Liquid chromatographic estimation of (S) - glycidyl butyrate in (R) - glycidyl butyrate

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Abstract

Aim: The present study was undertaken out of a commercial need for the synthesis of Linezolid with impurity limits within the specification. Materials and Methods: (R)-Glycidyl butyrate (RGB) is raw material for the synthesis of Linezolid drug substance. This RGB contains (S)-(+)Glycidyl butyrate (SGB) and SGB appears in same concentration in the final Active Pharmaceutical Ingredient. So, a normal phase high-performance liquid chromatography (HPLC) method has been developed to determine the SGB level in the raw material. RGB and SGB were separated using an HPLC system equipped with quaternary gradient pumps on a Daicel chiralpak AD-H (250 × 4.6 mm) column with a mobile phase consisting 2.0 ml of ethanol in 1 000 ml of n-hexane. A 0.5 ml/minute flow rate and a 10 μl injection volume was used and the compounds were detected at 215 nm. Results: Method validation parameters demonstrated the same to be reliable, reproducible, and accurate one. Conclusion: Thus, the present study may be used for regular quality control of RGB to improve commercial feasibility for the synthesis of Linezolid.

Key words: (R)--Glycidyl butyrate, (S)--(+)--glycidyl butyrate, chiral--HPLC, Linezolid, validation

INTRODUCTION

The eventual purpose of any analytical method in general and liquid chromatographic method in particular is to separate and quantify the compounds of interest. These compounds may either be drug substances or an unwanted substance in drug substance, its raw material, or intermediates.[1] The developed method should be a reproducible, accurate, sensitive, and commercially viable one. To ensure these parameters, validation of the method is performed as per guidelines like International Conference on Harmonization (ICH).[2]

Optically pure 2,3-epoxy-1-propanol (glycidol) and its derivatives are versatile intermediates in organic synthesis because the epoxide ring is reactive toward nucleophiles for the synthesis of asymmetric alcohols.[3-5] Particularly, both enantiomers of the (R)-glycidyl butyrate (RGB) and (S)-glycidyl butyrate (SGB) [Figure 1] are chiral synthesis units for the production of biologically active compounds of commercial interest. RGB has been used to introduce a stereogenic center in the synthesis of Linezolid,[6,7] which is currently marketed for the treatment of multidrug-resistant Gram-positive infections such as nosocomial, community-acquired pneumonia, and skin infections. SGB is also very useful as starting material in the synthesis of many drugs, such as (R)-argentilactone,[8] which exhibits both antileishmanial activity and cytotoxic activity against mouse leukemia cells.[9]

RGB is an intermediate in the synthesis of Linezolid drug substance. The importance of the study lies in the fact that SGB is inherently present in RGB and it appears in

Figure 1: Chemical structure of SGB and RGB
almost same percentage in the Linezolid drug substance as was present in the intermediate (RGB). Thus, the content of SGB, if not controlled in the raw material itself, will appear in more than specified limit in the finished drug substance (Linezolid). Literature survey on this revealed absence of any such reports or publications. Thus, the current study was undertaken out of the need for successful commercialization of Linezolid. A chiral high-performance liquid chromatography (HPLC) method has been developed for the estimation of SGB in RGB sample.[10,11]

EXPERIMENTAL

Reagents and chemicals
Standard SGB was obtained from Sigma Aldrich, United States of America (USA). RGB (Glenmark Generics Ltd, Glenmark Research Centre, Navi Mumbai, Maharashtra, India) was obtained as in-house standard. n-Hexane (HPLC Grade) was purchased from Ranbaxy Fine Chemicals Limited (RFCL), New Delhi, India, and ethanol (Changshu Yangyuan Chemical, China) was used.

Instrumentation and liquid chromatographic conditions
HPLC method was performed using a Waters 2695 quaternary gradient pumps, Waters 2489 Dual λ absorbance detector (Waters, USA). Separation was achieved with ethanol: n-Hexane (0.1 : 50) as eluent at flow rate 0.5 ml/ min. The column temperature was maintained at 25°C. Ultraviolet detection was performed at 215 nm. The mobile phase was used as diluent. The run times were used 105 minute and 65 minute for sample and reference standard, respectively, because of the fact that a peak at retention time of 95 minutes was appearing in sample.

Optimization of mobile phase and selection of column
During method development trials, three different chiral columns have been used—Daicel chiralpak AD-H (250 x 4.6 mm), Daicel chiralpak OD-H (250 x 4.6 mm), and Daicel chiralpak AS-H (250 x 4.6 mm). These columns were tried with different mobile phases consisting 2.0 ml and 4.0 ml of ethanol in 1 000 ml of n-hexane. Mobile phase itself was used as the diluent for preparation of reference standards and sample.

Preparation of standard and sample solutions for assay
Preparation of reference solutions (a) and (b)
About 1 00.0 mg of SGB standard was accurately weighed into 20-ml volumetric flask. 10 to 15 ml of diluent was added and Elma Elmasonic S 300 H (Germany) sonicator was used for sonication to dissolve. Volume was made up with diluent and mixed well. 5.0 ml of this solution was diluted to 50 ml with diluent and mixed well (reference solution a). 2.0 ml of reference solution (a) was diluted to 20 ml with diluent and mixed (reference solution b).

Preparation of Reference solution (c)
200.0 mg of RGB sample was accurately weighed into a 20-ml volumetric flask. 10 to 15 ml of diluent was used to dissolve by sonication. 2.0 ml of reference solution (a) was added and volume was made up with diluent.

Preparation of test solution
200.0 mg of sample was accurately weighed into a 20-ml volumetric flask. They were dissolved in diluent by sonication and made up the volume with diluent.

System suitability
The relative standard deviation (RSD) of six replicate injections of reference solution (b) was not more than 5% and resolution between the main peak and SGB peak of reference solution (c) was more than 1.0 set as the system suitability criteria.

Method validation
Method validation is closely related to method development. When a new method is being developed, some parameters are already being evaluated during the “development stage,” while in fact, this forms part of the “validation stage.” The ICH guidelines achieved a great deal in harmonizing the definitions of required validation parameters, their calculation and interpretation. The international conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has harmonized the requirements in two guidelines.[2,12] The first one summarizes and defines the validation characteristics needed for various types of test procedures and the second one extends the previous test to include the experimental data required and some statistical interpretation.

Specificity
The ability of the method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix (that may be expected to be present in the sample matrix) under the stated conditions of the test (specificity = 100% selectivity).

Solution stability
Drug stability in Active Pharmaceutical Ingredient is a function of storage conditions and chemical properties of the drug and its impurities. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data are required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling.
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RESULTS AND DISCUSSIONS

Optimization of mobile phase and selection of column
Two different compositions of mobile phases were tried out in three different columns and it was observed that 2.0 ml ethanol in 1 000 ml n-hexane on Chiralpak AD-H produced best resolution and peak shape.

System suitability
Resolution between the main peak and SGB peak of reference solution (c) was found to be 1.19 [Figure 2].

Method validation
Specificity
Specificity of the method was evidenced by comparing blank, SGB, and SGB spiked into RGB solutions [Figures 2-4]. From the experimental data, there are no interfering peaks at the retention times of RGB and SGB from the chromatogram [Figure 2]. Good separation is also seen between RGB and SGB from the spiked chromatogram.

Solution stability
The solution stability was ascertained from HPLC peak area of reference standard samples. The standard sample solutions were kept at room temperature for 48 hours; it was observed that there was no change in peak area of these solutions.

Linearity
Under the experimental conditions, the peak area–
concentration plot for the proposed method was found to be rectilinear over the range of 50 to 150% of the specified limit with a correlation coefficient of 0.999 [Figure 5].

Accuracy
Analytical method may be considered validated in terms of accuracy if the mean value is within ±10% of the actual value. Recovery of SGB was found in the range of 91.83 to 95.11% [Table 1], which was well within the acceptance criteria.

Limit of detection and Limit of quantitation
The Limit of detection of SGB was found to be 0.23 microgram / milliliter (µg/ml). The Limit of quantification of SGB was found to be 0.69 microgram / milliliter (µg/ml)

Ruggedness
The ruggedness of the method was evaluated by estimating % RSD of reference solution (b) by two different analysts using different HPLC columns on different days. The average % RSD was found to be 1.27%.

Robustness
Robustness of the method was determined by analyzing the sample with deliberate change in the parameters like (a) flow rate of mobile phase (± 0.1 ml/min), (b) column temperature (± 5°C), (c) wavelength of detection (± 2 nm), and (d) mobile phase composition (± 0.5 ml of ethanol).

(a) Change in flow rate
It was observed that at a flow rate of 0.4 ml/min and 0.6 ml/min, resolution between the main peak and SGB peak of reference solution (c) were found 1.10 and 2.57, respectively. The % RSD values were within desired limits.

(b) Change in column temperature
It was observed that resolutions between RGB and SGB in reference solution (c) were shifted from 1.06 to 1.52 when column temperature changed from 20°C to 30°C.

(c) Change in wavelength of detection
It was observed that changing the wavelength from 213 to 217 nm did not change the % RSD values significantly when compared with injections under normal condition. The resolution main peak and SGB peak of reference solution (c) were found to be 1.07 and 1.15, respectively.

(d) Mobile phase composition
It was observed that resolutions between RGB and SGB in reference solution (c) were shifted from 1.10 to 1.29 when volume of ethanol increased from 1.5 to 2.5 ml with 1 000 ml n-Hexane and the % RSD values were within desired limits.

CONCLUSIONS
The proposed method does not require any laborious clean up procedure before measurement. The validation data demonstrate good accuracy, which proves the reliability of the proposed method. Hence, the validated method can be used for routine determination of RGB present in SGB in quality control laboratories in the pharmaceutical industry and thus commercial viability for synthesis of Linezolid has been enhanced.

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