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Method Development and Validation of Labetalol by RP-HPLC

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ABSTRACT:
Labetalol is a competitive antagonist of alpha 1-, beta 1-, and beta 2-adrenergic receptors. The hemodynamic effects of the drug include reduced blood pressure, heart rate, and peripheral resistance, with little change in resting cardiac output or stroke volume. In open trials and controlled studies, labetalol was an effective antihypertensive. Labetalol compared favorably with beta-blockers alone or in combination with vasodilators, for the treatment of hypertension. Reductions in heart rate are less pronounced with labetalol as compared with propranolol. Labetalol produces rapid reductions in blood pressure when administered intravenously for severe hypertension. The most frequent adverse reactions to the drug include fatigue, postural symptoms, headache, and gastrointestinal complaints. Labetalol may prove advantageous when vasodilation in addition to beta-blockade is desired, or for selected patients experiencing adverse effects attributable to beta-blockade. Until the clnical profile of labetalol is better defined, the use of the drug should be limited.(1)

KEYWORDS: chromotography, HPLC, instrumentation, pharmacokinetics, ultra voilet (UV)(2)

I. INTRODUCTION

Pharmaceutical analysis is a specilized branch of analytical chemistry derivies its principles from various branches of science like physics, microbiology, nuclear science, and electronics etc.. Qualittive analysis is required before a quanttitative analysis can be undertaken. A separation step is usally a necessary part of both a qualitative and quantitative analysis. The result of typical quantitative analysis can be computed from two measurements. One is the mass or volume of samples to be analysied and the second one is the measurment of some quantity that is propotional to the amount of analyte in the sample and complete the analysis.(3)

TYPICAL INSTRUMENTAL TECHNIQUES

The method of estimation of drugs are divided into a physical, chemical,physicochemical and biological one of them physical and physicochemical method are used mostly. Physical method of analysis of involve the studying of the physical properties of a substtance. The include determination of the solubility, transparency or degree of turbidity, color density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. Physicochemical method methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physiocochemical are optical refractometry polarimetry, emission and fluorescent methods of analysis, photometry including photocolorimetry, spectrophotometry, nephelometry and turbidometry electro chemical (potentiometry, amperometry, coulometer, polarography) and chromotography (column, paper, thin layer, gas, high performance liquid) methods are generally preferable. Methods involving nuclear reaction such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming popularthe combination of spectroscopy with gas chromotagraphy is one of the most powerful tools available.(4)

CHROMATOGRAPHY

Chromatography is based on the principle where molecules is mixture applied on to the surface or into the solid, and fluid stationary phase (stable

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phase) is sepersting from each other while moving with the aid of the mobile phase. The effective on this seperstion process include moluculer charecteristics related to adsorption (liqud-solid),partition (liqud-solid), and affinity or differences among their molecular weights.because of this differences,some components of the mixture stay longer in the stationary phase, and they move slowly in the chromotography system, while others pass rapdly into mobile phase,and leve the system faster. Based on this approach three components from the basiss of chromotography thechnique.(5)

- **Stationary phase**: it is phase is always composed of a "solid" phase or "a layer of a liqud adsorbed on the surface a solid support"
- **Mobile phase**: it is phase is always composed of "liqud"or a "gaseous components"
- **Seperated molecules**: The method based on partition are very effective on seperation, and identification of small molecules as amino acids, carbohydrates, and fatty acids.

HIGH PERFORMANCE LIQUID CHROMATOGRAPY (RP-HPLC)

High-performance liquid chromatography (or High-pressure liqid chromatography,HPLC) is a specific from of column chromatography generally used in biochemistry and analysis to separate, identify the active compounds. HPLC mainly utilize a column that holds packing material (stationary phase),a pump that molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s)used.The sample to be analyzed introduced in small volume to the stream of mobile phase and is retared by specific chemical or physical interaction with the stationary phase. The amount of retardation depends on the nature of the analyt and composition of both stationary and mobile phase.The time at which a specific analyte clutes (comes out of the end of the column) is called retention time. Common solvents used incluude any misibile combinations of water or organic liquids (the most common are methonal and acetonitrile). Seperation as been done to vary the mobile phase consumption during dianlysis; this is known as gradient elution. The gradient seperates the analyte mixtures as function of the affinity of the analyte for the current mobile phase. The choice of the solvents, additives and gradient depend on the nature of the stationary phase and the analyte.(6)

INSTRUMENTATION

HPLC Solvent Reservoir, Pump, Detector:-
HPLC Include between 1-4 reservoirs for storeing mobile phase solvents.(7)

A Flow scheme for HPLC

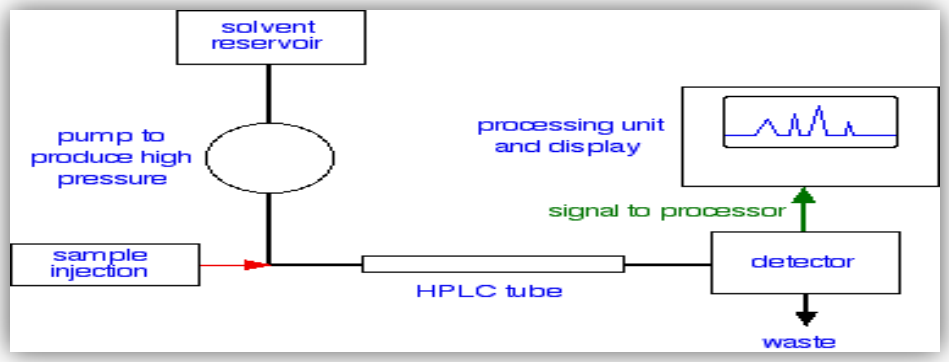

Fig: 1.1 Flow chart of HPLC

- Mobile phase
- Pumps
- Injector port
- Stationary phase
- Detector

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Mobile phase, Pumps:-

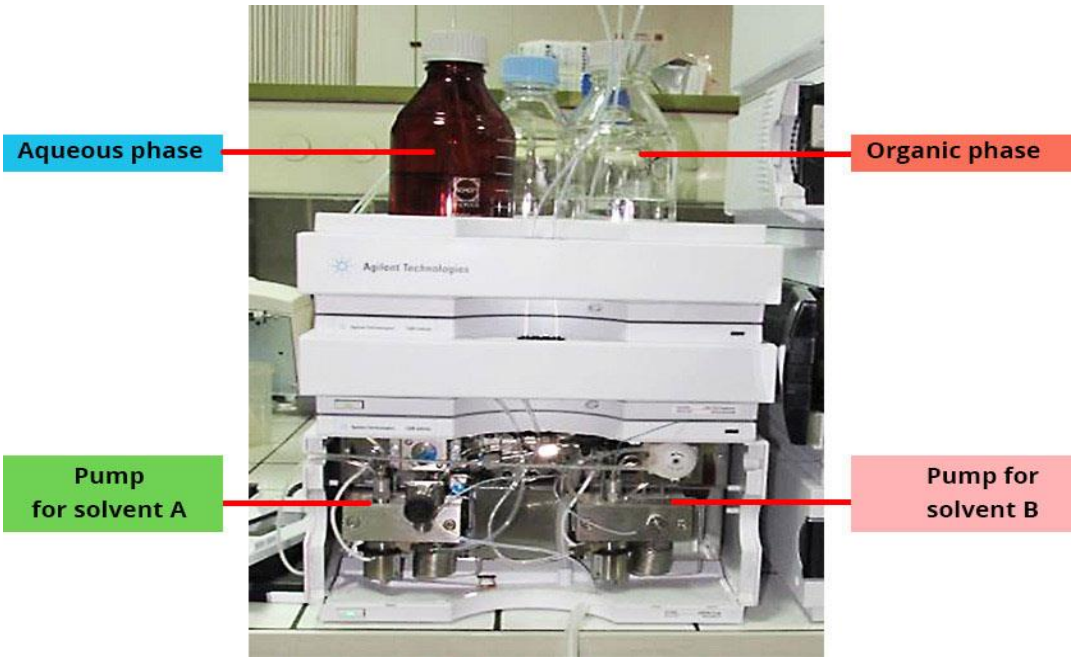

Fig: 1.2 mobile phase and pumps

Mobile phase:

- The mobile phase in HPLC refers to the solvents being continuously applied to the column, or stationary phase.
- The mobile phase act as a carrier for the sample solution. A sample solution is injected into the mobile phase of an assay through the injector port.
- As a sample solution flows through a column with the mobile phase, the components of that solution migrate according to the non-covalent interactions of the compound with the column.(8)
- The chemical interactions of the mobile phase and sample,with the column, determine the degree of migration and seperation of components contained in the sample.
- In isocratic elution compounds are eluted using constant mobile phase composition. The separation of compounds can be described using several equations.

EQUATIONS FOR ISOCRATIC ELUTION	
RETENTION TIME (s)	(1) $t_R = t_0k' + t_0$
BANDWIDTH (ml)	(2) $\sigma = V_m(1 + k')N^{-1/2}$
RESOLUTION ($\Delta t_R/4\sigma$ or $\Delta t_g/4\sigma_g$)	(3) $R_S = [(1/4)(\alpha - 1)N^{1/2}][k'/(1 + k')]$
CAPACITY FACTOR	(4) $k' = (t_R - t_0)/t_0$

➢ All compounds being migration through the column at onset. However, each migrates at a different rate, resulting in faster or slower elution rate.

➢ This type of elution is both simple and inexpensive, but resolution of some compounds is questionable and elution may not to be obtained in a reasonable amount of time.

HPLC PUMPS:

➢ There are several types og pumps avabile for use with HPLC analysis, they are reciprocating piston pumps, syring type pumps, and constant pressure pumps.

Stationary phase:-(column)

- The stationary phase in HPLC refers to the solids support contained within the the column over which the mobile phase continuously flows.

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- The sample solution is injected into the mobile phase of the assay through the injector port.
- As the sample solution flows with the mobile phase through the stationary phase, the components of the solution will migrate according to the non-covalent interactions of the compounds with the stationary phase.
- The chemical interaction of the stationary phase and the sample with the mobile phase, determines the degree of migration and separation of the components contained in the sample.(9)

ULTRA-VIOLATE(UV)

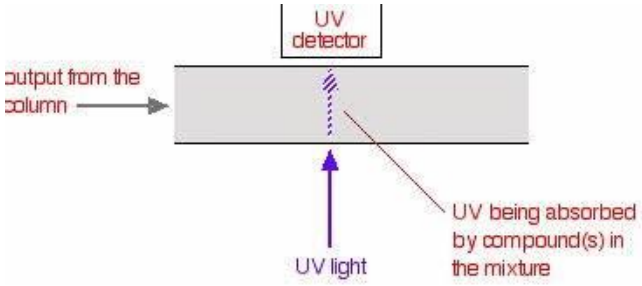
- It is called also as universals detector.
- UV-detector measures the ability of a sample to absorb light. This can be accomplished at one or several wavelengths.
- There are several ways of detecting when a substance has passed through the column. A common method which is easy to explain uses ultra-violet absorption.
- May organic compounds absorb UV light of various wavelengths. Ig you have a beam of UV light shinning through the stream of liquid coming out of the column, and a UV detector on the opposite side of the stream, you can get a direct reading of how much of the light is absorbed.
- The amount of light absorbed will depend on the amount of a particular compountes that is passing through the beam at the time.
- All the following methods use this formula that measures N, or number of theoretical plates.(10)

N = a $\frac{t_r^2}{W^2}$

A = constant dependent on height where peak width measured

t_r = retention time

W = peak width



DRUG PROFILE :

STRUCTURE:

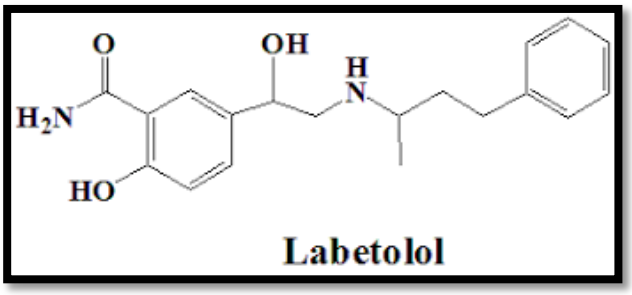


Fig 2.1 Structure of labetalol

MOLECULAR FORMULA : C19H24N2O3
IUPACNAME:**2**-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]benzamide monohydrochloride.
MOLECULAR WEIGHT:364.9 g/mol
STATE: Liquid
MELTING POINT: 188 °C
BRAND NAME: Normodyne, Trandate

DRUG DESCRIPTION:

- LABETOLOL 100MG TABLET may be used alone or in combination with other medicines. It should be taken with food.
- You can take it at any time of day but try to take it at the same time each day. Most people with high blood pressure do not feel ill, but if you stop taking this medicine, your condition could get worse.
- This may lead to your blood pressure rising again and increase your risk of heart disease and stroke.(11)

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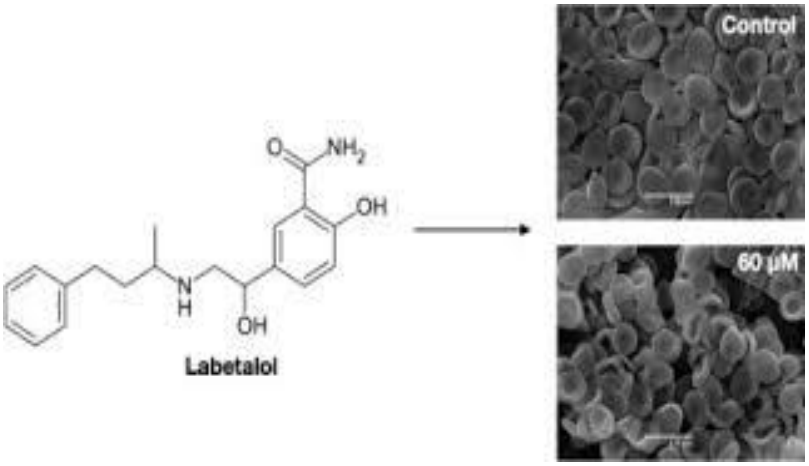

MODE OF ACTION:

- Labetalol is a unique alpha- and beta-adrenergic-receptor blocking agent that has recently been approved for the treatment of hypertensive emergencies and urgencies.
- This agent lowers peripheral vascular resistance by vasodilatation with little or no effect on cardiac output.
- Labetalol is a beta blocker, or an antagonist of the β-adrenergic receptors.
- It is specifically a non-selective antagonist of the β_1- and β_2-adrenergic receptors.
- Labetalol has intrinsic sympathomimetic activity.

PHARMACOKINETICS :

Labetalol is metabolized by the liver resulting in an inactive glucuronide conjugate. It has an onset of action within 2 to 5 minutes, reaches its peak effects at 5 to 15 minutes, has an elimination half-life of 5.5 hours, and a duration of action up to four hours.

DRUG INTERACTIONS :

Drug	**Interaction**
Integrate drug-drug interactions in your software	
Atenolol	Atenolol may increase the orthostatic hypotensive activities of Labetalol.
Atomoxetine	The metabolism of Atomoxetine can be decreased when combined with Labetalol.
Atropine	Atropine may decrease the antihypertensive activities of Labetalol.

- Medicines for your heart, such as amiodarone, flecainide or digoxin. non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen – they can stop labetalol working properly. medicines for diabetes, particularly insulin – labetalol may make it more difficult to recognise the warning signs of low blood sugar.(12)

II. CONCLUSION:

The developed method can be successfully applied for routine analysis, quality control analysis and also suitable for stability analysis of the simultaneous determination of labetalol and its degradation products in tablet dosage forms as per the regulatory requirements.

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