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Development and Validation of RP-HPLC Method of Analysis for Assay of Spironolactone

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ABSTRACT: This research paper is about to determine the assay of spironolactone with the help of RP- HPLC method. The various parameters are considered for assay of spironolactone determination .The ICH published the guidlines such as accuracy, precision, range and many other. These research determine the specificity, accuracy, precision ruggedness, robustness method precision and many other parameters. These research method is useful in find out the assay of other drugs.

KEYWORDS: ICH, RP- HPLC , Assay, Spironolactone, accuracy, precision.

I. INTRODUCTION

The measurement science is a type analytical chemistry consisting a set of powerful ideas and methods that are useful in all fields of science and medicine. The example demonstrates that both qualitative information and quantitative information are required in an analysis. Qualitative analysis establishes a chemical identity of the species in the sample. Quantitative analysis determines the relative amounts of the sespecies, or analyte, in numerical terms. Quantitative analytical measurements also play a vital role in many research areas in chemistry, biochemistry, biology, geology, physics and the other sciences Analytical chemistry seeks ever improved means of measuring the chemical composition of natural and artificial materials. The methods of this science are used to detect the substances which may be present in a material and to determine the exact quantity of the identified substance. Analytical chemists work to progress the reliability of existing methods to meet the demands for improved chemical measurements which arise constantly in our society. They adapt proven methodologies to new types of materials or to response new questions about their arrangement and their reactivity mechanism. They carry out the investigation to discover completely new ethics of measurement and are at the forefront of the use or major discoveries such as lasers and micro cheap devices for practical purposes

Chromatography is critical a group of techniques for the separation of the mixes of mixtures by their persistent distribution between two phases, one of which is moving past the other. The systems associated with these definitions are:

Adsorption chromatography
Partition Chromatography
Ion exchange chromatography
An inert gel which acts as a molecular sieve, and a liquid mobile phase (gel chromatography)

The basis of the separation of the components of a mixture may be defined in terms of one of these four modes of separation, or buy a combination.

Advances in technology since the first simple applications of chromatography were recorded have resulted in a wide range of techniques varying in com ability, sensitivity and cost. The modern instrumental techniques of gas-liquid chromatography and high performance liquid chromatography provides excellent separation and allow the accurate assay of very low concentrations of a wide variety of substances in complex mixtures. The older inexpensive chromatography techniques, such as column chromatography are used in analytical and preparative separations which do not require the resolution and sensitivity or justify the expense of the instrumental techniques.

METHOD

HPLC METHOD:
HPLC Method Development and its
Validation of Spironolactone in tablet dosage form

Preparation of mobile phase:

Mix Methanol and Water in the ratio of 700:300(v/v), mixed well an degas by sonication for 10 minutes.

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Methodology:
Assay (By HPLC method):
Reagents:
Acetonitrile: HPLC grade
Methanol: HPLC grade
Water: HPLC grade/ Purified water

Chromatographic parameters:
Use suitable High Performance Liquid Chromatograph equipped with following:

Column	:	ZorbaxSBC8,5µ 4.6X 150 mm
Flow rate	:	1.5 mL/min
Wavelength	:	230 nm
Injection volume	:	20 µL
Column oven temperature	:	30ºC
Sample compartment temperature	:	25ºC
Run time	:	10 minutes

Preparation of Diluent:
Mix Acetonitrile and Water in the ratio of 600:400(v/v).

Preparation of Blank solution:
Use diluent as a blank.

Preparation of standard solution:
Weigh and transfer accurately about 50mg of Spironolactone working standard into a 100mL volumetric flask, add 70mL of diluent, sonicate to dissolve and dilute upto the volume with diluent. Further, transfer 5.0mL of this solution into a 50mL volumetric flask and dilute up to the volume with diluent.

Preparation of sample solution for 100 mg:
Weigh and transfer 5 tablets into a 500mL volumetric flask, add about 350mL of diluent and shake mechanically for 20minutes and sonicate for 20minutes with intermediate shaking and make up to the volume with the diluent. Centrifuge this solution at 5000RPM for 10 minutes to get the clear solution.
Further transfer 5.0mL of this clear solution into a 100mL volumetric flask and make up to the volume with the diluent.

Preparation of sample solution for 25 mg:
Weigh and transfer 10 tablets into a250mLvolumetricflask, add about 170mLof diluent and shake mechanically for 20minutes and sonicate for 20minutes with intermediate shaking and make up to the volume with the diluent. Centrifuge this solution at 5000RPM for 10minutes to get the clear solution.
Further, transfer 5.0mL of this clear solution into a 100mL volumetric flask and make up to the volume with the diluent.
Procedure: Separately inject 10 µL of blank, standard solution and sample solution into the chromatographic system. Record the chromatograms and measure the peak area count for Spironolactone peak.

EXPERIMENTATION
In order to validate the HPLC method for the assay of Spironolactone in Spironolactone tablets USP by considering following parameters.
Specificity
By Interference
By Forced degradation
Linearity
Precision
System precision
Method precision
Intermediate precision (Ruggedness)
Accuracy
Range
Solution stability
Robustness
Filter validation

Specificity:
To ensure the absence of interference from blank and placebo those are likely to be present in Spironolactone drug product.
Analyze blank, standard solution(five replicate injections),worst case placebo (duplicate preparation), test solution and test solution spiked with impurities:

Preparation of solutions:
Prepare the blank solution, diluent, mobile phase, standard solution, test solution as per the proposed method of analysis

Preparation of worst case placebo solution:
Weigh and transfer worst case placebo equivalent to 500mg of Spironolactone into a 500ml. volumetric flask, add about 350ml. of diluent and shake mechanically for 20minutes and

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sonicate for 20minutes with intermediate shaking and make up to the volume with the diluent. Centrifuge this solution at 5000RPM for 10minutes to get the clear solution. Further transfer 5.0mL of this clear solution into a 100ml. volumetric flask and make up to the volume with the diluent.

Preparation of canrenone stock solution:

Weigh and transfer accurately about 2mg of canrenone impurity into a 50mL volumetric flask. add 1 mL of Tetrahydrofuran, sonicate to dissolve and dilute up to the volume with diluent.

Preparation of 7-Beta Spironolactone stock solution:

Weigh and transfer accurately about 2mg of 7-Beta Spironolactone impurity into 50mLvolumetric flask, add mL of Tetrahydrofuran, sonicate to dissolve and dilute up to the volume with diluent.

Preparation of test solution spiked with impurities at 1%:

Weigh and transfer 5 tablets into a 500ml. volumetric flask, add about 350mL of diluent and shake mechanically for 20minutes and sonicate for 20 minutes with intermediate shaking and make up to the volume with the diluent. Centrifuge this solution at 5000RPM for 10minutes to get the clear solution.

Linearity:

Evaluate the linearity from 50% to 150%. The working level for Spironolactone is 50µg/mL with respect to standard solution.

Preparation of solutions:

Prepare the blank solution (diluent), mobile phase, standard solution as per the test method .

Preparation of linearity stock solution:

Weigh and transfer accurately about 50mg of Spironolactone working standard into a 100mL volumetric flask, add 70mL of diluent, sonicate to dissolve and dilute up to the volume with diluent.

Range:

For range data shall be considered from linearity and accuracy.

Acceptance criteria:

1. **Linearity:** Correlation coefficient 'r' should not be less than 0.999. Bias at 100% response should be between + 2.0%.
2. **Accuracy**: Individual % recovery of Spironolactone at each level should be between 98.0 and 102.0.

Robustness:

Determine the robustness by making deliberate alterations to the chromatographic conditions by minor variation and check the effect of the same on system suitability criteria. Prepare three sample solutions as per proposed method of analysis mentioned under section 6.3 and analyze under the following conditions.

By changing the flow rate by ±10%.

By changing the column oven temperature by +5 °C.

By changing the organic content by ±5% absolute.

II. CONCLUSION

All the parameters are determine and recorded

The % purity of spironolactone is find out with the Help of RP- HPLC method and all parameters are recorded.

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