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Short communication

Separation and quantitation of eight isomers in a molecule with three stereogenic centers by normal phase liquid chromatography☆

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ABSTRACT

A normal phase liquid chromatography method was developed for the separation and detection of eight stereoisomers of the key intermediate, CORE + OMe, having three chiral centers. The stereochemistry of this intermediate dictates the stereochemistry of the active pharmaceutical ingredient generated by an additional six synthetic steps. Multiple columns and mobile phases were screened during the development based on a platform approach. The use of dichloromethane as mobile phase additive and adjustment of flow rate and column temperature contributed in achieving resolution of these eight stereoisomers.

The separation and detection of these stereoisomers was achieved using a Chiralcel OD-H, 4.6 × 250 mm, 5 μm d_p column with heptane: ethanol: dichloromethane in a ratio of 95:3:2 (v:v:v) as mobile phase at a flow rate of 0.7 mL/min. UV detection was carried out at 245 nm and the column temperature was maintained at 15 °C. The analytical method was phase appropriately validated. The limit of detection and limit of quantification were found to be 0.035 and 0.07 μg, respectively. The newly developed method has been implemented for routine utilization to monitor the chiral control during process development and used as the quality control method for chiral purity of the desired compound.

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1. Introduction

Currently more than 50% of the drugs on the market are chiral compounds [1,2]. The enantiomers of chiral drugs can differ in their interactions with enzymes, proteins, receptors, and other chiral molecules which result in differences in biological activity [1–5]. The effect on biological activity can be further extended to differences in pharmacology, pharmacokinetics, metabolism, and toxicity. Therefore, one isomer may produce the desired therapeutic effect while another may be inactive or produce adverse effects. Due to these factors, typically a single enantiomer is required to meet the desired therapeutic outcome.

Thus, the process development and analytical control of chiral molecules plays an important role in pharmaceutical development due to the potential impact on the biological activity and safety profile of an active pharmaceutical ingredient (API). Regulatory agencies have issued guidance on the acceptable manufacturing control of the synthesis and impurities, pharmacological and toxicological assessment, and proper characterization of metabolism [6–9]. Thus it is clear that control of chiral impurities is crucial for ensuring novel therapeutics reach the patient. Chiral impurities may be introduced from starting materials, byproducts of the synthetic process, and as a result of inversion of chiral centers due to chemical degradation which can carry through to the drug substance [10,11].

In order to ensure that chiral impurities are adequately controlled, suitable analytical methods are required. Numerous analytical techniques have been used for the determination of chiral impurities, such as high performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC), and nuclear magnetic resonance (NMR) spectroscopy [12–15]. Since enantiomers are physiochemically identical in achiral environments, enantiomers have to be converted to diastereomers in order to resolve them from each other. To avoid derivatization, the use of chiral mobile phase additives or the use of chiral stationary phases (CSP) are more favorable [12,16–18]. Additionally, the resolution of diastereomers can be challenging using reverse phase chromatography based on the typical properties governing retention using non-chiral stationary phases. For this reason, chiral stationary phases have been employed to achieve resolu-

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Table 1
The structures of the eight stereoisomers of CORE + OMe.

Name	Structure
CORE + OMe ISOMER 1 (SSR Isomer)	
CORE + OMe ISOMER 2 RSR Isomer	
CORE + OMe ISOMER 3 RRS Isomer	
CORE + OMe Desired SRR Desired	
CORE + OMe ISOMER 4 RRR Isomer	
CORE + OMe ISOMER 5 RSS Isomer	
CORE + OMe ISOMER 6 SRS Isomer	
CORE + OMe ISOMER 7 SSS Isomer	

tion [15,19,20]. The studies discussed here utilized chiral stationary phases in combination with HPLC.

In the present work, a normal phase HPLC method was developed for CORE + OMe, a chiral intermediate possessing three stereogenic centers leading to eight isomers: SSR, RSR, RRS, SRR (desired), RRR, RSS, SRS and SSS, Table 1. A quality control method which can resolve and quantitate these eight stereoisomers is critical to the understanding of the stereospecificity of the synthetic reaction and to the chiral control of the drug substance. The chiral centers of CORE + OMe are fixed and are not affected by the downstream chemistry. The current work discusses a condensed platform column and mobile phase screen in order to achieve the required method parameters. Other potential combinations of mobile phase solvents and stationary phase can be evaluated as needed to meet the method requirements. Also, the effect of column temperature and flow rate was evaluated. The final method was phase appropriately validated for specificity, sensitivity, linearity, precision, accuracy, and solution stability.

2. Material and methods

2.1. Solvents, reagents, and standards

All eights stereoisomer standards were synthesised by the process development group at Syngene Amgen Research and development Centre, Bangalore, India. Heptane, hexane, and dichloromethane (DCM) were purchased from Finar and Sigma Aldrich. Ethanol was purchased from Changshu Hongsheng Fine Chemical Co. Ethane sulphonic acid (ESA) and methane sulphonic acid (MSA) were purchased from Sigma Aldrich. All solvents were HPLC grade.

2.2. Chromatography

A Agilent 1260 Series HPLC screening system (Agilent Technologies, Germany) equipped with autosampler G1367C, column compartment G1316B, and PDA detector G1315C was used. The ChemStation Open lab software version 1.2.0 was used for acquisition and data processing. The chiral columns (4.6 × 250 mm, 5 μm d_p), Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak AD-H, and Chiralcel OD-H were purchased from Daicel (Japan). The initial instrument conditions for the column screening used a mobile phase of hexane:ethanol in the ratio 90:10 (v:v), 1.0 mL/min flow rate, column temperature at 20 °C, and UV detection at 245 nm [21]. Additional mobile phase conditions, column temperatures, and flow rates were evaluated during the method development as discussed in the results and discussion section.

2.3. Sample preparation

All the sample and standard solutions were dissolved in ethanol. The typical concentration of the standard and sample was 0.5 mg/mL. For the method development trials, a mixture of the eight stereoisomers was used with each isomer at 0.5 mg/mL. Individual stereoisomer standards were prepared to assess specificity. The limit of detection (LOD) and limit of quantitation (LOQ) solutions were prepared at 7.0 μg/mL and 14 μg/mL (0.035 and 0.07 μg), respectively, for each stereoisomer. The linearity solutions were prepared over the range of 14 μg/mL–2.0 mg/mL.

3. Results and discussion

3.1. Method development strategy

Chiral column screening followed by mobile phase screening was performed. Resolution of the stereoisomers was further improved through modification of the flow rate and column temperature.

3.1.1. Column screening

Several chiral columns Chiralpak IA, IB, IC, AD-H, and Chiralcel OD-H were screened in an effort to achieve the desired stereospecific separation. The immobilized series of columns, IA, IB, IC, have the advantage over coated columns of being able to tolerate a wider range of solvents which can help provide additional selectivity. [16,22] These columns were selected as the majority of chiral compounds can be resolved using either IA/AD-H or IB/OD-H. [14,15] Additionally, the IC column has a different chiral selector than the other columns evaluated and has been shown to provide improved selectivity in some cases. [22]

With the Chiralpak IA column, only two out of the eight stereoisomers were resolved. For the Chiralpak IB and IC columns, two and four stereoisomer peaks were resolved, respectively. The different chiral selectivity of the Chiralpak IC was found to provide improved resolution, but the peaks were found to be broad resulting in poor sensitivity. With the Chiralpak AD-H and Chiralcel OD-H columns, increased resolution was obtained and the peak shape was slightly better than what was observed with the Chiralpak IA, IB, and IC columns. The amount of improvement in selectivity with the Chiralpak AD-H as compared to the Chiralpak IA was unexpected. Typically only small selectivity differences are observed between the immobilized and free chiral stationary phase. [22] The increased enantioselectivity of the coated stationary phases over

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