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Analysis of Cardiovascular Drugs Using Rp-Hplc and Mlc; A REVIEW

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ABSTRACT
Reverse phase high performance liquid chromatography is a method used to perform drug assays for its profound results. The methodology following the RP-HPLC is not only one of the most user-friendly analytical technique available, but also is one of the most sustainable. It is only impressive to find this technique being deployed on so many aspects and at various levels. This review paper aims to serve as a compilation of the latest techniques and methods (Matrixes, in terms of HPLC) deployed in the drug assay industry of a few cardiovascular drugs. Namely, Atenolol, Metoprolol, Felodipine and Diltiazem along with similar drugs are found to be consumed at a very large scale all over the globe.
Micellar liquid chromatography is also a technique of recent advancement. MLC is a method derived from RP-HPLC is known for its green design. MLC is not only greener out of the two, but is also cost effective. It is said to have mobile phase of 90% or more water (v/v), which can even be recycled if the injection volumes are few and distantly made. A few modifications in the RP-HPLC system serves as a gate way to this analytical technique. MLC has the same setup as RP-HPLC system yet the methodology involved is different. The basic machinery of pumps and injection needles, along with the detectors used are similar. The difference lies in the mobile and stationary phase development during the analysis. With few modifications, the interactions involved in the separation are heightened, resulting in quantification. The chromatograms produced are yet blunt and/or broad. With the issue of reduced efficiency, it has only the green factor at its disposal to be turned into a suitable technology for long run. MLC is a technique being explored for ways to enhance its efficiency. After almost 45 years of this technique's discovery, it still captures attention of eager scientists. The working principle involved in the techniques are discussed along with the recent method development and discussions.
The quantification of the potent drugs lead to newly proposed ideas being scribbled and brought to life every day. The latest potential matrixes (All the necessary chromatographic conditions) for the mentioned drugs are taken in account in this review paper, keeping in mind their versatility, cost-effectiveness and their green approach.
KEY WORDS: Drug analysis, HPLC, RP-HPLC, MLC, Atenolol, Felodipine, Metoprolol, Diltiazem, hypertension, BP, Assay

I. INTRODUCTION

With the ever-increasing number of heart patients around the planet and the country, the analysis and use of cardiovascular drugs is a topic of great significance. India stands 2nd in most deaths due to cardiovascular diseases (CVD); the casualties have grown in the past years. Recent ongoing pandemic also witnessed a greater than ever demand of cardiovascular drugs. The life in a metro city, not to mention the food and lack of physical activities are the initial changes leading to great complications for the body. Genetic disposition and metabolic deregulation also contribute to the growing number of cardiovascular drugs in takers.

CVD are diseases that involve the heart and blood vessels associated with it. It involves arterial diseases like angina and myocardial infraction, commonly known as the heart attack. The complications are termed cardiac arrest. The specific mechanisms vary depending on the type of disease. General symptoms include chest pain, shortness of breath.

Cardiovascular drugs are agents that affect the functioning of heart and bool vessels. One of the most widely used drugs, the cardiovascular drugs are very important class of drugs. The acting mechanisms of the drug are different based on the specification of the diseases but it can be generalized in three ways.
They may affect the force of contraction of the heart muscle, causing inotropic effects.
They may affect the frequency of heartbeat or the heart rate, causing chronotropic effects
Lastly, they may affect the regularity of heart beat, causing rhythmic effects.

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Among CVDs, hypertension and arrhythmias are most common.

Significance of drug analysis

The significance of drug analysis is to have information about qualitative and quantitative composition of substances constituting it. The assay will provide us with qualitative and quantitative analysis of the drugs. This helps in research, mass production of the desired drug. The method described in analysis cardiovascular drugs in this project is high performance liquid chromatography (HPLC). HPLC is an extended form of liquid chromatography. A greener approach of MLC combined with the basics of HPLC and modifications done on par for lowering the risks faced. This not only prevents the use of various carcinogenic, toxic solvents but also provides a better alternative in waste production.

Class of drugs

Describing the drugs analyzed, most commonly found class of cardiovascular drugs are β-blockers. They are the class of drugs used to subside the CVDs such as hypertension, angina pectoris, cardiac arrythmias and myocardial infraction etc. They are also used to treat high blood pressure although better first choice options are not available in the market.

These are competitive antagonists that block the receptor sites for epinephrine and norepinephrine on adrenergic beta receptors. These are the very catecholamines that are responsible for our fight and flight response. Beta receptors are present on cells of heart muscles, smooth muscles and other tissues in the sympathetic nervous system. Beta blockers inhibit the binding with the receptor of epinephrine or other stress hormones in order to reduce their effects.

Another class of drugs mentioned in this project work on the mechanism of calcium channel blocking. Also called calcium channel antagonists or calcium antagonists. These are the group of drugs that disrupt the movement of calcium channels. This class of drugs can act by decreasing the blood pressure in patients with hypertension, that's why they are often called antihypertensive drugs. These are specifically effective against stiffness of large vessels. Calcium channel blockers are also frequently used to alter heart rate or to prevent vasospasm. They also find use in reducing chest pain caused due to angina pectoris.

Cardiovascular drugs

Atenolol ((RS)-2-{4-[2-Hydroxy-3(propan-2-ylamino)propoxy]phenyl}acetamide) is a drug based on the β-blocker mechanism. Used to treat high blood pressure and heart associated chest pain, it was in huge demand. Although it doesn't seem to improve the mortality in cases of high blood pressure. Belonging to the drug class β1 receptor antagonist category, it is taken via mouth or intravenous injection. Side effects which are considered along with atenolol includes feeling tired, heart failure, dizziness, depression etc. The use of this drug is prohibited during pregnancy and alternate drugs are recommended during breastfeeding. Patented in 1969 and medically approved in 1975, it is on the list of essential medicines by world health organization. It was 39th in the 2019 list of most prescribed drugs in US. It is used in a number of conditions such as hyperthyroidism, hypertension, angina, long syndrome, acute myocardial infraction, supraventricular tachycardia etc.
Atenolol has molecular weight of 266. It is relatively polar hydrophilic compound with a water solubility of 26.5 mg/mL at 35°C. Atenolol tablets, USP is available as 25, 50 and 100 mg tablets for oral intake. Inactive Ingredients include Magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycolate, hydroxypropyl methylcellulose, titanium dioxide and glycerin.

Metoprolol ((RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(propan-2-yl)amino]propan-2-ol) works on the same mechanism as atenolol. Other than the complications mentioned under atenolol, this drug can be recommended under cases of chest pain due to poor blood flow to the heart, or number of conditions involving an abnormally fast heart rate. It inhibits the beta 1 receptors present in the cardiac muscle's cells, causing both chronotropic and inotropic effect. It also prevents migraines after myocardial infarction. It also falls under the category of beta blocker and is administered by mouth or intravenous injection. It is commonly combined with a diuretic like hydrochlorothiazide to form a single tablet. Common side effects include trouble in sleeping, faint and tired feeling. Large quantity can be toxic and is advised to avoid during pregnancy and breastfeeding. Metoprolol should only be registered if the benefits outweigh the risks. Stopping the use of the drug should also be done slowly to avoid withdrawal syndrome and further complicate the health condition. made in 1969, patented in 1970, it came into market use by 1982. Generic drug that is listed on world health

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organization's list of essential medicines, in 2019, it was the 5th most prescribed drug in US.

Felodipine ((RS)-3-ethyl5-methyl4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) is a drug that works on the calcium channel blocker mechanism, used to treat high blood pressure. Used to treat high blood pressure and stable angina. It is not prescribed to pregnant women, people who have acute heart failure, people who are having a heart attack, those who having obstructed heart valve or have obstructions that block blood flow out of the heart. Because felodipine is cleared by the liver, lower doses are prescribed to people with liver failure. Common side effects include head ache, heart palpitations, dizziness and fatigue. Diseases like gingivitis can be exacerbated from felodipine.

Diltiazem,(cis-(+)-[2-(2-dimethylaminoethyl)-5-(4-methoxyphenyl)-3-oxo-6-thia-2-azabicyclo[5.4.0]undeca7,9,11-trien-4-yl]ethanoate) also known as Cardizem, is again a calcium channel blocker. It also finds its use in hyperthyroidism if beta blocker drugs can't be used. it can be administered both orally and by intravenous injection. When deployed in veins, the effect can be seen within minutes and also lasts for few hours. It falls under the drug category of Non dihydropyridine calcium channel blocker. Metabolized in liver, it is eliminated in approximately. The common side effects related to this drug include headaches, low blood pressure. Overly slow heart beat along with liver and heart problems are also witnessed in some cases. also, it is said to be avoided in pregnancy and breast feeding. The drugs cause relaxing of the smooth muscles in the walls of arteries, resulting in them opening and thus causing blood flow easier.

It is an important class of drug falling in the essential category because of the fact that it can prolong the period so that the heart can find its pumping rhythm again, which it causes by blocking calcium in the heart and blood vessels. It is a class iv antiarrhythmic drug. Approved for medical use in the US in 1982, it falls under generic medication. It is 72nd most prescribed drug in US by 2019, with more than 10 million prescriptions.

Other cardiovascular drugs are also considered for this review paper such as Amlodipine, Ramipril and Aliskiren etc. The method of analysis are recorded in this review paper.

II. METHOD OF ANALYSIS

The method of analysis opted for the project is called high performance liquid chromatography (HPLC). It was earlier known as high pressure liquid chromatography. It is an analytical technique which is used to separate, identify and quantify various components in a given mixture. The mixture added to a suitable solvent, also known as the mobile phase is passed through a column filled with solid adsorbent. Column is also known as the stationary phase. Various components in the mixture interact differently with the stationary phase i.e., the column. Due to these varied interactions produced in the column, there are variations observed in flow rates, thus causing separation of the components. The components being separated based on their interactions with the stationary phase column are keenly monitored and are converted to electrical signals within detectors. The detector used in HPLC are of various kinds.

The method involved can be sub divided into two according to the type of mixture obtained for segregation. Based on that, HPLC can further be divided into two chromatography systems.

i) Normal phase chromatography

When the mixture to be separated contains polar solute particles, then non-polar mobile phase is used to carry out the separation. This is named normal for the obvious reason as it follows the accepted norms.

ii) Reverse phase chromatography

When the mixture contains non-polar components, non-polar stationary phase with polar mobile phase is used. This is termed as reverse phase for the polarity is reversed from what we usually use. This gave to the name.

Principle

Basic chromatography involves mass transfer of mixture using a mobile phase over a stationary phase. The separation is caused by the resulting adsorption. HPLC involves various pumps that creates a pressure, leading to pressurized liquid passing through the stationary phase. The various components of the column, which mainly consists of long chains of carbon bonded along with silica, come in contact with the mobile phase which contains the mixture of components. Due to the different degrees of interaction of various components in the mixture, the components get separated. The interactions are physical in nature such as dipole-dipole interaction, hydrogen bonding, Vanderwall's forces etc. Mobile

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

phase consists of various chemicals depending upon its interaction with the adsorbate being quantified. Chemicals can be water, acetonitrile, methanol in various proportions.

Temperature also plays a very crucial role in the adsorption occurring in the column.it highly influences the interactions between absorbate and adsorbent. Therefore, many of the matrices involve columns temperature being raised to a certain degree.

With operational pressure being around 50 to 350 bar, it is said to be different than traditional chromatography. The traditional chromatography relies on gravity and adsorption on stationary phase which makes the separation possible. The amount of mixture taken in HPLC is very low and is very carefully monitored before being deployed in the system. Since the columns used in HPLC are having very low dimensions(2-50µm), it is seen that the components are thoroughly dissolved n no particle remains suspended in the analyzing mixture, filled in small transparent Vials.

Various components of an HPLC system

Degasser

Detector

It a device designed to remove gas from the mobile phase of the HPLC system. The degasser uses ultrasonic waves to remove traces of gas from the mobile phase. The bottle containing the mobile phase is mixed well and is kept in the degasser, also known as the sonicator. The ultrasonic waves are passed through and the gas bubbles are evolved. The solvent in the mobile phase is mixed thoroughly. The bubble formation is prevented in that case. Removing bubbles is of utter importance, because it brings the mobile phase below supersaturation level.

Solvent reservoir

It contains the solvent which is to be used a mobile phase. After being filtered and degassed, the solvent resides in the solvent reservoir in which the pumps are used to proceed in the liquid. The pumps are adjusted to different flow rates according to the sample being processed. The pumps are to be washed with the mobile phase in order to remove any particles from previous mobile phase and are dipped in them. The pumps are cleaned after every use and are maintained clean.

Sampler/ Auto sampler

It contains a machine with injection needle. The sampler is where the mixture to be separated is introduced in the mobile phase. The set volume of the injection is the amount of mixture that will be injected in the stream of mobile phase. The auto sampler is designed to take the requisite amount of the mixture and inject in in the mobile phase stream. In auto sampler, the needle pierces through the capped vial and takes the mixture.

Column

Column is the most important part of the HPLC system. The column is the part in which the separation of different components of the mixture. The sample passes through the column in which different components interact with the column. The difference in the rate of adsorption causes the separation. The columns are filled with silica. It not only has huge surface are with porosity which helps in separation, but also is inert. It doesn't interact with the components thus making it an ideal material for separation. There are basically three types of columns based on the interactions between and properties of the mobile phase and components being quantified. Liquid- liquid, liquid- solid, and ion exchange. The stationary phase in the liquid-liquid column is also liquid. These are not very popular due to less stability of liquid. In the liquid-solid columns, the solid stationary phase adsorbs and separates components. In ion-exchange columns, there is an ion-exchange resin which further helps in partitioning.

Detector – A detector is a device that detects the degree of absorbance shown by various components in the mixture. The degree of absorbance is reflected how slowly or how fast various components are ejected out of the column. Detectors are of various types. Most commonly used detectors in HPLC are UV detector and PDA detector. Ultra- violet detectors work by radiating UV light on the flowing mixture along with the mobile phase and absorbs the UV light. The Photo diode array works on a similar principle but it can radiate UV of variable range, thus obtaining a wider range of results. The differences in absorbance are recorded and converted into electrical signals. The electric signals are converted to a visible graph and is displayed on the screen. There are several other types of detectors as well which are found equally useful like refractive index detector (RI), fluorescence detector (FL), chemiluminescence detector (CL), optical rotation detector (OR), electrochemical detector (EC), conductivity detector (CD) etc. All of these detectors monitor separated components of the mixture are expressed electronically.

Another technology deployed for the analysis is Micellar liquid chromatography.

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Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

Micellar liquid chromatography or MLC is considered to be an effective and better suited version of high-performance liquid chromatography (HPLC). It is often regarded as the greener technique out of the two. The micelles added to the mobile phase introduces a pseudo stationary phase. Now in this pseudo stationary phase, the partition of the solutes is comparatively much smoother, reducing the need of an organic modifiers usually added to the mobile phase in HPLC.

Solvents involved are ethanol and isopropanol which are safer than usually used methanol and acetonitrile. The mobile phases are 90% or more water proves it to be much greener technique. It improves separation efficiency drastically. The wetting of the stationary phase is achieved in a better way, improving mass transfer between micelles and stationary phase.

Micellar liquid chromatography works when the mobile phase is altered using surfactants etc. The mobile phase is less toxic when compared to mobile phases of RP-HPLC. The mobile phase contains surfactant above it's critical micelle concentration (CMC). This not only produces more accurate results, but also proves to be greener than conventional HPLC system. It is cost effective. The interactions in the column are modified and also the organic solvent in the mobile phase is reduced. The mobile phase can later be recycled, given that the number of injections made during the analysis werr small and distant.

Micelles provide hydrophobic and electrostatic sites for interactions.

Mobile phases

Mostly hybrid micellar mobile phases are used for the analysis. They also contain micelles, surfactant monomers, organic solvent molecules as well as water.

One of the most peculiar feature of the mobile phase in MLC is that the mobile phase are 90% water or more. Thus being the most suitable for the process.

Features of Mobile phase

1. Low Critical micelle concentration is the required feature for the mobile phase. High CMC would involve greater amount of surfactant, resulting in increased viscosity of the mobile phase. This would further lead to enhanced interaction between the detector and the mixture, resulting in sensitive detection. The chromatograms would be displaying peaks along with the required peak. Most commonly used surfactants are SDS,CTAB and Brij-35 with the CMC's as $8.2*10^{-3}$, $9*10^{-5}$ and $9*10^{-4}$.
2. Krafft's point is the temperature at which solubility of the surfactant monomer becomes equal to CMC. This temperature should be maintained in order to avoid surfactant precipitation. The precipitation of the surfactant can result in degradation of the column, ruining column as well as the whole analytical process. Selecting the surfactant with Krafft's point below the room temperature is advised to avoid precipitation.
3. pH of mobile phase has a working range from 2.5 to 7.5. Various matrixes involve different pH range, depending of the type of separation being initiated.

Modified stationary phase

1. Surfactant adsorption- C18 columns which are alkyl bonded are deployed. Other columns used are C8 and cyanopropyl. The columns are affected by the adsorption of surfactant. This leads to the interaction of the mobile phase in a better suited way,. leading to better results.
2. Presence of organic solvent is necessary for efficient, sharp peaks and reduction in retention time. This is what comprises of hybrid micellar mobile phase.

MLC system- precautions and storage

1. Mobile phase saturation- Pure or hybrid micellar mobile phases contain high amounts of water, more or equal to 90% water.

Critical at 30°C, most ambient pH being at 6, but the working range may differ (2.5-7.5).

2. Column conditioning- Columns are stored in 100%MeOH. Before using the column, the columns are supposed to be washed with 100% water. At least 30 washes with water, followed by the mobile phase being used are supposed to be done before one work on the MLC system. These washes are necessary for removal of the components from last matrix, any undesirable particles from organic solvents etc.
3. Mobile phase flushing- Mobile phase is to be continuously flushed from the system.. Mobile phase should not interact with the stationary phase i.e. the column in an idle system. If mobile phase interacts with the column for a long time, it results in crystal formation, thus degrading the efficiency of the column. The mobile phases can be left overnight if the system is working but it is advised not to leave it otherwise. The mobile phase can be recycled

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

if the number of injections made were small and distinct.

Cleaning of the column

Columns are stirred in 100% MeOH and are supposed to be washed thoroughly before use.

The washes are to be initiated with water. Approximately 30 washes from the volume of column are to be made. After that, the washes are made with the mobile phase of the system.

This eliminates the undesirable components from previous runs made. The crystallization is also prevented in the column.

III. LITERATURE REVIEW

Compared to quantification of Atenolol in drug production, many methods have been developed in recent years. Lately, methods to identify atenolol in human plasma have been in rise. Besides that, quantifying atenolol in solution form, in diet form and in other biological fluids are popular methods showing incredible development. Methods like HPLC is already deployed at various levels, in analyzing atenolol. **1-6**

Besides that, liquid chromatography tandem mass spectroscopy, capillary electrophoresis and gas chromatography mass spectroscopy are also used.

Using blood spot collection card, sample of blood and plasma were collected for determination of atenolol. The method was progressed under high resolution liquid chromatography TOF mass spectroscopy (LC-HRMS). Atenolol was calculated in dried blood samples. The method was derived and validated. The assay turned out to be efficient and the method is being promoted to be an assay for atenolol retention. Since only 30µL of blood is required, it is a very useful and proficient method. **7**

As per a clinical trial done by scientists in 1998, 14 pregnant women who were consuming Atenolol on a regular basis, voluntarily enrolled themselves for this study. Out of 14, 10 were able to complete the study. The study was done in the hospital campus. The patients were monitored for 12 hours in third trimester and for 6 weeks after giving the birth to the child. The postpartum involved both infants and mothers heart rates to be monitored and kept under constant surveillance. The method of determining the action of atenolol on both child and mother was done by studying the relation between amount of atenolol consumed and the monitoring the heart rate of both. The results of this study proved that atenolol caused higher heart rates during pregnancy compared to postpartum stages. **8**

A simple yet sensitive method was developed, which relied on the quenching of Atenolol. This method is based on quenching of Atenolol on photoluminescence of gold nano particles at wavelength of emission at 705nm. The method developed was proposed to be used in the quantification of Atenolol because of its efficiency. **9**

Pulverized bone samples from human corpses were used in the determination of Bisoprolol and Atenolol. Method was based on gas chromatography- mass spectroscopy. The determination is carried at respective matrix effect, 61-69% for bisoprolol and 70% for atenolol. **10**

Metoprolol in human plasms was determined using HPLC method for limited blood volume. Limited blood volume refers to reduced volume of blood in a body. The body can either be suffering or an infant etc. The method proposed required 500 µL of blood sample. Chromatographic conditions involved Spheris orb C6 (Pour size-5µm). Fluorometric detection was achieved by excitation wavelength set as 225nm and emission wavelength of 310 nm. The mobile phase used contained 30% Acetonitrile and 70%of 0.25M potassium acetate buffer. PH was maintained at 4. This method was primarily developed for metoprolol quantification in pediatric patients, specifically for children around or below age of 2 years. **11**

An RP-HPLC method for estimation of metoprolol succinate in bulk and dosage form was done. The column used was 150nm*4.6nm with pour size of 5µm. The mobile phase used contained 75 parts of phosphate buffer at pH-3 and 25 parts of acetonitrile. The retention time for metoprolol succinate was found to be around 3.7-3.8 minutes. 12

Following is a table that records various drugs being quantified by RP-HPLC system.

DRUG	COLOUMN LENGTH	MOBILE PHASE	WAVELENGTH (nm)	RETENTION TIME (minutes)	REFERENCE

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

Atenolol	250*4.6mm,5µm	ACN:water 6:4	UV-270	1.3	**13**
Atenolol	250*4.6mm,5µm	Methanol: water 7:3	UV-226	5	**14**
Atenolol	250*4.6mm,5µm	10mMKH2PO4 :methanol 7:3	UV- 225	5	**15**
Atenolol	250*4.6mm,5µm	Phosphate buffer:ACN	UV- 230	2.1	**16**
Metoprolol succinate	250*4.6mm,5µm	50mM di-sodium hydrogen phosphate:Methanol:ACN (21:9:10)	UV- 222	5.3	**17**
Metoprolol	250*4.6mm,5µm	Phosphate buffer(pH-3):ACN (6:4)	UV- 262	4.7	**18**
Hydrochlorothiazide	250*4.6mm,5µm	50mM di-sodium hydrogen phosphate:Methanol:ACN (21:9:10)	UV- 222	3	**19**
Diltiazem	300mm*4.6, 5µm	Buffer:MeOH: ACN 50:25:25	UV- 240	-	**20**
cimetidine	250*4.6mm,5µm	MeOH:H3PO4: Sod,hex sulphonate 240mL:0.3mL:940mg	UV- 220	-	**21**
cimetidine	250*4.6mm,5µm	Buffer(sod,hex sulphonate in 780mL H20 +0.4 mL diethyl amine, pH=2.8)+250mL MeOH	UV- 220	-	**22**
Ranitidine	200*4.6mm,5µm	MeOH:Buffer (85:15)	UV- 322	-	**23**
Amlodipine	250nm*4mm, 3µm	60mM sodium phosphate buffer:ACN (20:80) (pH=2.6)	UV- 210	11.7	**24**
Ramipril	250nm*4mm, 3µm	60mM sodium phosphate buffer:ACN	UV- 210	9.3	**25**

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

		(20:80) (pH=3.6)			
Aliskiren	150nm*4.6nm,5 µm	Phosphate buffer: ACN (40:60) (pH=3)	UV- 237	3.98	**26**
Amlodipine	125nm*4.6nm, 5µm	0.01M sodium dihydrogen phosphate buffer: ACN (63:37) pH -3.5	UV- 239	-	**27**
Metoprolol	250nm*4.6nm,5 µm	MeOH:Water (1:1), 0.1%TFA	UV- 250-300	4.7 (approx.)	**28**
Atenolol	250nm*4.6nm,5 µm	Phosphate buffer5mM/L, pH -7	UV- 280	-	**29**
Amiloride hydrochloride	250nm*4.6nm,5 µm	Phosphate buffer5mM/L, pH -7	UV- 280	-	**30**
Hydrochlorothiazide	250nm*4.6nm,5 µm	Phosphate buffer5mM/L, pH -7	UV- 280	-	**31**
Metoprolol	50nm*4.6nm, 5µm	Buffer(0.06%OPA, .0045M Sodiun lauryl sulphate):ACN 1:1	UV-210	1.3	**32**
Atorvastatin	50nm*4.6nm, 5µm	Buffer(0.06%OPA, .0045M Sodiun lauryl sulphate):ACN 1:1	UV-210	2.1	**33**
Ramipril	50nm*4.6nm, 5µm	Buffer(0.06%OPA, .0045M Sodiun lauryl sulphate):ACN 1:1	UV-210	2.6	**34**
Meloxicam	250nm*4.6nm,5 µm	15mM Phosphate buffer: MeOH 4:6, pH- 4.7	UV- 357	-	**35**

Following are the researches and developments done in the recent times in field of drug assay using Micellar liquid chromatography.

MLC is used in determination of number of compounds in pharmaceutical industry, biological samples such as serum, urine, food analysis and environmental samples. 36

Many β-antagonist drugs such as Atenolol, Metoprolol etc. Were determined using micellar

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Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

liquid chromatography. The mobile phase used was 0.1M sodium dodecyl sulphate(SDS) with 15% propanol and 1%trimethylamine. The pH was maintained at 3. The samples of urine were detected using fluorometric detector. 37

Verapamil, an equally famous cardiovascular drug was quantified using a method developed. The method was for serum and urine samples without any pretreatment. Spectrofluorometric detection was done with excitation at 230nm and emission at 310 nm. Since verapamil is itself hydrophobic compound, use of SDS often lead to non-efficient analysis. Mobile phase contained 0.15M SDS and 5% pentanol maintained at pH7-. C-18 column with pH range of around 3-7 were the other chromatographic conditions involved. 38

following are the chromatographic conditions used for the analysis of cardiovascular drugs using MLC.

DRUG	COLUMN LENGTH	MOBILE PHASE	WAVELENTH (nm)	RETENTION TIME (minutes)	REFERENCE
Metoprolol	ODS-3 150*4.6mm,5µm	Buffer(0.15M SDS, 25mM Na2HPO4):1-butanol:TEA 93:6:1, pH-3	FLD Excitation-230nm Emission-311nm	3.6	39
verapamil	ODS-3 150*4.6mm,5µm	Buffer(0.15M SDS, 25mM Na2HPO4):1-butanol:TEA 93:6:1, pH-3		6.4	40
Furosemide	ODS-3 150*4.6mm,5µm	Buffer(0.15M SDS, 25mM Na2HPO4):1-butanol:TEA 93:6:1, pH-3	UV- 240	11.6	41
Atenolol	ODS-2 120*4.6mm, 5µm	0.1M SDS,15% propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	FLD Ex-225 Em- 300	3.2	42
Metoprolol	ODS-2 120*4.6mm, 5µm	0.1M SDS,15% propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	FLD Ex-225 Em- 300	6.9	43
Nodolol	ODS-2 120*4.6mm, 5µm	0.1M SDS,15% propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	FLD Ex-220 Em- 300	4.1	44
Propranolol	ODS-2 120*4.6mm, 5µm	0.1M SDS,15% propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	FLD Ex-225 Em- 338	12.2	45
Acebutolo	ODS-2	0.1M SDS,15%	FLD	5.5-5.7	46

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1886-1898 www.ijprajournal.com ISSN: 2456-4494

l	120*4.6mm, 5µm	propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	Ex-240 Em- 454		
Celiprolol	ODS-2 120*4.6mm, 5µm	0.1M SDS,15% propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	FLD Ex-240 Em- 484	6	47
Labetalol	ODS-2 120*4.6mm, 5µm	0.1M SDS,15% propanol,1% triethylamine, 0.02M phosphate buffer. pH -3	FLD Ex-210 Em- 434	11.8	48
Atenolol	C-18 250*4.6mm,5µm	0.07M SDS, Phosphate buffer, 15%1-propanol, pH-3	UV-225	6.6	49
1Hydrochlorothiazide	250*4.6mm,5µm	0.07M SDS, Phosphate buffer, 15%1-propanol, pH-3	UV-225	2.4	50
Metoprolol	ODS-3 150*4.6mm,5µm	100mM SDS, 20mM sodium dihydrogen phosphate buffer of pH-3, 10% n-butanol	FLD Ex-275 Em-303	-	51
Amlodipine	ODS-3 150*4.6mm,5µm	100mM SDS, 20mM sodium dihydrogen phosphate buffer of pH-3, 10% n-butanol	FLD Ex-364 Em-455	-	52

Several methods for estimating the partitioning of membranes in biological samples, thus interfering with biological activity of potential oral drug molecules. Studies have suggested that fast MLC on monolithic columns could be advancement in technology if it is channeled in a better suited way.

IV. FUTURE SCOPE

Every day, newly developed methods surrounding the analysis of drugs are being made. Since atenolol forms an important class of drug, its use is and quantification is an important matter. There are always efforts being made to find a more efficient method for drug analysis. Drug industry relies in proper quantification and administration of the drugs.

Now looking at HPLC system, HPLC has become an integral part of the human society when quantifying quantities in very small concentrations is necessary. Looking at the role HPLC plays in a food and pharmaceutical lab, it can be concluded that it is one of many methods of great importance. Methods where the use of solvent or the mobile phase are being developed, and some have been promoted. Methods like Micellar liquid chromatography, super critical liquid chromatography are being adopted in a much faster rate. These methods have been derived from the HPLC system and some of the advanced liquid chromatography's also use the same machines and setup as the HPLC.

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