ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ISONIAZID AND ETHAMBUTOL BY USING REVERSE PHASE HPLC METHOD

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ABSTRACT

Developed a simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method for the simultaneous determination of Bicalutamide and Atenolol from combined dosage form by reverse phase C18 column (Agilent C18, 5µm, 4.6*250mm). The sample was analysed using Water: Acetonitrile the ratio of 70:30 as a mobile phase at a flow rate of 1ml/min and detection at 254nm. The retention time for Bicalutamide and Atenolol was found to be 4.626 min and 2.528 min respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form

1. INTRODUCTION

1.1 Analytical Chemistry [1-4]

Analytical chemistry is the branch of chemistry that deals with the analysis of chemical molecules, either in qualitative or quantitative manner. The former identifies the nature of substance, and if it is mixture, the nature of the components present, whereas the later determines the elemental composition of the substance and/or the quantitative distribution of each component.

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest.

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use"

To know information concerning the compound or analyte is worth. Understand its physical and chemical characteristics allow us to select the most appropriate HPLC method development from the vast literature. Information concerning sample, for example, molecular mass, structure and functionality, P^{Ka} values and UV spectra, solubility of compound(s) should be compiled, the requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc., Should be checked. For pure compound, determine sample solubility whether it's organic soluble or water soluble, as this helps to select the best mobile phase and column to be used in the HPLC Method development.

Various detectors include: UV/Visible photodiode array detector, fluorescence detector, conductivity detector, refractive index detector, electrochemical detector, Mass spectrometer detector, evaporative light scattering detector. UV-Vis detectors are typical in many laboratories as they can detect a wide array of compounds.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients.

The validation of an analytic method demonstrates the scientific soundness of the measurement or characterization. It is required to varying extents throughout the regulatory submission process. The validation practice demonstrates that an analytic method measures the correct substance, in the correct amount, and in the appropriate range for the intended samples. It allows the analyst to understand the behavior of the method and to establish the performance limits of the method. The goal is to identify the critical parameters and to establish acceptance criteria for method system suitability.

1.2 High Performance Liquid Chromatography (HPLC)[5]

The acronym HPLC, coined by the late Prof.Csaba Horvath for his 1970 Pittcon paper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi. This was called high pressure liquid chromatography, or HPLC.

New HPLC instruments could develop up to 6,000 psi of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time (smaller particles, even higher pressure), the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

- Speed(min)
- Greater sensitivity
- Improved resolution (wide variety of stationary phases)
- Reusable columns
- Needs a small sample with a high accuracy and precise

- Easy sample recovery, handling and maintenance.
- Reproducibility of +/- 1% (not so for LC)
- Non-destructed sample during operation compared to GC.
- Controls and automates chromatography instrumentation.
- Provides data management, security features, and reporting and instrument validation.

Major types of HPLC:

Normal Phase Chromatography

The IUPAC Compendium of Chemical Technology defines 'Normal Phase' as an elution procedure in which the stationary phase is more polar than the mobile phase. Adsorption chromatography, which was classified as a separation mode called "solid-liquid chromatography," is now considered to be normal-phase chromatography. In most cases, the solid phase used for normal-phase chromatography is an untreated porous silica-gel column (SIL column) or a column containing silica gel chemically bonded at the surface to polar functional groups, such as the aminopropyl group (NH2 column) or cyanopropyl group (CN column). The mobile phase used is generally ethanol or another polar solvent added to a non polar solvent such as n-hexane. However, a mobile phase containing water is sometimes used for the analysis of highly polar components. The separation of each component differs according to the distribution ratio between the solid phase and mobile phase. The interaction between the solid phase and target components during normal-phase chromatography are mainly hydrophilic interactions, such as hydrogen bond interactions and electrostatic interactions. Consequently, normal-phase chromatography generally offers different separation selectivity to reversed-phase chromatography, which mainly involves hydrophobic interactions.

Normal-phase chromatography can easily separate tocopherol isomers that are difficult to separate by reversed-phase chromatography and sugars that are difficult to retain by reversed-phase chromatography. It can elute together all components with different alkyl chain lengths and branches during the analysis of alkyl benzene sulfonate. These properties arise as the regions involved in retaining compounds differ from those in reversed-phase chromatography. In addition, as the mobile phase used for normal-phase chromatography generally contains no water, this technique is ideal for the separation of easily hydrolyzed compounds, such as acid anhydride; concentration after fractioning; or preparative separation and purification that requires drying. Normal phase chromatography can also be advantageous from the viewpoints of quantum yield for fluorescence detection, molar absorptive and detection wavelength in absorption detection. With normal-phase chromatography, increasing the mobile phase polarity generally accelerates elution. For example, if an n-hexane/ ethanol mixture is used as the mobile phase, elution occurs faster if the proportion of ethanol, which has higher polarity, is increased. Care is required, as this is the reverse relationship to reversed-phase chromatography, whereby the rate of elution increases when the mobile phase polarity is decreased. Using a low-viscosity solvent in the mobile phase sometimes permits high flow rates and rapid column equilibration. If an NH2 column or CN column is used, some mobile phase compositions result in reversed polarity of the mobile phase and solid phase, such that the column functions as a reversed-phase chromatography column. Therefore, it is important to be aware of the possibility that the elution behavior may fluctuate wildly, especially if a mobile phase with a high proportion of water is used. **1.3 Instrumentation** [6-10]

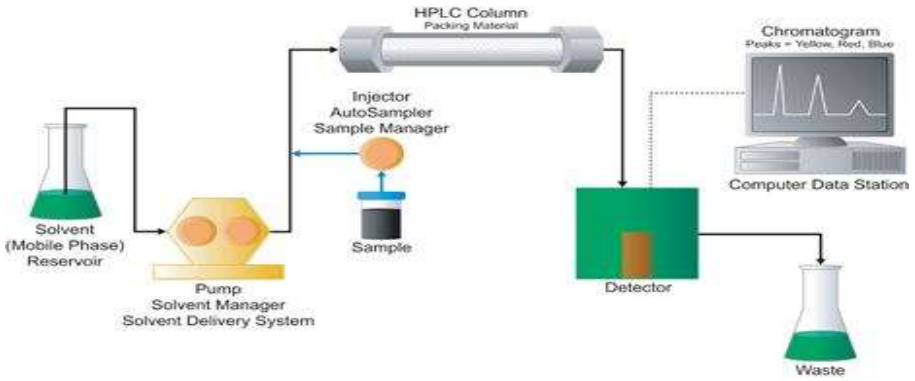

Fig No.1.1 High Performance Liquid Chromatography system

The basic components of a High Performance Liquid Chromatographic system are shown in Fig.1. The instrument consists of

- Mobile Phase Reservoir
- A pump to move the eluent and sample through the system.
- An injection device to allow sample introduction**.**
- A Column(s) to provide solute separation.

- A Detector to visualize the separated components.
- A Data collection device to assist in interpretation and storage of results.

Compounds are separated by injecting a plug of the sample mixture usually 5- micro liters onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Mobile phase reservoir:

The most common type of solvent reservoir is a glass bottle. Most of the manufacturer's supply these bottles with special caps, Teflon tubing and filters to connect to the pump inlet and to the spurge gas (Helium) used to Remove Dissolved air. Filtration is needed to eliminate suspended Particles and organic impurities.

Solvent System:

The mobile phases used in Reversed-Phase Chromatography are based on a polar solvent, typically water, to which a less polar solvent such as acetonitrile or methanol is added. Solvent selectivity is controlled by the nature of the added solvent in the same way as described for Normal-Phase Chromatography. Solvents with large dipole moments, such as methylene chloride and 1, 2-dichloroethane interacts preferentially with solutes that have large dipole moments such as nitro-compounds nitriles amines and sulfoxides. Solvents that are good proton donors such as chloroform, m-cresol, and water interact preferentially with basic solutes such as amines and sulfoxides and solvents that are good proton acceptors such as alcohols, ethers, and amines, tend to interact best with hydroxylated molecules such as acids and phenols.

BIBLIOGRAPHY

1. Sharma BK. Instrumental Methods of Chemical Analysis. 7th ed. Meerut: Goel Publishing House; 2000. p. 1-2.
2. David CL, Michael Webb. Pharmaceutical Analysis. London: Black well publishing; 1994. p. 2-4.
3. Chatten LG. Pharmaceutical Chemistry. Vol. II. New York: Marcel Dekker Inc; 1996. p. 23-25.
4. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. Vol. II. New Delhi: CBS Publisher and Distributors; 1986. p. 13-17.
5. B.K. Sharma, Textbook of Instrumental Methods of Chemical Analysis,GOEL publishing house, Meerut, 23rd Ed, p.288-289.
6. G.Vidyasagar, Textbook of Instrumental Methods of Drug Analysis, Pharmam. ed Press, 2009, p.106-120.
7. H. H Willard, L. L Merritt, J. A Dean, and F. A Settle, Textbook of Instrumenta Methods of Analysis, CBS publishers and distributors, New Delhi, 7th Ed,1986, p.592-596.
8. H.H.Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and direct acting types, Proceedings of the twenty-fourth international pump user's symposium,2008, p.45-58.
9. LR.Snyder, JJ Kirkland, LG.Joseph, Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Ed, 1997, p. 1-56.
10. D.A.Skoog, F.J.Holler, S.R.Crouch, Textbook of Instrumental Analysis, Brooks/Cole, Cengage Learning India Private Limited, 2007, p.900-906.
11. R.E.Schirmer, Textbook of Modern Methods of Pharmaceuticals, CRC press,2nd Ed, P.242-244.
12. LR.Snyder, JJ Kirkland, LG.Joseph, Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Ed, 1997, p. 1-56.

13. Ranjith singh, HPLC Method Development and Validation- an Overview, J Pharm Educ. Res.4 (2013) 26-33.

14. ICH: Q2B, Analytical Validation – Methodology (1996)

15. Kumar GVS Development and Validation of RP-HPLC Method for Simultaneou Estimation of Amlodipine and Indapamide in Bulk and Tablet Dosage Form, Asian journal of biomedical and pharmaceutical sciences.

16. S.D.KAYAL F.A.Khan, A.G.Tated ,R.L.Bakal,A.V.Chandewar Method development and validation for the simultaneous determination of amlodipine besylate and telmisartan in tablet dosage form by rp-hplc, Internationa journal of pharmaceutical research and development.

17. Chandanam Sreedhar, Sowmya Manala, Sreenivasa Rao T, Anusha Vemuri, Naresh kumar et al,Development And Validation Of Rp-Hplc Method For The Estimation Of Bicalutamide In Pure And Pharmaceutical Dosage Forms. International Journal of PharmTech Research, Vol.4, No.4, pp 1686-1690.

18. B. Sowmya*, A. Elphine Prabahar, N. Ramarao Ananlytical method development and validation of Bicalutamide in pharmaceutical formulation using RP-HPLC,International Journal of Pharmaceutical Research and Novel Sciences,vol1(2), 2014.

25. Lakshmi thulasi. K*, Janardhan. M, Nandakishore Agarwal Development and validation of RP-HPLC method for estimation of Bicalutamide in pure and pharmaceutical dosage forms, International Research Journal of harmaceutical And Applied Sciences,vol2(5),2012,165-169.

26. Palleshwar Rao G, JVLNS Rao, Lanka A. Rama Prasad and Srinivasu Pamidi

. Development And validation of new stability indicating HPLC method for quatantification of process related and degradation impurities of Bicalutamide in tablet dosage form, International Journal of Pharmacy,vol2(1),2012,218-223.