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Stereoselective HPLC separation of alvimopan on cellulose-based immobilized polysaccharide as a chiral stationary phase

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Abstract

Chiral separation by normal phase high performance liquid chromatography is one of the most powerful technique to quantify the chiral purity of the compounds. In this study, a novel, simple, and specific analytical method was proposed to ascertain the chiral purity of alvimopan (ALV). The normal phase HPLC method was developed based on cellulose tris (3,5-dichlorophenylcarbamate) stationary phase. The separation of ALV isomers achieved by using column CHIRALPAK IC (250 × 4.6 mm, 5 μm), mobile phase n-hexane: isopropyl alcohol: ethanol: diethylamine (650:200:150:5 *v*/v), column oven temperature 30°C, flow rate 1.0 mL min^{-1}, injection volume was 10 μL, chromatographic response monitored at 273 nm. The developed method was validated as per the ICH guidelines and found precise, accurate, and linear. The advantage of the method is a good separation of ALV isomers within 35 minutes of the analysis time. Therefore, this method is suitable for routine determination of chiral purity of ALV active pharmaceutical ingredient.

KEYWORDS

alvimopan, HPLC, method development, method validation, stereoselective

1 | INTRODUCTION

Alvimopan is a potent selective peripherally acting antagonist of the human μ-opioid receptor[1-4] that is being developed for the management of acute postoperative ileus and for the reversal of the delayed gastrointestinal and colonic transit that result in symptoms such as constipation, nausea, and motility disorders in patients treated with opiate analgesics.[1] It is orally available, but its activity is peripherally restricted because its moderately large, zwitterions form and polarity limit gastrointestinal absorption and prevent passage through the blood-brain barrier.[2-4] Preclinical and small-scale clinical studies demonstrated that ALV administered orally can selectively antagonize the gastrointestinal effects of opioid agonists such as morphine without affecting analgesia.[2-5] The adverse effects of opioids on the gastrointestinal tract can be reversed by μ-opioid-receptor antagonists such as naloxone and nalmefene, but these drugs are also active in the central nervous system, and so inhibit the analgesic effects of systemic opioids. To overcome this issue, efforts have been directed at identifying μ-opioid receptor antagonists with peripherally restricted activity.[6-8]

Chirality infuses the natural world and is of particular importance to life, as the molecules that comprise living things are chiral and their chirality is crucial to their biological function.[9-11] Recent trend of pharmaceutical research and development is most inclined to the developing the selective isomer and testing of individual enantiomers become a growing priority. So far, no method has been published for the determination of the ALV chiral purity. By considering an inherent criticality of reversed phase mode for chiral separation, normal phase mode preferred for separation of ALV chiral isomers.[12-14] The objective of the present study was to develop a stereoselective HPLC-UV method with cellulose tris-(3,5-dichlorophenylcarbamate) as a stationary phase. This method allows to determine the chiral purity of ALV without any derivatization. In this study, the developed method was validated as per the ICH guidelines. The proposed method is novel, simple, and accurate for quantification of ALV isomeric impurities. The advantage of the method is a good separation of ALV isomers within 35 minutes of analysis time. Therefore, this method is suitable for routine determination of chiral purity of ALV active pharmaceutical ingredient.

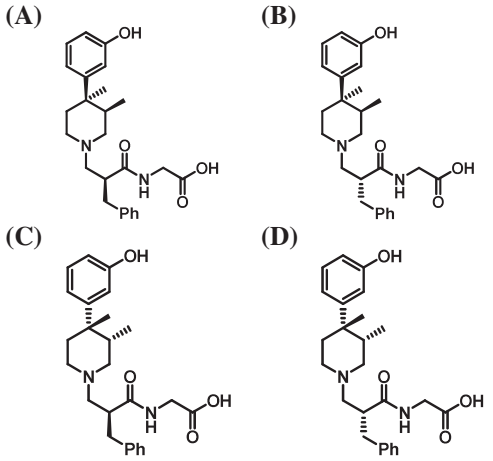
2 | MATERIALS AND METHODS

2.1 | Reagents and chemicals

All the chemical reagents are of analytical reagent grade and used as received without further purification throughout the experiments. n-Hexane and isopropyl alcohol were purchased from Rankem (Mumbai), ethanol was purchased from (Hyman), and diethylamine was purchased from Merck (India). ALV and isomeric impurities were supplied by Dr. Reddy's Laboratories (Figure 1)

2.2 | Instrumentation and chromatographic conditions

A Waters HPLC model 2695 equipped with pump, automated injector, column oven, and photo diode array detector was set at 273 nm. Experiment was carried out by using Diacel CHIRALPAK-IC column (250 mm × 4.6 mm, 5 μm). The mobile phase consists of n-hexane: isopropyl alcohol, ethanol, and diethylamine (650:200:150:5 *v*/v) which is operated at 30°C column oven temperature at isocratic mode. The mobile phase flow of the method was 1.0 mL min^{-1}. Injection volume was 10 μL. Data acquisition and interpretation were performed by using empower-2 software.



(a) 2-((S)-2-benzyl-3-((3R, 4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2S,3R,4R)
(b) 2-((R)-2-benzyl-3-((3R, 4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2R,3R,4R)
(c) 2-((S)-2-benzyl-3-((3S, 4S)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2S,3S,4S).
(d) 2-((R)-2-benzyl-3-((3S, 4S)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2R,3S,4S).

FIGURE 1 Structures of ALV and stereoisomers. A, 2-((S)-2-benzyl-3-((3R, 4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2S,3R,4R). B, 2-((R)-2-benzyl-3-((3R, 4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2R,3R,4R). C, 2-((S)-2-benzyl-3-((3S, 4S)-4-(3-hydroxyphenyl)-3,4- dimethylpiperidin-1-yl) propanamido) acetic acid. (2S,3S,4S). D, 2-((R)-2-benzyl-3-((3S, 4S)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl) propanamido) acetic acid. (2R,3S,4S)

3 | EXPERIMENTAL DETAILS

3.1 | Preparation of solutions

3.1.1 | Selection of diluents

Special attention should be paid on the solubility of the enantiomers as well as racemate while preparing of sample, because the separation shall be decided based on the solubility of enantiomers into the particular solvents.[15-] As part of solubility study, different solvents were investigated. It was found that the enantiomers are soluble in the methanol and mixture of methanol and ethanol, whereas it is practically insoluble in n-hexane, isopropyl alcohol, and ethyl acetate.

The mode of separation was normal phase (non-polar); therefore, the n-hexane and alcohols are selected as solvents for the mobile phase. Initially, the sample was dissolved in methanol. Due to the difference in polarity of mobile phase and diluent, the peak splitting of ALV was observed. Typical chromatogram of ALV isomers using methanol as diluent is shown in Figure 2A,C. Further ALV isomer was dissolved in methanol: isopropyl alcohol (20:80 *v*/v). The isomers were dissolved in methanol and

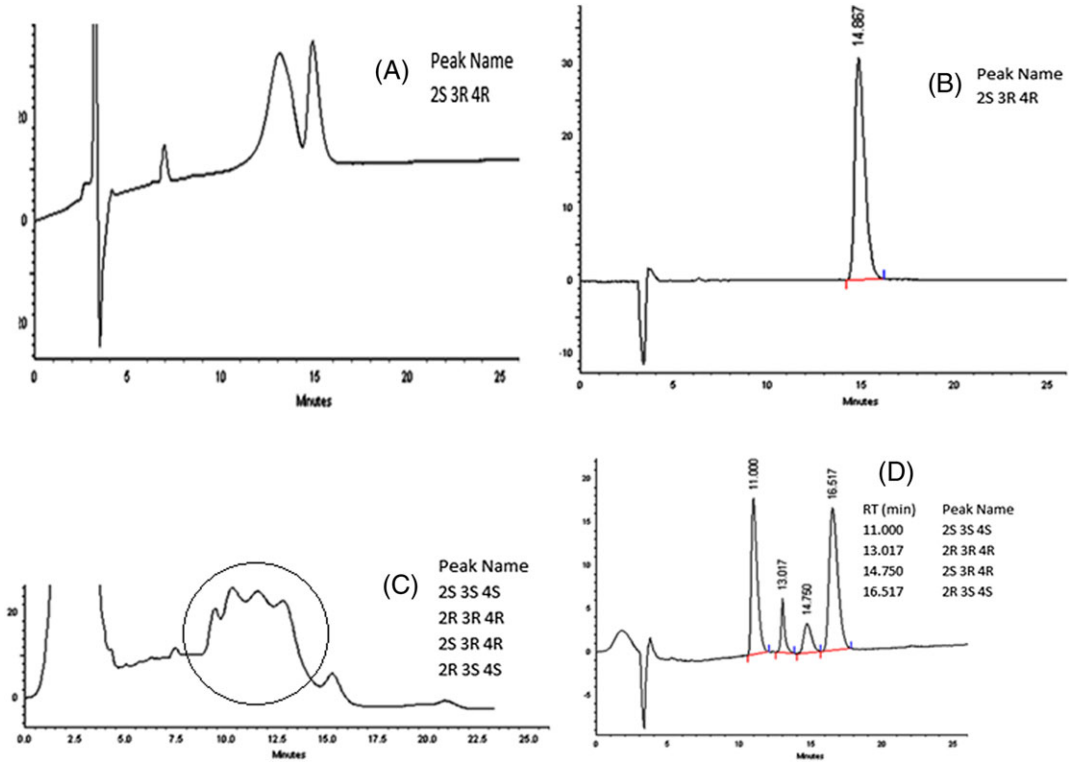


FIGURE 2 A, The chromatogram of ALV stereoisomer 2S 3R 4R using methanol (100%) diluent. B, The chromatogram of ALV stereoisomer 2S 3R 4R using methanol and isopropyl alcohol (20:80 v/v). C, The chromatogram of ALV stereoisomers 2S 3S 4S, 2R 3R 4R, 2S 3R 4R, and 2R 3S 4S using methanol (100%) diluent. D, The chromatogram of ALV stereoisomers 2S 3S 4S, 2R 3R 4R, 2S 3R 4R, and 2R 3S 4S using methanol and isopropyl alcohol (20:80 *v*/v)

diluted volume with isopropyl alcohol. The symmetric peak shape of ALV was found. Typical chromatogram of ALV isomers using methanol and isopropyl alcohol as diluent shown in Figure 2B,D. Therefore, the methanol and isopropyl alcohol combination (20:80 *v*/v) was finalized as diluent in ALV chiral analysis.

3.1.2 | Sample and standard solution preparation

The details of ALV and isomeric impurities are summarized in Figure 1. The ALV chiral isomers were dissolved in the diluent (methanol: isopropanol 20:80 v/v) as stock solutions. Initially, samples are dissolved in methanol and diluted volume in isopropanol and sonicated for 3 minutes.

ALV isomers were spiked in ALV sample (0.5 mg mL^{-1}).

3.1.3 | Evaluation of system suitability and method validation

Based on the criticality of the method, peak tailing and peak resolution (Rs) were considered as system suitability criteria for this method. The developed method was validated as per ICH guidelines.[15,16]

4 | RESULTS AND DISCUSSION

4.1 | Method development and optimization

Chiral separation and estimation are a very complex phenomenon. Normal phase chromatography with chiral stationary phase considered for separation of ALV and isomeric impurities.[17]

4.2 | Theory of chiral separation

There are several articles available in literature describing about the separation mechanism of enantiomers on polysaccharide phases.[14,17-21] In this chiral separation study, specific interactions are most important like, π-π interaction between the aromatic rings of the ALV enantiomers and chiral stationary phase, hydrogen bond interaction between carbonyl group of chiral stationary phase and acidic proton of ALV isomers, dipole-dipole interaction and the steric effect when nonpolar group of ALV isomer comes contact with chiral stationary phase. Wainer et al reported that the analyte-containing aromatic functionalities could provide additional stabilizing effect to the isomer-chiral stationary phase complex by insertion of the

aromatic portion into the chiral cavity.[14,18,21] This type of stabilization effect may also exist due to the presence of aromatic function in Alvimopan molecule.

4.3 | Experiments

4.3.1 | Experiment no. 1

During development, the initial experiment was performed on Chiracel OD-H with the combination of 50:50 *v*/v n-hexane and isopropyl alcohol. Spiked chiral isomers in ALV were injected. ALV and isomers peak was eluted with 3 minutes and no resolution was found between the ALV and isomers.

4.3.2 | Experiment no.2

To resolve the ALV isomers, mobile phase combination was changed to n-hexane, ethanol, and isopropyl alcohol and performed the experiment using CHIRALPAK-IC column and methanol used as diluent. The ALV isomers were not resolved well and asymmetric peak shape was found.

4.3.3 | Experiment no.3

This experiment was performed using mobile phase consist of n-hexane: isopropyl alcohol, ethanol, and diethylamine (650:200:150:1 *v*/v) and CHIRALPAK-IC (250 x 4.6 mm, 5 μm) column. Resolution was found between the ALV and isomers peak but the peak shape was asymmetric (split peak). Upon literature survey, it was understood that the cause of peak splitting was difference in the polarity of the diluent (methanol) and mobile phase solvents. Hence, the diluent was changed from methanol to combinaion of methanol and isopropyl alcohol (20:80 *v*/v). To improve the peak symmetry/peak shape and resolution between isomers, further experiments were carried out with modifying the camposition of mobile phase. The resolution between the peaks improved with increasing the volume of diethylamine in the mobile phase because it improves the resolution, peak symmetry, and plate number.[10]

The mobile phase combination, n-hexane: isopropyl alcohol, ethanol, and diethylamine (650:200:150:5 *v*/v) and keeping chromatographic conditions same as per the above, obtained a good resolution between ALV and isomers and peak symmetry.

4.4 | Study of column oven temperature

Thorough literature survey reveals that the improvement of enantiomeric resolution at sub-ambient temperature by using polysaccharide based CSPs.[9] During optimization of the method, the effect of column oven temperature on stereoisomers was studied in the range of 20 to 50°C. The retention of chiral isomers can be expressed by using Van't Hoff's equation. The temperature dependence of retention can be expressed using Van't Hoff's equation.[9,10] Multiple type of interaction making contributions on the chiral recognition. At lower temperature, the viscosity of the mobile phase was high compared with higher temperature; therefore, slow elution of the ALV isomer observed at lower temperature and increased the resolution and decreased USP plate count. Figure 3 shows the dependence of resolution on the column oven temperature, and Figure 4 shows the dependence of USP plate count on the column oven temperature.

4.5 | Elution order of ALV isomers

During chiral method development, order of elution of ALV isomers was monitored by injecting individual pure

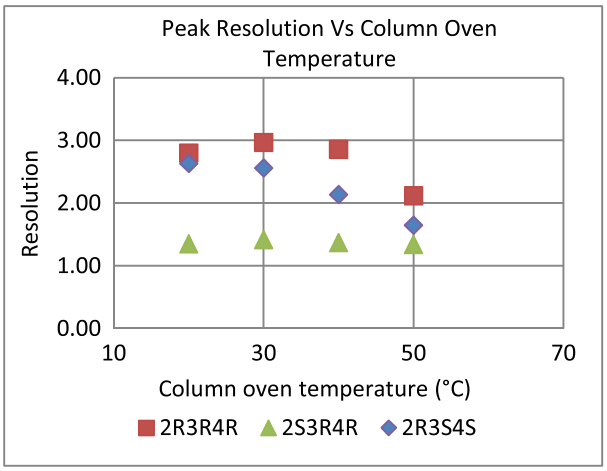


FIGURE 3 Graphical presentation of effect of the column oven temperature on resolution of alvimopan stereoisomers

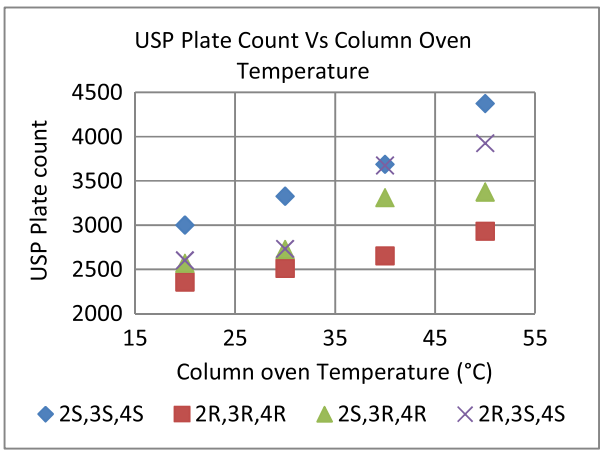


FIGURE 4 Graphical presentation of effect of the column oven temperature on plate count of alvimopan stereoisomers

isomer (Chiral purity mentioned in Table 1) because the elution order of isomers are most crucial for chiral method development. A typical system suitability chromatogram of ALV chiral separation is shown in Figure 5.

The system suitability of the method was monitored by using mobile phase as n-hexane, isopropyl alcohol, ethanol, and diethylamine in the ratio of 650:250:100:5 (*v*/v) with flow rate of 1.0 mL min^{-1} and column oven temperature 30°C on CHIRALPK IC column (250 × 4.6 mm, 5 μm).

4.6 | Method validation

Blank solution (20:80 methanol: isopropyl alcohol v/v) was injected to ensure the absence of any peak at retention time of ALV-2S,3R,4R, ALV-2R,3R,4R, ALV-2S,3S,4S, ALV-2R,3S,4S ALV isomer peak. Straight baseline indicated that there was no carryover or any other peak at retention time of analytes/isomers peak.

4.6.1 | System suitability

System suitability criteria were evaluated to ensure the satisfactory performance of the method as well as ensure the analytical method can be used within established chromatographic conditions. System suitability results are summarized in Table 2.

4.6.2 | Precision study

Precision study of the method was performed. The percentage relative standard deviation of the area of the 2S,3S,4S, 2R,3R,4R, 2R,3S,4S isomer in the precision study at 50% level obtained was 0.97%, 1.29%, and 0.65% whereas at 100% level obtained was 1.23%, 0.96%, and 0.71%, respectively, which confirms the good precision of the method. The resolution between ALV-2R,3R,4R and ALV-2S,3R,4R ($n = 6$) obtained was 1.4.

TABLE 1 Chiral purity of alvimopan isomers

Sr. No.	Isomer Name	Chiral Purity (% area)
1	2S,3R,4R	99.2
2	2R,3R,4R	68.5
3	2S,3S,4S	99.2
4	2R,3S,4S	99.6

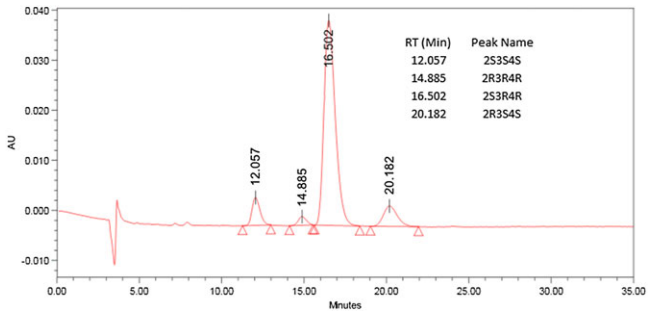


FIGURE 5 Typical system suitability chromatogram of alvimopan chiral separation

TABLE 2 Summarized data of system suitability

Peak	Name of Isomer	Resolution	Tailing Factor
Peak-1	2S,3S,4S	NA	1.23
Peak-2	2R,3R,4R	2.97	1.03
Peak-3	2S,3R,4R	1.42	1.33
Peak-4	2R,3S,4S	2.56	1.25

4.6.3 | Limit of detection and limit of quantification

Limit of detection and limit of quantification of the method was calculated based on the signal to noise ratio The retention time region of ALV isomers in blank was considered for baseline noise measurement by using empower 2. For 2S,3S,4S, 2R,3R,4R, 2R,3S,4S isomers, limit of quantification were found 0.136, 0.285, and 0.153 μg/mL, respectively, and limit of detection were found 0.048, 0.109, and 0.061 μg/mL, respectively. The %RSD for the area of the 2S,3S,4S, 2R,3R,4R, 2R,3S,4S isomer in the precision study at LOQ level was 1.86, 1.41, and 1.56%, respectively.

4.6.4 | Linearity study

All the 3 isomers of the ALV were prepared in the range from LOQ to 150% of the target concentration 0.15% was prepared. Linear curve was plotted with a peak area of ALV-2R,3R,4R, ALV-2S,3S,4S, ALV-2R,3S,4S isomer against its concentration. The correlation coefficient obtained was 0.9991, 0.9995, and 0.9999, respectively.

4.6.5 | Accuracy

Accuracy study for ALV isomers was evaluated in triplicate at 3 concentration levels (0.10%, 0.15% and 0.20% with respect to ALV). The percentage recovery of 2S,3S,4S, 2R,3R,4R, 2R,3S,4S isomers in the samples at LOQ level was 100.6, 98.0 and 100.9%, respectively, at 50% level 99.1, 98.8, 98.7%, respectively, at 100% level 98.7%, 99.3%, and 99.9%, respectively, and at 150% level 99.6, 98.4, and 101.2%, respectively. From the above results, the method was found accurate.

5 | CONCLUSION

A novel, simple chiral analysis HPLC method was developed for analysis of ALV isomers ALV-2S,3R,4R, ALV-2R,3R,4R, ALV-2S,3S,4S, ALV-2R,3S,4S using cellulose-based immobilized polysaccharide chiral stationary phase, CHIRALPAK IC column. Separation was achieved using n-hexane: isopropyl alcohol: ethanol: diethylamine 650:250:100:5 (*v*/v) as mobile phase within 35 minutes with a resolution not less than 1.4 for all the peaks. Based on validation data, this method was found precise, linear, and accurate. Therefore, this method is suitable for routine determination of chiral purity of ALV active pharmaceutical ingredient.

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REFERENCES

1. Camilleri M. Alvimopan, a selective peripherally acting μ-opioid antagonist. *Neurogas Motility*. 2005;17(2):157-165.
2. Schmidt WK. Alvimopan (ADL 8-2698) is a novel peripheral opioid antagonist. *The American J of Surg*. 2001;182(suppl):27S-38S.
3. Zimmerman DM, Gidda JS, Cantrell BE. Discovery of a potent, peripherally selective trans-3, 4-Dimethyl-4-(3-hydroxyphenyl) piperidine opioid antagonist for the treatment of gastrointestinal motility disorders. *J of Medi Chem*. 1994;37:2262-2265.
4. Liu SS, Hodgson PS, Carpenter RL, Fricke JR. Jr ADL 8-2698, a trans-3,4-dimethyl-4-(3-hydroxyphenyl) piperidine, prevents gastro-intestinal effects of intravenous morphine without affecting analgesia. *Clin Pharmacol Ther*. 2001;69:66-71.
5. Food and Drug Administration. FDA labeling information. (2008).
6. Taguchi A, Sharma N, Saleem RM. Selective postoperative inhibition of gastrointestinal opioid receptors. *The New Eng J of Medicine*. 2001;13:935-940.
7. Maron DJ, Fry RD. New therapies in the treatment of postoperative ileus after gastrointestinal surgery. *Am J of Ther*. 2008;15:59-65.
8. Delaney CP, Wolff B, Viscusi E. Alvimopan, for postoperative ileus following bowel resection: a pooled analysis of phase III studies. *Ann Surg*. 2007;245:355-363.
9. Lough WJ, Wainer IW. *Chirality in Natural and Applied Science*. Oxford: Blackwell; 2002.
10. Barron LD. *Molecular Light Scattering and Optical Activity*. Cambridge: Cambridge University Press; 2004.
11. Blackmond DG. The origin of biological homochirality. *Cold Spring Harb Perspect Biol*. 2010;2:a002147.
12. Matthijs N, Perrin C, Maftouh M, Massart DL, Vander HY. Definition and system implementation of strategies for method development of chiral separations in normal- or reversed-phase liquid chromatography using polysaccharide-based stationary phases. *J of Chromatogr A*. 2004;1041:119-133.
13. McCarthy JP. Direct enantiomeric separation of the four stereoisomers of nadolol using normal phase and reversal-cease high performance liquid chromatography with Chiralpak AD. *J Chromatogr*. 1994;685:349-355.
14. Dousa M, Lehnert P, Adamusova H, Bosakova Z. Fundamental study of enantioselective HPLC separation of tapentadol enantiomers using cellulose-based chiral stationary phase in normal phase mode. *J Phar Bio Ana*. 2013;74:111-116.
15. Zhai Z, Shi Y, Wang T. Development and validation of HPLC methods for enantioseparation of mirtazapine enantiomers at analytical and semipreparative scale using polysaccharide chiral stationary phases. *Anal Chim Acta*. 2005;550:123-129.
16. ICH harmonized tripartite guideline, validation of analytical procedures: text and methodology, Q2 (R1). Current step 4 version, parent guideline dated 27 October 1994, (Complementary Guideline on Methodology dated 6 November 1996, incorporated in November **2005**). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
17. Younes AA, Mangelings D, Heyden YV. Chiral separations in normal-phase liquid chromatography: enantioselectivity of recently commercialized polysaccharide based selectors. Part II. Optimization of enantioselectivity. *J Pharm Biomed Anal*. 2011;56:521-537.
18. Istvan H, Richard E, Jürgen A. Ultimate limits in high-pressure liquid chromatography. 1975;112:37-60.
19. Okamoto Y, Kaid YA. Resolution by high-performance liquid chromatography using polysaccharide carbamates and benzoates as chiral stationary phases. *J Chrom A*. 1994;666:403-419.
20. Okamoto Y, Yashima E. Polysaccharide derivatives for chromatographic separation of enantiomers. *Angew Chem Int Ed*. 1998;37:1020.
21. Wainer IW, Stiffin RM, 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*. 1987;411:139-151.

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