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# Development and Validation of RP-HPLC Method for Estimation of Indoramin in Pharmaceutical Dosage Form

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**ABSTRACT**
RP-HPLC technique is a simple, Precised, Accurate method for quantitative analysis of API and pharmaceutical dosage form. High Performance Liquid Chromatography which is also known as High Pressure Liquid Chromatography. This technique involves two phases stationary and mobile phases. The separation of constituents is based on the difference between partition coefficients of the two phases.The interaction of each sample component will be varies and this causes difference in flow rates of each component and finally leads to separation of components. This review mainly focuses on the HPLC technique, its principle, types, instrumentation, applications and method development and validation of method.

In the current study, A simple, precise and economic UV and stability indicating RP- HPLC method was developed and validated for estimation of Indoramin in tablet dosage form. Inthe current study, this approach was used to estimate the Indoramin tablet formulation.The method shows good reproducibility; moreover the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The findings in the table show that the RP-HPLC technology may be used to accurately estimate the above-mentioned medicines in theirformulatio

**Keywords :-** forced degredation ,Indoramin.

## I. INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs.Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data.Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities

.The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.

Pharmaceutical Analysis is used to determining the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more of this component. For analyzing the drug samples in bulk, pharmaceutical formulations and biological fluids, different analytical methods are routinely being used. In non-instrumental, the conventional and physicochemical property are use to analyze the sample.

The instrumental methods of analysis are based upon the measurements of some physical property of substance using instrument to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical methods. Therefore, analytical methods developed using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC have wide applications in assuring the quality and quantity of raw materials and finished products.

### Chromatography

Chromatography (Chroma means ‗colour' and graphein means to ‗write') is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a

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stationary phase, (2-4) which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases.
Differences in compounds partition coefficient results in differential retention on the stationary phase and thus changing the separation.

**High Performance Liquid Chromatography (Hplc)**

Liquid chromatography is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the time of the solutes through a column. During 1970‘s, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography and thin layer chromatography (TLC). However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds

**Reversed Phase - High Performance Liquid Chromatography (RP-HPLC)**

As opposed to NP-HPLC, RP-HPLC employs mainly dispersive forces (hydrophobic or vanderwal‘s interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. RP-HPLC is by far the most popular mode of chromatography. Almost 90% of all analyses of low-molecular-weight samples are carried out using RP-HPLC. Dispersive forces employed in this separation mode are the weakest intermolecular forces, thereby making the overall background interaction energy in the chromatographic system very low compared to other separation techniques.

This low background energy allows for distinguishing very small differences in molecular interactions of closely related analytes. Adsorbents employed in this mode of chromatography are porous rigid materials with hydrophobic surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous silica.

Indoramin is an alpha-1 adrenergic receptor antagonists blocking agent shown to be capable of reducing blood pressure in humans. It is postsynaptic Adrenergic antagonist and same time an antagonist of histamine H1 and 5HT receptor as well.It is used to treat hypertension.it is also used in prostatic hypertrophy, Raynauds phenomenon etc Blood pressure lowering effect results from relaxation of peripheral article as a consequence of a blockade of postsynaptic alpha-1 adrenoreceptar Intrestingly unlike some other alpha blocker , lowering of blood pressure by Indoramin is rarely associated with reflux tachycardia.

Indoramin is a postsynaptic selective alpha 1-adrenoceptor antagonist used in the treatment of hypertension. In contrast to some other alpha-blockers, animal studies suggest that its blood pressure lowering effect results from relaxation of peripheral arterioles as a consequence of blockade of postsynaptic alpha 1-adrenoceptors. Further more, unlike some other alpha-blockers, this lowering of blood pressure is rarely associated with reflex tachycardia or postural hypotension.

Therapeutic trials have shown indoramin to be effective in lowering blood pressure in all grades of hypertension: mild and moderate hypertension when used alone, but generally in combination with a thiazide diuretic, and in moderate to moderately severe hypertension when used in combination with a beta-blocker and diuretic. In a few small comparative studies, no significant difference was found in the blood pressure lowering effects between indoramin and methyldopa, propranolol and prazosin. Side effects were similar for indoramin, propranolol and methyldopa; however in the 1 comparative study with prazosin, prazosin produced a lower incidence of sedation.

Indeed, the most common side effect with indoramin therapy has been sedation of a mild to moderate and/or transient nature, reported in about 19% of cases. Other side effects which have sometimes led to a withdrawal of indoramin treatment have been dry mouth, dizziness, and in males, failure of ejaculation; however, side effects may be reduced by starting therapy with smaller doses and titrating more gradually.

**Drug Profile**

- Indoramin
- Structure :-
- Chemical name :- N-{1-[2-(1H-indol-3-yl)ethyl]piperidin-4-yl}benzamide
- Category : Anti hypertensive,Anti Adrenergic agent
- Molecular Formula : C22H25N3O

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- Solubility: slightly soluble in water ,sparingly soluble in alcohol,
- very slightly soluble in ether ,soluble in ethyl alcohol
- Description : A white or almost white powder. It exhibits polymorphism.

**Methods and materials:-**

**Method development:**

Analytical method development and validation play important roles in the discovery development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physicochemical properties (pKa, log P, solubility) and determining which mode of detection would be suitable for analysis (i.e., suitable wavelength in case of UV detection) .The majority of the analytical development effort goes into validating a stability indicating HPLC–method. The goal of the HPLC-method is to try & separate quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.

**Materials and Methods**

**Chemicals and Reagents**

Indoramin,Acetonitrile and Phosphoric acid 88%,. 0.45 µm Millipore syringe filters, Sodium dihydrogen phosphate, Hydrochloric Acid,Sodium Hydroxide,Peroxide Solution.

**Instruments**

HPLC, UV-Spectrophotometer,Analytical balance, Digital pH Meter

**Chromatographic Equipment and Conditions**

The optimized chromatographic conditions are as follows:

Column used for chromatographic separation was GL-Science, Inertsil ODS 3VC18, 5µ, 4.6 x 150mm using 5 trials. pH 3.2 Phosphate Buffer: Acetonitrile (70:30 v/v) was used asmobile phase. Water and Acetonitrile in the ratio of 80:20 v/v respectively was used as diluent. Flow rate was set to 1mL/min and injection volume to 10µl. Detection was carried out at 233 nm in UV detector at 300 C. Retention time was 3.5 min and run time was 12min.

**High performance reverse phase development and optimization of liquid**

chromatography method For method development trials, the standard solution of Indoramin was utilised to optimise the technique for determining Indoramin 20 mg. Systematic forced degradation studies were utilised to create degraded samples, which were then employed in method development experiments to optimise the method as stability indicator.

**Preparation of solution Preparation of Buffer solution:**

Weigh and transfer 1.19 g of sodium dihydrogen phosphate in 1000 mL volumetric flask. Add 700 mL water, sonicate to dissolve and dilute up to the mark with water. Mix well and adjust pH 3.2 ± 0.05 with Phosphoric acid. Filter through 0.45µ nylon membrane disc filter.

**Preparation of Mobile phase:**

Prepare mixture of Buffer pH 3.2 and Acetonitrile in the ratio of 70:30 v/v respectively, mix well.

**Preparation of Diluent**:

Preare mixture of water and Acetonitrile in the ratio of 80:20 v/v respectively, mix well.

**Preparation of Blank:**

Use diluent as blank.

**Preparation of Standard solution:**

Weighed and transferred accurately about 40 mg of Indoramin working standard into 100 mLclean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 15 minutesto dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 5.0 mL of this solution to 50 mL with diluent and mix well.

Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.(Concentration of Indoramin standard solution:40 ppm)

**Preparation of Sample solution:**

Weighed and transferred 5 Indoramin tablets in to 200 mL clean and dry volumetric flask. Added about 150 mL of diluent, sonicate for 30 minutes with intermittent shaking, at control room temperature and make up volume upto mark with diluent and mix. Further diluted above stock solution 4.0 mL of this solution to 50 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45µ

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membrane PVDF filter.Discard first 4.0 mL of filtrate and then collected the sample. (Concentration of Sample Solution: 40 ppm)

**Selection of Stationary phase:**

On the basis of reversed phase HPLC mode and number of carbon present inmolecule (analyte) stationary phase with C18 bonded phase i.e.Inertsil C18 (150 mmX 4.6 mm), 5µm was selected.

**Selection of Mobile Phase:**

The selection of mobile phase was done after assessing the solubility of drug in different solvent as well on the basis of literature survey and finally mixture of Buffer Solution pH 3.2 and Acetonitrile was selected as a mobile phase.

**Selection of Detector and Detection wavelength:**

UV-visible 2487 detector was selected, as it is reliable and easy to set at the correct wavelength and 233 nm wavelengths was selected as detection wavelength.

**Selection of oven temperature:**

An inclusion of column temperature (30°C) minimized day to day variation ofretention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

**Selection of Sample temperature:**

An inclusion of sample temperature (25°C) minimized day to day variation ofretention time due to fluctuations in the ambient temperature.

## II. CONCLUSION :-

This review describes the general technique of HPLC method development and validation of optimized method. The general approach for the method development for the separation of pharmaceutical compounds was discussed. The knowledge of the pKa, pH and solubility of the primary compound is of utmost importance prior to the HPLC method development. Knowledge of pH can help to discern the ionizable nature of the other impurities (i.e., synthetic byproducts, metabolites, degradation products, etc.) in the mixture. Selection of buffer and mobile phase composition (organic and pH) plays a dramatic role on the separation selectivity. Final optimization can be performed by changing the temperature, gradient slope, and flow rate as well as the type and concentration of mobile-phase modifiers. Optimized method is validated with various parameters (e.g. accuracy, precision, specificity, linearity, detection limit etc.) as per ICH guideline

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