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Analytical method development and validation for the simultaneous estimation of doxepin by using rp-hplc technique

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ABSTRACT

A simple and selective LC method is described for the determination of Doxepin dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Triethyl amine: ACN (60:40v/v/v), with detection of 250 nm. Linearity was observed in the range 75-150 µg /ml for Doxepin(r^2 =0.999) for drugs estimated by the proposed methods was in good agreement with the label claim quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

Keywords: Doxepin, Linearity, Quality control, Clinical Pharmacokintic

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals and manufactured exclusively in accordance with the formulae mentioned in authoritative books.[1] Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification, and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis– Qualitative and Quantitative analysis. Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in each sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc.

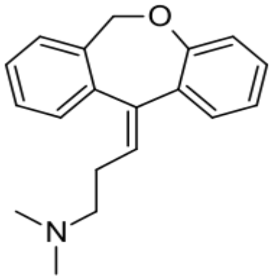
DRUG PROFILE

DOXEPIN

Doxepin hydrochloride is a dibenzoxepin-derivative tricyclic antidepressant (TCA). TCAs are structurally similar to phenothiazines. They contain a tricyclic ring system with an alkyl amine substituent on the central ring.

Category:
Adrenergic Uptake Inhibitors

Structure:



IUPAC Name:
dimethyl(3-{9-oxatricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,11,13-hexaen-2-ylidene}propyl)amine.
Chemical formula: $C_{19}H_{21}NO$
Molecular weight: 279.3761

AIM

To develop new RP HPLC method for the estimation of Doxepin in pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSILcolumn,C18(150x4.6 ID) 5µm

Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen ortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade

Drug used

Doxepin	Gift Samples obtained from Chandra labs, Hyd.
label claims	Obtained from local pharmacy

METHOD DEVELOPMENT AND VALIDATION

Introduction to Method Development

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias.

Method Development Using HPLC

In method development, an attempt to select the best chromatographic conditions like the best column, the best mobile phase, the detection wavelength etc. to be used for routine analysis of any drug is done. For the method development by HPLC method some information about the sample is very essential.

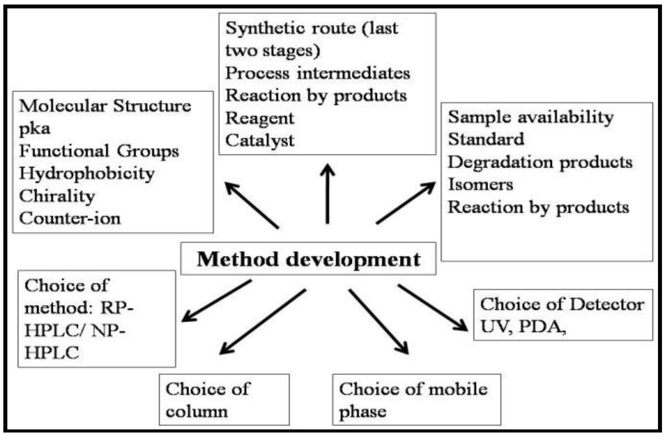


Fig 1: Outline of the process involved in method development

Method Validation (ICH Guidelines)

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be established across the specified range of the analytical procedure.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Specificity

Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix. Typically these might include impurities, degradants, matrix, etc

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. It is a limit test that specifies whether or not an analyte is above or below a certain value.

5.3.5 Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

5.3.6 Linearity and Range

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the (inclusive) interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method. The range is normally expressed in the same units as the test results obtained by the method.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated varying method parameters such as percent organic solvent, pH, ionic strength, or temperature and determining the effect (if any) on the results of the method.

System Suitability

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as a whole.

RESULTS AND DISCUSSION

Doxepin

It is slightly soluble in water. It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.

Preparation of standard stock solution of Doxepin

25mg of Doxepin was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.4ml to 10ml with methanol.

Method Development Of Doxepin

Preparation of mixed standard solution

Weigh accurately 25mg of Doxepin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 250µg/ml of Doxepin is prepared by diluting 2.5ml of Doxepin to 10ml with mobile phase. This solution is used for recording chromatogram. Efficiency of the peaks was more. Hence this method was optimized. The amount of Doxepin present in the taken dosage form was found to be 99.52%.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 25mg of Doxepin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of Doxepin is prepared by diluting 1ml of Doxepin to 10ml with mobile phase

Preparation of sample solution

Tablets (each tablet contains 100mg of Doxepin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 100µg/ml were prepared by dissolving weight equivalent to 100mg of Doxepin dissolved in sufficient mobile phase. It is observed from the above data, diluents or excipient peaks are not interfering with the Doxepin peaks.

Linearity and range

Preparation of mixed standard solution

Weigh accurately 50mg of Doxepin in 100 ml of volumetric flask and from this, 5ml dissolve in 10ml of mobile phase and make up the volume with mobile phase.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 75%, 100%, 125%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug. The % recovery of Doxepin should lie between 98% and 102%. The percentage mean recovery of Doxepin is 99.89%.

Method precision

Prepared sample preparations of Doxepin as per test method and injected 5 times in to the column. The % Relative standard deviation of Assay preparations of Doxepin should be not more than 2.0%. Test results for Doxepin are showing that the %RSD of Assay results are within limits. The results were shown in table 8.5.7.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision. The system suitability should pass as per the test method at variable conditions. From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%. From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

DISCUSSION

A simple and selective LC method is described for the determination of Doxepindosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Triethyl amine: ACN (60:40v/v/v), with detection of 250 nm. Linearity was observed in the range 75-150 µg /ml for Doxepin (r^2 =0.999) for drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Doxepin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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