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Amlodipine and Metoprolol: A Review on Analytical and Bio-Analytical Methods

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ABSTRACT

Hypertension also known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure typically does not cause symptoms. It can lead to severe health complications and increase the risk of heart disease, stroke, and sometimes death. Blood pressure is the force that a person's blood exerts against the walls of their blood vessels. The literature entitles the various analytical techniques like UV spectroscopy, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography, Liquid Chromatography-Mass spectrometry. In this literature we reviewed the various analytical and bio-analytical methods used for the estimation of anti-hypertensive drugs. This review gives the concise and collective information about the analytical validative parameters like Limit of detection (LOD), Limit of Quantification (LOQ), Standard Curve, Accuracy & Precision for the analysis of amlodipine, metoprolol alone or combination with other drugs. This review helps to carry out further analytical studies on the mentioned drugs.

Keywords: Hypertension, Metoprolol, Amlodipine, Bioanalytical

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1. INTRODUCTION:

High blood pressure (BP), also known as hypertension, as a clinic blood pressure of 140/90 mmHg or higher confirmed by a subsequent ambulatory blood pressure monitoring daytime average (or home blood pressure monitoring average) of 135/ 85 mmHg or higher. Hypertension is a key risk factor for cardiovascular disease. Currently, around a third of people with hypertension are undiagnosed, and of those diagnosed, around half are not taking antihypertensive medications. The World Health Organisation estimates that high blood pressure directly or indirectly causes deaths of at least nine million people globally every year [1]. Hypertension or high blood pressure is defined as abnormally high arterial blood pressure. According to the Joint National Committee 7 (JNC7), normal blood pressure is a systolic BP < 120 mmHg and diastolic BP < 80 mm Hg. Hypertension is defined as systolic BP level of ≥140 mmHg and/or diastolic BP level ≥ 90 mmHg. The grey area falling between 120–139 mmHg systolic BP and 80–89 mmHg diastolic BP is defined as "prehypertension". Although prehypertension is not a medical condition in itself, prehypertensive subjects are at more risk of developing HTN [2]. It is a silent killer as very rarely any symptom can be seen in its early stages until a severe medical crisis takes place like heart attack, stroke, or chronic kidney disease [3]. Since people are unaware of excessive blood pressure, it is only through measurements that detection can be done. Although majority of patients with hypertension remain asymptomatic, some people with HTN report headaches, light headedness, vertigo, altered vision, or fainting episode. Metoprolol is a beta1-selective (cardioselective) adrenergic receptor blocking agent. This preferential effect is not absolute, however, and at higher plasma concentrations, metoprolol also inhibits beta2-adrenoreceptors, chiefly located in the bronchial and vascular musculature. Metoprolol has no intrinsic sympathomimetic activity, and membrane-stabilizing activity is detectable only at plasma concentrations much greater than required for betablockade. Amlodipine besylate is the besylate salt of amlodipine, a long-acting calcium channel blocker. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure [25]. The literature entitles the various analytical techniques like UV spectroscopy, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography, Liquid Chromatography-Mass spectrometry.

2. METHODOLOGY:

2.1 *U V Spectroscopic methods:*

UV spectroscopy is a preliminary approach in the analysis of drug molecules it's simple and effective in analysis of molecules. It gives the brief information about the solubility, lambda max of the entity and UV absorbance pattern so it's helpful in the identification and quantification of the dug substance. Only few methods were reported for the analysis of AM and METO and they are reviewed in this section.

Table 1: Ultra violet Spectrophotometric methods

Sl.no	Drugs	Method	Parameters	Author
1.	Amlodipine, HCT and TEL	UV zero order spectral method	Solvent: ethanol and water Wavelength: 371,279.4,314.7nm Linearity: 1-9,1-20,2-40 µg/ml Correlation coefficient: 0.999	Mahesh Attimarad [4]
2.	Metoprolol succinate and Olmesartan	UV simultaneous equation method	Solvent: water Wavelength: 221,257nm Linearity: 5-25,4-20 µg/ml Correlation coefficient: 0.999 and 0.16	Bindi N. Vora [5]
3.	Amlodipine and BZ	UV simultaneous equation method	Solvent: HCL Wavelength: 366,257 nm Linearity:2-24 µg/ml Correlation coefficient:0.999	Abhi Kavathia [6]

2.2 *HPLC Methods:*

Liquid chromatographic methods have the benefit of high separation capacity, hence their repeated use for the analysis of antihypertensive drugs. HPLC (High Performance Liquid Chromatography) is the advanced form of LC employed for separating specific molecules in complex mixtures such as biological fluids. Owing to its remarkable selectivity and sufficient precision, HPLC is the most applied LC method for the analysis of drugs; reversed-phase HPLC is certainly the most common technique in pharmaceutical drug development such as analysis of drug substances in biological samples. Of note, most of the methods reviewed in this study employed reversed-phase columns such as C18 or C8. Furthermore, HPLC columns are usually filled with 3-5 µm particles.

2.2.1 Analytical Methods

In analytical method development all the reported methods carried out by the reverse phase technique for the LC method development and these methods helps in estimation of the drugs and selection of mobile phase for the estimation of the drugs and methods are validated according to the ICH Q2 R1 guidelines.

Hyeon Woo Moon et al., has developed a HPLC method for the determination of fimasartan and amlodipine in tablet dosage form. The isocratic elution was accomplished by Nucleosil C18 column (250 mm × 4.6 mm, 5 mm) at 40 C. The mobile phase consisted of ACN and 0.02 M monopotassium phosphate buffer (pH 2.2) in the ratio of 50:50 (v/v) was eluted at 1.0 ml/min [7]. Shi-Ying Dai has developed a RP-HPLC method for estimation of RP and AM in tablets was developed and validated by Chinese Pharmacopeia 2010. The linearity in the range of 0.01-0.25 mg/mL for RP and 0.014-0.36 mg/mL for AL. The column was an Inertsil ODS-3 column (250 mm×4.0 mm, 3 µm). The mobile phase A consisted of 60 mM sodium perchlorate buffer (containing 7.2 mM triethylamine) - acetonitrile (60:40, v/v) and mobile phase B was 60 mM sodium perchlorate buffer - acetonitrile (20:80, v/v) was eluted at 1 ml/min [8]. Another liquid chromatographic method with UV detection 230 nm by Anandkumar R et al., has developed for estimation of HCT, AM and LOSART in tablet dosage form. Separation was achieved with a phenomenex luna 5m CN 100R, 250 × 4.60 mm 5-micron size column. Mobile phase containing acetonitrile, water and 0.4% of potassium dihydrogen phosphate buffer pH 2.7 (45:35:20). The flow rate was 1 ml/min [9]. Abhi Kavathia has developed a High-performance liquid chromatographic (HPLC) method and validated for the quantitative determination of AB and BH. Validated according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on a reversed-phase Shodex C18 5e column. The mobile phase used was potassium dihydrogen phosphate buffer: Acetonitrile (55:45 v/v) was eluted at 1 ml /min. The retention time for AM and BZ was 4.43 min and 5.70 min respectively at 237 nm detection [10]. Another isocratic reversed-phase stability-indicating HPLC method by A. Mohammadi has developed and validated for the simultaneous determination of AT and AM in commercial tablets. Separation was achieved on a Perfectsil® Target ODS-3, 5 m, 250 mm × 4.6 mm i.d. column using a mobile phase consisting of acetonitrile–0.025 M NaH2PO4 buffer (pH 4.5) (55:45, v/v) at a flow rate of 1 ml/min. The drugs were subjected to oxidation, hydrolysis, photolysis and heat to apply stress conditions. The linearity in the range of 2–30g/ml for AT and 1–20 g/ml for AM [11]. An isocratic reversed phase high-performance liquid chromatographic (HPLC) method with UV detection at 268 nm has been

developed by Deepak Sharma for the determination of amlodipine besylate and nebivolol hydrochloride in dosage formulation. chromatographic separation was achieved by the Lichrospher ODS RP-18 column (250 × 4 mm), particle size 5 µm. mobile phase consisting of ACN and a phosphate buffer (pH 3.0), mixed in a ratio of 40: 60 at a flow rate of 0.8 ml/min [12]. Vaijanath G. Dongre has developed a RP- HPLC method for estimation of MS and AB in tablet dosage form. The chromatographic separation was achieved on Hypersil BDS cyano 250 mm × 4.6 mm, 5m column using PDA detector. The mobile phase consisting of buffer (aqueous triethylamine pH 3) and ACN in the ratio of 85:15 (v/v). The elution flow rate was 1.0 mL/min. The method was validated according to the ICH guidelines [13]. Shaikh Javed Shaikh Afzal et al., has developed a HPLC method for estimation of AM, VALSAT and HCT drugs in the pharmaceuticals tablet formulations developed and validated. Acetonitrile + methanol and Ammonium acetate buffer in the volume ratio of 20:80 v/v. An Hibar RP-18e, 250 X 4.6mm, 5µ as chromatographic column used at a flow rate of 1.300 mLmin-1, injection volume 10 µL and at a wavelength 235 nm with UV detector. Linearity of the analytical method was evaluated at a concentration range of 2.5 µg/ml to 45.3 µg/ml for AM, 32.0 µg/ml to 720.1 µg/ml for VALSAT and 5.0 µg/ml to 112.6 µg/ml for HCT respectively [14]. Another isocratic RP-HPLC for the simultaneous estimation of AM and TELMI in combined dosage form is developed and validated by Saurabh K Sinha. The separation was achieved by using mobile phase ACN and 0.05M sodium dihydrogen phosphate buffer (60:40) adjusted to pH 6.0 at a flow rate of 0.8 mL/min, a C-18 column, perfectsil target ODS3 (150 mm× 4.6 mm i.d., 5 µm). Linearity for amlodipine besylate and telmisartan was established in the range of 5-30 and 10-60 µg/mL, respectively[15]. A stability indicating reversed-phase HPLC method has been developed and validated by K. Raghu Naidu et al., for estimation of AB, and BH from their combination product. The proposed RP-HPLC method utilizes a Zorbax SB C18, 5 µm, 250 mm × 4.6 mm column, mobile phase consisting of phosphate buffer and acetonitrile in the proportion of 65:35 (v/v) pH 7.0 and UV detection at 240 nm. AB, BH, and their combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions. The described method was linear over a range of 6–14µg/ml for AM and 12–28µg/ml for BH [16]. Shaikh Javed Shaikh Afzal et al., has developed a High-Performance Liquid Chromatographic method of analysis for simultaneous determination of assay of Amlodipine, Valsartan and Hydrochlorothiazide drugs in the pharmaceuticals tablet formulations. The assay was accomplished using a mixture of ACN & methanol in the volume ratio of 20:80 v/v (mobile phase B) and Ammonium acetate buffer (Mobile phase A) in gradient flow as mobile phase on an Hibar RP-18e, 250 X 4.6mm, 5µ as chromatographic column. Linearity of the analytical method was evaluated at a concentration range of 2.5 µg/ml to 45.3 µg/ml for Amlodipine,

32.0 µg/ml to 720.1 µg/ml for valsartan and 5.0 µg/ml to 112.6 µg/ml for Hydrochlorothiazide respectively with Correlation coefficient (r) value more than 0.9997[17] .

Table 2: High performance liquid chromotography methods

S.No	Drugs	Method	Chromatographic conditions	Author
1.	AM and Femasartan	RP-HPLC	Mobile phase: ACN and 0.02 M monopotassium phosphate buffer (pH 2.2) Wavelength:237nm Flow rate:1.0 ml/min Retention time: 5.22 and 2.68 min Temp: 40°C	Hyeon Woo Moon et al.,[7]
2.	RP and AM	RP-HPLC	Mobile phase A: 60 mM sodium perchlorate buffer - ACN (60:40, v/v) Wavelength: 210 nm Flow rate:1.0 ml/min Rtention time: 10 min Temp: 55°C	Shi-Ying Dai [8]
3.	AM, HCT And LOSART	RP-HPLC	Mobile phase: ACN, water and 0.4% of potassium dihydrogen phosphate buffer pH 2.7 (45:35:20) Wavelength:230 nm Flow rate: 1.0 ml/min Retention Time:4.9 , 3.9 and 5.8 min Temp: 30°C	Anandkumar R et al [9]
4.	AB And BH	stability indicating RP-HPLC	Mobile phase: potassium dihydrogen phosphate buffer: ACN (55:45 v/v) Wavelength: 237nm Flow rate: 1.0 ml/min Retention time: 4.43 and 5.70 min Temperature: 40°C	Abhi Kavathia [10]
5.	AT And AM	RP-HPLC	Mobile phase: ACN–0.025 M NaH_2PO_4 buffer (pH 4.5) (55:45, v/v) Wavelength:237 nm Flow rate: 1.0 ml/min Retention Time: 9.5 and 4.3 min Temp: Ambient	A. Mohammadi [11]
6.	AB And Nebivolol hydrochloride	RP-HPLC	Mobile phase: ACN and a phosphate buffer (pH 3.0)	Deepak Sharma [12]

			Wavelength: 268 nm Flow rate: 0.8 ml/min Retention Time: 7.42 and 10.2 min Temp: Ambient	
7.	AB And MS	RP- HPLC	Mobile phase buffer (aqueous triethylamine pH 3) and ACN in the ratio of 85:15 (v/v) Wavelength:254 nm Flow rate: 1 ml/min Retention time: 17.3 and 4.3 min Temp: Ambient	Vaijanath G. Dongre[13]
8.	AM , VALSAT And HCT	RP-HPLC	Mobile phase: ACN + methanol and Ammonium acetate buffer (20:80 v/v) Wavelength:235 nm Flow rate: 1.3 ml/min Retention time: Temp: Ambient	Shaikh javed shaikh Afzal, et al., [14]
9.	AM And TELMI	RP-HPLC	Mobile phase: ACN and 0.05M sodium dihydrogen phosphate buffer (60:40) Wavelength:254 nm Flow rate: 1.0 ml/min Retention time:4.0 And 8.2 min Temp: Ambient	Saurabh K Sinha.[15]
10.	AB And BH	RP-HPLC	Mobile phase: buffer and ACN in the proportion of 65:35 (v/v) pH 7.0 Wavelength:240 nm Flow rate: 1.0 ml/min Retention time:21.1 and 8.3 Temp: 28°C	K. Raghu Naidu et al.,[16]
11.	AM, VAL and HCT	RP-HPLC	Mobile phase: ACN + Methanol and Ammonium acetate buffer (20:80 v/v) Wavelength:235 nm Flow rate: 1.3 ml/min Retention time:21.1 and 8.3 Temp: 30°C	Shaikh Javed Shaikh Afzal et al.,[17]

2.2.2 Bio analytical methods

There are only few methods are reported in the bio analysis of the drugs. Bio analytical methods gives the estimation or analysis of the samples from the blood, serum, plasma, urine and other biological fluids this helps in the clinical and preclinical studies of the drugs.

Gh Bahrami et al., has Developed a high-performance liquid chromatographic method using fluorescence detection for analysis of amlodipine in human serum. Amlodipine is extracted from serum by ethyl acetate and involves precolumn derivatization with 4-chloro-7-nitrobenzofurazan (NBD-Cl) and reverse-phase chromatography on C18 column. The mobile phase was sodium phosphate buffer (pH 2.5) containing 1 ml/l triethylamine and methanol. Propranolol was used as internal standard. The retention time was found at 3.7 min. The standard curve was linear over the range 0.25–16 ng/ml of amlodipine in human serum [18]. A simple, sensitive HPLC method was developed and validated by Pawan K. Porwal, Gokul S. Talele for simultaneous estimation of two fixed dose combinations frequently prescribed in diabetes (Metformin plus Glibenclamide) and hypertension with dyslipidemia (Amlodipine plus Atorvastatin) in Human plasma. mobile phase consisting of 0.1% Phosphoric acid (pH 3.0) and AC) in gradient mode with column oven temperature maintained at 30°C. Protein precipitation was employed to extract the selected analyte form human plasma. The recoveries were more than 90% for all analytes in cold aqueous 10% trichloroacetic acid and ACN [19]. Zarghi et al., has developed a sensitive high-performance liquid chromatography (HPLC) method for quantification of amlodipine in plasma. The method involves one-step extraction procedure and analytical recovery was about 97%. The separation was performed on an analytical 125 × 4.6 mm i.d. Nucleosil C8 column. The mobile phase was a mixture of 0.01 M sodium dihydrogen phosphate buffer and ACN (63:37, v/v) adjusted to pH 3.5. The calibration curve was linear over the concentration range 0.5–16 ng ml−1. The coefficients of variation for inter-day and intra-day assay were found to be less than 10% [20]. Another sensitive HPLC method has been developed for the determination of METO and its two metabolites by Tao Xu, Shihui Bao et al., in human plasma and urine. An Agilent XDB-C18 column (150 mm × 4.6 mm, 5 m) using fluorescence detection with Ex 216 nm and Em 312 nm. The mobile phase consisted of ACN–H2O-0.1%TFA. The assay was linear over the concentration range of 5–600 ng/mL and 2.5–300 ng/mL for metoprolol and its metabolites, respectively. The extraction recoveries were found to be more than 86.91% both in plasma and urine [21]. Shah SK, Asnani AJ et al., has developed a sensitive HPLC method for determination of OLM and AM in plasma. The method involves one-step extraction procedure and analytical recovery was about 50%. The separation was performed on an analytical

250mm×4.6 mm Eurospher 100^{-5} C18 coloumn. The Mobile phase consist of ACN: 0.05 ammonium acetate buffer: 0.01 triethyleamine (6.8 ph)[22]. A simple and selective HPLC-DAD stability indicating method has developed for the simultaneous determination of the three antihypertensive drugs AML, VAL and HCT in their combined formulation. Effective chromatographic separation was achieved using Zorbax SB-C8 column (4.6 · 250 mm, 5 lm ps) with gradient elution of the mobile phase composed of 0.025 M phosphoric acid and ACN. The retention times found to be 4.9, 6.4 and 8.3 min for HCT, AML and VAL respectively [23].

Table 3: High performance liquid chromotography methods

S.No	Drugs	Method	Chromatographic conditions	Author
12.	Amlodipine	RP-HPLC with fluorescence detection	Stationary phase: column (150 mm × 6 mm ,5µm) Mobile phase: sodium phosphate buffer (pH 2.5) and methanol. Wavelength:537nm Flow rate:2.8 ml/min Biological fluid: Human serum Temp: 62°C	Gh Bahrami et al., [18]
13.	MET, AM, AT and Glibenclamide	RP-HPLC	Stationary phase: Water's Novapack Phenyl (150 mm × 4.6 mm, 5.0 µm) column Mobile phase: 0.1% Ortho phosphoric acid and acetonitrile (45:55 v/v) Wavelength: 227 nm Flow rate:1.5 ml/min Biological fluid: Human plasma Temp: 30°C	Pawan K. Porwal, Gokul S. Talele et al.,[19]
14.	AM	stability indicating RP-HPLC	Stationary phase: l 125 × 4.6 mm Nucleosil C8 column Mobile phase: 0.01 M sodium dihydrogen phosphate buffer and acetonitrile (63:37, v/v) Wavelength: 239nm Flow rate: 1.5 ml/min Biological fluid: Human plasma	A. Zarghi et al., [20]

15.	METO	RP-HPLC with fluorescence detection	Stationary phase Agilent XDB-C18 column (150 mm × 4.6 mm, 5 µm Mobile phase: f ACN–H_2O-0.1%TFA (pH 3.4) Wavelength:312 nm Flow rate: 0.8 ml/min Biological fluid: Human plasma and urine Temp: 40°C	Tao Xu, Shihui Bao et al., [21]
16.	OLM and AB	RP-HPLC	Stationary phase: 250mm×4.6 mm Eurospher 100^{-5} C18 coloumn Mobile phase:ACN : 0.05 ammonium acetate buffer : 0.01 triethyleamine(6.8 ph) Wavelength: 239 nm Flow rate: 1.0 ml/min Biological fluid: Plasma Temp: Ambient	Shah SK, Asnani AJ et al., [22]
17.	AB, VAL and HCT	RP- HPLC-DAD stability indicating	Stationary phase: Zorbax SB-C8 column (4.6 · 250 mm, 5 µm) Mobile phase: 0.025 M phosphoric acid and CAN Wavelength:225 nm Flow rate: 1 ml/min Biological fluid: Plasma Temp: Ambient	Rasha A. Shaalan et al., [23]
18.	AM	RP-HPLC with amperometry.	Stationary phase: 4.6 x 150 mm, 5µm) column Mobile phase: 0.05 M phosphate buffer solution (pH 3.1)-ACN (65:35, v/v) Wavelength:212 nm Flow rate: 1 ml/min Biological fluid: Serum Temp: Ambient	K. SHIMOOKA, et al., [24]

2.3 LC-MS Methods:

Liquid chromatography coupled with mass spectrometry is one of advanced technique used in drug analysis and mass studies give the information about the molecular weight and fragmentation pattrens of molecule.

Amlan Kanti Sarkar et al., has developed a liquid chromatography–tandem mass spectrometry method and validated for quantification of MPS and AB using HCT as IS in human plasma. mobile phase consists of methanol–water containing 0.5% formic acid (8:2, v/v). The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer. The method was validated over the concentration range of 1–100 ng/ml for MPS and 1–15 ng/ml AM in human plasma. The MRM transition of m/z 268.10–103.10, m/z 409.10–334.20 and m/z 296.00–205.10 were used to measure MPS, AM and HCTZ (IS), respectively [25]. An automated method (XLC–MS/MS) was developed by Jianzhong Shentu for the estimation of amlodipine in human plasma. Automated pre-purification of plasma was performed using 10 mm × 2 mm HySphere C8 EC-SE online solid-phase extraction cartridges. Mobile phase consisted of water with 0.1% formic acid and methanol. Mass spectrometric detection was achieved in the multiple reaction monitoring mode using a quadrupole tandem mass spectrometer in the positive electrospray ionization mode. The XLC–MS/MS method was validated and yielded excellent specificity. The calibration curve ranged from 0.10 to 10.22 ng/mL[26]. Another LC–MS/MS method was developed and validated by Liliya Logoyda et al., for the estimation of AM, BIS, ENA in the presence of its metabolite ENT in real human plasma was developed and validated. The prepared samples were chromatographed using Eclipse C18 column (4.6 × 100 mm, 5 µm) and the mobile phase was pumped in an isocratic mode consisting of ACN: 0.01% formic acid (70:30, v/v) at a flow rate 0.7 mL min−1. The detection was achieved on an API 4500 triple quadrupole mass spectrometer using multiple reaction monitoring mode in the positive electrospray ionization interface. Linearity concentration range of 0.1–10, 0.5–50, 5–500 and 1–100 ng/mL for AM, BIS, ENA and ENT respectively[27]**.** The liquid chromatographic–tandem mass spectrometry method was developed for the accurate quantitation of MPS and SIM in human plasma by P. Senthamil Selvan, T.K. Pal. An isocratic mobile phase consisting of a mixture of ACN and 0.5% formic acid (90:10 (v/v), pH 3.5) flowing through C18 column at a flow rate of 0.2 ml/min. Electro spray ionization with MRM was used to acquire mass spectra. Ions were monitored in positive mode and the mass transitions measured were m/z 268.1→m/z 103.2, m/z 441.3→m/z 325.1 and m/z 260.0→m/z 129.5 for MET, SIM and WIS, respectively [28]. Ehab F. Elkady et al., has develop a method using liquid chromatography-tandem

mass spectrometry (LC-MS/MS) for the estimation of AB, OLM and HCT in human plasma. A mobile phase of 10 mM ammonium formate containing 0.1% formic acid: methanol: ACN (35:50:15, v/v/v). Polarity switching SRM transitions in positive-mode for AML, OLM, and negative-mode for HCT were applied for detection. Sequential double liquid-liquid extraction (SdLLE) was adopted for sample preparation. The developed method was fully validated. The developed method was linear (r2 > 0.99), accurate (105 - 90%), precise (CV% % <11.92) and specific for the determination of AB, OLM and HCT over the concentration range of 0.1–15, 5–1200 and 2–150 ng/mL. The adopted SdLLE resulted into reproducible extraction recoveries of 75%, 63% and 83% for AML, OLM and HCT, respectively [29].

2.4 HPTLC:

HPTLC is a simple and advanced technique of TLC; this method uses very less amount of organic solvents compared to other methods. A.P. Argekar has developed a high-performance thin-layer chromatographic (HPTLC) method for the simultaneous determination of atenolol and amlodipine in tablets, using methylene chloride: methanol: ammonia solution (25% NH_3) (8.8:1.3:0.1; v/v) as the mobile phase. The retention factors of ATL and AMLO were 0.33 and 0.75, respectively. Calibration curves were linear in the range 10–500 mg /ml for both. Assays of atenolol and amlodipine were 49.87 mg and 4.90 mg [30]**.** Another HPTLC method has been developed by Dimal A. Shah et al., for estimating of irbesartan and amlodipine besylate in combination. It involves TLC aluminium plates pre-coated with silica gel 60-F254 as the stationary phase and a solvent system of chloroform/toluene/methanol/acetic acid (6/2.5/1.5/0.5, v/v/v/v). The Rf values were 0.57 ± 0.02 for irbesartan and 0.30 ± 0.02 for amlodipine besylate. The separated spots were analysed densitometrically in absorbance mode at 244 nm. The results were linear in the range 50–500 ng per band for irbesartan and 400–900 ng per band for amlodipine besylate, with limits of detection of 14.4 ng per band and 12.7 ng per band, respectively [31].

3. RESULT AND CONCLUSION

The above-noted records is concise collective statistics approximately the analysis of the Amlodipine and metoprolol by myself or in mixture with different anti-high blood pressure tablets. All the techniques noted are verified in step with the ICH/USFDA guidelines and are usefull inside the analysis of the mentioned drugs. The distinguished method for the analysis of the medication is

carried out by using RP-HPLC in these strategies the majorly used solvents are Acetonitrile with potassium dihydrogen phosphate buffer and methanol with sodium phosphate buffer. In UV spectroscopic methods methanol, water and HCL solvents used, the lamda max for Amlodipine and Metoprolol changed into determined to be 366 nm and 221nm. In Stability indicating HPTLC method most they used the Methanol as mobile phase with other solvents. LC-MS methods with MRM techniques in high quality and poor mode below electro spray ionisation method had been pronounced for the evaluation. In the Bio-analysis the extraction of the drugs is finished through the Protein precipitation and Solid segment extraction, inside the protein precipitation technique Acetonitrile is majorly used because the precipitating agent. In solid segment extraction method MCX sorbent supply the nice consequences as compared to HLB and WCX sorbents.

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Conflict of Intrest: No Conflict of Intrest.

5. ABBREVIATIONS:

AM: Amlodipine

HCT: Hydrochlorothaizide

AT: Atorvastin

BH: Benazepril Hydrochloride

METO: Metoprolol

RP: Ramapril

MS: Metoprolol succinate

ACN: Acetonitrile

TELMI: Telmisartan

MET: Metformin

VAL: Valsartan

SIM: Simvastain

MRM: Multiple Reaction monitoring

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