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A Review on new Analytical method Development and Validation by RP-HPLC

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Abstract

A simple, precise, accurate, specific and RP-HPLC method was developed for determination of drug in pharmaceutical formulation. The presented study is simple, since samples are directly used without any preliminary chemical dramatization or purification steps. Generally a RP-HPLC assay utilize Symmetry C18 or equivalent with mobile phase composition of ph 7 buffer: acetonitrile [40:60] was used, and flow rate was 0.8 ml min^{-1} with UV detection at 285 nm. The proposed method was validated for various ICH parameters like linearity, limit of detection, accuracy, precision, ruggedness, robustness, and system suitability.

Key words: RP-HPLC, P^H 7 buffer: acetonitrile.

INTRODUCTION

Analysis is important in any product or service,but in drug it is very important as it involves life.In comparison to general consumer products, in drugs there is and there can be only quality/standard product and no other product [1]. This comes from series of tests from quality control, starting from raw materials in process during manufacture, finished product is the moral obligation to the patients, and hence the manufacture and quality of drugs should be taken care off. These tests may vary from single entity or combination of several potent drugs in formulation these tests of quality control may belong to the following types:

1. Chemical methods
2. Physicochemical methods
3. Microbiological methods
4. Biological methods

ANALYSIS CAN BE MAINLY CLASSIFIED [2]

QUANTITATIVE ANALYSIS

In general, quantitative analysis is the determination of the absolute or relative abundance often expressed as a concentration of one, several or all particular substance present in a sample. Once the presence of certain substance(s) in a sample is known, the study of their absolute or relative abundance can help in determining specific properties. Volumetric analysis can be simply a titration based in a neutralization reaction but it can also be a precipitation or a complex forming reaction as well as a titration based in a redox reaction. Complex forming titration is a reaction that occurs between metal ions and a standard solution that is in the most cases EDTA (ethylene diamine tetra aceticacid).

QUALITATIVE ANALYSIS

The general expression Qualitative Analysis refers to analyses in which substances are identified or classified on the basis of their chemical or physical properties, such as chemical reactivity, solubility, molecular weight, melting point, radioactive properties (emission, absorption), mass spectra, nuclear half-life, etc. Qualitative Analysis may take place without Quantitative Analysis, but Quantitative Analysis requires the identification (qualification) of the analyse for which numerical estimates are given.

INTRODUCTION TO CHROMATOGRAPHY

CHROMATOGRAPHY [4]

A method of separating and identifying the components of a complex mixture by differential movement through a two-phase system, in which the movement is effected by a flow of a liquid or a gas (mobile phase) which percolates through an adsorbent (stationary phase) or a second liquid phase.

TYPES OF CHROMATOGRAPHY

- PAPER CHROMATOGRAPHY
- COLUMN CHROMATOGRAPHY
- THIN LAYER CHROMATOGRAPHY
- GAS CHROMATOGRAPHY
- ION EXCHANGE CHROMATOGRAPHY
- TWO-DIMENSIONAL CHROMATOGRAPHY
- HIGH PERFORMANCE (PRESSURE) LIQUID CHROMATOGRAPHY
- HIGH PRESSURE THIN LAYER CHROMATOGRAPHY [4]

PRINCIPLE

The main principle involved in HPLC is adsorption. When a mixture of components is introduced into a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

TYPES OF HPLC TECHNIQUES

(A) BASED ON MODES OF CHROMATOGRAPHY

(1) Normal-phase chromatography

(2) Reversed-phase chromatography (RPC)

(B) BASED ON PRINCIPLE OF SEPARATION

(1) Adsorption chromatography

(2) Chiral Phase chromatography

(3) Size-exclusion (or) Gel Permeation chromatography

(4) Ion-exchange chromatography

(5) Ion – Pair chromatography

(6) Affinity chromatography

(C) ELUTION TECHNIQUE

(1) Isocratic Separation

(2) Gradient Separation

(D) BASED ON SCALE OF OPERATION

(1) Analytical HPLC

(2) Preparative HPLC

(E) BASED ON TYPE OF ANALYSIS

(1) Qualitative Analysis

(2) Quantitative Analysis

INSTRUMENTAL REQUIREMENTS

- Pumps- solvent delivery system
- Mixing unit, gradient controller and solvent degassing
- Injector- manual or auto injectors
- Guard column
- Analytical columns
- Detectors

THE SCHEMATIC DIAGRAM OF HPLC

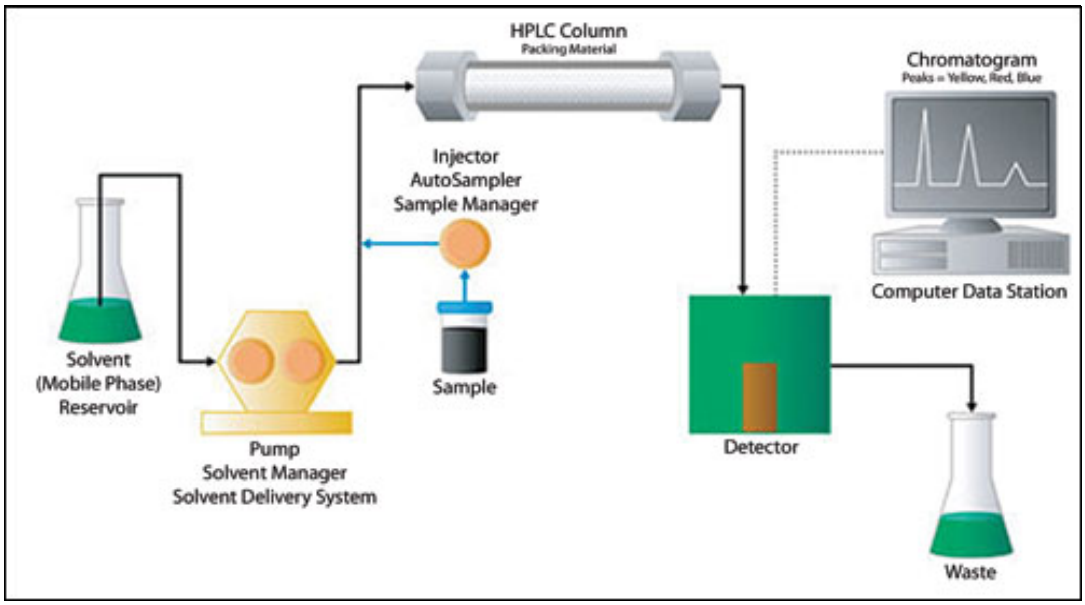

Fig 1: *Instrumentation of HPLC*

Fig 2: *HPLC equipment*

PARAMETERS USED IN HPLC [4]

- Retention time
- Retention volume
- Separation factor
- Resolution
- Theoretical plate
- HETP- Height Equivalent to a The original Plate
- Efficiency (no of theoretical plates)
- Asymmetry factor- Fronting Tailing

APPLICATIONS OF HPLC [5]

1) Qualitative analysis.
2) Quantitative analysis.
    (a) Direct Comparison Method
    (b) Calibration Curve Method
    (c) Internal Standard Method
3) Checking the purity of a compound.
4) Presence of impurities.
5) Determination of mixture of drugs.

RP-HPLC (Reverse Phase High Pressure Liquid Chromatography) [6]

Reversed-phase chromatography (RP-HPLC) separates molecules on the basis of differences in their hydrophobicity. The components of the analytic mixture pass over stationary-phase particles bearing pores large enough for them to enter, where interactions with the hydrophobic surface removes them from the flowing mobile-phase stream. The strength and nature of the interaction between the sample particles and the stationary phase depends on both hydrophobic interactions and polar interactions. As the concentration of organic solvent in the eluant increases, it reaches a critical value for each analyte which desorbs it from the hydrophobic stationary-phase surface and allows it to elute from the column in the flowing mobile phase.

- Interaction : Hydrophobic
- Packing materials **:** Non-polarEx: Silica-C18 ,Silica-C8, Polymer
- Mobile phase : PolarEx:MeOH/H2O, MeOH/Buffer sol.
- Sample : Having different length of carbon chain

Mobile phase solvents**:**

- Main solvent : MeOH-H2O, CH3CN -H2O
- Sub solvent : EtOH, IPA, THF, DMF
- Additive :Acid, Salt, Ion-pairing agent.

Comparison of Reversed Phase and Normal Phase

Table 1: Comparison of Reversed Phase and Normal Phase

	NORMAL PHASE	REVERSE PHASE
Stationary phase Mobile Phase Interaction Elution order	High polarity Low polarity Adsorption Low to High (Polarity)	Low polarity High polarity Hydrophobic Short to Long (Length of carbon chain)

STEPS FOR ANALYTICAL DEVELOPMENT [7, 8]

1. ANALYTE STANDARD CHARACTERIZATION
    a) All information about the analyte i.e., physical and chemical properties, toxicity, purity, hygroscopic nature, solubility and stability.
    b) The standard analyte (100% purity) is obtained. Made an arrangement for the proper storage (refrigerator, desiccators and freezer).

c) When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.

2. METHOD REQUIREMENTS

The goals of the analytical method that need to be developed are considered. The detection limits, selectivity, linearity, range, accuracy and precision are defined.

3. LITERATURE SEARCH AND PRIOR METHODOLOGY

The information related to the analyte is surveyed. For synthesis, physical and chemical properties, solubility and relevant analytical methods. Books, periodicals and USP / NF, and publications are reviewed. Chemical Abstracts Service (CAS) automated computerized literature searches are convenient.

4. CHOOSING A METHOD

Using the information in the literatures, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analytes and samples. There is usually one compound for which analytical method already exist that is similar to the analyte of interest.

5. INSTRUMENTAL SETUP AND INITIAL STUDIES

The required instrumentation is setup Installation, operational and performance qualifications of instrumentation using laboratory SOP's are verified. Always new solvents, filters are used the analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with, known standard rather than with a complex sample matrix.

6. OPTIMIZATION

During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan, and every step is documented (in a lab notebook) in case of dead ends.

7. DOCUMENTATION OF ANALYTICAL FIGURES OF MERIT

The originally determined analytical figures of merit limit of quantization (LOQ), Limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc., are documented.

8. EVALUATION OF METHOD DEVELOPMENT WITH ACTUAL

SAMPLES

The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

METHODOLOGY

PREPARATION OF MOBILE PHASE

Mix a mixture of above buffer 400mL (40%) and 600 mL of Acetonitrile HPLC (60%) anddegas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

PREPARATION OF STANDARD SOLUTION

Accurately weigh and transfer 10mg of drug Working standard into a 10 mL volumetric flask add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

MOBILE PHASE: Acetonitrile + Buffer (60:40)
CHROMATOGRAPHIC CONDITIONS

Equipment	: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column	: Symmetry C18 (4.6 x 150mm, 5 µm, Make: XTerra) or Equivalent
Flow rate	: 0.8mL per min
Wavelength	: 285 nm
Injection volume	: 20 µl
Column oven	: Ambient
Run time	: 5 min

METHOD VALIDATION [19]

It can be done by

(A) PRECISION

(B) INTERMEDIATE PRECISION/RUGGEDNESS

(C) ACCURACY

(D) LINEARITY

(E) LIMIT OF DETECTION

(F) LIMIT OF QUANTIFICATION

(G) ROBUSTNESS

DISCUSSION

RP-HPLC METHOD

The objective of the proposed work was to develop methods for the determination of drug and to validate the methods according to ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of any drug

In RP- HPLC method, HPLC conditions were optimized to obtain, adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to elute title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time, resolution. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions.

The low values of % R.S.D indicate the method is precise and accurate. Sample to sample precision and accuracy were evaluated using three samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using five concentrations analyzed on three trials over a period of three days. These results may show the accuracy and reproducibility of the assay.

Ruggedness of the proposed method was determined by analysis of aliquots from different environmental conditions; the % R.S.D. reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

CONCLUSION

RP-HPLC method: For routine analytical purpose it is desirable to establish methods capable of analysing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool.

The run time of the HPLC procedure is only 5 minutes. Good agreement was seen in the assay results of pharmaceutical formulation as well as in laboratory prepared mixtures by developed methods. We concluded that all the proposed methods are a good approach for obtaining reliable results and were found to be suitable for the routine estimation of drug in pharmaceutical formulation.

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