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Review article

Validation of a Normal Phase Chiral HPLC Method for Analysis of Afoxolaner

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Abstract:

Chiral HPLC (High Performance Liquid Chromatography) is a technique used to separate enantiomers of chiral compounds. Enantiomers are mirror-image molecules that have the same physical and chemical properties but differ in their three-dimensional orientation. Chiral compounds have at least one asymmetric center, which means they exist in two different forms that are non-superimposable mirror images of each other. These two forms are called enantiomers and they can have different biological activities, pharmacokinetics, and toxicities. Therefore, it is essential to separate and analyze them separately. The analysis of afoxolaner typically involves the use of a chiral HPLC method, which can separate the two enantiomers of afoxolaner based on their interactions with a chiral stationary phase. The development and validation of a chiral HPLC method for the analysis of afoxolaner are important to ensure accurate and reliable data on the enantiomeric composition of afoxolaner in various sample matrices. The validated method can be used for quality control, pharmacokinetic studies, and other applications related to afoxolaner.

Keywords: Afoxolaner , High Performance Liquid Chromatography, chiral compounds

Introduction:

Chiral HPLC (High Performance Liquid Chromatography) is a technique used to separate enantiomers of chiral compounds. Enantiomers are mirror-image molecules that have the same physical and chemical properties but differ in their three-dimensional orientation. Chiral compounds have at least one asymmetric center, which means they exist in two different forms that are non-superimposable mirror images of each other. These two forms are called enantiomers and they can have different biological activities, pharmacokinetics, and toxicities. Therefore, it is essential to separate and analyze them separately.[1] Chiral HPLC involves the use of a chiral stationary phase (CSP) column, which contains a chiral selector that interacts differently with the two enantiomers. The mobile phase used in chiral HPLC is typically a mixture of solvents, and the flow rate is carefully controlled to optimize separation.[2]

The validation of a chiral HPLC method involves the development and optimization of the HPLC method for the separation of the enantiomers, as well as the testing of its selectivity, sensitivity, linearity,

accuracy, precision, and robustness. A validated chiral HPLC method can be used for the analysis of chiral compounds in various sample matrices, including pharmaceuticals, food, and environmental samples.[3]

The aim of this study is to review validation of a normal phase chiral HPLC method for the analysis of afoxolaner, a chiral compound widely used as an insecticide and acaricide in veterinary medicine. As a chiral compound, the analysis of afoxolaner requires the use of a chiral HPLC method. In this study, we aim to validate the developed method for the analysis of afoxolaner in various sample matrices such as animal tissues, blood, and urine.[4]

Validation process: [5]

The validation of the method will involve testing the linearity, limit of detection, limit of quantitation, precision, accuracy, specificity, and robustness of the method. The results of this study will provide reliable and accurate data for the analysis of afoxolaner, and will contribute to the development of better analytical methods for chiral compounds.[6]

Afoxolaner and validation: [7]

Afoxolaner is a chiral compound that is widely used as an insecticide and acaricide in veterinary medicine. It is a member of the isoxazoline family of compounds and is effective against a variety of ectoparasites, including fleas and ticks. Afoxolaner works by inhibiting the neurotransmitter gamma-aminobutyric acid (GABA) and glutamate-gated chloride channels in the nervous system of the parasites, leading to paralysis and death.

Afoxolaner is a chiral compound with two enantiomers, which means it exists in two forms that are non-superimposable mirror images of each other. The two enantiomers of afoxolaner have different biological activities, with the (+)-enantiomer being more active than the (-)-enantiomer. Therefore, it is important to separate and analyze the enantiomers separately in order to understand their pharmacokinetics and toxicities.

The analysis of afoxolaner typically involves the use of a chiral HPLC method, which can separate the two enantiomers of afoxolaner based on their interactions with a chiral stationary phase. The development and validation of a chiral HPLC method for the analysis of afoxolaner is important to ensure accurate and reliable data on the enantiomeric composition of afoxolaner in various sample matrices.

Afoxolaner is a chiral compound that is commonly used as an insecticide and acaricide in veterinary medicine. As it is a chiral compound, it is important to use a chiral HPLC method for its analysis. Here are the steps for validating a normal phase chiral HPLC method for analysis of afoxolaner:[8]

1. Method development: The first step is to develop a normal phase chiral HPLC method for analysis of afoxolaner. This can be done by testing various chiral columns, mobile phases, and detection wavelengths. Once a suitable method has been developed, it is important to optimize the method parameters for resolution, sensitivity, and reproducibility.
2. Linearity: To test the linearity of the method, a series of standard solutions of afoxolaner should be prepared at different concentrations, and their peak areas should be measured. The peak areas should be plotted against the concentrations to generate a calibration curve. The linearity of the method can be assessed by determining the correlation coefficient (r^2) of the calibration curve.
3. Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ of the method can be determined by preparing a series of standard solutions of afoxolaner at concentrations below and above the expected levels of the compound in the sample matrix, respectively. The solutions should be

analyzed by the method, and the LOD and LOQ should be determined based on the signal-to-noise ratio.

4. Precision: The precision of the method can be determined by analyzing replicate injections of afoxolaner standard solutions at different concentrations. The %RSD (relative standard deviation) of the peak areas should be calculated to assess the intra-day and inter-day precision.
5. Accuracy: The accuracy of the method can be determined by analyzing spiked samples of the matrix with known amounts of afoxolaner at different concentrations. The recovery of afoxolaner should be determined by comparing the measured amounts to the known amounts.
6. Specificity: The specificity of the method can be determined by analyzing the sample matrix without afoxolaner and with other potentially interfering compounds. The retention time and peak shape of afoxolaner should be compared to those of the interfering compounds.
7. Robustness: The robustness of the method can be determined by testing the method under different conditions, such as changes in column temperature, mobile phase composition, and flow rate. The effect of these changes on the resolution and peak area of afoxolaner should be evaluated.

Once the method has been validated, it can be used for the analysis of afoxolaner in various sample matrices, such as animal tissues, blood, and urine. It is important to follow the validated method to ensure accurate and reliable results.

Validation of afoxolaner significance : [9]

The validation of a chiral HPLC method for the analysis of afoxolaner is significant because it ensures that the analytical method is accurate, precise, and reliable for the separation and quantification of the two enantiomers of afoxolaner.

Afoxolaner is a chiral compound, meaning it exists in two non-superimposable mirror-image forms that have different biological activities and pharmacokinetic properties. The accurate determination of the enantiomeric composition of afoxolaner is crucial for understanding its biological activity, toxicology, and pharmacokinetics. Inaccurate determination of the enantiomeric composition of afoxolaner can result in incorrect dosage recommendations, ineffective treatments, or potential toxicities.

Therefore, the validation of a chiral HPLC method for the analysis of afoxolaner is essential to ensure that the method is accurate and reliable for the analysis of afoxolaner in various sample matrices, including animal tissues, blood, and urine. The validation process includes testing the linearity, limit of detection, limit of quantitation, precision, accuracy, specificity, and robustness of the method, which ensures that the method is capable of providing accurate and reliable results for the determination of the enantiomeric composition of afoxolaner.

Analysis of Afoxolaner :

The analysis of afoxolaner typically involves the use of a chiral HPLC method, which can separate the two enantiomers of afoxolaner based on their interactions with a chiral stationary phase. The HPLC method typically involves the following steps:[1,4,5,610]

1. Sample Preparation: The sample, which can be animal tissues, blood, urine, or other matrices, is prepared by extraction or other sample preparation techniques.

2. Chromatographic Conditions: The chiral HPLC method utilizes a chiral stationary phase (CSP) column and a mobile phase consisting of a mixture of solvents. The chromatographic conditions are optimized to achieve optimal separation of the two enantiomers of afoxolaner.
3. Calibration Standards: Calibration standards containing known concentrations of the two enantiomers of afoxolaner are prepared and analyzed using the chiral HPLC method. Calibration curves are generated to determine the limit of detection, limit of quantitation, and linearity of the method.
4. Analysis of Samples: The prepared samples are injected into the chiral HPLC system, and the two enantiomers of afoxolaner are separated and quantified based on their retention times and peak areas.
5. Data Analysis: The results are analyzed using appropriate statistical methods to determine the enantiomeric composition of afoxolaner in the samples.

Conclusion:

The development and validation of a chiral HPLC method for the analysis of afoxolaner are important to ensure accurate and reliable data on the enantiomeric composition of afoxolaner in various sample matrices. The validated method can be used for quality control, pharmacokinetic studies, and other applications related to afoxolaner.

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