Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 7, 2015

Original Article

SEPARATION AND DETERMINATION OF THE S-ISOMER OF (10-CAMPHORSULFONYL) OXAZIRIDINE IN A BULK DRUG SUBSTANCE BY NORMAL-PHASE LIQUID CHROMATOGRAPHY

HEMSAGAR P. JADHAV, DNYANDEO B. PATHARE

Department of Chemistry, Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu 333001 Rajasthan, India
Email; hemsagar_p@rediffmail.com

Received: 21 Apr 2015 Revised and Accepted: 21 May 2015

ABSTRACT

Objective: To develop novel, simple and accurate enantiomeric separation of (10-Camphorsulfonyl) oxaziridine by normal-phase high-performance liquid chromatographic method as per ICH guidelines.

Methods: The S-isomer and R-isomer of (10-Camphorsulfonyl) oxaziridine were baseline resolved on a Chiralcel OD-H (250 x 4.0 mm i. d, 5 µm) column using a mobile phase system containing n-Hexane: ethanol: trifluoroacetic acid (90:10:0.1 v/v/v.) at detector wavelength 210 nm and column temperature 30 °C. The chromatographic resolutions between S-isomer and R-isomer were found three. The developed method was extensively validated according to ICH guidelines.

Results: Good linearity was observed for S-isomer over the concentration range of 900–9000 ng/ml, with the linear regression (Correlation coefficient R = 0.999) and proved to be robust. The limit of detection and limit of quantification of S-isomer was found to be 400 and 900 ng/ml, respectively for 20 µl injection volume. The percentage recovery of S-isomer was ranged from 97.0 to 102.0 in bulk drug samples of (10-Camphorsulfonyl) oxaziridine. (10-Camphorsulfonyl) oxaziridine sample solution and mobile, phase was found to be stable for at least 48 hours. The proposed method was found to be suitable and accurate for the quantitative determination of S-isomer in bulk drugs.

Conclusion: A novel, simple and accurate normal phase LC method was described for the enantiomeric separation of 10-Camphorsulfonyl Oxaziridine is precise and specific.

Keywords: (10-Camphorsulfonyl) oxaziridine, Chiralcel OD, Chiral HPLC, Validation, Solution and mobile phase stability.

INTRODUCTION

The (10-Camphorsulfonyl) oxaziridine is widely used as oxidizing reagent in synthesis of enantioenriches sulfoxides [1]. Enantiopure sulfoxides used in pharmaceutical industries due to their biological activity. Omeprazole, the world highest selling drug in 1997 [2], is to treat acid-induced inflammation and ulcers of stomach and duodenum. Esomeprazole the (*S*)-form of omeprazole. (1S)-(+)-(10-Camphorsulfonyl) oxaziridine is used as a oxidizing reagent in synthetic reactions for Omeprazole, Rebeprazole, Lansoprazole and Pantoprazole [1]. (10-Camphorsulfonyl) oxaziridine is racemic mixture of (1R)-(-) and (1S)-(+)-(10-Camphorsulfonyl) oxaziridine. It is a camphor based reagent. Camphorsulfonyl oxaziridine a chiral reagent has great effect in the synthesis of many natural products [3]. Chiral oxaziridine can be manufactured by oxidation of chiral imine [4].

In the literature, there is no method reported for separation of S-isomer and R-isomer of (10-Camphorsulfonyl) oxaziridine using normal phase and reverse phase high performance liquid chromatography. It is very important to know the accurate percentage of isomer of reagents during the manufacturing process of an active pharmaceutical ingredient (API's) in bulk drugs pharmaceutical industry. Normal-phase chromatography is the most popular mode of liquid chromatography at present. Briefly, the major disadvantages of normal phase HPLC stretch out in the highly non-polar nature of the mobile phase, the possibility of column inactivation by water, contamination by polar compounds and lower potential in terms of selectivity.

This report describes a normal phase LC method for the rapid separation of S-isomer and R-isomer (10-Camphorsulfonyl) oxaziridine. The developed HPLC method was validated for quantification of S-isomer in (10-Camphorsulfonyl) Oxaziridine.

MATERIALS AND METHODS

Chemicals

(1*S*)-(+)-(10-Camphorsulfonyl) oxaziridine and (1*R*)-(-)-(10-Camphorsulfonyl) oxaziridine were kindly supplied by Lavender Laboratories Private Limited, Pune, India and the chemical structure of (10-Camphorsulfonyl) oxaziridine given in (fig. 1), chemical structure of (1*S*)-(+)-(10-Camphor sulfonyl) oxaziridine given in (fig. 2) and chemical structure of (1*R*)-(-)-(10-Camphorsulfonyl) oxaziridine given in (fig. 3). HPLC grade n-Hexane purchased from Merck, HPLC grade Absolute Ethanol purchased from Hayman speciality products UK, HPLC grade Trifluoroacetic acid purchased from Sigma-Aldrich.

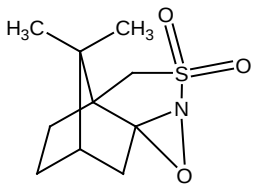

Fig. 1: Chemical structure (10-Camphorsulfonyl) oxaziridine

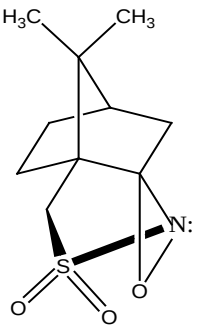

Fig. 2: Chemical structure (1*S*)-(+)-(10-Camphorsulfonyl) oxaziridine

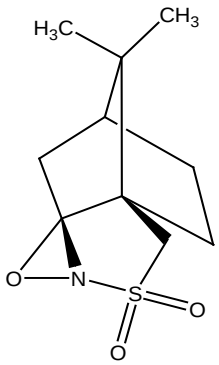

Fig. 3: Chemical structure (1*R*)-(-)-(10-Camphorsulfonyl) oxaziridine

Equipment

A shimadzu 2010 series LC systems with UV detector and inbuilt auto injector were utilized for method development and validation. LC Solution software was used for data acquisition and system suitability calculations.

Sample preparation

Stock solutions of Racemic (10-Camphorsulfonyl) oxaziridine (10.0 mg/ml) were prepared by dissolving the appropriate amount of the substances in diluent. The analyte concentration of R-isomer was fixed as 10.0 mg/ml. working solutions of S-isomer and R-isomer were prepared in diluent.

Chromatographic conditions

The chromatographic conditions were optimized using a Chiralcel OD-H (250 x4.0 mm i. d, 5 µm) column. The mobile phase was n-hexane: ethanol: trifluoroacetic acid (90:10:0.1, v/v/v). The diluent was ethanol: trifluoroacetic acid (100:0.1, v/v), the flow rate was set at 1.0 ml/min. The column was maintained at 30 °C and the detection was carried out at a wavelength of 210 nm. The injection volume was 20 µl.

Validation of the method

Method reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-days precision) of retention times and peak areas for S–isomer and R-isomer.

In order to determine the repeatability of the method, replicate injections (n=6) of a 10.0 mg/ml solution containing R-isomer spiked with S-isomer (0.5 %) was carried out. The intermediate precision was also evaluated over three days by performing six successive injections each day.

Limit of detection and limit of quantification of S-isomer

The limit of detection, defined as the lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal to noise ratio [5]. The limit of quantitation, defined as the lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as ten times the signal to noise ratio [5]. LOD and LOQ were achieved by injecting a series of dilute solutions of S-isomer.

The precision of the developed method for S-isomer at the limit of quantification was checked by analyzing six test solutions of S-isomer prepared at LOQ level and calculating the percentage relative standard deviation of an area.

Linearity of S-isomer

Detector response linearity was assessed by preparing six calibration sample solutions of S-isomer covering from 900 ng/ml (LOQ) to 4500 ng/ml (900 ng/ml, 1800 ng/ml, 2700 ng/ml, 3600 ng/ml, 4500 ng/ml and 9000 ng/ml), prepared in diluent from S-isomer stock solution.

Regression curve was obtained by plotting peak area versus concentration, using the least squares method. Linearity was checked for 3 consecutive days in the same concentration range from the same stock solution. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

Quantification of S-isomer in bulk sample

The (10-Camphorsulfonyl) oxaziridine bulk sample gift by Lavender Lab showed the presence of 0.50 % of S-isomer. Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of S-isomer in bulk drug samples.

The study was carried out in triplicate at 0.5, 1.0 and 1.5 percent of the (10-Camphorsulfonyl) oxaziridine target analyte concentration. The recovery of S-isomer was calculated from the slope and Y-intercept of the calibration curve.

Robustness

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, mobile phase composition and column temperature. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between S-isomer and R-isomer was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution of isomers, it was changed by 0.2 units from 0.8 ml/min to 1.2 ml/min. The effects of change in percent ethanol on resolution were studied by varying from-1 to+1 % while the other mobile phase components were held constant. The effect of column temperature on the resolution was studied at 25 °C and 35 °C instead of 30 °C while the other mobile phase components were held constant.

Solution stability and mobile phase stability

Stability of (10-Camphorsulfonyl) oxaziridine in solution at analyte concentration was studied by keeping the solutions in tightly capped volumetric flask at room temperature on a laboratory bench for two days. Content of S-isomer was checked for six hours interval up to the study period.

Mobile phase stability was carried out by evaluating the content of S-isomer in (10-Camphorsulfonyl) oxaziridine sample solutions prepared freshly at six hours interval for two days. Same mobile phase was used during the study period.

RESULTS AND DISCUSSION

Method development

The aim of this work is to separate the S-isomer and R-isomer of (10-Camphorsulfonyl) oxaziridine using normal phase HPLC within short run time, the analysis of (10-Camphorsulfonyl) oxaziridine sample using normal phase is time consuming. A 10.0 mg/ml solutions of isomeric mixture prepared in the mobile phase were used in the method development. To develop a rugged and suitable LC method for the separation of (10-Camphorsulfonyl) oxaziridine isomer, different mobile phases and stationary phases were employed. In an attempt to separate the isomer of (10-Camphorsulfonyl) oxaziridine. Various experiments were conducted, to select the best stationary and mobile phases that would give optimum resolution and selectivity for the two isomer. The chromatographic separation was achieved on a chiralcel OD-H (250 x 4.0 mm i. d, 5 µm) column using a mobile phase system containing n-hexane: ethanol: trifluoroacetic acid (90:10:0.1 v/v/v).

The flow rate of the mobile phase was 1.0 ml/min. At 30 ºC column temperature, the peak shape of (10-Camphorsulfonyl) oxaziridine was found symmetrical.

In the optimized method, the typical retention times of S-isomer and R-isomer of (10-Camphorsulfonyl) oxaziridine were about 7.4 and 8.4 min respectively. The isomeric separation of (10-

Camphorsulfonyl) oxaziridine is shown in system suitability chromatogram (fig. 4). HPLC chromatogram of (10-Camphorsulfonyl) oxaziridine bulk sample (1000 µg/ml) spiked with S-isomer (1.0 %) is shown in (fig. 5).

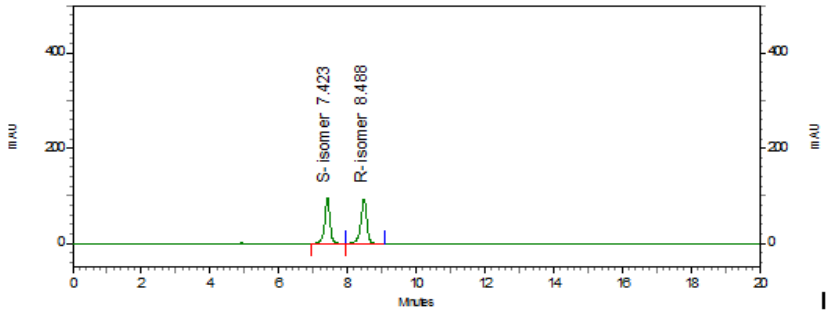

Fig. 4: Typical HPLC chromatogram of system suitability solution

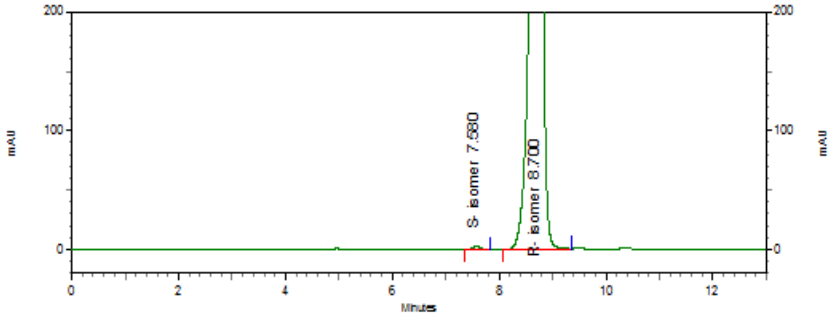

Fig. 5: Typical HPLC chromatogram (10-Camphorsulfonyl) oxaziridine bulk sample (1000 µg/ml) spiked with S-isomer (1.0 %)

Validation results of the method

The system suitability test results are presented in (table 1). In the repeatability study, the relative standard deviation (RSD) was less than 0.8 % for the retention times of the isomers, 1.8 % for (10-Camphorsulfonyl) oxaziridine peak area and 4.8 % for S-isomer peak area (table 2). In the intermediate precision study, results show that RSD values were in the same order of magnitude than those obtained for repeatability (table 2).

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were estimated to be 400 and 900 ng/ml for S-isomer, when a signal-to-noise ratio of 3 and 10 was used as the criteria. The method precision for S-isomer at the limit of quantification was less than 5 % RSD (table 2).

Good linearity was observed for S-isomer over the concentration range of 900–9000 ng/ml, with the linear regression equation y = 25.241X+948.11 (Correlation coefficient R = 0.999). Linearity was checked for S–isomer over the same concentration range for three consecutive days. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve were 2.8 and 2.3 respectively (table 2).

The standard addition and recovery experiments were conducted for S-isomer in bulk samples in triplicate at 0.5, 1.0 and 1.5 percent of analyte concentration. Recovery was calculated from the slope and Y-intercept of the calibration curve obtained in linearity study and percentage recovery was ranged from 97.0 to 102.0 (table 3).

The chromatographic resolution of S-isomer and R-isomer of (10-Camphorsulfonyl) oxaziridine peaks was used to evaluate the method robustness under modified conditions. The resolution between S-isomer and R-isomer of (10-Camphorsulfonyl) oxaziridine was greater than 2.5, under all separation conditions tested (table 4), demonstrating sufficient robustness.

No significant change in the S-isomer content was observed in (10-Camphorsulfonyl) oxaziridine sample during solution stability and mobile phase stability experiments. Hence (10-Camphorsulfonyl) oxaziridine sample solution and mobile phase are stable for at least 48 hours.

Table 1: System-suitability report

Compound (n=3)	Rt	R_S	N	T
S-isome	7.4	-	6500	0.9
R-isomer	8.4	3.1	6800	0.9

n =3 determinations, Rt-Retention time, R_S-USP resolution, N-number of theoretical plates (USP tangent method), T-USP tailing factor

Table 2: Validation results of the developed normal phase method

Validation parameter	Results
Repeatability (n=6, % RSD)	
Retention time (S-isomer)	0.6
Retention time (R-isomer)	0.8
Area (S-isomer)	4.8
Area (R-isomer)	1.5
Intermediate precision (n=18, % RSD)	
Retention time (S-isomer)	0.9
Retention time (R-isomer)	0.8
Area (S-isomer)	4.8
Area (R-isomer)	1.8
LOD-LOQ (S-isomer)	
Limit of detection (ng/ml)	400
Limit of quantification (ng/ml)	900
Precision at LOQ (% RSD)	4.5
Linearity (S-isomer)	
Calibration range (ng/ml)	900-9000
Calibration points	6
Correlation coefficient	0.999
Slope (%RSD)	2.8
Intercept (% RSD)	2.3

Table 3: Recovery results of S-isomer in bulk drugs

Added (ng) (n=3)	Recovered (ng)	% Recover	% RSD
5000	4850	97.0	3.8
10000	10200	102.0	4.1
15000	14700	98.0	4.3

n=3 determinations

Table 4: Robustness of the method

Parameter	USP resolution between S-isomer and R-isomer
Flow rate (ml/min)	
0.8	2.8
1.0	3.0
1.2	3.1
Column temperature (°C)	
25 25	3.1
30	3.0
35	2.8
Ethanol percentage in mobile phase	
9	3.0
10	3.0
11	2.6

CONCLUSION

A novel, simple and accurate normal phase LC method was described for the enantiomeric separation of 10-Camphorsulfonyl Oxaziridine is precise and specific. The method was completely validated showing satisfactory data for all the method validation parameters as per ICH guidelines. The developed method can be used for the quantitative determination of S-isomer in bulk drug materials in the pharmaceutical industry.

ACKNOWLEDGMENT

We would like to thank management of Lavender Lab Pune, India for gift sample.

CONFLICT OF INTERESTS

Declared None

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