A Novel Analytical Method Development by HPLC technique for Active Pharmaceutical Ingredients (API) in formulation.

ABSTRACT SUBMITED TO

Dr. BABASAHEB AMBEDKAR MARATHWADA UNIVERSITY
AURANGABAD - 431004, INDIA.

BY
VILAS RAMKRISHNA CHOUDHARI
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY

Under the guidance of

Dr. MILIND UBALE.
Head& Associate Professor
Department of Chemistry,
Vasantrao Naik Mahavidyalaya,
Aurangabad-431103(M.S)

Introduction:

Analytical method development and validation plays an important role in the discovery, development, and manufacture of pharmaceuticals. Quality control laboratories to ensure the identity, purity, potency, and performance of drug products use the official test methods that result from these processes.

The current trends in drug development emphasize high volume approaches to accelerate lead candidate generation and evaluation. The impact of these developments on the overall drugs development cycle has been significant in creating unprecedented opportunities for growth and. focus particularly in the field of analytical sciences.

An active pharmaceutical ingredient is any component that provides pharmacological activity, direct effect on the diagnosis, cure, mitigation, treatment, prevention of disease, and affect the structure or any function of the body of human beings or animals.

Importance of HPLC in Pharmaceutical drug Analysis

The ability to provide timely, accurate, and reliable data is the central role of analytical chemists and is especially true in the field of discovery, development, and manufacture of pharmaceuticals. Analytical data are used to screen potential drug candidates, aid in the development of drug synthesis, support formulation studies, monitor the stability of bulk pharmaceuticals and formulated products, and also to test final products for release. The quality of analytical data is a key factor in the success of a drug development program. The process of method development and validation has a direct impact on the quality of these data. Although a through validation cannot rule all potential problems, the process of method development and validation should address the most common ones. Examples of typical problems that can be minimized or avoided are synthesis of impurities that co elute with the analyte peak in an HPLC assay.

Aim of the proposed work

Pharmaceutical Industry is highly regulated Industry. It is under increased scrutiny from the Government regulatory authorities and Public Interest groups to curtail costs and consistently deliver safe and efficacious products to market. Quality of drug products has become the focus of both Industry and Regulatory Authorities. Physico-Chemical Attributes of drug substance and drug products are more closely monitored, both at the time of finished product release and throughout the shelf life of the product. Determining stability of drug substance and drug product under accelerated and real time stability study conditions is a mandatory requirement for registration of product. Very Sensitive and specific stability indicating analytical methods are required for analysis of stability study samples. The faster drug discovery and drug product development programs coupled with greater requirement from industry and regulatory authorities have resulted in increased pressures on pharmaceutical analyst to deliver accurate and precise analytical data in shortest possible time.

Keeping in view the needs of pharmaceutical industry modest attempt has been made to develop some new choral and reverse phase chromatographic methods of analysis for some important active pharmaceutical ingredients and formulations. The drugs related for the present study are enlisted as:

Zopiclone

Irbesartan

Piperaquine Phosphate

Meclizine hydrochloride

Divalproex sodium

S-Zopiclone

Zoledronic acid

Flucytosine

Clopidogrel Bisulfate

Artemether

Importance of Validation in Pharmaceutical drugs:

Validation is process to demonstrate that analytical method is suitable for its intended use. In the present study a method developed for analysis on HPLC, GC, XRD etc but due to the Validation procedure we can sure that this method is reproducible, precise and having ruggedness to anywhere we can trust on that method. At the time of submission of DMF and ANDA's FDA requires Validation of the method to show the adequacies of analytical method.

Specificity: The specificity of the method is evaluated by injecting which did not show any interference at the retention time of analyte. The specificity is also evaluated by spiking the known impurities to the analyte and it is quantified with standard it should match with the unspiked sample.

System Suitability: The system suitability is studied to determine the reproducibility if the chromatographic system and column performance inadequate' for the intended analytical application. In this we observe the peak area, RT, RSD, Theoretical plates, tailing factor and resolution.

Precision: The precision of an analytical procedure express the closeness of agreement between series of measurement obtained from multiple sample preparation of the same homogenous sample under the prescribed conditions.

Accuracy: The accuracy of an analytical procedure express the closeness of agreement between the values which is accepted either as a conventional true value and the value found. Accuracy of the method was studied by recovery experiment. The recovery of the analyte was obtained at three concentration level. At each concentration level three samples were prepared and their average recoveries were calculated it should within the limit.

Stability in analytical solution: Stability of solution is evaluated by keeping solution in the mobile phase and diluent and injecting the solution at periodic intervals.

Limit of quantification: It is a smallest concentration that can be quantified with specified level of accuracy and precision. The limit of quantification was determined by making serial dilutions and injecting in the chromatogram till the signal to noise ration 10:1 was obtained. The particular dilution was injection six times and RSD is calculated.

Limit of detection: The LOD was determined by dilution LOQ solution three times and RSD is calculated.

Linearity and **Range:** The Linearity of analytical method is its ability to obtain test results, which are directly proportional to the concentration of analyte in the test sample. Linearity of a method is the measure of how well calibration plot of a response Vs concentration is a straight line upper and lower data of linearity represents the range of linearity.

Ruggedness: The ruggedness is carried at two different locations by two analysts using two different columns.

Robustness: A measure of capacity to remain unaffected under a small deliberately changes to the analytical parameters. The analytical conditions are purposely altered to and the system suitability is measure. Robustness is carried out at following altered conditions.

Change in the mobile phase concentration ± 2 %

Change in pH of buffer ± 0.2

Change in flow rate ±10 %

Change in wavelength ± 5 %

Change in temp of column oven ± 2

Degradation: Forced degradation studies were performed to develop stability indicating HPLC method for the quantative determination and purity evaluation of analyte peak. In some times degradation is done under specificity. Degradation is done under different stress conditions and following are these conditions. :

Acid degradation

Alkali degradation

Oxidative degradation (peroxide degradation)

UV irradiation

Experimental Details

SrNo	Drugs	Column	Mobile phase
1	**Zopiclone Assay**	Cosmosil C18, 250 x 4.6 mm, 5 µm	Mixture of Acetonitrile, sodium lauryl sulphate, sodium dihydrogen phosphate and ortho phosphoric acid. (pH 4.0 ± 0.1)
2	**Irbesartan Assay and Related substances**	Merck Purospher star C18, 250x4mm, 5 µm	Phosphoric acid (pH 3.2) by Orthophosphoric acid, acetonitrile and methanol.
3	**Piperaquine Phosphate**	Waters Xterra, RP 18, 250 x 4.6 mm, 5 µm	Disodium hydrogen phosphate, triethylamine, Orthophosphoric acid, water Acetonitrile. (pH 7.0)
4	**Meclizine hydrochloride Assay**	Waters X-bridge pheny 250 x 4.6 mm, 5 µm	Ammonium dihydrogen phosphate, diammonium hydrogen phosphate, tetra-butyl ammonium hydrogen sulphate, water, Acetonitrile. (pH 6.50.)
5	**Divalproex sodium**	Phenyl Hypersil 150 x 4.6mm 5µm .	Citrate buffer ,Phosphate buffer Acetonitrile 35: 35 :30(pH 3.0)
6	**S-Zopiclone Assay**	Zorbax C18, 250 x 4.6mm 5µm.	Acetate buffer ,Acetonitrile 75:25 dilute acetic acid. (pH 5.5)

7	**Assay in Zoledronic acid**	Grace, anion exchange 150 x 4.6mm, 7µ.	0.05% formic acid and adjust the pH 3.5 with dilute sodium hydroxide
8	**Flucytosine Assay**	Phenomenex Luna 5 µ NH2 100A 250 x 4.6 mm, 5 µm	Acetonitrile and water (80 : 20)
9	**Clopidogrel Bisulfate Assay.**	Ultron ES-OVM 150 x 4.6 mm, 5 µm (C/LC/136)	Degassed mixture of phosphate buffer and acetonitrile in ratio of 80: 20.
10	**Artemether Assay**.	Purospher STAR C-18 250 x 4.0 mm 5µm	Acetonitrile and water (62: 38)

Calculation:

LOD, LOQ, Linearity, Recovery and method precision calculation done using computer program. The graph of Linearity plotted from obtained HPLC data to see the linear equation. From the calculation of LOD, LOQ detection limit for method precision set fixed.

Present work

The present work entitled: **"A Novel Analytical Method Development by HPLC Technique for Active Pharmaceutical Ingredients (API) in formulation."**

Chapter I - Introduction:

It deals with the brief introduction, basic principles of HPLC, literature survey pertaining to the proposed work, Pharmaceutical drug importance .Importance of Stability indicating validated HPLC method. The chapter concludes with the aims and objectives of the work and references.

Chapter II- Experimental Details:

It deals with all the experimental and mathematical details of Pharmaceutical drugs method development and validation.lt describes method precision, LOD, LOQ, Recovery, Linearity, Intermediate Precision and Solution stability data. Finally it deals with forced

degradation data.

Chapter III- Experimental Results:

It incorporates the studies of experimental results of Pharmaceutical drugs.

It is outlined in three parts:

Part one gives experimental results of Zopiclone, Irbesartan related substances and Piperaquine Phosphate. Its method development, validation study and forced degradation study for drug stability.

Part two gives experimental results Meclizine hydrochloride Divalproex sodium, S-Zopiclone. Its method development, validation study and forced degradation study for drug stability.

Part three gives experimental results Zoledronic acid Flucytosine Clopidogrel Bisulfate Artemether

Its method development, validation study and forced degradation study for drug stability.

Chapter IV- Discussion of the Results

The method developed for quantative determination for all above pharmaceutical drugs was rapid, precise, accurate and selective. The method was completely validated showing satisfactory data. The developed method can be conveniently used for assay determination, related substance and Enantiomeric determination. The chapter ends with the conclusion drawn from the present study and suggestions.

Original Work (rule 0.238)

A modest attempt has been made to develop the analytical method and validate the method as per ICH guideline. The drug used is the study has been used first time for validation and force degradation study. The method developed is totally interdisciplinary and it is equally important in the stability indicating for a drug and for formulated product. The method developed will play a key role not only for quality assurance of drugs and their formulation but it will assure to the consumer for safe and reliable product.

Vilas Ramakrishna Choudhari — **Research Student**

Dr. Milind B. Ubale — **Research Guide**