IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 11, Issue 9 Ver. I (September. 2018), PP 12-18
www.iosrjournals.org

A Validated Chiral HPLC Method For Enantiomeric Separation of Glycidyl Butyrate on Cellulose Based Stationary Phase

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Abstract: *A Chiral high-performance liquid chromatographic (HPLC) method was developed and validated for the enantiomeric separation of glycidyl butyrate and the quantification of S (+)-glycidyl butyrate. Separation was achieved on Chiralcel OD-H (250 × 4.6mm, 5µ) column using a mixture of Hexane: 2-Propanol (100:1, v/v) as a mobile phase with flow rate of 1.0 mL/min on UV detector. The resolution between S (+)-glycidyl butyrate (SGB) and R (-)-glycidyl butyrate (RGB) enantiomers was found to be more than 2. The proposed method was validated with respect to linearity, precession, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness. The SGB showed an excellent linearity over the concentrations range from 2 to 24 µg/mL, while the mean recovery of SGB was found to be 99.26%. The proposed method was found simple, precise, accurate and robust for quantitative determination of SGB in RGB. The developed method possess advantages such as shorter run time, better resolution between enantiomers, rapid analysis with less solvent consumption, which makes the method economical and environment friendly.*

Date of Submission: 31-08-2018 Date of acceptance: 15-09-2018

I. Introduction

Quality of key starting material is of prime important especially in the synthesis of chiral active pharmaceutical ingredient. Most of the optically active drug substances are synthesized from optically active key starting materials or intermediates. Thus, one of the most important and challenging areas in the analysis of pharmaceuticals is the assessment of chiral purity. The scientific and economic significance of chiral substances has favored the preeminent development in separation science in the last two decades. Moreover, the obligation from the regulatory authorities made mandatory for the availability of enantio-selective techniques to assess the stereo-isomeric composition of chiral substances. The measurement of chiral purity is an essential means of quality control for chiral substances to quantify the percentage abundance of the undesired enantiomer relative to the total peak area of both enantiomers [1, 2]. The analytical method development and their validations are mandatory for control of the enantiomeric purity of chiral key starting materials and products.

In recent time the regulatory committees are more stringent specifically for submission of the documentation regarding the synthetic process and controls mainly in New Drug Application (NDA) or Drug Master File (DMF). A significant regulatory burden is incurred for review of critical quality attributes of synthesized drug substance during regulatory review and GMP inspection. During the past decades analytical methodology has also improved dramatically for aiming to monitor the quality attributes for starting materials, intermediates, and reagents/solvents, which have the potential to affect the quality of the drug substance. The regulatory agencies such as United States Food and Drug Administration (US FDA) and International Conference on Harmonization (ICH) provided guidelines and acceptance criteria for establishing the specifications and enhancing the quality of the drugs substance synthesized [3].

As most of the drugs are synthesized as chiral substances using chiral starting materials or intermediates, the specifications for these key starting materials or intermediates should be adequate to assure that requisite degree of purity with the molecular architecture for the final product, have been attained. During this process, it is very important to perform the quantitative determination(s) of undesired materials as an impurity (e.g., isomers, by-products, starting materials, etc) by appropriate established analytical methodology.

The US FDA has established various guidance's like 'Guideline for submitting supporting documentation in drug applications for the manufacture of drug substances', 'Q11 Development and manufacture of drug substances' and 'Drug Substance Chemistry, Manufacturing, and Controls Information' for submitting information to regulatory agency. The International Conference on Harmonization (ICH) has also established guidance such as 'Impurities in new drug substances Q3A (R2)' and 'Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances Q6A [4-8].

However, the development of the analytical methods for the quantitative analysis of chiral compounds and the assessment of enantiomeric purity is extremely challenging, because the same physical and chemical properties of the two enantiomers make discriminating and separating them very difficult. The chromatographic has been shown to be very effective in separation, identification and quantification of isomers. The use of gas chromatography for the separation of stereoisomers is not very common as compared to liquid chromatography, whereas the advantage of HPLC is that, it can be used for separation of many chiral compounds that are non-volatile, polar, or ionic in nature [9, 10].

S(+)-glycidyl butyrate(SGB) and R (-)-glycidyl butyrate (RGB) (Figure 1) are used for the production of biologically active compounds of commercial interest. To introduce a stereogenic center in oxazolidinones derivatives, RGB has been used. Among various examples available in the literatures linezolide is one of the commonly used antibiotics for the treatment of multidrug resistant Gram-positive infections such as nosocomial, community-acquired pneumonia, and skin infections is synthesized using an intermediate RGB. RGB is a reagent also used in the synthesis of glycidol which is used as a stabilizer in manufacturing of vinyl polymers; intermediate in synthesis of glycerol, glycidyl ethers, and amines. The SGB is intrinsically present as a chiral impurity in RGB, which is used as starting key material for synthesis of oxazolidinone antibacterial agentsand it appears in almost same percentage in final drug substance as was present in the raw material (RGB). Hence, it is very important to monitor SGB in RGB raw material to maintain the quality of the final drug substance. SGB is also very useful as starting material in the synthesis of many drugs, such as R (-)-argentilactone which exhibits both antileishmanial activity and cytotoxic activity against mouse leukemia cells [11-14].

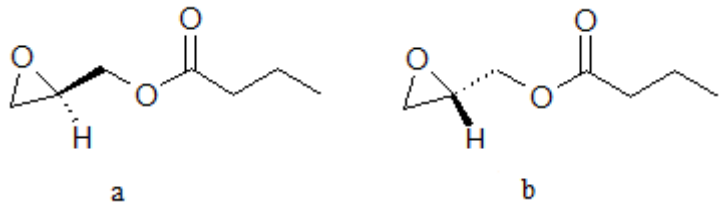

Fig. 1. Structures of a. R (-)-Glycidyl butyrate; b. S (+)-Glycidyl butyrate

The literature reveals several reported methods for resolution of glycidyl butyrate by GC and enzymatic resolution [15-18]. Khan et. al., reported liquid chromatographic method for estimation of (S)-glycidyl butyrate in R-glycidyl butyrate [19]. The reported method was utilized for estimation SGB in commercial synthesis of Linezolide. The reported method utilized mixture of ethanol and n-Hexane (0.1:50) at flow rate of 0.5 mL/min as mobile phase. In this published method SGB eluted at very high retention time (40 min) with very long run time for analyzing sample and standard (105 and 65 min respectively). The long run time of the method do not make it perfectly suitable for quality control unit in pharmaceutical industry and it ultimately creates an economical and environment burden because of more consumption of organic solvents.

Now a day, in the pharmaceutical industry, it is expected to obtain accurate and reliable analytical data with simpler, faster, cost-effective and environment friendly manner. The simpler approaches having advantages of more consistent methods, lower method diversity, reduced inventory costs for consumables and more easily transferable methods from R&D laboratories to manufacturing quality control (QC) laboratories [20].

Liquid chromatography with polysaccharide based stationary phases is popular and broadly applicable for separation of many chiral compounds. The derivatives of amylose and cellulose are the most commonly used chiral stationary phases [21, 22].

Attempts were made to develop chiral HPLC method for enantiomeric separation of glycidyl butyrate using cellulose based chiral stationary phase. The proposed method mainly deals with quantification of SGB in RGB. The separation was performed using normal phase high-performance liquid chromatographic method with UV visible detector. The proposed method was validated as per ICH Q2(R1) guidelines [23] and found to be highly specific, precise andaccurate. The developed method was successfully applied for monitoring the quality of RGB which is used as starting material for synthesis of oxazolidinone.

II. Material and Methods

Equipment and Reagents

The HPLC system used was an Agilent 1100 series, equipped with quaternary pump, degasser, auto sampler, column oven and UV detector. The out-put signal was monitored and processed using Chemstation software. S (+)-Glycidyl butyrate (98.88%) and R (-)-Glycidyl butyrate (99.41%) were procured from Fluka (USA). HPLC grade Hexane and 2-propanol were procured from Rankem fine chemicals (Mumbai, India).

Chromatographic condition

The separation of SGB and RGB was performed using Chiralcel OD-H (250.0 × 4.6 mm ID 5 μm particles size) column from Daicel Chemical Industries, Ltd., Tokyo, Japan. The column was maintained at 30 °C. The separation was performed using mixture of Hexane: 2-Propanol (100:1, *v/v*) as a mobile phase which was pumped at flow rate of the 1.0 mL/min in isochratic mode with the run time of 12 minutes. The column eluents were monitored using UV visible detector set at a wavelength 220 nm with the injection volume of 20 μL.

Standard solution preparation

About 100.0 mg of SGB and RGB were weighed separately in 10 mL of volumetric flask. Around 5.0 mL of mobile phase was added and sonicated to dissolve. The volume was made up to the mark using mobile phase to get concentration of 10 mg/mL of SGB and RGB as stock solution. Stock solutions were further diluted by pipetting out 5 mL in a separate 25 mL volumetric flask and volume was made up to the mark with mobile phase to achieve concentration of 2 mg/mL solution which was used as working stock solutions.

Sample preparation

The sample for analysis was prepared by weighing accurately about 50 mg of RGB in 25 mL of volumetric flask. Approximately 10 mL of mobile phase was added to dissolve and the volume was made up to the mark with mobile phase with proper mixing of solution. The prepared solution was analyzed using developed method and content of SGB was calculated against calibration curve.

Method Development

The objective of this study was to separate the enantiomers SGB and RGB using liquid chromatography. Solution of individual enantiomer (2 mg/mL) was prepared in mobile phase and used for the method development. Initially the efforts were made to develop a method on gas chromatography using β and γ-cyclodextrin based chiral stationary phases of dimension 30m × 0.25mm × 0.25μ (Supelco analytical). The various method optimization studies with respect to temperature and time programming did not result in separation of the enantiomers by GC method. Further experiments were tried on HPLC using different chiral stationary phases. To develop the suitable chiral HPLC method for the separation of the enantiomers of glycidyl butyrate, stationary phase Chiralcel OD-H along with different combination of mobile phases were employed. Various experimental trials were conducted to optimize the mobile phase composition which can provide an optimum resolution and selectivity for two enantiomers.

Method Validation

System suitability

System suitability is an integral part of chromatographic method and used to verify that the system is working adequately for the analysis to be performed. The critical pieces of information to evaluate the method performance on chiral assays are resolution, sensitivity and injector precision.

Resolution is important parameter in chromatographic analysis which reflects the ability of method to separate two components from each other.Equally essential is to demonstrate quantitation of a low-levels of the undesired enantiomeric impurity. System suitability also helps to assure that column loading is under control throughout the analysis, reproducible and precise.

The capacity factor, theoretical plates, and peak tailing reflects the performance of chromatographic column and hence, are considered as system suitability parameters. Sensitivity and resolution are two parameters which demonstrate that reasonable small change in retention time and/or peak shape due to column wear, the method can still be considered suitable for intended use.

In chiral chromatography, it is very important to demonstrate the system suitability in terms of resolution because the isomers under analysis form a critical band pair in chromatogram, their resolution and tailing factor were considered for monitoring the chromatographic performance. The system suitability of method was determined by injecting solution of racemic glycidyl butyrate.

Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of potential impurities expected to be present.

Specificity was evaluated by injecting diluent, individual isomers of glycidyl butyrate and spiked solution of the SGB in RGB. The method specificity was determined by monitoring the resolution between the enantiomers. By overlapping the blank chromatogram with standard spiked chromatogram, SGB was monitored for any potential interference.

Precision

Precision of the method is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple sampling of homogeneous sample. The precision of the method was determined by analyzing six replicate samples of RGB (at concentration 2 mg/mL) and determined the content of SGB.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analytes within a given range. Linearity of SGB was determined in the range of 2 to 24 µg/mL of RGB. Calibration curve was obtained by plotting the peak response against concentration. The coefficient of determination (r^2), slope and intercept were calculated and statistically evaluated.

Accuracy / Recovery

Accuracy of the method is its ability to quantify the analyte to its true value. Accuracy of the method was ensured by determining recovery of the spiked amount of SGB in pre-analyzed sample of RGB at five levels ranging from 50 to 200%.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is ability of analytical method to detect the lowest concentration of the analyte. LOQ is lowest concentration of the analyte which can be quantitatively determined with acceptable precision and accuracy.
In enantiomeric purity analysis LOD and LOQ are important in connection with estimation of chiral impurity. SGB is chiral impurity in RGB hence, it is very much necessary to define LOD and LOQ. The LOD and LOQ were determined by following equation according to ICH guideline, LOD= 3.3 × S/N, LOQ= 10 × S/N

Robustness

Robustness of a method is the ability of method to remain unaffected by small changes in chromatographic parameters such as flow rate and column temperature. Robustness was evaluated by determining chromatographic resolution between two enantiomers. Flow rate was varied by 10% and column temperature was varied by 5°C.

Solution stability

Stability of SGB and RGB in mobile phase was studied by keeping the solution in tightly capped volumetric flask at room temperature on laboratory bench for 48 hrs. Content of SGB in RGB was checked at every 6 hrs intervals up to 48 hrs.

III. Result

A representative chromatogram of glycidyl butyrate is shown in Fig. 2, showing an excellent resolution (Rs = 2.0) between enantiomers and symmetric peak with tailing factor of 1.2.The obtained results andsystem suitability parameters acceptance criteria are summarized in Table 1.

Table 1 System suitability data

Analyte	R_t	α	Rs	N	T
S (+) Glycidyl butyrate	7.7	-	-	15286	1.15
R (-) Glycidyl butyrate	8.2	1.07	2.0	15016	1.14

R_t – retention time, α - enantioselectivity, Rs - USP resolution, N -number of theoretical plates (USP tangent method) and T - USP tailing factor

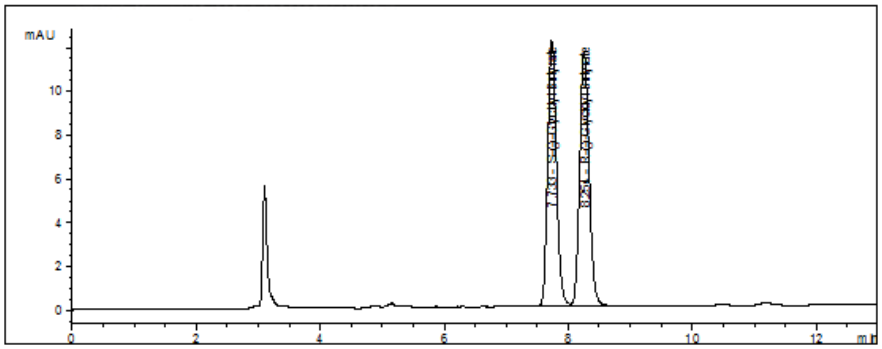

Fig. 2. Representative chromatographic resolution of Glycidyl butyrate enantiomers

The specificity of the method was performed by analyzing blank and standard solution of individual SGB and RGB isomer and SGB spiked in RGB solution. All the solutions were analyzed and chromatograms were overlaid and observed for any interference. It was observed that there was no interference at the retention time of SGB and RGB. The representative chromatogram of SGB spiked in RGB solution is shown in Fig. 3. The method was found to be precise with percent relative standard deviation (%RSD) less than 1.5% for SGB content. The method precision data is shown in Table 2.The chromatographic response was found to be linear in the range of 2 to 24 µg/mL for SGB. The calibration curve was plotted between concentration and peak area of SGB, and the correlation coefficient was found to be 0.9997 with the regression equation Y= 0.232x – 0.0231.

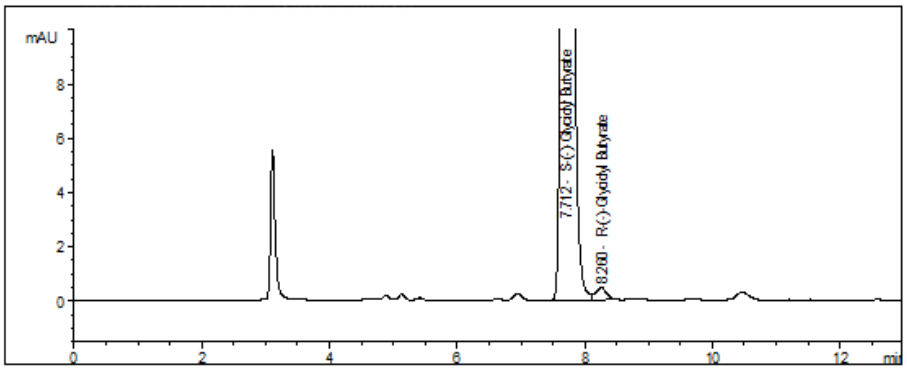

Figure 3. Representative chromatogram of standard S(+)-Glycidyl butyrate spiked with R (-)-Glycidyl butyrate

Table 2 Results of precision, LOD, LOQ and linearity studies

Validation parameter	**Results**
Precision (n = 6)	% R.S.D.
Retention time of S (+) enantiomer peak	0.5
Retention time of R (-) enantiomer peak	0.4
LOD and LOQ of SGB	
Limit of detection (µg/mL)	0.25
Limit of quantification (µg/mL)	1.21
Linearity of SGB	
Calibration range (µg/mL)	2 – 24
Correlation coefficient	0.9997

Accuracy of the method was checked by determining recovery of the spiked amount of SGB in pre-analysed sample of RGB. The recovery was found to be in the range from 97.6 to 101.8% for SGB at five levels with average recovery of 99.26%. The amount spiked and amount found for SGB are shown in Table 3. The results obtained for limit of detection (LOD) and limit of quantification (LOQ) for SGB are shown in Table 3. Both enantiomers were found to be stable in mobile phase, as no significant change was observed up to 48 hrs. The results of robustness study with respect to change in flow rate and robustness data is shown in Table 4.

Table 3 Results of recovery study for S (+)-Glycidyl butyrate (n = 3)

Level	**Amount spiked (%)**	**Amount found (%)**	**Recovery (%)**
0.0	0.0	0.97	-
50	0.56	1.54	101.8
75	0.84	1.81	100.0
100	1.12	2.07	98.2
150	1.68	2.61	97.6
200	2.24	3.18	98.7
Average % recovery			99.26

Table 4 Results of robustness study

Parameter	**Rs**	**% RSD**
Flow rate (mL/min)		
0.9	2.1	0.47
1.0	2.0	0.43
1.1	1.9	0.45
Column temperature (℃)		
25	2.1	0.49
30	2.0	0.40
35	1.8	0.51

IV. Discussion

There are several other techniques such as polarimetry, nuclear magnetic resonance (NMR), isotopic dilution, calorimetry and enzyme techniques which are used for enantiomeric purity analysis without separation of enantiomers. The major drawbacks of all these techniques are requirement of pure chiral samples, poor sensitivity, slowness and requirement of special instruments. The quantification of individual enantiomers is important and essential as per regulatory prospective.

The proposed method involves various experiments carried out using chiral stationary phases Chiralcel OJ-H (250 × 4.6mm, 5µ) and Chiralcel OD-H (250 × 4.6mm, 5µ) along with different combination of mobile phases. Initially mobile phase consisting hexane and 2-propanol in proportion of 90:10 v/v, was used with Chiralcel OJ-H (250 × 4.6mm, 5µ) for separation of SGB and RGB but the resolution was found to be poor. Further the experiments were carried out using Chiralcel OD-H (250 × 4.6mm, 5µ) with mobile phase comprising of hexane and 2-propanol in proportion of 90:10 v/v to get optimum resolution and selectivity for two enantiomers. This column showed some ray of hope as two isomers were eluted at about 4.5 min with resolution of 0.2. In order to achieve better resolution, percentage of 2-propanol in mobile phase was reduced step by step. Excellent separation was achieved on Chiralcel OD-H column using mobile phase of hexane: 2-propanol (100:1, v/v). By using of this mobile phase, resolution of more than 2.0 was achieved for the enantiomers with retention times 7.5 min and 8.5 min for SGB and RGB, respectively. The peaks obtained were symmetric (Tailing factor about 1.2) with high column efficiency (Theoretical plates about 15000).

In the present study the cellulose based Chiralcel OD-H (250.0 × 4.6 mm ID, 5 µm particles size) with mobile phase was hexane: 2-propanol (100:1, v/v) was used. The mechanism of chiral separations is the interaction of CSP with analytes to form short-lived, transient diastereomeric complexes. The complexes are formed as a result of hydrogen bonding, dipole-dipole interactions, pi bonding, electrostatic interactions and inclusion complex formation [24-26]. As discussed earlier, isomers of glycidyl butyrate could not be separated on Chiralcel OJ-H (Tris- 4-methyl benzoate ester derivative of cellulose) and better resolution was obtained on Chiralcel OD-H (Tris-3,5-dimethylphenyl carbamate derivative of cellulose). From these observations it can stated that separation of glycidyl butyrate isomers may be due to the interaction between the epoxide group of glycidyl butyrate isomers and the polar carbamate group of the CSP. The carbamate group on the CSP interacts with C-O of Glycidyl butyrate isomers through hydrogen bonding, in addition the dipole-dipole interaction occurs between the C=O group on the CSP and C=O group on Glycidyl butyrate isomers. Wainer et al [27] reported that solute having cyclic ring functionality could provide additional stabilizing effect on the solute-CSP complex by insertion of cyclic ring portion in to the chiral cavity. In case of glycidyl butyrate, epoxide ring form complex with CSP providing additional stabilizing effect. Cellulose phenyl carbamates forms a cylindrical structure and possess more defined cavity making it different than benzoate ester derivatives. These polysaccharides contain a large number of chiral active sites and thus a relative high probability of interaction with the solute leading to separation of two isomers.

Applicability of method

The proposed method was applied for estimation of SGB in RGB samples for monitoring of enantiomeric purity. The RGB is used a key starting material for synthesis of oxazolidinones derivatives. The proposed method can be successfully applied for analysis of commercial samples of RGB.

V. Conclusion

A simple, specific, sensitive, linear, precise and accurate normal phase chiral HPLC method was developed and validated for the separation of glycidyl butyrate enantiomers. Chiralcel OD-H (Celluose based stationary phase) CSP was found to be selective for the separation of analyte. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be used for the quantitative determination of enantiomeric impurity in the R-glycidyl butyrate in quality control laboratories. The developed method has dual advantage of short run time of less than 15 minutes and is highly sensitive as compared to reported methods. Due to high sensitivity the method can detect presence of 0.01% of S-glycidyl butyrate in R-glycidyl butyrate. The shorter method run time leads to rapid analysis of samples making the method economically feasible and environment friendly.

Acknowledgement

Authors thank to Department of Chemical Technology of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India for their support in conducting this research.

References

[1]. Maier, N.M., Franco, P., Lindner, W., Separation of enantiomers: needs, challenges, perspectives, *J Chromatogr A.*, 2001, vol.906, no.1, p. 3-33.

[2]. Wrezel, P.W., Chion, I., Pakula, R., Weissmueller, D.W., System suitability and validation for chiral purity assays of drug substances, *LC GC North America.*, 2006, vol.24, no.11, p.1216-1221.
[3]. Cupps, T., Fritschel, B., Mavroudakis, W., Mitchell, M., Ridge, D., Wyvratt, J., Starting material, reprocessing, retesting, and critical controls, *Pharm Technol.*, 2003, vol.27, no.2, p.34-53.
[4]. United States Food and Drug Administration, Center for Drug Evaluation and Research. Guideline for submitting supporting documentation in drug applications for the manufacture of drug substances, 1987.
[5]. United States Food and Drug Administration, Center for Drug Evaluation and Research. Q11 Development and manufacture of drug substances, 2012.
[6]. United States Food and Drug Administration, Center for Drug Evaluation and Research. Drug Substance Chemistry, Manufacturing, and Controls Information, 2010.
[7]. International Conference on Harmonization, Impurities in new drug substances Q3A(R2), 2006.
[8]. International Conference on Harmonization, Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances Q6A, 1999.
[9]. Cabusas, M.E.Y.,Chiral separations on HPLC derivatized polysaccharide csps: temperature, mobile phase and chiral recognition mechanism studies, Virginia Polytechnic Institute and State University Department (Doctoral dissertation), 1998.
[10]. Beesley, T.E., Scott, R.P., Chiral chromatography, John Wiley & Sons, 1998.
[11]. Yu, D., Wang, L., Gu, Q., et al., A two-step enzymatic resolution of glycidyl butyrate, *Process Biochem.*, 2007, vol. 42, no. 9, p.1319-1325.
[12]. Griera, R., Cantos-Llopart, C., Amat, M., Bosch, J., del Castillo, J.-C., Huguet, J.; New potential antibacterials: A synthetic route to N-aryloxazolidinone/3-aryltetrahydroisoquinoline hybrids, *Bioorg Med Chem Lett.*, 2006, vol.16, no.3, p.529-531.
[13]. Griera, R., Cantos-Llopart, C., Amat, M., Bosch, J., del Castillo, J.-C., Huguet, J., A synthetic route to a novel type of conformationally constrained N-aryloxazolidinones, *Bioorg Med Chem Lett.*, 2005, vol.15, no.10, p. 2515-2517.
[14]. Brickner, S.J., Hutchinson, D.K., Barbachyn, M.R., et al., Synthesis and antibacterial activity of U-100592 and U-100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections, *J Med Chem.*, 1996, vol. 39, no. 3,p.673-679.
[15]. Duchateau, A., Jacquemin, N., Straatman, H., Noorduin, A., Liquid chromatographic determination of chiral epoxides by derivatization with sodium sulphide, o-phthalaldehyde and an amino acid, *J Chromatogr A*, 1993, vol. 637, no.1, p.29-34.
[16]. Song, X., Qi, X., Qu, Y., Resolution of (R, S)-(±)-glycidyl butyrate with immobilized Y-11 Trichosporon capitatum lipase in gelatin-containing microemulsion-based organogels, *Coll Surf B: Biointerfaces*, 2008, vol. 67, no.1, p.127-131.
[17]. Palomo, J.M., Segura, R.L., Fernandez-Lorente, G., Guisán, J.M., Fernandez-Lafuente, R., Enzymatic resolution of (±)-glycidyl butyrate in aqueous media. Strong modulation of the properties of the lipase from Rhizopus oryzae via immobilization techniques, *Tetrahedron: Asymmetry*, 2004, vol. 15, no.7, p.1157-1161.
[18]. Wu, D.R., Cramer, S.M., Belfort, G., Kinetic resolution of racemic glycidyl butyrate using a multiphase membrane enzyme reactor: experiments and model verification, *Biotechnol Bioeng.*, 1993, vol. 41, no.10, p. 979-990.
[19]. Khan, M.A., Sinha, S., Khandekar, N., Parashar, V., Liquid chromatographic estimation of (S)-glycidyl butyrate in (R)-glycidyl butyrate, *J Nat Sc Biol Med.*, 2011, vol. 2, no.2, p.180-184.
[20]. Lee, D.C., Webb, M.L., Pharmaceutical analysis, John Wiley & Sons, 2009.
[21]. Dousa, M., Lehnert, P., Adamusová, H., Bosáková, Z., Fundamental study of enantioselective HPLC separation of tapentadol enantiomers using cellulose-based chiral stationary phase in normal phase mode, *J Pharm Bbiomed Anal.*, 2013, vol.74, p.111-116.
[22]. Tang, Y., Significance of mobile phase composition in enantioseparation of chiral drugs by HPLC on a cellulose-based chiral stationary phase, *Chirality*, 1996, vol. 8, no.1, p.136-142.
[23]. International Conference on Harmonization, Validation of analytical procedures: text and methodology Q2 (R1), 2005.
[24]. Sahajwalla CG., New drug development: Regulatory Paradigms for Clinical Pharmacology and Biopharmaceutics, 1st edition, Chpater 21 Marcel Dekker Inc., CRC press New York, 2004, vol. 141.
[25]. Kato, M., Fukushima, T., Shimba, N., Shimada, I., Kawakami, Y., Imai, K., A study of chiral recognition for NBD-derivatives on a Pirkle-type chiral stationary phase, *Biomed Chromatogr.*, 2001, vol.15, no.4, p.227-234.
[26]. Rane, V., Shinde, D., Development and validation of chiral LC method for the enantiomeric separation of duloxetine on amylose based stationary phase, *J Chromatogr Sci*, 2008, vol. 46, no.9, p.772-776.
[27]. Wainer, I.W., Stiffin, R.M., Shibata, T., Resolution of enantiomeric aromatic alcohols on a cellulose tribenzoate high-performance liquid chromatography chiral stationary phase: a proposed chiral recognition mechanism, *J Chromatogr A*, 1987, vol.411, p.139-151.

IOSR Journal of Applied Chemistry (IOSR-JAC) is UGC approved Journal with Sl. No. 4031, Journal no. 44190.

Udaykumar Rakibe1. " A Validated Chiral HPLC Method For Enantiomeric Separation of Glycidyl Butyrate on Cellulose Based Stationary Phase." IOSR Journal of Applied Chemistry (IOSR-JAC) 11.9 (2018): 12-18.