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Review Article

Review on Simultaneous Determination of Antihypertensive Analytes in Pharmaceutical Dosage Form by Different Analytical Methods

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ABSTRACT
The main objective of this review is to unify and interpret widely scattered information of reported studies on potential, reliable and efficient analytical methodologies which can estimate all the major components of antihypertensive drugs. The information and suggested outlined below may facilitate and guide further needed studies to optimize the use of analytical techniques like High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Gas Chromatography (GC) etc. for determination of antihypertensive analytes in formulation. Presented work is focused on the use of different analytical methods for the estimation of antihypertensive drugs in API as well as formulation. The first, Hypertension, Antihypertensive drugs their mechanism were described. From the reviewed literature it is obvious that HPLC is a commonly available method of testing in pharmaceutical laboratory so this method should be of choice for complete determination of all the components. Selection of analytical methods is determined by several factors such as speed, convenience, specificity, accuracy, precision, sensitivity, selectivity, cost, availability of instruments, technical expertise and the number of samples to be analyzed.

Key words: Anti hypertensive analytes, HPLC, HPTLC

INTRODUCTION

Hypertension

Hypertension is considered to be present when a person's systolic blood pressure is consistently 140 mm hg or more, and/or their diastolic blood pressure is consistently 90 mm hg or more[1]. Recent 'global burden of hypertension' data showed that more than a quarter of the world's adult population (nearly 1 billion) had hypertension in 2000 and this is expected to increase by about 60% (1.56 billion) in 2025; the population burden being greater in developing countries[2]. In United States, a total of 68.9% of people

With hypertension were aware of the diagnosis, 58.4% received treatment, and only in 31.0% the blood pressure was controlled [3].

Hypertension is already a highly prevalent risk factor for cardiovascular dieses throughout the world. It is becoming an increasingly common health problem worldwide because of contributing factors such as obesity, physical inactivity and an unhealthy diet. [4, 5, 6]

Hyper tension plays a major etiologic role in the development of cerebrovascular disease, ischemic heart disease and renal failure. Treating hypertension has been associated with about a 40% reduction in the risk of stroke and about a 15% reduction in the myocardial infarction [7]. Although hypertension has been shown to prevent Cardio Vascular Disease (CVS) and to extend and enhance life, hypertension remains inadequately managed everywhere. In addition, hypertension often coexists with other cardiovascular risk factors' such as tobacco use, diabetes, hyperlipidemia and obesity, which compound the cardiovascular risk attributable to hypertension. Worldwide, these coexistent risk factors are inadequately addressed in patients with hypertension, resulting in high morbidity and mortality [8]. Globally data indicated that about 62% cerebrovascular disease and 49 % of ischemic heart disease are attributed to the treatment of suboptimal blood pressure (systolic blood pressure >115 mm hg). A global capacity assessment survey conducted by World Health Origination (WHO) shows that there is wide variation in the capacity for management of hypertension in various countries. Of the 167 countries surveyed, national hypertension guidelines were not available in 61%, health professionals were not trained to manage hypertension in 45%, antihypertensive were not affordable in 25%, and basic equipment and drugs for the management of hypertension were not available in primary healthcare in 8 and 12% of countries, respectively [9].

Table 1: Classification of hypertension for adults [4]

Category	Systolic (mm hg)		Diastolic (mm hg)
Optimal BP [a]	<120	And	< 80
Normal BP	<130	And	< 85
High-normal BP	130-139	Or	85-89
Stage 1 (mild)	140-159	Or	90-99
Stage 2 (moderate)	160-179	Or	100-109
Stage 3 (severe)	≥ 180	Or	≥ 110

a : Blood Pressure

Patients with hypertension must take antihypertensive drugs on a long-term basis. Although such drugs cannot give a radical cure, they can prevent heart failure, kidney failure and acute stroke induced by hypertension and delay the development of atherosclerosis by controlling the blood pressure. Generally speaking, antihypertensive drugs must be taken for life.

History of antihypertensive drugs

In the 1930s and 1940s, three antihypertensive treatments were developed: *sympathectomy*, very *low*

sodium diet and *pyrogen therapy*. Sympathectomy, which involved cutting nerves to blood vessels, lowered blood pressure in some patients, but it required more than ordinary surgical skills, often produced life-threatening complications. Low sodium diet also were unpleasant because they limited food choices, but they were effective in lowering blood pressure. Pyrogen therapy was based on the observation that fever lowers blood pressure. Fever was induced by intravenous infusion of bacterial products. The treatment transiently reduced blood pressure but required hospitalization and had many unpleasant side effects [9-12].

The first successful drug treatment for hypertension were introduced after World War II. By that time, researchers had learned that blocking the sympathetic nervous system could lower blood pressure. In 1946, tetramethylammonium was introduced as treatment of hypertension. Hexamethonium, an improved version of tetraethylammonium, was available for use by 1951 [13]. Another effective blood pressure lowering drug, hydralazine, resulted from the search for anti-malarial compounds.

Table 2 Antihypertensive drugs [18]

Therapeutic class	Drugs	Mechanism of action
Angiotensin Converting Enzyme(ACE) inhibitors	Lisinopril, Enalapril Ramipril, Captopril Perindopril, Trandolapril Fosinopril, Quinalapril	Inhibit ACE, preventing conversion of angiotensin i to angiotensin ii, leading to reduce peripheral arterial resistance, aldosterone secretion, accumulation of bradykinine
Angiotensin ii receptor blockers	Candesartan, Losartan, Irbesartan, Valsartan, Olmesartan, Telmisartan Eprosartan	Antagonist at angiotensin ii receptor leading to reduced peripheral arterial resistance, aldosterone secretion
Renin inhibitors	Aliskiren	Directly inhibit rennin, which decreases plasma rennin activity and inhibit conversion of angiotensinogen to angiotensin i
Loop diuretics	Furosemide	Block the reabsorption of sodium and chloride in kidney tubules causing a profound increase in urine output
Thiazide diuretics	Bendroflumethiazide Chlorthalidone Indapamide	Direct inhibit the mechanism for transport of sodium from tubule lumen intom the cell of the tubule wall and hence enhance the amount of sodium lost in urine
Calcium channel blockers	Dihydropyridine, Amlodipine, Nifedipine, Femodipine, lercanidipine, Nisoldipine, Verapamil, Nor-dihydropyridine	Antagonist at calcium channel, blocks the influx of calcium ions via calcium channel into cardiac and vascular smooth muscle
Selective α1 adrenoreceptor antagonist	Doxazosin Prazocin Indoramin	Selectively inhibit the post synaptic α1 and α2 adrenergic receptors, causing aeterial and venous dilation
Non-selective α1 and α2 antagonist	Phenoxybenzamine Phentolamine	Blocks the effect of adrenaline and noradrenaline at α1 and α2 adrenergic receptors, leading to arterial and venous vesodilation and inhibition of catecholamine mediated vesoconstriction
B-adrenoreceptor antagonist	Propranolol, Bisoprolol, Atenolol, Metoprolol, Nebivolol, Carvedilol Labetalol	Competitively block β-adrenoreceptor in heart, bronchi, pancreas, uterus, kidney, brain and liver, resulting in reduced cardiac output
Vesodilators	Sodium nitropruside	A prodrug that release nitric oxide, a potent vasodilator that increase cyclic guanidine monophosphate (cgmp) levels, leading to phosphorylation of protein kinase, which causes relaxation of vascular smooth muscle
	Minoxidil, Hydralazine Diazoxide	Causing direct relaxation of vascular smooth muscles, leading to vasodilation in the periphery
Centrally acting sympatholytics	Methyldopa Clonidine	Directly stimulates α2 adrenergic receptors, in the vasomotor centre of medulla, leading to reduced sympathetic outflow from the brain
	Moxonidine	Agonist at central imidazoline α2 adrenergic receptors

Table 4 Contraindications and cautions for specific antihypertensive drugs [20]

Drug	Contriindications	Drug	Cautions
Aceis, arbs	Pregnency, Biletral renel artery stenosis, Hyperkalemia	α – blockers	Congestive heart failure
β- blockers	High degree heart block, Severe bradycardia <50/min, Obstructive airway disease	Clonidine	Withdrawal syndrome
Diuretics	Gout	Methyldopa	Hepatotoxicity
		calcium channel blockers	Congestive heart failure

Table 3 Combination drugs used for hypertension[19]

Classes	Drugs	Available strengths (mg/mg)
Diuretic / diuretic	Triamterene/hydrochlorothiazide	37.5/25, 50/25, 75/50
	Spironolactone / hydrochlorothiazide	25/25, 50/50
	Amiloride /hydrochlorothiazide	5/50
B-blocker / diuretic	Propranolol/hydrochlorothiazide	40/25, 80/25
	Metoprolol/hydrochlorothiazide	50/25, 100/25
	Atenolol/chlorthalidone	50/25, 100/25
	Nadolol/bendroflumethiazide	40/5, 80/5
	Timolol/hydrochlorothiazide	10/25
	Propranolol/hydrochlorothiazide	80/50, 120/50, 160/50
	Bisoprolol/hydrochlorothiazide	2.5/6.25, 5/6.25, 10/6.25
Adrenergic inhibitor / diuretic	Guanethidine / hydrochlorothiazide	10/25
	Methyldopa/hydrochlorothiazide	250/15, 250/25, 500/30, 500/50
	Methyldopa/chlorothiazide	250/150, 50/250
	Reserpine/chlorothiazide	0.125/250, 0.25/500
	Reserpine/chlorthalidone	0.125/25, 0.25/50
	Reserpine/hydrochlorothiazide	0.125/25, 0.125/50
	Clonidine/chlorthalidone	0.1/15, 0.2/15, 0.3/15
ACE inhibitor / diuretic	Captopril/hydrochlorothiazide	25/15, 25/25, 50/15, 50/25
	Enalapril/hydrochlorothiazide	5/12.5, 10/25
	Lisinopril/hydrochlorothiazide	10/12.5, 20/12.5, 20/25
	Fosinopril/hydrochlorothiazide	10/12.5, 20/12.5
	Quinapril/hydrochlorothiazide	10/12.5, 20/12.5, 20/25
	Benazepril/hydrochlorothiazide	5/6.25, 10/12.5, 20/12.5, 20/25
	Moexipril/hydrochlorothiazide	7.5/12.5, 15/25
Angiotensin II receptor blocker / diuretic	Losartan/hydrochlorothiazide	50/12.5, 100/25
	Valsartan/hydrochlorothiazide	80/12.5, 60/12.5
	Irbesartan/hydrochlorothiazide	75/12.5, 150/12.5 300/12.5
	Candesartan/hydrochlorothiazide	16/12.5, 32/12.5
	Telmisartan/hydrochlorothiazide	40/12.5, 80/12.5
Calcium channel blocker / ACE inhibitor	Amlodipine/benazepril	2.5/10, 5/10, 5/20, 10/20
	Verapamil (extended-release) / trandolapril	180/2, 240/1, 240/2, 240/4
	Felodipine (extended release) / enalapril	5/5
Vasodilator /diuretic	Hydralazine/hydrochlorothiazide	25/25, 50/25, 100/25
	Prazosin/polythiazide	1/0.5, 2/0.5, 5/0.5
Triple combination	Reserpine / hydralazine / hydrochlorothiazide	0.10/25/15

It was diverted to the treatment of hypertension when it was found to have no anti-malarial activity but to lower blood pressure and increase kidney blood flow. They were reasonably effective in lowering blood pressure but often caused severe side effectives [14, 15]. The final drug developed in those early days, reserpine, was derived from rauwolfia serpentine, a plant used for centuries by physicians and herbalists on the indian subcontinent [16, 17]. Success in treating hypertension has been aided by the development of progressively more effective, more specific and more easily tolerated drugs. Both laboratory and clinical research in academia and industry played pivotal roles in the development of these drugs.

Role for different analytical methods

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. This review contains the various simultaneous estimation methods (spectrophotometric, High Performance Liquid Chromatography (HPLC), & High Performance Thin Layer Chromatography (HPTLC) which are employed for the quantitative estimation of drug products containing antihypertensive analytes. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

Simultaneous determination of antihypertensive drug review

Information of different analytical methods for simultaneous determination of antihypertensive analytes in formulation and Active Pharmaceutical Ingredient.

Conclusion

Presented work is focused on the use of different analytical methods like High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Gas Chromatography (GC) etc. for determination of antihypertensive analytes in formulation as well as in API. First, Hypertension, Antihypertensive drugs their mechanism was described. From the reviewed literature it is obvious that HPLC is a commonly available method of testing in pharmaceutical laboratory so this method should be of choice for complete determination of all the components. Selection of analytical methods is determined by several factors such as speed, convenience, specificity, accuracy, precision, sensitivity, selectivity, cost, and availability of instruments,

technical expertise and the number of samples to be analyzed.

Table 5 Analytical Methods for Antihypertensive Analytes in Formulation and Active Pharmaceutical Ingredient.

Reported work	Matrix	Method	Description	Ref.
Nebivolol Hydrochloride, Hydrochlorthiazide	Tablet	UV [a]	Simultaneous equations based on measurement of absorbances at two wavelengths 290 nm and 317 nm for nebivolol hydrochloride and hydrochlorthiazide respectively	21
Valsartan, Amlodipine	Capsule	HPLC	Rp c-18 column (kromasil, 250 x 4.6 mm) using acetonitrile: phosphate buffer (0.02m, ph 3.0), (56:44 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 234 nm.	22
Irbesartan, Losartan, HydroChlorothiazide, Chlorthalidone	Tablet	HPLC	Hypersil bds (250 mm × 4.6 mm particle size 5 µm) column with gradient flow. The mobile phase at a flow rate of 1.0 ml min−1 consisted of 0.05 m sodium dihydrogen phosphate buffer and acetonitrile (gradient ratio). The UV detection was carried out at 220 nm.	23
Amlodipine Besylate, Valsartan, Hydrochlorothiazide	Tablet	UV	Methanol and distilled water were used as solvents. The wavelengths selected for the analysis were 365 nm, 250 nm and 315 nm for amlodipine besylate, valsartan and hydrochlorothiazide respectively.	24
Amlodipine Besylate, Atenolol	API [b] , Tablet	UV	The method is Based on simultaneous equation or vierodt's method. The λmax values for amlodipine besylate and atenolol was 238.4 nm and 273.4 nm respectively	25
Atenolol, Indapamide	Tablet	HPLC	Intelligent c18 column (200 x 4.6 nm) with a mobile phase composed of methanol :water : diethylamine : glacial acetic acid (70:30:0.12:0.08) in isocratic mode at flow rate of 1.2 ml/min.	26
Atenolol, Indapamide	Tablet	HPLC	Chromatographic separation was achieved isocratically on a waters c18 column (250×4.6 mm, 5 µ particle size)Using a mobile phase, methanol and water (adjusted to ph 2.7 with 1% orthophosphoric acid) in the ratio of 80:20. The flow rate was 1 ml/min and effluent was detected at 230 nm.	27
Atenolol, Indapamide	Tablet	UV	The signals were measured at 225.0 nm and 240.0 nm corresponding to absorbance maxima of atenolol and indapamide in methanol respectively.	28
Atenolol, Indapamide	Tablet	UV	First method employs formation and solving of simultaneous equation using 246.4 nm and 266 nm as two wavelengths for formation of simultaneous equations. Second method being dual wavelength method, in which two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Atenolol has equal absorbance at 246.4 nm and 254.2 nm, where the differences in absorbance were measured for the determination of indapamide; similarly differences in absorbance at 266 nm and 270.2 nm were measured for the determination of atenolol.	29
Amlodipine Besylate, Nebivolol HCl	Tablet	UV	The first method is absorbance correction method based on determination of amlodipine besylate at 365 nm using its absorptivity value and nebivolol hydrochloride at 280 nm after deduction of absorbance due to amlodipine besylate. The second method is based on absorbance ratio in which wavelengths selected were 269 nm, an isoabsorptive point and 280 nm as λmax of nebivolol hydrochloride.	30
Telmisartan, Ramipril	Tablet	HPTLC	Tlc precoated silica gel on aluminum plate 60 f 254, (10 cm ×10 cm, prewashed by methanol and activated At 60° c for 5 min prior to chromatography). The solvent system was acetone: benzene: ethyl acetate: glacial acetic acid in the proportion of 5:3:2:0.03, (v/v/v/v) with rf value for telmisartan and ramipril was 0.673 and 0.353 respectively.	31
Telmisartan, Indapamide	Tablet	HPLC	Separation was achieved on an amazone c18, 5µ 150 x 4.6 mm the mobile phase (buffer: acetonitrile: methanol) (45+25+30) kh2po4 & triethaylamine ph 3.0 With ortho phosphoric acid buffer flow rate of 1 ml/min and UV detection at 285 nm.Column Oven temperature is 25 °c	32

Perindropil Erbumine	API	HPTLC	Separation of perindopril from its degradation product on plates precoated with silica gel 60f254, the mobile phase used was dichloromethane: methanol : glacial acetic acid in the ratio of 9.0:8.0:0.1 v/v/v. The drug showed considerable at 215 nm	33
Perindopril Erbumine, Indapamide	Tablet	HPLC	Inertsil ods- 3v column having 250mm x 4.6mm i.d. with 5µm particle size and potassium dihydrogen phosphate buffer adjusted to ph 3.0 using ortho phosphoric acid and acetonitrile (60:40 v/v) was used as eluent at a constant flow rate of 1.0ml/min.	34
Perindopril Erbumine, Amlodipine Besylate	Tablet	Absorption factor method	Absorption factor method was performed for perindopril Erbumine and amlodipine besylate at UV detection wavelength 215 nm and 237 nm respectively. Amlodipine besylate was show liner at 237 nm but amlodipine besylate also showed absorbance at 215nm and give interference in determination of perindopril erbumine.	35
Perindopril Erbumine, Indapamide	Tablet	HPTLC	The method was based on the separation of two drugs on plates precoated with silica gel 60 F_{254}. The mobile phase used was dichloromethane:methanol : glacial acetic acid in the ratio of 9.5:0.5:0.1 v/v/v. Both the drugs showed considerable absorbance at 215 nm.	36
Indapamide	API	HPLC	The separation was achieved on a inertsil ods 3v, 5µm, 150 x 4.6 mm,5µ in the isocratic mode using mixture of 7 volume of acetoinitrile, 20 volume of tetrahydrofuran and 73 volumes of a 1.5g/l solution Of triethylamine adjusted ph 2.8 with ortho-phosphoric acid at a flow rate of 1.4 ml/min. The methods were performed at 305 nm	37
Telmisartan , Indapamide	Tablet	HPLC	Amazon c18, 5 micron, 150 x 4.6 mm the mobile phase (buffer: acetonitrile: methanol) (45+25+30) kh2po4 & triethaylamine ph 3.0 with ortho phosphoric acid buffer flow rate of 1 ml/min and UV detection at 285 nm	38
Perindopril, Indapamide	Tablet	Absorbance Correction method.	Measurement of absorbances at two wavelengths 210.4nm (λmax of perindopril) and 285.8nm (λmax of indapamide) in methanol for the Simultaneous quantitative determination of perindopril and indapamide in the binary mixture without previous Separation.	39
Metoprolol Succinate, Hydrochlorothiazide	Tablet	HPLC	C-18 column using 50mm di-sodium hydrogen Phosphate: methanol: acetonitrile in a ratio of 525:225:250 as mobile phase. The flow rate was 1 ml/min and the compounds were detected by a UV-detector at 222 nm at a column temperature of 24 ± 2ºc.	40
Nebivolol, Hydrochlorothiazide	Tablet	HPLC	A octadecyl silane (ods) c18 column (250 x 4.6 mm), with mobile phase methanol: water (60:40 v/v) adjusted to ph 3:2 with o-phosphoric acid was used. The flow rate was 1.0 ml /min and the effluent was monitored at 281.0 nm.	41
Ramipril And Olmesartan	Tablet	First derivative UV	First derivative UV method. Olmesartan Has zero crossing point at 240 nm in methanol and ramipril has zero crossing point at 246 nm in methanol.	42
Atorvastatine, Amlodipine	Tablet	HPLC	C18 column (phenomenex phenyl hexyl column, 250mm* 4.6mm i.d, 5um). Utilizing mobile phase of water with 0.4%v/v triethyl amine and acetonitrile with diluted orthophosphoric acid ph adjusted to 5.2 in a ratio of 52.5:47.5 of water and acetonitrile respectively. Mobile phase was delivered at the flow rate of 1.0ml/min. Ultraviolet detection was carried out at 229nm	43
Ramipril And Telmisartan	Tablet	HPTLC	Tlc plate precoated with silica gel 60 f254 and the mobile phase consisting of methanol: chloroform in the ratio of 1:6v/v. Telmisartan and ramipril were well resolved with rf 0.68 ± 0.03 and 0.38±0.03 respectively wavelength selected for quantization was 210nm.	44
Telmisartan And Ramipril	Tablet	HPTLC	Precoated with silica gel 60f254 on aluminium sheets and a mobile phase comprising of toluene: acetonitrile: formic acid: water (5:5:0.3:1). Densiometric analysis of both the drugs was	45

			carried out in the absorbance mode at 212 nm.	
Beta-Blockers And Diuretic	Tablet	UPLC[C] method	The chromatographic separations of all the drugs were achieved on a waters acquity beh c18, 50×2.1 mm, 1.7 µm uplc column within a short runtime of 3.3 min.	46
Atenolol And Amlodipine Basylate	Tablet	UV	The method is based on simultaneous equation or vieodrt's method. The λmax values for atenolol and amlodipine besylate were found to be 224.6 nm and 239.6 nm respectively	47

a: UV-Ultra Violet spectrophotometry, b: API-Active Pharmaceutical Ingredient,
c: UPLC-Ultra Performance Liquid Chromatography

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