat.

Competing interest: The author declares that they have no competing interests.

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