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Development of a stability– indicating HPLC method for simultaneous determination of ten related substances in vonoprazan fumarate drug substance

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ABSTRACT

Vonoprazan fumarate is a novel potassium-competitive acid blocker for the treatment of acid-related diseases. In the present study, a simple, fast, and economic reversed-phase liquid chromatography (LC) method was developed for the analysis of ten related substances (raw materials, by-products and degradants) in vonoprazan fumarate. The optimized separation was performed on a Phenomenex Kinetex EVO C_{18} (250 mm × 4.6 mm, 5.0 μm) column. The mobile phase consisted of (A) 0.03 M sodium phosphate buffer (pH adjusted to 6.5) – methanol – acetonitrile (72:25:3, v/v/v) and (B) 0.03 M sodium phosphate buffer (pH adjusted to 6.5) – acetonitrile (30:70, v/v). Detection of the analytes was conducted at 230 nm using a UV detector. The stability-indicating ability of this method was demonstrated by carrying out forced degradation studies. Vonoprazan underwent significant degradation when subjected to alkaline and oxidative stress conditions, while the drug proved to be stable to acidic, thermal and photolytic degradation. The degradants did not interfere with the detection of vonoprazan fumarate and its impurities. The performance of this method was validated in accordance to the regulatory guidelines recommended by the International Conference on Harmonisation (ICH) and this validation included specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness. The method proposed in this paper could be applied for process development as well as quality assurance of vonoprazan in bulk drug, since no monograph is available in official compendia.

1. Introduction

Vonoprazan fumarate, 1-(5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H–pyrrol −3-yl)-N-methylmethanamine fumarate, is an orally bioavailable potassium-competitive acid blocker (P-CAB) [1,2]. The drug works by competitively inhibiting the binding of potassium ion to H^+, K^+-ATPase (proton pump) in the final step of gastric acid secretion in gastric parietal cells. It was discovered by Takeda Pharmaceuticals and approved in Japan in 2014 for the treatment of gastroesophageal reflux disease, peptic ulcer, and other acid-related diseases. Compared with other current marketed proton pump inhibitors (PPIs), vonoprazan fumarate exerts more potent, sustained suppression of gastric acid secretion with a more favourable safety profile [3].

Currently, high-performance liquid chromatography (HPLC) has been considered the most appropriate technique for impurity analysis which is very important for drug approval and can exhibit a significant effect on process development [4–6]. A survey of the literature showed that limited methods based on liquid chromatography have been reported for the analysis of vonoprazan. Qiao et al. [2] have published a method, using liquid chromatography-tandem mass spectrometry (LC–MS), for the quantification of vonoprazan pyroglutamate in rat plasma and tissues. Yoneyama et al. [1] have

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developed a LC–MS method for the simultaneous quantification of vonoprazan and its 4 metabolites in human plasma. However, these trace analysis LC–MS methods were not suitable for routine impurity analysis of bulk materials of vonoprazan, because they were expensive, complex, and potential lack of robustness [7,8]. To the best of our knowledge, there was only one paper reporting a HPLC-UV method for the quantification of process-related impurities in vonoprazan fumarate [9]. This method was time-consuming (a total run time of 67 min) for practice and gave insufficient information about degradation products. Further, vonoprazan is not yet official in any of the pharmacopoeia for compendial applications. Under these circumstances, there is a great need to develop a simple, sensitive and effective HPLC method which can detect and separate all the possible degradants and process impurities in bulk drug materials to assure the safety and quality of vonoprazan fumarate.

Hence, the aim of the current research was to develop a stability-indicating analytical method based on HPLC-UV for the separation and determination of ten potential related impurities (namely Imp-1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) in vonoprazan fumarate (Table 1). This newly developed method was validated and proved to meet the requirements delineated by ICH guidelines. The method proposed here is a suitable mean for routine testing as well as stability analysis of vonoprazan fumarate.

2. Materials and methods

2.1. Materials and reagents

Samples of vonoprazan fumarate, raw materials and impurities were kindly provided by Chifeng Wanze Pharmaceutical Co., Ltd. (Chifeng, China) and ChemFuture PharmaTech (Jiangsu) Ltd (Wuxi, China). HPLC grade acetonitrile, methanol and formic acid were purchased from Fisher Scientific (Beijing, China). Deionized-distilled water was obtained from Watson's Food & Beverage (Guangzhou, China). All other reagents used throughout this work were of analytical grade and commercially available.

2.2. Instrumentation

A Shimadzu LC-16 series HPLC system consisting of a binary pump with an on-line degasser, an autosampler, a column thermostat, and an ultraviolet absorbance detector (Shimadzu, Kyoto, Japan) was used for method development and validation. The chromatograms were recorded and analyzed through SHIMADZU LabSolutions Essentia Version 5.62.

2.3. Chromatographic conditions

The analysis of all compounds was carried out at 35 °C using a Phenomenex Kinetex EVO C_{18} (250 mm × 4.6 mm, 5 μm) column (Phenomenex, Guangzhou, China). The mobile phase-A was composed of 0.03 M sodium phosphate buffer (pH adjusted to 6.5), methanol and acetonitrile in the ratio of 72:25:3, and the mobile phase-B was a mixture solvent of 0.03 M sodium phosphate buffer (pH adjusted to 6.5) and acetonitrile in the ratio of 30:70. The developed gradient program was 0.01 min–0% B, 10.0 min–26% B, 24.0 min–100% B, 26.0 min–100% B, 30.0 min–0% B and 35.0 min–0% B. The mobile phase was filtered through a 0.22 μm membrane filter and delivered at a constant flow rate of 1 mL min^{-1}. The injection volume was 10 μL and the analytes were detected by UV at 230 nm.

2.4. Preparation of standard solutions

An acetonitrile and water mixture (10:90, v/v) was used as the diluent in the sample preparation. A stock solution of vonoprazan was prepared at a concentration of 5 mg mL^{-1} by dissolving appropriate amount of reference standard in the diluent. Mixed and individual stock solutions of the related substances were also prepared in the diluent to a final concentration of 100 μg mL^{-1}. The combination solution used to investigate the system suitability was freshly prepared by spiking the vonoprazan fumarate sample (0.5 mg mL^{-1}) with ten impurities at 0.1% of the sample concentration (system suitability solution). All solutions were filtered through 0.22 μm membrane filters before use.

3. Results and discussion

3.1. Possible mechanisms for the formation of impurities

After a comprehensive investigation on manufacturing process of vonoprazan fumarate [9], the potential impurities originating in each stage were tracked (Fig. 1). Among them, the routes for the formation of Imp–3, 4, 5, 10 and vonoprazan fumarate had been described in detail by Liu et al. [9].The possible formation mechanisms of other impurities were described as follows. The unreacted SMA(Imp-8) and intermediate-2 (Imp-9) were quite possible to exist in the final vonoprazan fumarate samples. In addition, Imp-9 was speculated as a oxidative degradation product of vonoprazan, which was confirmed in degradation test (Route 8). In route 3, intermediate-1 may react with CH_3NH_2 to form Imp-1. Besides, Imp-1 was speculated as a base degradation product of vonoprazan, which was also confirmed in forced degradation studies (Route 7). In these two ways, Imp-1 was formed. In route 4, intermediate-1 may react with HCl to form Imp-7. In route 5, intermediate-2 may be hydrogenated by $NaHB_4$, affording Imp-6. Finally, recrystallization of vonoprazan furmarate by dissolving it into organic solvent at high temperatures afforded Imp-2 (Route 9).

3.2. Optimization of chromatographic conditions

The main criteria for developing a successful HPLC method for the quantitative analysis of vonoprazan fumarate and its impurities were as follows: the analytical method should be stability-indicating, robust and straightforward enough for routine analyses in quality control laboratories [5,10].

The first step of method development for the quantification of process-related impurities in vonoprazan fumarate drug substance is the appropriate selection of the wavelength to obtain good sensitivity with minimum noise. It was studied by measuring the ultraviolet absorption spectrum of all analytes. Vonoprazan and its impurities showed significant UV absorbance at wavelength of 230 nm (Fig. S1). Hence, the detector wavelength was kept at 230 nm throughout the analysis.

The column selectivity for the separation of all the related substances was critical because of similar chemical structure and polarities. Preliminary experiments were performed using three different columns including Waters XBridge Shield RP18 (250 × 4.6 mm, 5 μm), Phenomenex Kinetex EVO C_{18} (250 mm × 4.6 mm, 5 μm) and Waters XBridge C_{18} (250 mm × 4.6 mm, 5 μm) column. It was noticed that the studied compounds were well retained and separated with comparatively sharp peaks on Phenomenex Kinetex EVO C_{18} (250 mm × 4.6 mm, 5.0 μm) column (Fig. S2). So, further optimization was carried out on this column.

Several mobile phases with different compositions and polarities were examined for their efficiency in resolution e.g.: water – acetonitrile, water – methanol, sodium phosphate buffer – acetonitrile, sodium phosphate buffer – methanol. The mobile phase of sodium phosphate buffer– methanol – acetonitrile was finally selected for subsequent investigations as it yielded the best separation between the test compounds. Then trials were undertaken using different ratios of sodium phosphate buffer, methanol and