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Review Article

Simultaneous Analysis of Anti-Hypertensive Drugs by RP-HPLC: An Overview

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Abstract

High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. In now a day pharmaceutical market, many newer antihypertensive combination drugs are available to control hypertension as well as to keep society healthy and stress free. All presently available old drugs have frequent dosing produces various side effects. So, there is need to analyse such antihypertensive drugs. The aim of this review is to analysed such commonly used antihypertensive combination drugs by using reverse phase high performance liquid chromatography (RP-HPLC). Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase. The drugs like Amlodipine besylate and Valsartan, Irbesartan and Hydrochlorothiazide, Atenolol and Chlorthalidone, Hydrochlorothiazide and Candesartan, Ramipril and Amlodipine, Hydrochlorothiazide and Enalapril, Quinapril and Hydrochlorothiazide etc. About fifteen combination drugs are analysed by using RP-HPLC. This review assists in appropriate selection of column, mobile phase, pH, flow rate, detector and form of such combination drugs.

Keywords: Simultaneous Analysis; Antihypertensive; Combination; RP-HPLC

Introduction

High performance liquid chromatography (also known as high pressure liquid chromatography) is a type of column chromatography used to separate, identify, and quantify active ingredients in biochemistry and analysis [1].

HPLC mainly utilizes a column that holds packaging material (stationary phase), a pump that moves the mobile phase through the column and a detector that shows the retention time of the molecule. Retention time varies depending on the interaction between the stationary phases the molecule being analysed, and the solvent used [2].

A known amount of the material to be analysed is added to the mobile phase stream and evaluated by a chemical or physical interaction with the stationary phase. The amount of retardation is determined by the type of the analyte as well as the stationary and mobile phase composition. Retention time is the time it takes for a certain analyte to elute (come out of the end of the column). Any miscible combination of water and organic liquids is the most common mobile phase utilised (the most common are methanol and acetonitrile).

Gradient elution is used to change the mobile phase composition during the study [3].

Types of HPLC

The phase system employed in the process determines the type of HPLC [3,4]. The following HPLC types are commonly used in analysis.

Normal phase chromatography

This approach separates analytes based on polarity and is also known as Normal phase HPLC (NP-HPLC). A polar stationary phase and a non-polar mobile phase are used in NP-HPLC. The polar analyte interacts with the polar stationary phase and is retained by it. As the polarity of the analyte rises, so does the adsorption strength, and the interaction between the polar analyte and the polar stationary phase lengthens the elution time.

Reversed phase chromatography

Reversed phase high performance liquid chromatography (RP-HPLC) consists of a non-polar stationary phase and a moderately aqueous polar mobile phase. RP-HPLC works on the principle of hydrophobic interactions, the non-polar stationary phase is formed by repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar eluent. When the analyte molecule associates with the ligand in the aqueous eluent, the contact surface area around the non-polar segment of the analyte molecule is proportional to the contact surface area around the non-polar segment of the analyte molecule.

Size exclusion chromatography

Size Exclusion chromatography, also known as gel permeation chromatography or gel filtration chromatography, is a type of chromatography that separates particles based on their size. It can also be used to figure out the quaternary and tertiary structures of proteins and amino acids. This method is often used to determine the molecular weight of polysaccharides.

Ion exchange chromatography

Ion-exchange chromatography (IEC) depend on the attraction between solute ions and charged sites bound to the stationary phase. The ion exchange chromatography is mainly used for the purification of water.

Bio-affinity chromatography

In this method separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands. Proteins bound to a bio affinity column can be eluted in two ways:

- **Biospecific elution**: Inclusion of free ligand in elution buffer which competes with column bound ligand.
- **A specific elution:** Change in pH, salt, etc. which weakens interaction protein with column-bound substrate.

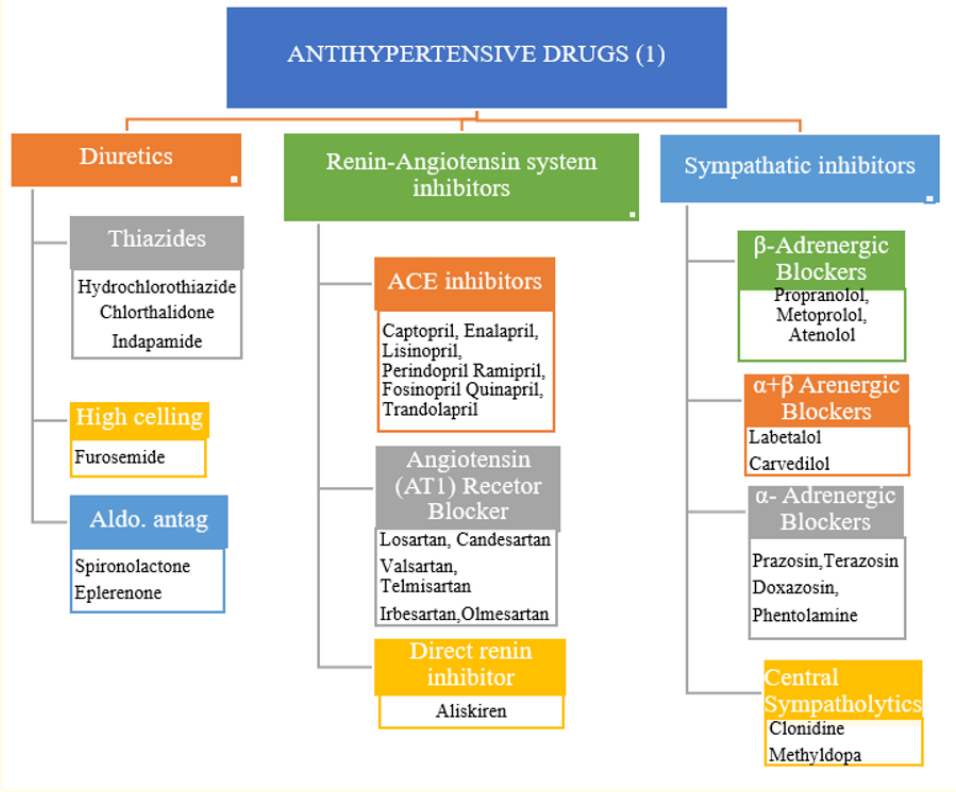
Hypertension

Hypertension is a very common disorder, particularly past middle age. It is not a disease in itself, but is an important risk factor for cardio-vascular mortality and morbidity. The cut-off manometric reading between normotensive and hypertensive is arbitrary. For practical purposes 'hypertension' could be that level of BP at or above which long-term antihypertensive treatment will reduce cardiovascular mortality. The JNC 7*(2003) and WHO-ISH@ guidelines (2003) have defined it to be 140 mm Hg systolic and 90 mm Hg diastolic, though risk appears to increase even above 120/80 mm Hg. Epidemiological studies have confirmed that higher the pressure (systolic or diastolic or both), greater is the risk of cardiovascular disease. Majority of cases are of essential (primary) hypertension, i.e., the cause is not known. Sympathetic and renin-angiotensin systems may or may not be overactive, but they do contribute to the tone of blood vessels. Many antihypertensive drugs interfere with these regulatory systems at one level or the other. Antihypertensive drugs, by chronically lowering BP, may reset the barostat to function at a lower level of BP [5].

As per the 2012 WHO report 1 out 4 adults i.e., 1.13 billion are suffering from hypertension worldwide and that causes 50% of death due to stroke and heart disease.

The combination therapy also reduces the risk of cardiovascular complications due to the rapid control of targeted BP further, the use of two or more drugs in lower dose instead of higher dose of single drug also reduces the side effects related with high dose of one drug [6].

Classification of Antihypertensive Drugs [5]



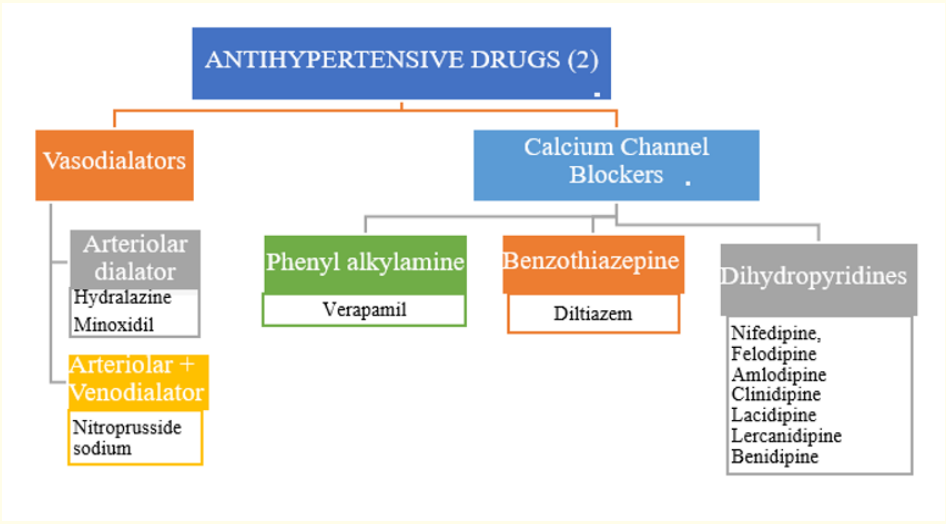


Figure 1

Antihypertensive combination drugs [7]

Sr. No	Drug name	Brand name	Approved year
1)	Amlodipine besylate and Valsartan	Exforge	2010
2)	Irbesartan and Hydrochlorothiazide	Avalide	1998
3)	Atenolol and Chlorthialidone	Tenoretic 50	1998
4)	Hydrochlorothiazide and Condesartan	Atacand HCT	2000
5)	Ramipril and Amlodipine	Car-Race AM	2010
6)	Hydrochlorothiazide and Enalapril	Lepril-H	2013
7)	Quinapril and Hydrochlorothiazide	Accuretic	1999
8)	Telmisartan and Hydrochlorothiazide	Teliska-H	2000
9)	Azilsartan medoxomil and Chlorthalidone	Azilsmart	2011
10)	Captopril and Hydrochlorothiazide	Captopril-H	1984
11)	Hydrochlorothiazide and losartan potassium	Luthozide-H	2003
12)	Amlodipine and Atenolol	Amlopres-AT	1996
13)	Trandolapril and Verapamil	Calaptin	1998
14)	Hydrochlorothiazide and Triamterene	Ditide	1965
15)	Hydralazine Hydrochloride and Isosorbide Dinitrate	Isorus	2005

Table 1: Antihypertensive combination drugs with brand name and approved year.

Amlodipine besylate and Valsartan:

Amlodipine besylate, 3-ethyl 5-methyl [4RS]-2-[[2-aminoethoxy] methyl]-4-[2-chlorophenyl]-6-methyl-1, 4-Dihydropyridine -3, 5-dicarboxylate benzene sulphonate, It is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris. Amlodipine besylate inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle.

Valsartan N-[p-[o-1H-tetrazol-5-ylphenyl] benzyl] –N-valeryl-L-valine is an orally active, potent and specific competitive angiotensin II antagonist acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardiovascular system. Valsartan is widely used in the treatment of hypertension [8].

Sr. No	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	RP-HPLC	C_{18} (150×4.6 mm,5µm)	Methanol: Potassium dihydrogen phosphate buffer (60:40 v/v) pH-2.5 Flow rate-1 ml/min	λ 238 nm	Tablet	[8]
2)	RP-HPLC	Phenomenex luna C_{18} (250×4.6) mm,5µm	Acetonitrile: water (75:25 v/v) pH-4.8 Flow rate-0.8 ml/min	λ 245 nm	API	[9]
3)	RP-HPLC	XTerua RP C_{18} (150×4.6) m,5µm	Water: Acetonitrile: Trifluoro acetic acid (40:60:10 v/v) pH-4.2 Flow rate-1.5ml/min	λ 237 nm	API	[10]

Table 2: Analysis of amlodipine besylate and valsartan by RP-HPLC.

Irbesartan and hydrochlorothiazide

Irbesartan, 2-butyl-3({4- [2- (2H-1, 2, 3, 4-tetrazol-5yl) phenyl] phenyl} methyl) 1, 3-diazaspirol [4, 4] non-1-en-4-one, it is mainly used for the treatment of hypertension. IRB is an angiotensin II Type 1 receptor antagonist that is highly selective for Type 1 angiotensin II receptor. Angiotensin II is the main presser agent for angiotensin system with the effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium.

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4-benzothiadiazine 1, 1-dioxide, Irbesartan and hydrochlorothiazide are used jointly to lower blood pressure. Irbesartan controls high blood pressure (hypertension) by relaxation of blood vessels. Thiazide affects the renal tubular mechanisms of electrolytes reabsorption, directly increasing excretion of sodium salt and chloride in approximately equivalent amounts. The combination is useful in the treatment of mild-to-moderate hypertension [11].

Sr.No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Irbesartan and Hydrochlorothiazide	RP-HPLC	C_{18} (250×4.6mm, 5µm)	Potassium dihydrogen phosphate: acetonitrile (55:45 v/v) pH-2.5 Flow rate-1.3ml/min	λ 210 nm	API	[11]
2)	Irbesartan and Hydrochlorothiazide	RP-HPLC	Hypersil pack BDS C_{18} (250×4.6mm,5µm)	Acetonitrile: buffer (sodium acetate anhydrous) (55:45 v/v) pH-3.5 Flow rate-1ml/min	λ 260 nm	API	[12]
3)	Irbesartan and Hydrochlorothiazide	RP-HPLC	C_{18} (250×4.6mm, 5µm)	Phosphate buffer: Acetonitrile (60:40 v/v) pH-6.4 Flow rate-1ml/min	λ 258 nm	API & Tablet	[13]

Table 3: Analysis of irbesartan and hydrochlorothiazide by RP-HPLC.

Atenolol and chlorthalidone

Atenolol, 2-(4-{2-hydroxy-3-[(propan-2-yl) Amino] propoxy] phenyl) acetamide, Molecular formula C14H22N2O3 and Molecular weight 266.34 g/mol. Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at B-1 adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension. Higher doses of atenolol also competitively block B- 2 adrenergic responses in the bronchial and vascular smooth muscles.

Chlorthalidone, 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide, Molecular formula C14H11ClN2O4S and Molecular weight 338.766 g/mol. Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism [14].

Hydrochlorothiazide and candesartan

Hydrochlorothiazide, it is a prototypical member of the thiazide diuretic. It helps in reduction of reabsorption of various electrolytes through renal tubules resulting in excretion of water along with different electrolytes like sodium, potassium, chloride, magnesium etc. It is widely used in the treatment of oedema, hypertension, hyperparathyroidism, and diabetes insipidus.

Candesartan, 2-ethoxy-1-({4- [2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-)-1H-1, 3-benzodiazole-7-carboxylic acid, it is an angiotensin receptor blocking agent which can be used alone or in combination with other drugs for the treatment of hypertension. It competes with angiotensin-II for its receptors there

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Atenolol and Chlorthalidone	RP-HPLC	Xterra Rp C_8(150×4.6 mm, 5µm)	Potassium dihydrogen phosphate buffer: methanol (50:50 v/v) pH-3.6 Flow rate-0.5 ml/min	λ 240 nm	API	[14]
2)	Atenolol and Chlorthalidone	RP-HPLC	Agilent C_8 (150×4.6 mm,5µm)	Ammonium acetate buffer: methanol (60:40 v/v) pH-7.00 Flow rate-1.0ml/min	λ 228 nm	Tablet	[15]
3)	Atenolol and Chlorthalidone	RP-HPLC	THERMO C_{18} (250×4.6 mm,5µm)	Dipotassium phosphate: methanol (65:35 v/v) pH-3.8 Flow rate-1.0 ml/min	λ 266 nm	API	[16]

Table 4: Analysis of atenolol and chlorthalidone by RP-HPLC.

by lowering blood pressure. It is also used as an effective alternative for the treatment of heart failure, myocardial infarction, coronary diseases and systolic dysfunction [17].

Ramipril and amlodipine

Ramipril, 2-[N-[(S)-1-(ethoxy carbonyl)-3-phenylpropyl)] – L-alanyl] -(1S, 3S, 5S)-2-azabicyclo [3-3-0] octane carboxylic acid, is

Sr.No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Hydrochlorothiazide and Candesartan	RP-HPLC	Silanol BDS C_{18} (250×4.6 mm, 5µm)	Orthophosphoric acid: Acetonitrile (30:70 v/v) pH-2.8 Flow rate-1 ml/min	λ 210 nm	API	[18]
2)	Hydrochlorothiazide and Candesartan	RP-HPLC	Phenomenex C_{18} (250×4.6 mm, 5µm)	Ammonium acetate: acetonitrile (65:35 v/v) pH-2.6 Flow rate-1.2 ml/min	λ 260 nm	API	[19]
3)	Hydrochlorothiazide and Candesartan	RP-HPLC	Kromasil,ODS C_{18} (250×4.5 mm, 5µm)	Methanol: Acetonitrile: Disodium hydrogen phosphate (20:30:50 v/v/v) pH-2.5 Flow rate-1 ml/min	λ240 nm	API & Tablet	[20]

Table 5: Analysis of hydrochloride and candesartan by RP-HPLC.

an angiotensin converting enzyme (ACE) inhibitor. It acts on the renin angiotensin aldosterone system. It inhibits the conversion of the Inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduces the degradation of bradykinin. It is to treat high blood pressure and heart failure. Amlodipine, it is a long-acting dihydropyridine calcium channel blocker (CCB) with dose-related antihypertensive efficacy. It inhibits calcium ions transport into vascular smooth muscle and cardiac muscle to protect the target organs. However, it would also cause peripheral oedema as a side effect. It is used in the treatment of hypertension and angina [21].

Hydrochlorothiazide and enalapril

Enalapril, N-[(S)-1-ethoxycarbonyl-3-phenyl propyl]-L-alanyl}-L-proline, it is an angiotensin converting enzyme inhibitor used in the treatment of hypertension and heart failure. It is also used to reduce the incidence of coronary ischemic events, including myocardial infarction.

Hydrochlorothiazide, it is diuretic of benzothiadiazide class, extremely useful in the treatment of edema, hypertension and hypercalcaemia. A new combination dosage form containing enalapril and hydrochlorothiazide is indicated for the treatment and management of edema and hypertension [24].

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Ramipril and Amlodipine	RP-HPLC	Inertsil ODS-3 C_{18} (250×4.0 mm, 3µm)	Sodium perchlorate (containing triethylamine): acetonitrile (60:40 v/v) pH-2.6 Flow rate-1.0 ml/min	λ 210 nm	Tablet	[21]
2)	Ramipril and Amlodipine	RP-HPLC	BDS C_{18} (250×4.6 mm, 5µm)	Phosphate buffer: Acetonitrile (45:55 v/v) pH-6.5 Flow rate-1 ml/min	λ 230 nm	API	[22]
3)	Ramipril and Amlodipine	RP-HPLC	C_{18} (250×4.6 mm, 5µm)	Acetonitrile: Sodium phosphate buffer: Methanol (50:20:25 v/v/v) pH-6.8 Flow rate-0.8 ml/min	λ 210 nm	API and Tablet	[23]

Table 6: Analysis of ramipril and amlodipine by RP-HPLC.

Sr.No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref.No
1)	Hydrochlorothiazide and Enalapril	RP-HPLC	ODS UG – C_{18} (250×4.5 mm, 5µm)	Acetate buffer: methanol: Acetonitrile (60:20:20 v/v/v) pH-5 Flow rate-0.8 ml/min	λ 232 nm	Tablet	[25]
2)	Hydrochlorothiazide and Enalapril	RP-HPLC	C_{18} (150×3.9 mm, 5µm)	Phosphate buffer: Acetonitrile (80:20 v/v) pH-3.4 Flow rate-1ml/min	λ 265 nm	API and Human Plasma	[26]
3)	Hydrochlorothiazide and Enalapril	RP-HPLC	Inertial ODS C_{18} (250×4.6 mm, 5µm)	Phosphate buffer: Acetonitrile (55:45 v/v) pH-3.7 Flow rate-1ml/min	λ 215 nm	API	[27]

Table 7: Analysis of hydrochlorothiazide and enalapril by RP-HPLC.

Quinapril and hydrochlorothiazide

Quinapril, (3S)-2-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenyl-butan-2-yl]-amino] propanoyl]-3, 4-dihydro-1H-isoquinoline-3-carboxylic acid, it is a prodrug and an angiotensin converting enzyme inhibitor. The esterases of the liver transform quinapril into quinapril at, an active metabolite. Quinapril is used in the treatment of congestive heart failure and hypertension. Angiotensin converting enzyme catalyses the formation of angiotensin II, a powerful vasoconstrictor and increases blood pressure, from angiotensin I. The inhibition of angiotensin converting enzyme by quinapril leads to the reduced production of angiotensin II. The result is the reduced plasma concentrations of aldosterone, increased sodium excretion in urine and increase potassium concentration in blood.

Hydrochlorothiazide, it is a diuretic belonging to the thiazide class of drugs. The combination of quinapril and hydrochlorothiazide is useful in treatment of hypertension [28].

Telmisartan and hydrochlorothiazide

Telmisartan, 4'- [(1, 4-dimethyl-2'-propyl [2, 6'-1H-benzimidazol]-1'-yl) methyl]- [1, 1'-biphenyl]-2-carboxylic acid, it is a non-peptide molecule under the class of angiotensin II receptor antagonist. It is used for the treatment of essential hypertension as alone or in combination with other agents.

Hydrochlorothiazide, it is a widely used thiazide diuretic. The combination of telmisartan and hydrochlorothiazide is useful mainly in the Treatment of mild to moderate hypertension [31].

Azilsartan medoxomil and Chlorthalidone

Azilsartan medoxomil and Chlorthalidone, (5–Methyl–2–Oxo-1, 3–dioxol-4–yl) Methyl 2–ethoxy-1– {[2'-(5-Oxo-4, 5–dihydro-1, 2, 4–oxadiazol-3-yl) Biphenyl–4–yl] methyl}-1H–benzimidazole-7-carboxylate mono-potassium salt, Fixed-dose combination is found to show superior antihypertensive efficacy in blood pressure reduction in patients with stage 2 hypertension. Azilsartan

Sr.No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref.No
1)	Quinapril and Hydrochlorothiazide	RP-HPLC	Hypersil BDS C_{18} (150×4.6 mm, 5µm)	Triethylamine buffer: acetonitrile (60:40 v/v) pH-3.5 Flow rate-1 ml/min	λ 220 nm	API	[29]
2)	Quinapril and Hydrochlorothiazide	RP-HPLC	Hichrom C_{18} (250×4.6 mm, 10µm)	Acetonitrile: Potassium dihydrogen phosphate (40:60 v/v) pH-2.5 Flow rate-1 ml/min	λ 211 nm	API	[30]
3)	Quinapril and Hydrochlorothiazide	RP-HPLC	Agilent C_{18} (250×4.6 mm, 5µm)	Potassium dihydrogen phosphate: Methanol (65:35 v/v) pH-4.5 Flow rate-1ml/min	λ 210 nm	Tablet	[28]

Table 8: Analysis of quinapril and hydrochlorothiazide by RP-HPLC.

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref.No
1)	Telmisartan and Hydrochlorothiazide	RP-HPLC	Inertsil L11- (250×4.6 mm, 5µm)	Acetonitrile: Methanol (50:50 v/v) pH-3.0 Flow rate-1.2 ml/min	λ 298 nm	Tablet	[31]
2)	Telmisartan and Hydrochlorothiazide	RP-HPLC	ODS Hypersil C_{18} (25 cm ×4.6 mm, 5µm)	Acetonitrile: Potassium dihydrogen phosphate (60:40 v/v) pH-3.0 Flow rate-1.0 ml/min	λ 271 nm	Tablet	[32]
3)	Telmisartan and Hydrochlorothiazide	RP-HPLC	Kromasil C_{18} (250×4.6 mm, 5µm)	Acetonitrile: Methanol (80:20 v/v) pH-3.0 Flow rate-1.0 ml/min	λ 270 nm	Tablet	[33]

Table 9: Analysis of telmisartan and hydrochlorothiazide by RP-HPLC.

medoxomil is an angiotensin II receptor antagonist. It is a white crystalline powder which is practically insoluble in water, freely soluble in methanol, dimethyl Sulfoxide and dimethylformamide, soluble in acetic acid, slightly soluble in acetone and acetonitrile and very slightly soluble in tetrahydro furan and 1-octanol [34].

Captopril and Hydrochlorothiazide

Captopril, 1-[(2S)-3-mercapto-2-methylpropionyl]-l-pro-line, is an angiotensin-converting enzyme inhibitor that is used in the treatment of hypertension and congestive heart failure.

Hydrochlorothiazide, is a diuretic agent belonging to the class of benzothiadiazine drugs. A new combination dosage form of captopril and hydrochlorothiazide is indicated for the treatment and management of hypertension [37].

Hydrochlorothiazide and losartan potassium

Hydrochlorothiazide, it widely used diuretic drug of the thiazide class. It is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and in the prevention of kidney stones.

Losartan 2-butyl-4-Chloro-1-{[2'-(1H-tetrazol-5-yl) biphenyl-4-yl] Methyl} imidazol-4-yl) methanol, it is an Angiotensin II receptor antagonist used mainly to treat Hypertension.

Combination of hydrochlorothiazide and losartan potassium widely prescribed by the physicians due to simple dosing regimens, improved hypertension control, fewer dose-dependent side effects and low-cost treatment of hypertension. So, it is essential to develop a simple method for simultaneous estimation of hydrochlorothiazide and losartan in combined formulation [40].

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref.No
1)	Azilsartan medoxomil and Chlorthalidone	RP-HPLC	BDS C_{18} (100×4.6 mm, 5µm)	Phosphate buffer: Acetonitrile (90:10 v/v) pH-3.2 Flow rate-0.9 ml/min	λ 260 nm	Tablet	[35]
2)	Azilsartan medoxomil and Chlorthalidone	RP-HPLC	C_8 (150×4.6 mm, 5µm)	Acetonitrile: Potassium dihydrogen phosphate buffer (90:10 v/v) pH-2.8 Flow rate-0.8 ml/min	λ 220 nm	API	[36]
3)	Azilsartan medoxomil and Chlorthalidone	RP-HPLC	Cosmosil C_{18} (250×4.6 mm, 5µm)	Acetonitrile:Water (70:30 v/v) pH-2.8 Flow rate-0.9 ml/min	λ 244 nm	API	[34]

Table 10: Analysis of azilsartan medoxomil and chlorthalidone by RP-HPLC.

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Captopril and Hydrochlorothiazide	RP-HPLC	Phenomenex Luna C_{18} (150×4.6 mm, 5µm)	Acetonitrile: water (30:70 v/v) pH-2.8 Flow rate-1.0 ml/min	λ 210 nm	API	[38]
2)	Captopril and Hydrochlorothiazide	RP-HPLC	Diamonsil C_{18} (150×4 mm, 5µm)	Trifluroacetic acid: Acetonitrile (87:13 v/v) pH-1.8 Flow rate-1.2ml/min	λ 263 nm	Tablet	[37]
3)	Captopril and Hydrochlorothiazide	RP-HPLC	ODS C_{18} (15 cm×4.6 mm, 5µm)	Methanol: Water (45:55 v/v) pH-3.8 Flow rate-1ml/min	λ 210 nm	Tablet	[39]

Table 11: Analysis of captopril and hydrochlorothiazide by RP-HPLC.

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Hydrochlorothiazide and losartan potassium	RP-HPLC	Microbondapak C_{18} (300×3.9 mm, 10µm)	Sodium dihydrogen orthophosphate: methanol (80:20 v/v) pH-3 Flow rate-1.0 ml/min	λ 270 nm	Tablet	[41]
2)	Hydrochlorothiazide and losartan potassium	RP-HPLC	LC C_{18} (150×4.6 mm, 5µm)	Potassium dihydrogen phosphate: Acetonitrile (65:35 v/v) pH-3.1 Flow rate-1.0 ml/min	λ 232 nm	Tablet	[42]
3)	Hydrochlorothiazide and losartan potassium	RP-HPLC	Shim Pack CLC-ODS (250×4.6 mm, 5µm)	Phosphoric acid solution: Acetonitrile (60:40 v/v) pH-3.0 Flow rate-1.5 ml/min	λ 254 nm	Tablet	[40]

Table 12: Analysis of hydrochlorothiazide and losartan potassium by RP-HPLC.

Amlodipine and atenolol

Amlodipine, Amlodipine is long-acting calcium channel blocker used as anti-hypertensive and in treatment of angina. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels; in angina it decreases blood flow to the heart muscle.

Atenolol, it is a β-blocker see to be equally effective as an antihypertensive, antianginal and antiarrhythmic drug widely used as cardiovascular drug in combination with Amlodipine. Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation [43].

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Amlodipine and Atenolol	RP-HPLC	BDS C_{18} (250×4.6 mm, 5µm)	Phosphate buffer: methanol: Acetonitrile (40:30:30 v/v/v) pH-5.0 Flow rate-3.0	λ 213	Capsule and Tablet	[43]
2)	Amlodipine and Atenolol	RP-HPLC	Intertie C_{18} (250×4.6 mm, 5µm)	Phosphate buffer: Acetonitrile: Methanol (40:35:25 v/v/v) pH-3.0 Flow rate-1.0 ml/min	λ 225	API	[44]
3)	Amlodipine and Atenolol	RP-HPLC	C_{18} (150×4.6 mm, 5µm)	Phosphate buffer: Methanol: Acetonitrile (35:55:10 v/v/v) pH-3.0 Flow rate-1.0 ml/mi	λ 237	API	[45]

Table 13: Analysis of amlodipine and atenolol by RP-HPLC.

Trandolapril and verapamil

Trandolapril, (2S, 3aR, 7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-octahydro-1H-indole-2-carboxylic acid, it is a non-sulfhydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to its biologically active diacid form trandolapril in the liver. Trandolapril at inhibits ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS).

Verapamil, 2-(3, 4-dimethoxyphenyl)-5- {[2-(3, 4-dimethoxyphenyl) ethyl] (methyl) amino}-2-(propan-2-yl) pentane nitrile, Verapamil inhibits voltage-dependent calcium channels. Specifically, its effect on L-type calcium channels in the heart causes a reduction in ionotropy and chronotropy, thus reducing heart rate and blood pressure. Verapamil mechanism of effect in cluster headache is thought to be linked to its calcium-channel blocker effect [46].

Hydrochlorothiazide and triamterene

Hydrochlorothiazide, it widely used diuretic drug of the thiazide class. It is often used in the treatment of hypertension. It is white crystalline powder and it is soluble in dilute ammonia, slightly soluble in water.

Triamterene, 2, 7-Diamino-6-phenyl-1, 2, 3, 7-tetrahydro-4-pteridinamine. Triamterene is a potassium-sparing diuretic is used in combination with thiazide diuretics for the treatment of high blood pressure. It is white crystalline powder, soluble in formic acid. The combination of hydrochlorothiazide and triamterene is used to treat hypertension [49].

Hydralazine hydrochloride and isosorbide dinitrate

Isosorbide dinitrate, 1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol or (3R,3aS,6S,6aS)-6-(nitrox)-hexahydrofuro[3,2] furan-3-yl nitrate, Isosorbide dinitrate is a moderate to long acting oral organic nitrate which acts as a vasodilator. It is profoundly used in the

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Trandolapril and Verapamil	RP-HPLC	Intertsil ODS 3V (150×4.6 mm, 5µm)	Acetonitrile: Triethylamine buffer (40:60 v/v) pH-3.0 Flow rate-1.3ml/min	λ 216	API	[46]
2)	Trandolapril and Verapamil	RP-HPLC	Intertsil C_{18} (250×4.6 mm, 5µm)	Methanol: Phosphate buffer (55:45 v/v) pH-4.8 Flow rate-1.0 ml/min	λ 282	API	[47]
3)	Trandolapril and Verapamil	RP-HPLC	Symmetrical C_{18} (150×4.6 mm,3.5µm	Potassium dihydrogen phosphate buffer: Acetonitrile (35:65 v/v) pH-2.2 Flow rate-0.6 ml/min	λ 230	API	[48]

Table 14: Analysis of trandolapril and verapamil by RP-HPLC.

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Hydrochlorothiazide and Triamterene	RP-HPLC	Luna C_{18} (250×4.6 mm, 5µm)	Acetonitrile: Ortho phosphoric acid (25:75 v/v) pH-2.5 Flow rate-1.0 ml/min	λ 272	API	[49]
2)	Hydrochlorothiazide and Triamterene	RP-HPLC	C_{18} (250×4.6 mm, 5µm)	Phosphate buffer: methanol: Acetonitrile (55:35:10 v/v/v) pH-3.5 Flow rate-1.0 ml/min	λ 270	Tablet	[50]
3)	Hydrochlorothiazide and Triamterene	RP-HPLC	Zorbax eclipse plus RRDH C_{18} (50×2.1 mm,1.7 µm	Formic acid: methanol: water (50:40:10 v/v/v) pH-2.7 Flow rate-0.4 ml/min	λ 254	Tablet	[51]

Table 15: Analysis of hydrochlorothiazide and triamterene by RP-HPLC.

treatment of angina pectoris, a condition which occurs when the oxygen supply to the myocardium is insufficient. The vasodilating action is through the relaxing action in blood vessels by nitrates, particularly nitric oxide. This will decrease the oxygen demand of the heart and chest pain.

Hydralazine, 1-hydrazinylphthalazine, is a direct-acting smooth muscle relaxant. It is used as an antihypertensive agent in cases like preeclampsia (a condition in pregnancy characterized by high blood pressure). Hydralazine acts by increasing cyclic guanosine mono-phosphate (cGMP) levels which causes an increase in the activity of protein kinase G (PKG). Active PKG adds an inhibitory phosphate to myosin light-chain kinase which is a protein involved in the activation of cross-bridge cycling (i.e., contraction) in smooth muscle. This results in blood vessel relaxation and causes dilation of arteries and arterioles. It also functions as an antioxidant. It inhibits membrane-bound enzymes that form reactive oxygen species, such as superoxides. Excessive superoxide counteracts nitric oxide-induced vasodilation.

Isosorbide dinitrate and Hydralazine in combination are used with other medications to treat heart failure. As both the drugs are vasodilators, they work by relaxing and widening blood vessels so that can flow more easily to the heart [52].

Sr. No	Drug combination	Method	Column	Mobile phase	Detection	Form	Ref. No
1)	Hydralazine Hydrochloride and Isosorbide Dinitrate	RP-HPLC	Phenomenex C_{18} (250×4.6 mm, 5µm)	Acetonitrile: Triethylamine water (35:65 v/v) pH-3.5 Flow rate-1.0 ml/min	λ 273	API	[53]
2)	Hydralazine Hydrochloride and Isosorbide Dinitrate	RP-HPLC	Zorbax C_{18} (250×4.6 mm, 5µm)	Orthophosphoric acid: methanol (60:40 v/v) pH-2.1 Flow rate-1 ml/min	λ 278	API	[54]

3)	Hydralazine Hydrochloride and Isosorbide Dinitrate	RP-HPLC	Zodiac C_{18} (250×4.6 mm, 5µm)	Ammonium acetate: Acetonitrile: methanol (50:30:20 v/v/v) pH-3 Flow rate-1 ml/min	λ 270	API and Tablet	[52]

Table 16: Analysis of hydralazine hydrochloride and isosorbide dinitrate by RP-HPLC.

Conclusion

This review work is a comprehensive and critical review of the analytical methods reported in the literature for the determination of selected antihypertensive combination. Overall, it should be noted that a number of reverse phase high performance liquid chromatographic (RP-HPLC) methods. These methods constitute useful tools for pharmacokinetic and toxicological studies or for quality control tests. Moreover, some of them may support the routine therapeutic drug monitoring of these antihypertensive drugs in clinical practice.

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