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Review article:

Development of a Normal Phase Chiral HPLC Method for Analysis of Afoxolaner: Review

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Abstract:

Afoxolaner is a chiral drug that is commonly used for the treatment and prevention of flea and tick infestations in dogs and cats. The two enantiomers of afoxolaner may have different pharmacokinetic and pharmacodynamic properties, making it important to develop an analytical method for the analysis of the individual enantiomers. Normal phase chiral HPLC is a commonly used method for the separation of chiral compounds, and in this case, the amylose tris(3,5-dimethylphenylcarbamate) (ADMC) column has been selected as the chiral stationary phase (CSP). The mobile phase for normal phase chiral HPLC typically consists of a non-polar solvent and a polar modifier, and the specific conditions for the separation of afoxolaner enantiomers will be discussed in the following sections. The development of a normal phase chiral HPLC method for the analysis of afoxolaner enantiomers is crucial for understanding the pharmacokinetics and pharmacodynamics of this important drug.

Keywords: Chiral HPLC Method , Afoxolaner , pharmacokinetics

Introduction:

Afoxolaner is a chiral drug that is commonly used for the treatment and prevention of flea and tick infestations in dogs and cats.[1] The two enantiomers of afoxolaner may have different pharmacokinetic and pharmacodynamic properties, making it important to develop an analytical method for the analysis of the individual enantiomers. Normal phase chiral HPLC is a commonly used method for the separation of chiral compounds, and in this case, the amylose tris(3,5-dimethylphenylcarbamate) (ADMC) column has been selected as the chiral stationary phase (CSP). The mobile phase for normal phase chiral HPLC typically consists of a non-polar solvent and a polar modifier, and the specific conditions for the separation of afoxolaner enantiomers will be discussed in the following sections. The development of a normal phase chiral HPLC method for the analysis of afoxolaner enantiomers is crucial for understanding the pharmacokinetics and pharmacodynamics of this important drug.[2]

To develop a normal phase chiral HPLC method for the analysis of Afoxolaner, the following steps can be followed:[3]

Selection of Chiral Stationary Phase (CSP):

The selection of an appropriate Chiral Stationary Phase (CSP) is a critical step in the development of a normal phase chiral HPLC method for the analysis of Afoxolaner. Cellulose-based, amylose-based, and cyclodextrin-based CSPs are commonly used in normal phase chiral HPLC. Among these, cellulose-based CSPs, such as Chiralpak IC, Chiralcel OD, and Chiralcel OJ, are widely used for the separation of a wide range of chiral compounds. Amylose-based CSPs, such as Chiralpak AD and Chiralpak AS, are also commonly used for the separation of chiral compounds with a high degree of enantiomeric selectivity. Cyclodextrin-based CSPs, such as Chiralpak IB, are used for the separation of chiral compounds with moderate selectivity.

The selection of the CSP should be based on its selectivity and resolving power towards Afoxolaner. It is recommended to evaluate several CSPs to determine the optimal separation conditions for Afoxolaner. The choice of CSP can also be influenced by the nature of the sample matrix and the intended use of the analytical method. Selection of mobile phase: A suitable mobile phase consisting of an organic solvent and a polar modifier can be selected. The choice of mobile phase depends on the properties of the CSP and the compound being analyzed. Commonly used organic solvents are hexane, heptane, and isopropanol, and polar modifiers include methanol, ethanol, and acetonitrile. Optimization of chromatographic conditions: The conditions of the HPLC run, such as flow rate, column temperature, and injection volume, can be optimized to obtain the best separation and resolution of the enantiomers.

Preparation of sample: [4]

The preparation of the sample is a critical step in the development of a normal phase chiral HPLC method for the analysis of Afoxolaner. The following steps can be followed for the preparation of the sample:

1. Selection of solvent: A suitable solvent for Afoxolaner can be selected based on its solubility and compatibility with the mobile phase. The solvent should be miscible with the mobile phase and not interfere with the separation of the enantiomers. Commonly used solvents for normal phase chiral HPLC include hexane, heptane, and isooctane.
2. Weighing and dissolving the sample: Accurately weigh the required amount of Afoxolaner and dissolve it in the selected solvent. The concentration of the sample solution will depend on the sensitivity of the detector and the expected concentration range of the analyte in the sample. Typically, a concentration range of 0.1 to 1.0 mg/mL is used for chiral HPLC analysis.
3. Filtering the sample: The prepared sample solution can be filtered through a 0.45 µm filter to remove any particulate matter or impurities that can interfere with the HPLC analysis.
4. Storage of the sample: The prepared sample solution can be stored in a clean, dry container and protected from light until it is ready for injection. It is recommended to prepare the sample solution immediately before analysis to minimize any potential degradation or evaporation of the sample.

Overall, the sample preparation should be optimized to ensure the accuracy and precision of the HPLC analysis of Afoxolaner. Care should be taken to avoid any potential contamination of the sample or introduction of impurities during the preparation process.

Method validation: [5,6,7]

Method validation is an essential step in the development of a normal phase chiral HPLC method for the analysis of Afoxolaner. The validation process ensures that the method is reliable, accurate, and reproducible for the intended use. The following parameters should be evaluated during method validation:

1. Specificity: The ability of the method to detect and quantify Afoxolaner in the presence of potential impurities or matrix components should be evaluated.
2. Linearity: The linearity of the calibration curve over a range of concentrations should be determined. This allows for the determination of the limit of quantification (LOQ) and the limit of detection (LOD) of the method.
3. Accuracy: The accuracy of the method should be determined by spiking known concentrations of Afoxolaner into a matrix and comparing the measured concentrations to the expected values.
4. Precision: The precision of the method should be evaluated by measuring the repeatability and intermediate precision of the method. Repeatability is the variation in results obtained by the same analyst using the same equipment and methodology. Intermediate precision is the variation in results obtained by different analysts or instruments under different conditions.
5. Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ of the method should be determined based on the signal-to-noise ratio of the analyte.
6. Robustness: The robustness of the method should be evaluated by introducing small variations in the method conditions to determine their impact on the method performance.
7. System suitability: The suitability of the HPLC system for the intended use should be demonstrated by evaluating parameters such as retention time, resolution, and peak shape.

Overall, method validation should be performed according to established guidelines, such as those provided by the International Conference on Harmonisation (ICH), to ensure that the method is suitable for its intended use. The validation data should be reported in a clear and concise manner, and the limitations and assumptions of the method should be discussed.

Data analysis:[8,9]

Data analysis is a critical step in the development of a normal phase chiral HPLC method for the analysis of Afoxolaner. The following steps can be followed for data analysis:

1. Calculation of retention times: The retention times of the Afoxolaner enantiomers and any potential impurities should be calculated based on the HPLC chromatogram.
2. Calculation of resolution: The resolution between the Afoxolaner enantiomers should be calculated as the difference between their retention times divided by the average of their peak widths at half-height.
3. Calibration curve: A calibration curve should be constructed by plotting the peak area or peak height of Afoxolaner enantiomers against their respective concentrations. The curve should be linear, and the correlation coefficient should be calculated.
4. Sample quantification: The concentration of Afoxolaner enantiomers in the sample can be determined by comparing their peak areas or peak heights to the calibration curve.

5. Statistical analysis: Statistical analysis can be performed to evaluate the precision and accuracy of the method. The coefficient of variation (CV) can be calculated to determine the precision of the method, and the percentage recovery can be calculated to determine the accuracy of the method.
6. Data interpretation: The data obtained from the HPLC analysis should be interpreted in the context of the sample matrix and the intended use of the method. The results should be reported with appropriate units and significant figures.

Overall, the data analysis should be conducted according to established guidelines and best practices to ensure that the results are reliable, accurate, and reproducible. Any potential sources of error or variability should be identified and addressed to minimize their impact on the results.

Reporting: [11]

The results can be reported in a suitable format, such as a chromatogram or a table, along with the method conditions and validation parameters. Overall, the development of a normal phase chiral HPLC method for the analysis of Afoxolaner requires careful optimization and validation to ensure accurate and reliable results.

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