Pravara Med Rev; June 2023, 15 (02), 30 - 33
DOI: 10.36848/PMR/2022/99100.510235

Research article

# Normal Phase Chiral HPLC Methods for Analysis of Afoxolaner

***Kavya sri , Dr Virendra Kumar Sharma**

Department of Pharmaceutical Sciences, LNCT University, kolar road, Bhopal, (M. P.)
Corresponding author *

**Abstract:**
Afoxolaner, an isoxazoline derivative, is widely used as an antiparasitic agent in veterinary medicine. Due to its chiral nature, the development of an efficient chiral high-performance liquid chromatography (HPLC) method for the analysis of afoxolaner is crucial for pharmaceutical research, quality control, and pharmacokinetic studies. Afoxolaner belongs to the class of isoxazoline compounds and has gained significant attention as an effective treatment for various parasitic infestations in animals. However, due to its chiral nature, enantioselective analysis is essential to determine the concentration of individual enantiomers, as they may exhibit different pharmacokinetic and pharmacodynamic properties. Therefore, the development of robust and sensitive chiral HPLC methods for afoxolaner analysis has become a topic of great interest. This review aims to provide a comprehensive overview of the normal phase chiral HPLC methods employed for the analysis of afoxolaner, highlighting their principles, instrumentation, column selection, mobile phase composition, detection techniques, and validation parameters. Additionally, the review discusses the applications and limitations of these methods, and potential future perspectives in the field.
**Keywords:** Chiral HPLC Methods, Afoxolaner, antiparasitic agent

## Introduction

Afoxolaner, an isoxazoline derivative, has emerged as a potent antiparasitic agent widely used in veterinary medicine for the treatment and prevention of various parasitic infestations in animals. [1] As a chiral compound, afoxolaner exists as a pair of enantiomers, which are mirror images of each other. The individual enantiomers of afoxolaner may exhibit distinct pharmacokinetic and pharmacodynamic properties, making enantioselective analysis crucial for understanding its behavior in biological systems.[2,3]

High-performance liquid chromatography (HPLC) is a widely employed analytical technique for the separation and quantification of chiral compounds. Chiral HPLC offers the advantage of separating enantiomers with high efficiency and selectivity, making it an indispensable tool in pharmaceutical research, quality control, and pharmacokinetic studies. [4]The development of an efficient and robust chiral HPLC method for the analysis of afoxolaner is of utmost importance to ensure the accurate determination of individual enantiomers, which, in turn, aids in understanding their biological activities and optimizing drug formulations.[5,6] This comprehensive review aims to provide an in-depth analysis of the normal phase chiral HPLC methods employed for the analysis of afoxolaner. It encompasses the principles, instrumentation, column selection, mobile phase composition, detection techniques, and validation parameters associated with chiral HPLC analysis of afoxolaner. [7]Moreover, this review discusses the applications and limitations of these methods and explores potential future perspectives in the field. By consolidating the existing knowledge and advancements in the area of chiral HPLC analysis of afoxolaner, this review aims to serve as a valuable resource for researchers, analysts, and pharmaceutical scientists involved in the study of afoxolaner and related chiral compounds.[8]

## Chiral Separation Techniques : [9,10,11]

Chiral separation techniques aim to separate enantiomers, the mirror-image isomers of chiral compounds. Various techniques are available, including chromatography, electrophoresis, and crystallization. Among these techniques, chiral high-performance liquid chromatography (HPLC) has gained significant prominence in pharmaceutical analysis. Chiral HPLC relies on the use of chiral stationary phases (CSPs), which selectively interact with enantiomers to achieve their separation. The chiral recognition mechanisms, such as adsorption, inclusion, and



PMR P ISSN: 0975-0533, E ISSN: 0976-0164

ion-exchange, enable the differential retention and elution of enantiomers. Chiral HPLC offers excellent resolution, high sensitivity, and wide applicability, making it a preferred technique for the analysis of chiral compounds like afoxolaner.

**Chiral Stationary Phases (CSPs) for Afoxolaner Analysis :** [12]

1. Chiral stationary phases (CSPs) play a pivotal role in achieving efficient chiral separation in high-performance liquid chromatography (HPLC). Various types of CSPs have been investigated for the analysis of afoxolaner, each exhibiting different chiral recognition abilities and selectivities.
2. Polysaccharide-based CSPs, such as amylose and cellulose derivatives, are widely employed for chiral separations. They offer excellent enantioselectivity and compatibility with a wide range of mobile phases. Amylose-based CSPs, such as Chiralpak® and Chiralcel®, have shown successful enantioseparation of afoxolaner enantiomers with good resolution and peak shape.
3. Protein-based CSPs, such as bovine serum albumin (BSA) and α1-acid glycoprotein (AGP), have also been explored for afoxolaner analysis. Protein-based CSPs exhibit excellent chiral recognition capabilities due to their complex three-dimensional structures. However, their limited stability and sensitivity to environmental factors pose challenges in practical applications.
4. Macrocyclic CSPs, including cyclodextrins (CDs) and crown ethers, have demonstrated efficient chiral separations of afoxolaner enantiomers. CDs, such as β-CD and γ-CD, form inclusion complexes with afoxolaner, leading to enhanced chiral recognition. Crown ethers, on the other hand, utilize host-guest interactions to achieve enantioseparation.

The selection of a suitable CSP depends on various factors, including the specific chiral properties of the analyte, column stability, and compatibility with the mobile phase. A comprehensive evaluation of different CSPs for afoxolaner analysis is crucial to identify the optimal chiral selector that provides the desired enantioselectivity, resolution, and robustness.

It is worth noting that advancements in CSP technology, such as the development of new chiral selectors and immobilization techniques, continue to expand the options for chiral separations. The future exploration of novel CSPs holds promise for further enhancing the chiral separation efficiency and selectivity in afoxolaner analysis and other chiral compounds.[2]

**Mobile Phase Composition and Optimization**

The selection and optimization of the mobile phase composition are critical steps in achieving efficient chiral separation of afoxolaner enantiomers in high-performance liquid chromatography (HPLC). The mobile phase composition includes the choice of organic modifiers, additives, and pH. Organic modifiers, such as methanol, acetonitrile, or ethanol, play a crucial role in solubilizing the analyte and modulating the selectivity of the separation. Additives like salts or acids/bases can further enhance the enantioseparation by altering the ionic strength or pH of the mobile phase. Optimization of the mobile phase involves systematic variation of these parameters to improve the resolution, selectivity, and efficiency of the separation. The development of an optimized mobile phase composition is crucial for obtaining accurate and reliable quantitative analysis of afoxolaner enantiomers, ensuring robust chiral HPLC methods for pharmaceutical research and quality control applications.[3-7]

**Instrumentation and Detection Techniques**

Instrumentation and detection techniques play a vital role in the analysis of afoxolaner enantiomers using chiral high-performance liquid chromatography (HPLC). Commonly used detectors include UV-Vis detectors, fluorescence detectors, and mass spectrometers. UV-Vis detectors offer broad applicability and are suitable for routine analysis. Fluorescence detectors provide enhanced sensitivity and selectivity for compounds with inherent fluorescence properties. Mass spectrometers enable precise identification and quantification of analytes, offering high sensitivity and selectivity. The choice of detector depends on the specific requirements of the analysis. Additionally, the selection of appropriate detection wavelengths and strategies for trace level detection contribute to the accuracy and reliability of the afoxolaner enantiomeric analysis. The utilization of advanced instrumentation and detection techniques

enhances the capabilities of chiral HPLC for the analysis of afoxolaner and facilitates comprehensive characterization of its enantiomeric profiles.[4]

**Method Validation and Applications**

Method validation is a crucial aspect of chiral high-performance liquid chromatography (HPLC) for the analysis of afoxolaner enantiomers. Validation parameters, including selectivity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), are assessed to ensure the reliability and robustness of the method. Validation studies establish the method's suitability for specific applications, such as pharmacokinetic studies, bioequivalence assessments, and stability studies. Accurate determination of afoxolaner enantiomers is essential for understanding their pharmacological behavior, optimizing dosage forms, and ensuring product quality. Rigorous method validation provides confidence in the accuracy and precision of the chiral HPLC method, enabling its successful application in various pharmaceutical research and quality control settings.[9]

**Comparison with Other Analytical Techniques**

Chiral high-performance liquid chromatography (HPLC) offers distinct advantages over other analytical techniques for the analysis of afoxolaner enantiomers. Compared to capillary electrophoresis (CE) and supercritical fluid chromatography (SFC), chiral HPLC provides superior resolution, flexibility in column selection, and compatibility with a wide range of sample matrices. Additionally, chiral HPLC coupled with mass spectrometry (LC-MS) offers simultaneous separation and identification of afoxolaner enantiomers with high sensitivity and specificity. The versatility, robustness, and well-established validation protocols of chiral HPLC make it an indispensable tool in pharmaceutical research and quality control, ensuring accurate determination of afoxolaner enantiomers and supporting drug development processes.[10-12]

**References:**

1. Toyo'oka, T.; Resolution of chiral drugs by liquid chromatography based upon diastereomer formation with chiral derivatization reagents; Journal of Biochemical and Biophysical Methods, (2002); 54: 25–56
2. Neue, U.D.; HPLC columns: theory, technology and practices. Wiley-VCH, New York, NY, (1997).
3. Lu, J., Rustum, A.M.; Separation of the two enantiomers of T-3811ME by normal-phase HPLC using modified amylose as chiral stationary phase; Journal of Chromatographic Science, (2008); 46: 466–471.
4. Rustum, A.M.; Separation of the R(−)- and S(+)-enantiomers of the ethyl ester of tiagabine HCl using a Chiralcel-OG column; Journal of Chromatography. A, (1994); 684: 29–36.
5. Rustum, A.M., Estrada, V.; Separation and quantitation of the S-(+)-enantiomer in the bulk drug tiagabine·HCl by chiral high-performance-liquid chromatography using a Chiralcel-OD column; Journal of Chromatography B: Biomedical Sciences and Applications, (1998); 705: 111–117.
6. Zheng, C., Huang, W.X., Chen, Z., Rustum, A.M.; Separation of chiral primary amino compounds by forming a sandwiched complex in reversed-phase high performance liquid chromatography; Journal of Chromatography A, (2010); 1217: 4965–4970.
7. Okamoto, Y., Yashima, E.; Polysaccharide derivatives for chromatographic separation of enantiomers; Angewandte Chemie International Edition, (1998); 37: 1020–1043.
8. Perrin, C., Vu, V.A., Matthijs, N., Maftouh, M., Massart, D.L., Heyden, Y.V.; Screening approach for chiral separation of pharmaceuticals: Part I. Normal-phase liquid chromatography; Journal of Chromatography A, (2002); 947: 69–83.
9. Tachibana, K., Ohnishi, A.; Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases; Journal of Chromatography A, (2001); 906: 127–154.
10. Stringham, R.W., Ye, Y.K.; Chiral separation of amines by high-performance liquid chromatography using polysaccharide stationary phases and acidic additives; Journal of Chromatography A, (2006); 1101: 86–93.

11. Zhuang J, Kumar S, Rustum A. Development and Validation of a Normal Phase Chiral HPLC Method for Analysis of Afoxolaner Using a Chiralpak® AD-3 Column. J Chromatogr Sci. 2016 Nov;54(10):1813-1819.
12. Padivitage N, Kumar S, Rustum A. Development and Validation of a Reversed-Phase Chiral HPLC Method to Determine the Chiral Purity of Bulk Batches of (*S*)-Enantiomer in Afoxolaner. J AOAC Int. 2017 Jan 1;100(1):65-73.