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Review on Quantification of Some Selected Cardiovascular Drugs and Their Metabolites in Pharmaceuticals

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ABSTRACT

The researches on drugs are increased due to development in pharmacology and clinical science with the help of chemist that contribute the progress in medical science. The understanding of metabolism in terms biochemical action gave us the pathway and function of drugs as well as the structure of biological compound which can guided a new noble structures. The investigation on human blood and serum bring about the physiochemical properties of drug. The important factor is the interaction between the active pharmaceutical ingredients and excipients present on the desired pharmaceutical formulation products that can be keep in mind so that after taking the medicine does not have any side effects. A description of the classification of drugs based on pharmacological action on human organs is included and finally a brief literature and chemical structures of the four drugs, i.e., metoprolol tartrate, enalapril maleate, labetalol hydrochloride and amiodarone hydrochloride are presented.

Keywords: Cardiovascular drugs, Metoprolol, Enalapril, Labetalol, Amiodarone, UV-visible, HPLC, LCMS

INTRODUCTION

The main importance for the discovery as well as development of a pharmaceutical product needed a complex pathway which is leading through several scientific expertises to make quality that leads a successful drug. Therefore it is necessary for every company to select products that have more probability to achieve the target. The company has to be keeping in mind about the chemical, physical properties of drug product and active pharmaceutical product will approve by the regulatory authorities in terms of therapeutic action. All the drugs according to their chemical nature can be divided into organic and inorganic compounds. They can be prepared synthetically or reconstituted from natural sources product. All the drugs having medicinal importance can be broadly divided into two classes.

Chemical classification

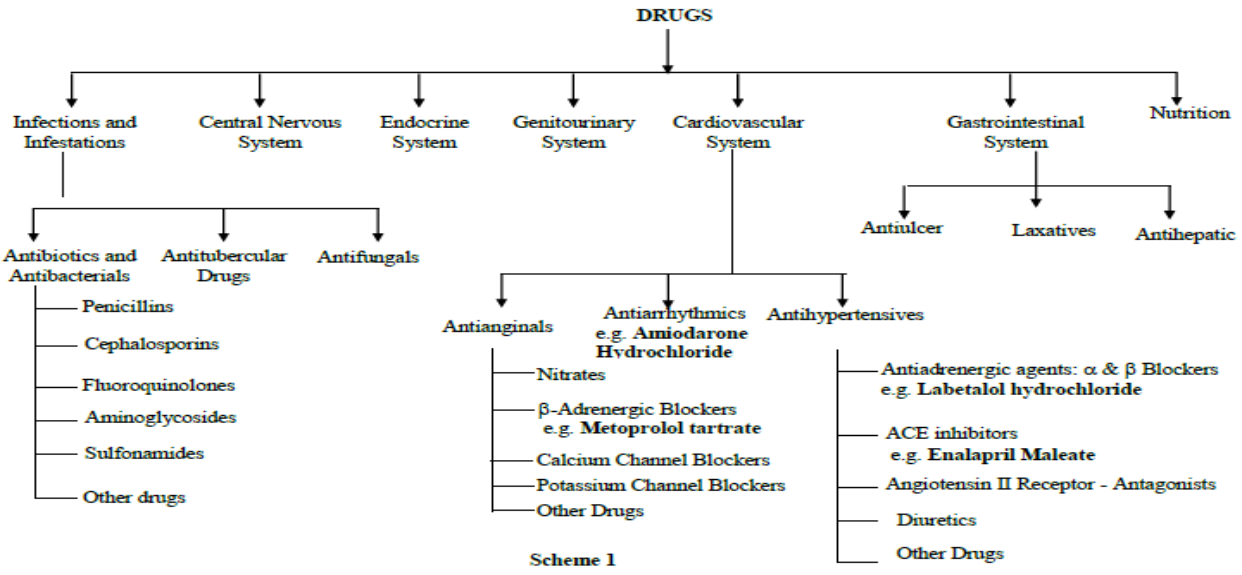
The drugs are classified according to their chemical structure and properties without taking the pharmacological action. In this class most of the drugs are having at least an organic substrate; further classification is done in the relevant manner.

Pharmacological classification

In this class the drugs are divided according to their action on the organism's organ (viz. heart, brain, lymphatic system, respiratory system, endocrine system, central nervous system etc.). Hence, these drugs are called cardiovascular, narcotics, analgesics, antibiotics, diuretics, and anesthetics etc. Further classification of each group is done according to the therapeutic/pharmacological specificity with the relevant organ. A detailed classification of drugs based on pharmacological action on human organs has been given in Scheme 1. This thesis deals with the determination of the cardiovascular drugs namely metoprolol tartrate, enalapril maleate, labetalol hydrochloride and amiodarone hydrochloride.

Metoprolol tartrate

Metoprolol tartarate, 2-Propanol,1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, [R-(R*, R*)]-2, 3-dihydroxybutanedioate (2:1) (salt) [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$, 56392-17-7, M.W. 684.81] is known as a cardioselective β-adrenergic receptor blocker. It is used for the treatment of hypertension, angina pectoris, supraventricular and ventricular arrhythmias and to reduce the frequency and intensity of migraine headache, among other applications. Administration of successive large doses might lead to some side effects such as cardiac depression, blockade of cardiac vascular or bronchial β-adrenoreceptor and visual disturbance. Metoprolol is administered as a racemic mixture. (S)-(-)-metoprolol has been reported to be significantly greater β-adrenergic receptor affinity by >25-fold than (R)-(-)-metoprolol [1].



Scheme 1: Pharmacological action on human organs

It undergoes extensive first pass metabolism with about 95% of the dose being metabolized in humans (Figure 1). The hydroxylated metabolite *α*-hydroxy metoprolol (OH-met) results from oxidation of the benzylic carbon atom of metoprolol by the cytochrome P-450 isozyme system in liver. There are marked interindividual differences in the pharmacokinetics partially due to the rate of drug oxidation by cytochrome P-450 isozymes. β-blockers (e.g. metoprolol, atenolol) are misused as doping agents in sports and hence these drugs have been added to the list of forbidden drugs by the International Olympic Committee [2].

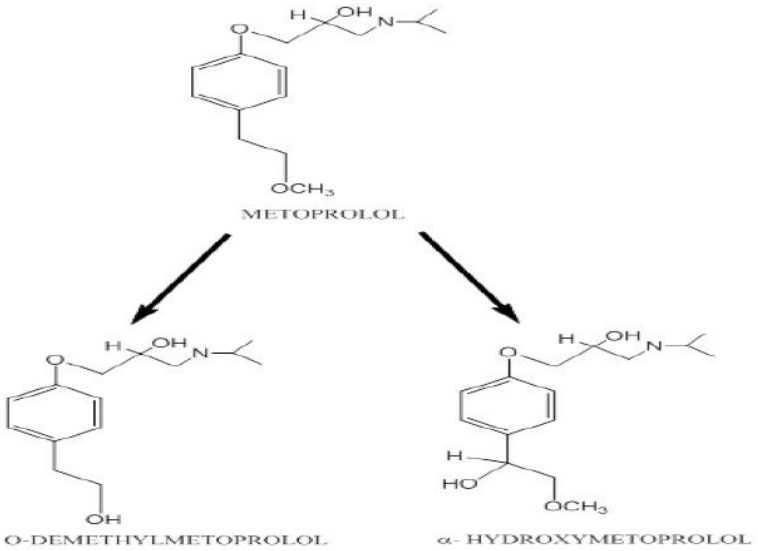


Figure 1: Major pathways of metoprolol metabolism in man

Enalapril maleate

Enalapril maleate ((S)-1-{N-[1-(ethoxycarbonyl)-3-phenyl-propyl]-L-alanyl}-L-proline, (Z)-2-butenedioate (1: 1) is a salt of enalapril and maleic acid. Its empirical formula is $C_{20}H_{28}N_2O_5.C_4H_4O_4$, and its structural formula is in Figure 2.

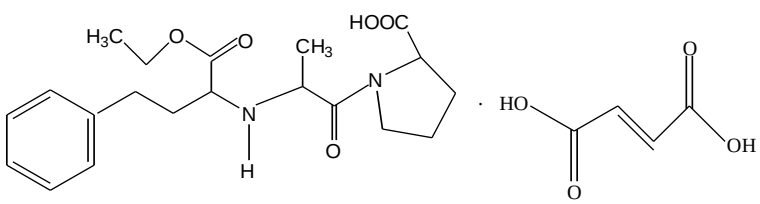


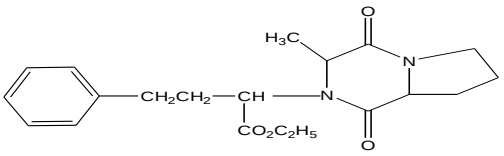
Figure 2: Structural formula of enalapril maleate

Enalapril maleate is a white to off-white, crystalline powder with a molecular weight of 492.53. It is sparingly soluble in water, soluble in ethanol, and freely soluble in methanol. Enalapril maleate is supplied as 2.5, 5, 10 and 20 mg tablets for oral administration.

In addition to the active ingredient enalapril maleate, each tablet contains the following inactive ingredients: lactose, magnesium state, starch, and other ingredients. The 2.5, 10 and 20 mg tablets also contain iron oxides. Enalapril is a pro-drug; following oral administration, it is bioactivated by hydrolysis of the ethyl ester to enalaprilat, which is the active Angiotensin Converting Enzyme Inhibitor (ACE). ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II, which stimulates aldosterone secretion by the adrenal cortex. Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decrease aldosterone secretion [3].

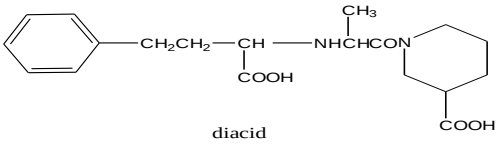
The enalapril is a widely used drug in clinical practice for various treatments such as arterial hypertension in all its grades, rennin-dependent hypertension and congestive cardiac insufficiency. In this last disease, this substance improves the symptoms, decreases the mortality and diminishes the frequency of patient hospitalizations.

On the other hand, it can be observed that enalapril is a very photosensitive drug and it degrades in an evident way through diketopiperazine (I) by dehydration process and enalaprilate diacid (II) by hydrolysis (Figure 3) [4]. These products of degradation increase with temperature and pH of solution. Therefore, there is a need to develop analytical methods for its quantitation in commercial dosage forms.



diketopiprazine

I



diacid

II

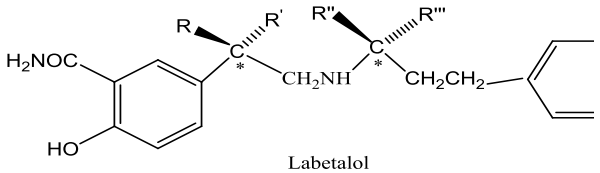
Figure 3: Hydrolysis of enalapril maleate after degradation

Labetalol hydrochloride

Labetalol hydrochloride is chemically known as 2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl]-benzamide hydrochloride (CAS No. 32780-64-6, M.W. 364.87). It is a white or almost white powder and soluble in water and alcohol; insoluble in dichloromethane and ether. A 1.0% solution (w/v) in water is clear and its pH varies between 4.0 and 5.0. Labetalol is unique drug with complex pharmacological properties exhibiting combined α_1 and β-adrenoceptor antagonist properties and partial β_2-agonist activity [5-7]. Labetalol, an antihypertensive, has been used in the management of pregnancy-induced hypertension or pre-eclampsia.

Labetalol has two asymmetric centers resulting in four stereoisomers, designated RR, SS, SR and RS (Figure 4). The drug is supplied as an approximately equicomponent mixture of all four isomers. Labetalol is a weak base (pKa=8.3) and is administered as the hydrochloride salt. Labetalol exerts reversible and competitive α_1- and β-blocking activity [8,9] and is approximately one- fourth as potent as propranolol in blocking β-receptors and one- tenth as potent as phentolamine in blocking α_1-receptors.

Labetalol is metabolized predominantly in the liver, the metabolites being excreted in the urine together with only small amounts of unchanged labetalol; its major metabolite has not been found to have significant α- or β-adrenoceptor blocking effect. Excretion also occurs in the feaces via the bile. The elimination half-life at steady state is reported to be 8 h. Following intravenous infusion, the elimination of half- life is about 5.5 h. Labetalol is not removed by dialysis.



Labetalol

	R	R'	R"	R"'
RR- isomer:	OH	H	H	CH_3
SS- isomer:	H	OH	CH_3	H
SR-isomer:	H	OH	H	CH_3
RS-isomer:	OH	H	CH_3	H

Figure 4: Structure of labetalol and proposed pharmacological activity of its 4 stereoisomers

β-Non-selective-β-adrenoceptor blocking activity, β_2-Selective β_2-adrenoceptor agonist activity, α_1-Selective β_1-adrenoceptor blocking activity, Denotes chiral center.

Amiodarone hydrochloride

Amiodarone Hydrochloride (AD) is a potent III antiarrhythmic drug and is chemically known as 2-butyl-3-benzofuranyl4-[2-(diethyl amino)ethoxy]-3,5-diidophenyl methanone hydrochloride. It is used to treat ventricular and supraventricular arrhythmias, especially when they are resistant to other conventional antiarrhythmic drugs [10,11]. However, its use is sometimes complicated by serious adverse effects, including occasionally life threatening pulmonary fibrosis and hepatitis. Amiodarone has a long serum elimination of 40-50 days, which was attributed to its huge distribution [12]. Therefore, the amiodarone concentrations in patients treated with therapeutic doses of the drug vary considerably and therapeutic drug monitoring of amiodarone may assist in individualizing the dosage regimen.

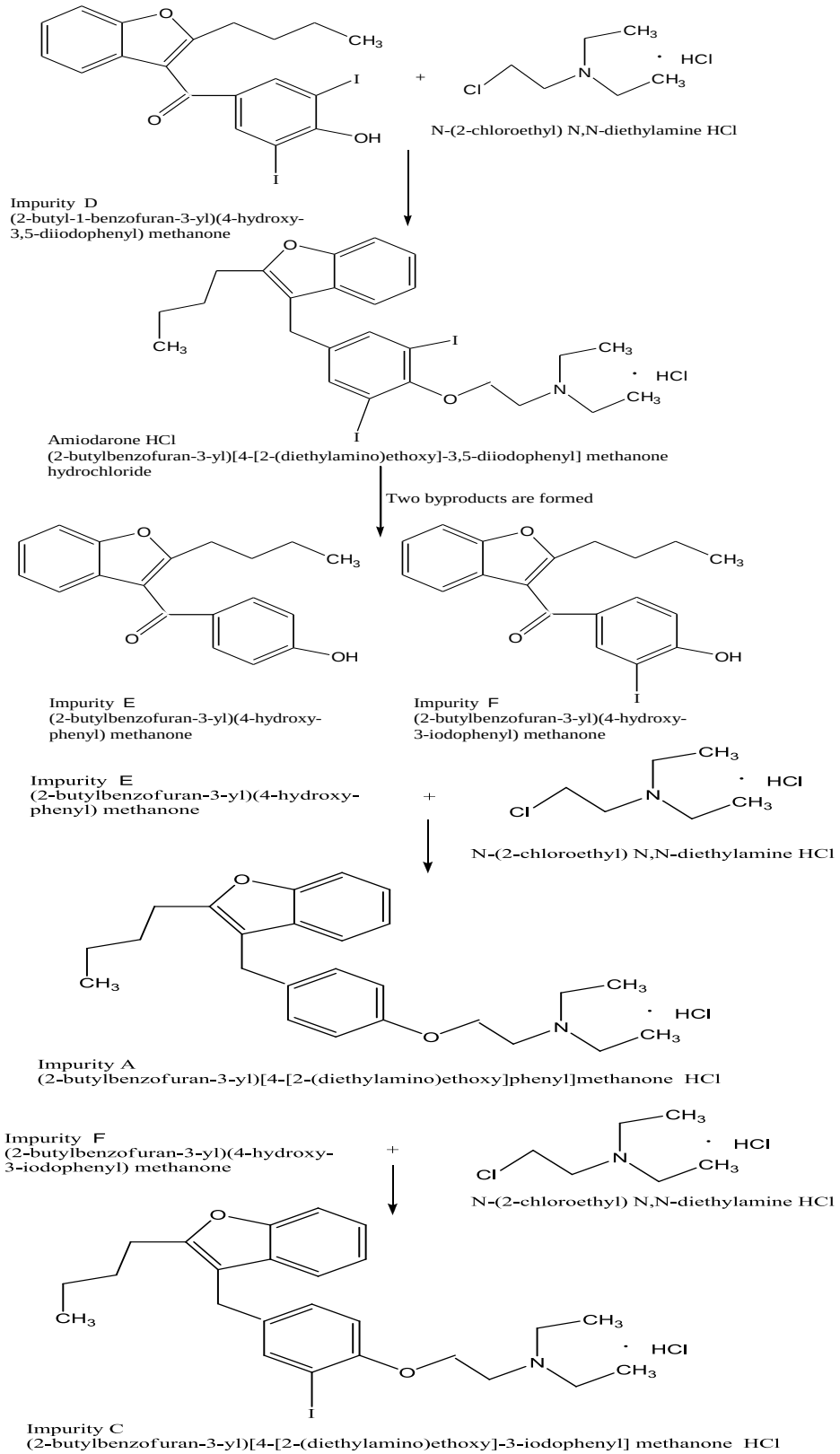


Figure 5: Proposed pathway for impurity substances of amiodarone hydrochloride

For the synthesis of AD, 2-butylbenzofuran-3-yl(4-hydroxy-3,5-diiodophenyl) methanone, a key intermediate, is condensed with N-(2-chloroethyl)-N,N-dietylamine Hydrochloride. The unreacted intermediate appears as impurity D ((2-butyl-1-benzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl) methanone) in the final product of AD (Figure 5). During the synthesis of the key intermediate two more byproducts are formed which appear as impurity E ((2-butylbenzofuran-3-yl)(4-hydroxy-phenyl) methanone) and F ((2-butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl) methanone). The impurity E and F may also condense with N-(2-chloroethyl)-N,N-diethylamine HCl to give impurity A ((2-butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]phenyl]methanone HCl) and C((2-butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]-3-iodophenyl] methanone HCl). The authentic standards of impurity D and E are commercially available.

Analytical techniques

UV-Visible spectrophotometer

The spectrophotometric methods have been widely used for the determination of drugs.

The main advantage of this technique is low cost and available in research laboratory, pharmaceutical industry and hospitals. The uses of UV-Vis spectrophotometry for the analysis pharmaceutical formulation are increased due to its high precision and simplicity. The spectrophotometry has high demand for qualitative as well as quantitative determination of drugs. The method does not require any pretreatment step prior to analysis. The disadvantage for this technique, sometime it is showing identical spectra as drug sample during the analysis of formulation products due to degradation products or may be from the excipients. Visible spectrophotometric method is carried out in the region of visible light. The methods are based on oxidation reduction process, complex formation and catalytic reaction. The absorbance of colored product is measured for the analysis using visible spectrophotometry. Representative examples of UV-visible methods for the analysis of metoprolol, enalapril, labetalol and amiodarone that have been published [13-37] are given in Table 1.

Table 1: UV–Visible techniques used for determination drugs

Name of drug	Reagents used	λ_{max} (nm)	Reference
Metoprolol	$KMnO_4$ in alkaline medium	610	[13]
	Ninhydrin	595	[14]
	1 -chIoro-2,4-dinitrobenzene	420	[15]
	-	209.5	[16]
	Cu (II)	675	[17]
	-	204	[18]
Enalapril	p-chloranilic acid	534	[19]
	Picric acid	370	[19]
	Bromocresol green	412	[19]
	$KI + KOI_3$	352	[20]
	$KMnO_4$	550	[21]
	Bromocresol purple	408	[22]
	Bromophenol blue	414	[22]
	-	208	[23]
	-	267	[24]
	-	206.8	[25]
Labetalol	4-aminobenzenesulfonic acid	395	[26]
	Fe^{+3} + 1,10-phenanthroline	510	[27]
	$KMnO_4$	605	[28]
	Ferric ammonium sulphate	535	[29]
	-	245.3	[30]
	-	246	[31]
	Potassium permanganate+ magnesium sulphate	520	[32]
Amiodarone	p-Chloranilic acid	535	[33]
	2,3-dichloro-5,6-dicyano-1,4-benzoquinone	575	[33]
	-	263.2	[34]
	p-N-methyl amino phenol sulphate-sulphanilic acid	520	[35]
	Potassium thiocyanate	465	[35]
	Cobalt nitrate-ethylene diamine tetraacetic acid disodium salt complex	840	[35]
	Tropaeolin 00	420	[36]
	Tropaeolin 000	480	[36]
	Wool fast blue	580	[36]
	Cobalt thiocyanate	620	[36]
	N-bromosuccimide	353	[37]
	Bromothymol blue	400	[37]

High performance liquid chromatography

The chromatography technique is based on the separation of compounds between stationary phase and mobile phase. Different modes of chromatography are developed depending on the interaction of solute with stationary phase. The interaction is due to hydrogen bonding, hydrophobic forces and Vander walls forces. In HPLC, two different types of column can be used namely reversed and normal phase. The non-polar and moderately polar drugs can be separate with help normal phase chromatography (Table 2). The separation of polar drugs is excellent using non polar column in reverse phase chromatography.

The common stationary phase used for the analysis of drugs is C18 ODS column in high performance liquid chromatography. The advantage is most pharmaceutical products are soluble in water because polar in nature. The HPLC technique is applicable for quantitative analysis, stability, degradation studies for bulk drugs and their formulated products [38-65].

Table 2: Important parameters for the determination of drugs and metabolites using HPLC

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Detector	Application	Reference
Metoprolol	Acetonitrile:methanol:0.5% acetic acid: triethylamine (56:18:26:0.1 v/v)	ODS C18 (25 × 4.6 mm)	1	UV 280 nm	Pharmaceutical formulations	[38]
	Acetonitrile–water–triethylamine 18:81:1 (v/v)	250 × 4 mm, 10 µm particle, Novapack C18 column	1	UV 275 nm	Human Plasma	[39]
	0.02 M phosphate buffer solution: acetonitrile (70:30 v/v, pH 3.0)	Kromasil C18 (250 × 4.6 mm, 5 µm)	1	UV 221 nm	Marketed formulation	[40]
	Methanol-water (50:50 v/v) containing 0.1% TFA (v/v)	Phenomenex C18 column with particle size of (250 × 4.6 mm, 5 µm) with a guard column (4 × 3 mm i.d., Phenomenex)	1	Fluorescence detection at 276 (excitation) and 296 nm (emission)	Commercial metoprolol dosage forms	[41]
	Potassium phosphate buffer and methanol (60:40 v/v)	Inertsil ODS-3, 250 mm, 4.6 mm ID, 5 µm	1	UV 226 nm	Oral dosage form	[42]
	0.03 M phosphate buffer and acetonitrile in the ratio of 32: 68 (pH 3.5).	RP stainless steel column ODS C18 250 × 4.6, 5 µm	1.2	UV 230 nm	Tablet	[43]
	Methanol, acetonitrile and 0.05 M phosphate buffer (adjusted to pH 4.5 with ortho-phosphoric acid) at a ratio of 60:20:20 v/v/v	C18 (250 × 4.6 mm, 5 µm)	1	UV 254 nm	Tablet dosage form	[44]
	Acetonitrile and Phosphate buffer (35 : 65 v/v)	4.6 × 250 mm column Luna 5U C18, 100 A, Phenomenex ODS, 5 µm	1.5	UV 226 nm	Pharmaceutical preparation	[18]
	55% methanol, 45% of 12.5 mM potassium dihydrogen phosphate (KH_2PO_4) aqueous solution, adjusted pH 7.0 with 0.2 N sodium hydroxide (NaOH) solution and 0.3% (v/v) trimethylamine.	ACE 5 C18 column (150 × 4.6 mm, 5 µm)	1	UV 205 nm	Bulk powder	[45]
Enalapril	Acetonitrile–phosphate buffer (pH 6.5) (50:50 v/v)	Luna C18 column	1	UV 215 nm	Tablets	[46]
	Phosphate buffer: Acetonitrile (80: 20) v/v pH 3.4 adjusted with orthophosphoric acid	C18 reversed-phase column, waters associate 3.9 i.d × 150 mm.	1	UV 265 nm	Human plasma	[47]
	Acetonitrile: water (pH 4.7 adjusted with ortho phosphoric acid) in the ratio of (25:75% v/v)	C18 reverse phase column (Hi-Q 250 × 4.6 mm ID; particle size 5 µm)	1.2	UV 220 nm	Pure and its Pharmaceutical Dosage form	[48]
	Buffer solution and Acetonitrile (40:60)	Hypersil MOS, 5 µ (250 mm × 4.6 mm)	1.5	UV 215 nm	Pharmaceutical formulation	[49]
Labetalol	5 mM acetate buffer (pH 4.5)–acetonitrile (70:30, v/v)	Base deactivated silica and an alkylamide bonded reverse phase C18 Precolumn	1	Electrochemical detector	Urine	[50]
	0.05 M phosphate buffer/acetonitrile of pH 4 (7:3, v/v)	Microbondapak C18 column (4.6 i.d. × 250 nm)	0.7	UV 302 nm	Spiked human plasma	[51]
	$H_2PO_4^-$ -HPO_4^{2-} buffer pH 7 and methanol, 49:51 (v/v)	Nova-Pak C18 column (4 m,3.9 mm 150 mm)	1	UV 250 nm	Powder	[52]
	Acetonitrile-phosphate buffer pH 3.8 with gradient	C18 (250 mm × 4.6 mm, 5 µm)	1	UV 220 nm	Human plasma	[53]
	Methanol: water (70:30, v/v)	C18 Column (4.6 × 250 mm; 5 µm)	1	UV 246 nm	Pure and tablet form	[54]
	Phase-A 0.1% TFA (v/v) in 1000 ml of water and phase-B composed of 0.1% TFA (v/v) in 1000 ml of ACN: methanol (1:1)	Zorbax Eclipse Plus C18 (100 × 4.6 mm, 3.5 µm)	1	UV 230 nm	Tablet dosage forms	[55]
Amiodarone	25 mM KH_2PO_4: 3 mM sulfuric acid: 3.6 mM trimethylamine (63:12:25, v/v/v)	Nova Pak C8 (100 × 8 mm i.d.)	1.5	UV 242 nm	Rat plasma	[56]
	Acetic acid/ammonia buffer solution pH 4.9, methanol, acetonitrile (30:30:40 v/v/v)	Hypersil BDS C18 (150 × 4.6 mm, 3 µm)	1	UV 240 nm	Commercial formulations	[57]
	Acetonitrile : 10 mM ammonium acetate pH 3.5 (60:40, v/v)	Luna C18 (250 × 4.6 mm, 5 µm)	1	UV 242 nm	Human Plasma	[58]
	Buffer solution pH 5.0, methanol, acetonitrile (30:30:40, v/v/v)	C18 column (5 µm, 150 × 4.6 mm)	1	UV 240 nm	Tablet	[59]
	Acetate buffer-Acetonitrile (15:85 v/v)	C2 column (4.6 × 250	1	UV 240 nm	Pharmaceutical	[60]

		mm, 5.0 µ)			formulations	
	Acetonitrile: 0.5% Triethylamine buffer pH 6.5 (75:25, v/v)	C18 Column (4.6 × 150 mm; 5 µm)	2	UV 240 nm	Pharmaceutical formulations	[61]
	Phosphate buffer (50 mM) with 0.1% formic acid pH 3.1–methanol–acetonitrile (45:5:50, v/v/v)	LiChroCART Purospher Star (C18; 55 ×4 mm; 3 mm)	1.2	UV 254 nm	Rat Plasma and Tissues	[62]
	Formic acid 0.5% in phosphate buffer solution pH 7.6 and methanol (25:75, v/v)	Hypersil Betasil C18, 150 × 4.6 mm; 5 µm	0.7	UV 254 nm	commercial tablets	[63]
	Acetonitrile: Triethylamine buffer pH 6.5 (75:25, v/v)	Hypersil BDS C18 (150 × 4.6 mm, 5 µm)	2	UV 240 nm	Pharmaceutical dosage form	[64]
	Potassium dihydrogen phosphate buffer with 0.1% triethylamine pH 6–methanol (10:90, v/v)	BDS Hypersil C18 (150 × 4.6 mm, 5 µm)	2	UV 254 nm	Pharmaceutical preparations	[65]

Liquid and gas chromatography with mass spectrophotometry

Liquid and Gas Chromatography-Mass Spectrometry (LC-MS, GC-MS) are analytical techniques that combines liquid chromatography and gas chromatography with mass spectrophotometer respectively. The impurity is developed during in the synthesis process or from the raw material so it is very important to quantify with lower limit at the finished product. MS is a powerful technique with high sensitivity and selectivity. The techniques are commonly applicable for the pharmacokinetics studies as well as bioavailability (Table 3). Applications of these techniques are in the field include drug detection, environmental analysis, explosives investigation, identification of unknown samples and biological fluids. The LC/MS and GC-MS are used for the determination of metoprolol, enalapril, labetalol, amiodarone and their metabolites in bulk, pharmaceutical formulations and biological samples [66-90].

Table 3: Determination of drugs using LC/MS and GC-MS

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Application	Reference
Metoprolol	1% acetic acid solution/acetonitrile (4:1, v/v)	LiChrospher RP 5 µm C8 (125 x 4 mm)	1	Powder	[66]
	10mM ammonium formate–methanol (3:97, v/v)	Phenomenex C8 (50mm×3mm, 3µm)	1	Human plasma	[67]
	Methanol-water containing 0.2% formic acid (65:35, v/v)	XB-C18 column (150 × 2.1 mm ID, 5 µm)	0.2	Beagle dog plasma	[68]
	0.2% formic acid in water–acetonitrile (25:75, v/v)	C18 column (50×4.6mm, 3 mm)	0.7 – 1.5	Rat plasma	[69]
	10 mM ammonium acetate - acetonitrile (10:90 v/v)	Unison US C18 column (50 x 4.6mm, 3.5µm)	0.8	Human plasma	[70]
	15 mM ammonium acetate, pH 5.0 and 0.1% (v/v) diethyl amine in acetonitrile (50:50, v/v).	Phenomenex LuxAmylose-2 (250mm×4.6 mm,5 mm)	1	Human Plasma	[71]
	0.1% ammonium hydroxide in hexane–isopropanol (80:20, *v*/v)	Cellulose-SB column (150 × 4.6 mm, 5 µm)	0.8	Plasma and saliva	[72]
	Helium	Agilent HP-5 MS column with 0.25 µm film thickness (30 m × 0.25 mm)	1	Human urine	[73]
Enalapril	2 mM ammonium formate buffer , pH 2.4: acetonitrile (67:33, v/v)	Waters Xterra C18 column (3.5µm, 2.1×100 mm)	0.3	Human plasma	[74]
	0.1% formic acid in ACN (Solvent A) and aqueous 0.1% formic acid solution (Solvent B)	Zorbax SB C18 (50mm×4.6mm, 1.8 micron)	0.8	Human plasma	[75]
	Methanol :Acetonitrile:Water: Formic acid (70:20 :10: 0.01 %v/v)	Phenomenex C18, 5µm, 50×4.6 mm	1	Human serum	[76]
	Phase A (0.1 % formic acid in water) and phase B (acetonitrile: methanol, 3:1v/v)	Thermo Scientific C18 (250 mm ×2.1 mm, 5 µm)	0.3	River water	[77]
	Methanol: water: formic acid 74:24:2 (v/v/v)	Zorbax XDB ODS C18 column (2.1mm×30mm, 3.5 µm)	0.2	Human plasma	[78]
	2 mM ammonium formate buffered with formic acid (1%, v/v) and methanol (60 : 40, v/v)	XBridge BEH C18 3.5 *µ*m column (3.0mm × 150 mm)	0.4	Biological fluid	[79]
	Methanol: water: formic acid 74:24:2 (v/v/v)	Zorbax XDB-ODS C18 (2.1mm×30mm, 3.5 micron)	0.2	Human plasma	[80]
	Phase A (0.1% formic acid in methanol) and phase B (0.1% formic acid in	Phenomenex kinetex C18 (50 mm × 3 mm, 5 µm)	0.5	Human plasma	[81]

	water).				
	Helium	5% phenyl methylpolysiloxane non polar (30m×0.25, 0.25µm)	0.8	Human plasma	[82]
Labetalol	Acetonitrile and ammonium acetate (10 mmol/l, 0.1% formic acid) with gradient elution	Genesis C18 (4µm, 100 x 2.1 mm)	0.2	Human urine	[83]
	Acetonitrile-phosphate buffer pH 3.8 with gradient	C_{18} (250 mm x 4.6 mm, 5 µm)	1	Human plasma	[53]
	2 mM ammonium formate (pH 5.0) / methanol (20:80 v/v)	Phenomenax Luna C18 (5µm, 100 x 4.6 mm)	0.5	Human plasma	[84]
	Solvent A 0.1% formic acid, and solvent B 0.1% formic acid in acetonitrile	UPLC BEH C18 (1.7 µm, 2.1×50mm)	0.4	Human liver microsomes	[85]
Amiodarone	0.1% formic acid and acetonitrile (80:20)	ODS Hypersil Gold column (5µm, 100 mm × 2.1 mm)	0.2	Horse plasma and urine	[86]
	Methanol and 0.2% aqueous formic acid	C 18 (3.5 µm, 2.1 x 50 mm)	0.2	Rat plasma	[87]
	Gradient mixture of acetonitrile and water (both containing 0.02% formic acid)	Capcell C18 (50mmx2.0mm,5microm)	0.3	Human plasma	[88]
	97.5%/2.5% water/methanol containing 0.1% formic acid and 2 mmol/L ammonium acetate for 1.0 min, followed by 100% methanol containing 0.1% formic acid and 2 mmol/L ammonium acetate	Phenomenx (2 mm×20 mm, 2.5 µm)	0.8	Human plasma	[89]
	Phase A (water acidified with 0.1% formic acid) and phase B (methanol acidified with 0.1% formic acid) with gradient elution	Prodigy Phenyl-3 (5 µm, 2.0-mm i.d.×100 mm)	0.2 – 0.4	Human whole blood	[90]

CONCLUSION

The main objective of this review is to provide an instant background of the drug metabolism and application of different analytical techniques/methods used in the area of drug analysis. This review allows researchers to find information of analytical techniques that are commonly used in the analysis of some cardiovascular drugs.

Additionally our aim with the help of this review, that the chemist, analyst, student who are going to do work in the area of drug analysis, they will develop the skills necessary to analyze the pure drug content and in pharmaceutical formulations and biological fluids. The UV-Visible spectrophotometer is simple and low cost method. But it has some restriction for the trace analysis as well as for the biological fluids sample because the analyte are present with very less concentration in the matrix. Then the HPLC combined mass spectrophotometer is very important techniques for the determination. The gas chromatography with mass spectrophotometer is very accurate and sensitive technique for the determination of drugs in pharmaceutical formulation as well as in plasma and serum samples. So we can conclude that the present review will give an idea for the analysis of drugs and its metabolites using UV-Visible, HPLC, LC/MS and GC-MS.

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