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

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ABSTRACT

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. It can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Keywords: Atomoxetine, repeatability analysis, high resolution, retention time

INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable.

REVIEW OF LITERATURE

Gurmeet Chhabra, Chandraprakash Jain, Saurabh K Banerjee: A simple, reliable, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the determination of Atomoxetine HCl in pharmaceutical dosage form. Chromatographic separation was carried on RP-C8 column (Phenomenex, size: 250×4.60 mm, particle size 5µm) with a mobile phase composed of acetonitrile and 10 mM disodium hydrogen phosphate buffer with 0.1% TEA (pH 3.0, adjusted with OPA) (55:45, v/v) in isocratic mode at a flow rate of 1mL/min. The detection was monitored at 271nm.

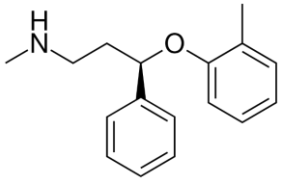
Zubaidur Rahman, Vijey Aanandhi M, Sumithra M: A simple, novel, sensitive, rapid high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for quantitative determination of atomoxetine HCl (ATH) in bulk and formulations. The chromatographic development was carried out on RP-HPLC. The column used as Xterra RP 18 (250 mm — 4.6 mm, particle size), with mobile phase consisting of methanol: water 80:20 V/V. The flow rate was 1.0 mL/min and the effluents were monitored at 270 nm. The retention time was found to be 5.350 min.

Structure:

DRUG PROFILE

Atomoxetine

Atomoxetine is a nonstimulant medication marketed in the form of the R (-) isomer as this structure seems to have approximately nine-fold more potency than the S (+) isomer. It is a phenylpropanolamine derivative that presents a similar structure to the tricyclic antidepressants.



IUPAC Name: (3*R*)-*N*-Methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine
Molecular Formula: $C_{17}H_{21}NO$
Molecular Weight: 255.3547

AIM

To develop and validate new HPLC method for Atomoxetine in pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Lab Solution
HPLC	SHIMADZU 2010
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Mettler Toledo
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(150x4.6mm) 4µm

Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen Phosphate	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen phosphate	AR Grade
Orthophosphoric acid	HPLC Grade

Drugs used

Atomoxetine (API)	Gift Samples obtained from Chandra labs, Hyd.
Strattera (60 mg)	Obtained from local pharmacy

Mobile Phase Preparation

Sodium Buffer

About 0.5g of 1-Heptane Sulphonic acid and 0.5g of Sodium chloride was weighed and transferred into 1000ml volumetric flask and add sufficient water for dissolving and make upto mark with water, sonicate for 15mins. Add 2.5ml of triethylamine and adjust the p^H to 3.8 using orthophosphoric acid and filter the solution through 0.45micron membrane filter.

Mixed Phosphate Buffer

Weigh 2.95g of Potassium Dihydrogen Ortho Phosphate and 0.5g of Dipotassium Hydrogen Ortho Phosphate into 1000ml volumetric flask and add sufficient water for dissolving, then make upto mark with water, sonicate for 15mins. Add 2.5ml of triethylamine and adjust the p^H to 3.5 using orthophosphoric acid and filter the solution through 0.45micron membrane filter.

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.

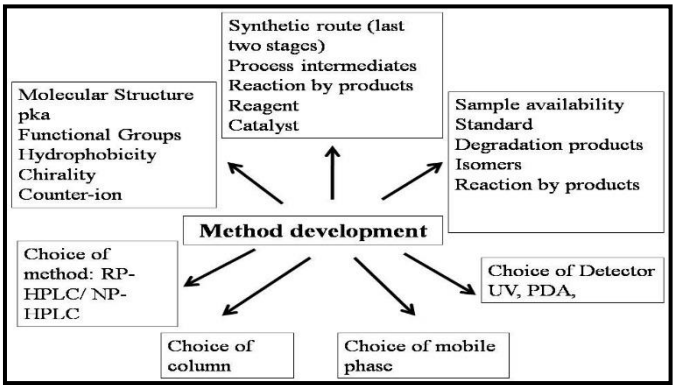


Fig 1: Outline of the process involved in method development

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. it is a measure of the degree of interference from such other things such as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to a single component only.

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.

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.

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.

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

Solubility Studies

These studies are carried out at 25 ^{0} C

Atomoxetine

Freely soluble in Methanol, Acetone and insoluble in Water

Determination of Working Wavelength (λmax)

Preparation of standard stock solution of Atomoxetine

10 mg of Atomoxetine 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 1ml to 10ml with Methanol.

Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drug in Methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against Methanol as blank. The resulting spectra are shown in the 8.3 and the absorption curve shows characteristic absorption maxima at 253 nm for Atomoxetine, selected as detector wavelength for the HPLC chromatographic method.

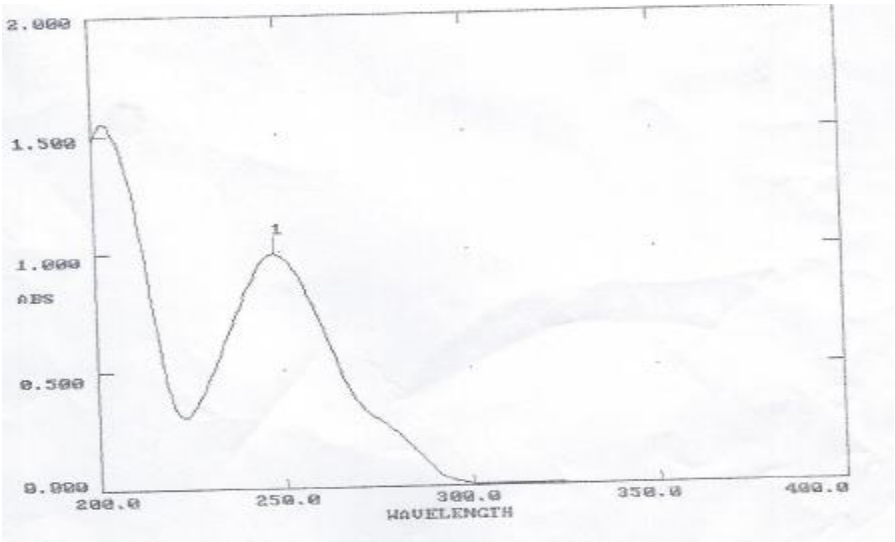


Fig 2: UV-VIS spectrum of Atomoxetine at 253nm

METHOD DEVELOPMENT OF ATOMOXETINE

Preparation of standard solution

weigh accurately 10 mg of Atomoxetine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Atomoxetine is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

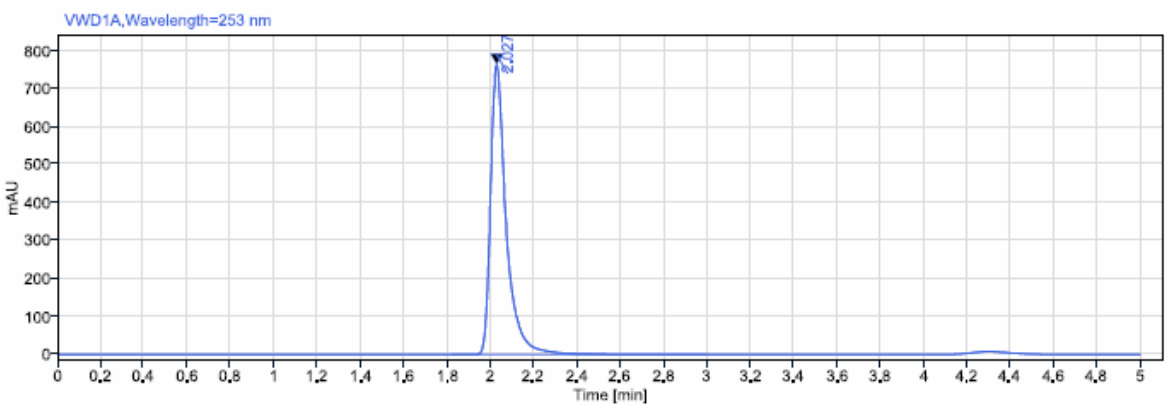


Fig 3: Chromatogram of Atomoxetine

Assay

Preparation of samples for Assay

Preparation of standard solution: weigh accurately 10 mg of Atomoxetine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Atomoxetine is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation: weigh accurately 10 Tablets (Atomoxetine -60 mg) weigh accurately 10 mg of Atomoxetine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Atomoxetine is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

VALIDATION

HPLC METHOD VALIDATION

System Suitability& System precision

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 20µg/mL of ATOMOXETINE was injected six times and the chromatograms were recorded for the same.

Method precision

Method precision was determined by injecting sample solutions of concentration ATOMOXETINE (20µg/mL) for six timesare prepared separately. The %RSD of determinations of ATOMOXETINE found to be within the acceptance criteria (less than 2.0%). %Assay Also within the limits (95.0 to 105.0) hence method is precise.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of ATOMOXETINE were prepared by dissolving 100 mg of ATOMOXETINE in 100 mL of Diluent. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min.

The relationship between the concentration (in %) of ATOMOXETINE and area of ATOMOXETINE should be linear in the specified range and the correlation should not be less than 0.99. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparation 0.990.

Specificity

A study to establish & determine the interference of blank and placebo as conducted. Analysis was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of blank and placebo solutions had shown no peaks at the retention times of ATOMOXETINE

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (reanalyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table 7.28 & 7.29.

Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters.

Table 9.7: Results for Robustness of ATOMOXETINE

Chromatographic changes		Rt(min)	Tailing Factor	Theoretical Plates	%RSD for Standard
Flow rate (mL/min)	0.8	2.429	1.74	5550	0.028
	1.2	1.738	1.28	2042	0.036
Temperature (°C)	35	2.031	1.53	3852	0.04
	45	2.031	1.59	4436	0.33

The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

Intermediate Precision (Ruggedness)

Intermediate precision (also called within-laboratory or within-device in different days, different analysts) is a measure of precision under a defined set of conditions: same measurement procedure, same measuring system, same location, and replicate measurements on the same or similar objects over an extended period.

DISCUSSION

A simple and selective HPLC method is described for the determination of Atomoxetine Chromatographic separation was achieved on a C18 column using mobile phase consisting of a mixture of 40 volumes of Methanol, 40 volumes of Acetonitrile and 20 volumes of Water with detection of 253 nm. Linearity was observed in the range 50-150 µg /ml for Atomoxetine (r^2 =0.990) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Atomoxetine 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 meant in industries, approved testing laboratories studies in near future.

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