Advances in Bioresearch
Adv. Biores., Special Issue (3) 2025: 198-211
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585. SPL3.25.198211

Advances in Bioresearch

Analytical Approaches for The Detection and Evaluation of Antihypertensive Drugs in Pharmaceutical Formulations: A Review

Chandrashekhar Patil, Yogita Yadnik, Shweta Gunjal, Shounak Mande, Pritam Dube*
Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik-422213, Maharashtra, India
***Corresponding Author Email:** pritamdube@gmail.com

ABSTRACT
This review explores recent advancements in the development and validation of analytical methods for a wide range of antihypertensive drugs. These methods, including Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), High-Performance Thin Layer Chromatography (HPTLC), and UV-spectrophotometry, are critical for ensuring drug quality, efficacy, and safety in pharmaceutical formulations. Key findings from various studies are summarized, demonstrating the use of modern analytical techniques to measure drugs like Torsemide, Aliskiren, Atenolol, Minoxidil, Lisinopril, and others in tablet, bulk, and solution forms. The review highlights specific parameters such as retention times, mobile phases, detection wavelengths, and linearity ranges optimized for these drugs. For example, RP-HPLC was widely used due to its high precision and reproducibility, with retention times ranging from 2 to 10 minutes depending on the drug and formulation. Mobile phases often comprised mixtures of phosphate buffers, methanol, and acetonitrile adjusted to specific pH values for enhanced separation and detection. Validation of these methods followed International Conference on Harmonization (ICH) guidelines, ensuring reliability through assessments of accuracy, precision, linearity, and robustness.

Keywords: Analytical method development, RP-HPLC, HPTLC, UV-spectrophotometry, Antihypertensive drugs, Validation, Quality control, ICH guidelines, pharmaceutical formulations, Stability testing.

Received 16.05.2025 Revised 19.06.2025 Accepted 18.07.2025

How to cite this article:
Chandrashekhar P, Yogita Y, Shweta G, Shounak M, Pritam D. Analytical Approaches for The Detection and Evaluation of Antihypertensive Drugs in Pharmaceutical Formulations: A Review. Adv. Biores. Special Issue [3] 2025. 198-211

INTRODUCTION

Hypertension, commonly known as high blood pressure, remains a major global health concern, contributing to cardiovascular diseases and increasing mortality rates worldwide [1,2]. The development and quality assessment of antihypertensive drugs are crucial for ensuring their efficacy and safety in clinical applications. Pharmaceutical formulations undergo rigorous analytical evaluation to determine drug potency, purity, stability, and bioavailability. This review explores the various analytical approaches employed for the detection and evaluation of antihypertensive drugs, highlighting advancements in chromatographic and spectroscopic techniques. By examining the principles, methodologies, and applications of these techniques, this study aims to provide a comprehensive understanding of how analytical methods contribute to the effective monitoring of antihypertensive pharmaceuticals, ensuring their therapeutic reliability and regulatory compliance [3].

METHODOLOGY

This review systematically evaluates analytical approaches for the detection and evaluation of antihypertensive drugs in pharmaceutical formulations. The methodology involves an extensive literature survey, data extraction, and synthesis from multiple research papers, emphasizing drug type, analytical method, specifications, mobile phase composition and retention times. Data from selected research papers were extracted and categorized based on drug type, analytical technique, mobile phase composition, retention time, and validation parameters (e.g., accuracy, precision, linearity, and robustness). The extracted information was systematically tabulated to facilitate comparative analysis.

The study critically analyzed the efficiency, sensitivity, and reproducibility of various analytical methods used for detecting antihypertensive drugs.

Analytical Methods

Analytical methods play a vital role in the evaluation and quality assurance of antihypertensive drugs throughout their development and production. The selection of an appropriate analytical approach depends on several key factors, including the chemical nature of the drug, its formulation type, and the specific purpose of the analysis. For instance, the polarity, solubility, and stability of the drug substance influence the type of solvents, detection techniques, and sample preparation steps required. In formulations that contain multiple active ingredients, such as fixed-dose combinations of antihypertensive and diuretics, the analytical method must be capable of simultaneously separating and quantifying each component with high specificity [4]. Sensitivity is another critical factor, particularly in applications such as bioanalytical studies [5], where trace levels of a drug or its metabolites need to be accurately measured in biological fluids. Analytical methods must also be selective enough to distinguish the active drug from its degradation products, impurities, and excipients. Furthermore, all methods must comply with regulatory guidelines and be validated for parameters like accuracy, precision, linearity, robustness, and reproducibility. Ultimately, well-developed and validated analytical methods ensure the safety, efficacy, and quality of antihypertensive drugs and are essential for routine quality control, stability testing, pharmacokinetic evaluation, and regulatory approval.

High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is one of the most widely used and reliable analytical techniques for the analysis of antihypertensive drugs in pharmaceutical formulations and biological samples. Its high sensitivity, selectivity, and reproducibility make it suitable for routine quality control, stability studies, and dissolution testing of various antihypertensive agents, including calcium channel blockers, beta-blockers, ACE inhibitors, ARBs, and diuretics [6]. HPLC is capable of accurately separating active ingredients from impurities, degradation products, and excipients, which is essential for fixed-dose combination formulations [7-9].

Application

Calcium channel blockers such as amlodipine and nifedipine, HPLC is commonly used to analyze formulations, study degradation behavior, and ensure dosage accuracy, especially given their sensitivity to light and oxidation. Beta-blockers like atenolol and metoprolol, HPLC methods are employed not only for tablet analysis but also for bioanalytical purposes, including plasma level monitoring in pharmacokinetic studies. ACE inhibitors, including enalapril and lisinopril, often undergo forced degradation testing using HPLC to establish stability-indicating methods. These drugs are also frequently analyzed in combination with diuretics, requiring precise separation and quantification [10]. Angiotensin II receptor blockers (ARBs) such as losartan and telmisartan are analyzed using HPLC to detect impurities and confirm active content in fixed-dose combinations. Similarly, diuretics like hydrochlorothiazide are analyzed alongside other antihypertensive in multi-component formulations. Alpha blockers and centrally acting agents, such as prazosin and clonidine respectively, are typically evaluated using HPLC for dosage uniformity and stability, particularly in oral dosage forms.

Advantages

Useful for Fixed-Dose Combinations-Capable of simultaneously analyzing multiple drugs in combination formulations. Stability-Indicating Capability -Detects changes in drug content under stress conditions (e.g., light, heat, humidity). Quantification at Low Concentrations-Enables detection in microgram or nanogram levels (especially with sensitive detectors). Non-Destructive Method- Sample remains intact for further analysis or retesting.

Challenges

Complex Formulations-Fixed-dose combinations (e.g., ARB + diuretic) require careful method development to separate multiple drugs and excipients. Matrix Interference-Biological samples (e.g., plasma, serum) may contain interfering substances that affect accuracy and sensitivity. Sample Preparation - Some drugs need extensive pre-treatment (e.g., filtration, extraction), which adds time and potential for error.

High-Performance Thin Layer Chromatography (TLC)

HPTLC is an advanced form of Thin Layer Chromatography (TLC) that offers enhanced resolution, sensitivity, and reproducibility. It operates on the principle of adsorption chromatography, where components of a mixture migrate at different rates on a pre-coated stationary phase (usually silica gel) under the influence of a mobile phase. The sample components separate due to differences in polarity, solubility, or molecular interactions with the stationary and mobile phases. Once separation is achieved, components are visualized, quantified, and compared using densitometry or scanning [11-13].

Application

High-Performance Thin-Layer Chromatography (HPTLC) is a versatile analytical technique widely employed in the pharmaceutical industry for various cardiovascular medications. It is utilized for quantitative estimation of Calcium Channel Blockers in tablets and capsules, and is suitable for stability-indicating methods under stress conditions. For Beta-Blockers, HPTLC is applied in content uniformity testing and detection of degradation products. In the case of ACE Inhibitors, it is commonly used for assay and degradation profiling. For Angiotensin II Receptor Blockers (ARBs), HPTLC is employed for assay and impurity profiling in finished dosage forms, enables simultaneous estimation with hydrochlorothiazide in combination tablets, and is applied in stress degradation studies to evaluate formulation stability [14, 15].

Advantages

Suitable for Multi-Drug Formulations-Capable of analyzing fixed-dose combinations (e.g., ARB + Diuretic). Minimal Sample Preparation-Often requires less complex preparation compared to other chromatographic techniques. Ideal for Herbal and Traditional Formulations -Useful in standardizing polyherbal antihypertensive products.

Challenges

Lower Sensitivity - Compared to HPLC/LC-MS-Not ideal for trace-level or bioanalytical studies (e.g., plasma drug levels).

Manual Errors-Results can vary with manual sample application or development if not automated.

Resolution Limitations-May struggle to separate structurally similar compounds in complex matrices.

Limited Detection Techniques-Primarily relies on UV/Vis densitometry; lacks advanced detection like MS [16].

UV-Visible Spectroscopy:

Principle of UV-Visible Spectroscopy

UV-Visible spectroscopy works by shining light through a drug solution and measuring how much of that light is absorbed. When light in the ultraviolet or visible range passes through the sample, certain wavelengths are absorbed by the electrons in the drug molecule, causing those electrons to move to a higher energy level. This absorption depends on the structure of the molecule, especially if it has aromatic rings or double bonds, which are known as chromophores. The amount of light absorbed is directly related to how much of the drug is present in the solution, which is calculated using Beer–Lambert's law [17-19].

Application

Ultraviolet (UV) spectrophotometry serves as a versatile analytical technique for various antihypertensive drug classes. Calcium channel blockers, such as Amlodipine and Nifedipine, are quantified using absorbance measurements at 238-365 nm, particularly for dissolution profile monitoring in quality control testing [20]. Beta-blockers like Atenolol and Propranolol are analyzed using simple UV methods, typically at wavelengths between 225-290 nm, for content uniformity and degradation studies [21]. Diuretics, including Hydrochlorothiazide and Furosemide, are easily detected at approximately 270 nm for tablet assay and dissolution studies. Alpha blockers (e.g., Prazosin) and central-acting drugs (e.g., Clonidine) are estimated using their UV maxima, usually in the range of 240-280 nm, for formulation analysis and degradation profiling [22]. These UV spectrophotometric methods provide efficient and reliable means for quantitative analysis of antihypertensive medications across different drug classes.

Advantages

Spectrophotometry is particularly advantageous for analyzing light-absorbing drugs, especially those containing structural elements such as double bonds or aromatic rings that interact with UV or visible light. This method's versatility extends to multi-drug formulations, allowing for precise simultaneous measurement of different compounds, provided they absorb light at distinct wavelengths. This dual capability makes spectrophotometry a valuable analytical tool in pharmaceutical research and quality control, enabling efficient and accurate quantification of both single-drug and complex multi-drug systems.

RESULT AND DISCUSSION

The comprehensive review of analytical approaches for the detection and evaluation of antihypertensive drugs in pharmaceutical formulations has provided valuable insights into the effectiveness, reliability, and advancements of various analytical techniques. The extracted information was systematically tabulated to facilitate comparative analysis as you can see in *Table 1*. The gathered data from multiple research papers were systematically categorized, highlighting key parameters such as drug type, analytical method, mobile phase composition, and retention times. The findings demonstrate that Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) remains the most widely employed

technique due to its high sensitivity, precision, and reproducibility in pharmaceutical analysis. High-Performance Thin-Layer Chromatography (HPTLC) has also been shown to offer significant advantages in drug separation and quantification, particularly in quality control applications. Additionally, UV-Spectrophotometry continues to be a simple and cost-effective method for the routine analysis of antihypertensive drugs in bulk and dosage formulations.

This review establishes a strong foundation for researchers and professionals seeking effective analytical techniques for antihypertensive drug evaluation, emphasizing the importance of continual methodological advancements to meet evolving pharmaceutical and regulatory demands.

Table 1: Analytical method validation of various antihypertensive drugs by HPLC method.

Drug	Method	Specification	Mobile phase	Retention time	References
Torsemide	RP-HPLC	• Column x terra c8 (4.6 x 150 mm, 3.5 mm id), • Flow rate 0.8 ml/min • The injection 20 µl volume	40:60% v/v of phosphate buffer and acetonitrile	2.406 min	[23]
	HPTLC	• 10 cm × 10 cm aluminium foil HPTLC plates	Toluene–methanol 8:2 (v/v)		[24]
	RPHPLC	• Injection volume 20 µl • Flow rate 1.5 ml/min • Detection wavelength 288 nm • Column dimensions 4.6 mm x 150 mm	Acetonitrile: phosphate buffer (0.05 m) 70:30 (ph 2.4)		[25]
	RP-HPLC	• Zorbax c18 (250x4.6mm) • Flow rate 1.3 ml/min • The injection 20 µl volume • Wavelength at 288nm	Phosphate buffer and methanol (50:50) (v/v)	6.0±0.2min.	[26]
	HPLC	• C8 (150 mm × 4.6 mm i.d.), 5 µm • Flow rate; 1 ml min−1 • The injection 20 µl volume • Wavelength at 287nm	Phosphate buffer ph 4 : acetonitrile (3:2, v/v)	2.2 ± 0.2 min	[27]
	HPLC	• Luna c column (4.6 mm i.d.) • Flow rate of 1 ml/min. • The injection 20 µl volume • Wavelength at 238nm	Methanol: acetonitrile: phosphate buffer with ph 3.5 ± 0.1 (60: 20: 20 v/v)	3.2 min	[28]
Aliskiren	RP-HPLC	• Hypersil BDS, 250 x 4.6 mm • Flow rate was 1.0 ml/min • Injection volume was 10 µl	Ltea-acetonitrile (60: 40)	5.9 min	[29]
	RP-HPLC	• Phenomenaxlunac18 (ods) column (150 x 4.6 mm i.d., particle	Phosphate buffer ph 3.0: acetonitrile (60:40, v/v)	5.02 min	[30]

		size 5m) • Flow rate 1 ml/ min			
	HPLC	• C8 column (4.6 x 250 mm, 5 µm) • Flow rate 1 ml/min • Wavelength: 220 nm • Injection volume: 10 m	Acetonitrile and phosphate buffer and methanol 45:40:15	3.407 min	[31]
	UV	• Range between 40 and 100 µg ml-1 • UV 1601 pc • Absorbance 279 nm			[32]
Atenolol	RPHPLC	• Inertsil® (ods-3 250 mm x 4.6 mm 5 µm siloxan) • Flow rate 1 ml/min • Injection volume 20 µl • Wavelength detection 276 nm	Acetonitrile and water (v:v%).	1.5 min	[33]
	UV	• (Model 1700) with 1 cm matched quartz cuvettes • Λmax 226nm • Linearity range, µg/ml 4.0-48 • Correlation coefficient (r2) 0.995	Distilled water, methanol, ethanol, 0.1 hcl and phosphate buffer		[34]
	HPLC	• Column (c18; 5µ, 4.6 x 150 mm) • Flow rate 1.0 ml/min • Λmax 237 nm	Methanol-acetonitrile buffer (solution of ammonium acetate and sodium pentanesulphonate ratio, 55:10:35 v/v	1.676 min	[35]
	RP-HPLC	• C18 princeton spher (250 × 4.6 mm) • Flow rate of 1.0 ml/min • Wavelength 270 nm	Acetonitrile : 50 mm potassium dihydrogen orthophosphate (ph 3.5) in the ratio (50 : 50)	15 min	[36]
Metoprolol	HPLC	• Altima (150 x 4.6 mm, 5µ • Flow rate of 1.0 ml/min • Wavelength 225nm • Injection volume : 10µl	Buffer (0.1%opa) and methanol in the ratio of 45:55 v/v	2.249min	[37]
	HPLC	• Novapack c-18 column a250 mm × 4 mm • Injection volume : 10µl • Flow rate of 1.0 ml min−1 . • Wavelength 254 nm.	Acetonitrile–water–triethylamine 18:81:1 (v/v)	1.5 min	[38]
	HPLC	• C18 column • Flow rate of 1.0 ml min−1 • Wavelength 274 nm • Injection volume	Sodium dihydrogen phosphate buffer–acetonitrile (70 + 30) mobile phase	6.32 min	[39]

		was 20 l.			
	HPLC	• Spherisorb® analytical column (5µm, 4.6 x 250mm) • Flow rate 2.0ml/min • Injection volume : 10µl	Acn: orthophosphoric acid: water; ph 3.0		[40]
	UV	• Absorbance 222 nm • Concentration range of 2–16 µg/ml • Molar absorptivity of 3.67 x 104 l/mol.cm	Methanol		[41]
	UV	• Absorption maxima at 222 nm • Concentration range of 5 to 30 µg/m	Distilled water, phosphate buffer 6.8 and 0.1 n hc		[42]
Minoxidil	HPLC	• X-bridge c18 column (150 mm x 4.6 mm id, 5 µm). • Flow rate of 1.0 ml/min • Wavelength of 280 nm • An injection volume of 10 µl	Buffer and acetonitrile mixture in a 55:45 (v/v)	6.6 min	[43]
	RP-HPLC	• Ods c18 column (25 cm × 4.6 mm, 5 µ particle size) • Wavelength 210 nm • Injection volume was 20 µl • Flow rate of 1 ml/min	0.5 % triethyl amine (tea), ph 6.38 ,ortho phosphoric acid (opa) 70:30 v/v	10.005 min	[44]
	UV	• Absorbance maxima at 280.4nm • Concentration range of 0.1-2.5µg/ml.	N-butanol as a solvent ,water		[45]
	RP-HPLC	• C18 column (250 mm × 4.6 mm i.d., particle size 5 µm, thermohypersil bds) • Flow rate of 1 ml/min • Uv detection at 250 nm • Injection volume 5 µl	Methanol-phosphate buffer (ph 3.0, 60:40, v/v)	3.640 min	[46]
Lisinopril	HPLC	• Xterra c8 column (150 mm x 4.6 mm; 3.5 µm) • Flow rate of 0.8 ml/min • Injection volume 20 µl	Phosphate buffer and methanol in the ratio of 35:65v/v	2.298 min	[47]
	UV	• Absorption maxima 206nm • Concentration range 10-50 µg/ml • Correlation coefficient value (r2) 0.999			[48]

	HPLC	• Nucleosil (125 x 4.0mm, 5mm • Flow rate 1.0 ml/min • Wavelength 215 nm • Injection volume 20 µl.	Buffer (ph 2.0), isopropyl alcohol and triethylamine in the ratio (95: 5: 0.1)	7 min	[49]
	RP-HPLC	• Star rp18e 150 mm x 4.6 mm • Flow rate 1.1 ml/min • Injection volume of 20 µl	Potassium dihydrogen phosphate buffer (ph 7.3; 20 mm)-methanol (70:30, v/v)	4.8 min	[50]
	RP-HPLC	• Agilent zorbax bonus-rp column (250 x 4.6 mm, 5µ) • Flow rate: 1-ml/min • Injection volume: 10 µl • Wavelength: 215 nm	Methanol and trifluoroacetic acid (50:50 v/v)	2.28 min	[51]
Ramipril	HPLC	• Fortis c18 (100 mm × 4.6 mm; 2.5 µm particle size). • Flow rate 1.0 ml/min. • Detection wavelength 270 nm, • Injection volume 20 µl	Methanol and citric acid sodium citrate buffer solution (50:50 v/v) ph 3.0	3.645 min	[52]
	HPLC	• Inertsil ods c18 column, 5 µ, 250mm x 4.60mm • Flow rate of 1.5 ml/min • Injection volume 100µl	Potassium di hydrogen phosphate buffer having ph 2.8 and acetonitrile of 60:40 v/v	10.8min	[53]
	RP-HPLC	• Hyperchom c18 column (250 mm, 4.6 mm i.d., 5 µm • Flow rate of 1.5 ml/min. • Wavelength 210 nm. • Injection volume was 10.0 µl.	Potassium dihydrogen phosphate (adjusted to ph = 3.4 using orthophosphoric acid): methanol: acetonitrile in the ratio 70:15:15 (v:v:v)	2.522 min	[54]
Hydrochlorothiazide	RP-HPLC	• Sun fire c18 column (4.6mm x 50, 5µm) • Flow rate of 1.0 ml/min • Wavelength at 225nm • Injection volume 5µl	Tri fluoro acetic acid : acetonitrile	3.07 ± 0.05 min	[55]
	RP-HPLC	• Shim-pack clc-ods column (250 mm x 4.6 mm, 5µ • Wavelength of 254 nm. • Flow rate was 1.5 ml/min	0.025 m phosphoric acid solution: acetonitrile (60:40 v/v, ph 3.0 adjusted with 80% phosphoric acid)	3.748 min	[56]
	RP-HPLC	• Bds c18 column 250x4.6 mm 5µ	Acetonitrile and buffer in the ratio	10 min	[57]

		• Flow rate was 1.0 ml/min • Wavelength 260 nm • Injection volume 20µl	of 55:45 (v/v).		
	UV	• Concentration range 10-50 µg/ml • Correlation coefficient of 0.9916 • Absorption maxima 224-nm.	Methanol		[58]
	HPLC	• C18 column (100 mm 4.6 mm) • Wavelength 233 nm • Flow rate 0.7 ml/min • Injection volume 20µl	60:40 methanol: water	3.440 min	[59]
	RP HPLC	• Younglin, 150mm×4.6mm • Flow rate of 0.7 ml / min • Injection volume of 20 µl	Potassium dihydrogen orthophosphate as buffer and buffer: acetonitrile: methanol (30:10:60)	2.1000 min	[60]
	RP-HPLC	• C18zorbax (eclipse plus, 4.6 × 250 mm, 5 μm) • Injection volume of 20 µ • Flow rate of 1ml/min • Wavelength 272 nm	50 : 50 acetonitrile : water,	3.5 min	[61]
Hydralazine	HPLC	• µbondapak phenyl column (30 cm x 3.9 mm i.d., 10 µm) • Flow rate of 1ml/min • Wavelength 295 nm	Methanol: 2% acetic acid solution (60:40, v/v)	7.4 min	[62]
	HPLC	• Bds hypersil c18 column (250 ×4.6 mm, 5µ) • Flow rate 1.2ml/min • Wavelength 270nm • Injection volume : 20µl	Methanol and acetonitrile solvent ratio : 40:60	2.58min	[63]
	HPLC	• Inertsil ods-3v, 250mm length, 4.6mm • Wavelength 230nm. • Injection volume is 10 µl • Flow rate 1.0 ml/min	Mobile phase-a: buffer and mobile phase-b: methanol	1.0 min	[64]
Spironolactone	HPLC	• Symmetry c-8 analytical column (150x3.9) mm • Injection volume 20µl • Flow rate 1 ml/min • Wavelength 254nm, 283nm	Water, tetrahydrofuran (thf), and acetonitrile (acn) in the ratio of 77:21:2 v/v	1.00 min	[65]

	RP-HPLC	• Inertsil c18 (250 x 4.6mm), 5µ column • Flow rate of 1.0 ml/min • Wavelength 236 nm • Injection volume 20 µl.	Methanol:water (70:30 v/v)	6.69 min	[66]
	HPLC	• Sge 150 × 4.6 mm ss wakosil ii 5c8rs 5-µm • Flow rate of 1.0 ml/min. • Wavelength 254 nm • Injection volume was 20 µl	Acetonitrile–ammonium acetate buffer (50:50, v/v)	3.328 min	[67]
	HPLC	• Symmetry® c18 (150 mm × 4.6 mm, 5m) • A flow rate of 1 ml/min. • Injection volume was 40 µl • Wavelength 238 nm	Methanol:water (60:40, v/v)	9.5 min	[68]
Telmisartan	RP-HPLC	• Symmetry c18 (4.6 x 150 mm, 5 mm) • Flow rate of 1 ml/min. • Wavelength 290 nm • Injection volume was 10 µl	Buffer 0.01 n kh2po: acetonitrile 45:55% v/v	2.593 min	[69]
	RP-HPLC	• C18 column with 250mm x 4.6mm • Wavelength 237nm • Flow rate 0.9 ml/min • Injection volume was 20 µl	Acetonitrile: ph buffer (80:20v/v)	11.97min	[70]
	RP-HPLC	• C-18 (phenyl) column (25 cm x 4.6 mm) • Flow rate: 0.8 ml/min • Injection volume: 10µl • Wavelength 296nm	Acetonitrile: phosphate buffer 90:10 v/v	5.37 min	[71]
	RP-HPLC	• Chromosil c18 (250 mm x 4.6 mm, 5 µm) • Flow rate was 1.5 ml/min • Wavelength 256 nm • Injection volume 20 µ	Methanol : 0.1% orthophosphoric acid : acetonitrile 80:05:15 v/v/v	2.7 min	[72]
Propranolol	RP-HPLC	• C-18 column (3.9 mm x 300 mm, particle size 5 µm) • Flow rate, 1 ml/min • Wavelength 214 nm	Acetonitrile: ph 4.5 phosphate buffer (35:65)	6.6 min	[73]
	RP-HPLC	• Phenomenex luna c18 column (250 x 4.6 mm, 5 µm) • Flow rate of 1.0 ml/min	Methanol: water in the ratio of 40:60 (0.01%o-phosphoric acid)	2.76 min	[74]

		• Wavelength 293 nm			
	RP-HPLC	• Xterra rp18 (150×4.6 mm, 5 µm) • Flow rate 1 ml/min • Wavelength 214 nm. • Injection volume 10 µl	50 mm pyrrolidine (ph 11.5) – acetonitrile (50:50, v/v)	2.86 min	[75]
	RP-HPLC	• Hypersil ods c-18 column (250*4.6 mm, i.d., 5 µm particle size) • Flow rate 1 ml/min • Injection volume 20 µl	Acetonitrile, methanol, and 0.01 m disodium hydrogen phosphate (ph 3.5) 50:35:15 v/v	6.62±0.003 min	[76]
	RP-UPLC	• Acquity beh c18 column (30 x 2.1 mm, 1.7µm) • Flow rate was 0.3 ml/min • Wavelength 230 nm • Injection volume 1µl	Trifluoroacetic acid (0.1%) and acetonitrile in the ratio 80:20 v/v	0.98 min	[77]
Diltiazem	RP-HPLC	• Flow rate of 1.0 ml/min • Column hiber, 250-4.6 rp-18 column • Injection volume of 20 µl • Wavelength 230 nm.	Acetonitrile–methanol–water (30:20:50, v/v, ph 2.59 ± 0.02	2.34 min	[78]
	RP-HPLC	• Zorbax [c8 (5µ, 4.6 mm×250)] • Flow rate of 1.0ml/min. • Wavelength 240 nm	Buffer and acetonitrile in the ratio of (60:40).	4.66 min	[79]
	HPLC	• Bds c18 (150 mm 4.6 mm, 5.0 mm) • Flow rate of 1.0 ml/min • Injection volume of 10 ml	Acn and/or methanol)	0.28 min	[80]
	HPLC	• A zorbax sb-c18 (4.6 mm x 250 mm, 5 µm) • Flow rate was 0.7 ml/min. • Wavelegth 240 nm	0.2 m ammonium dihydrogen phosphate, acetonitrile, isopropyl alcohol and triethylamine (55:43:1.7:0.3, v/v)	4 min	[81]
	RP-HPLC	• C18 column (250 mm ×4.6 mm, 5 μm particle size) • Flow rate of 2.0 ml/min • Injection volume of 20 µl • Wavelegth 240 nm	Ethanol: phosphoric acidsolution (ph = 2.5) (35 : 65, v/v	5.8 min	[82]

CONCLUSION

The reviewed advancements in analytical methods for antihypertensive drugs highlight the growing importance of precision, robustness, and validation in pharmaceutical quality control. As hypertension remains a major global health concern, ensuring the safety, efficacy, and consistency of antihypertensive formulations is paramount. Modern analytical techniques, including RP-HPLC, HPTLC, and UV-

spectrophotometry, have demonstrated exceptional versatility in the quantitative and qualitative analysis of various pharmaceutical preparations, ranging from finished dosage forms like tablets and capsules to raw bulk materials.

A critical aspect of these analytical methods is their adherence to the rigorous standards established by the International Council for Harmonisation (ICH) guidelines. Compliance with these guidelines guarantees that analytical techniques are not only reliable and reproducible but also suitable for routine application in pharmaceutical industries and regulatory settings. The validation parameters—such as accuracy, precision, specificity, linearity, and robustness play a crucial role in ensuring that antihypertensive drugs meet the required quality standards before reaching consumers.

Despite the significant advancements in analytical methodologies, there is a continuous need for innovation and refinement to address emerging challenges in pharmaceutical analysis. Future research should focus on enhancing analytical efficiency, optimizing separation techniques, and developing eco-friendly methodologies to minimize environmental impact.

This review serves as a comprehensive resource for researchers, analysts, and pharmaceutical professionals committed to ensuring the safety, efficacy, and quality of antihypertensive medications. By leveraging the latest analytical approaches and embracing technological innovations, the pharmaceutical industry can continue to evolve and meet the dynamic demands of modern healthcare, ultimately contributing to improved patient outcomes and global health advancements.

REFERENCES

1. Fathani HC, Perdhana F, Husain TA. (2024). Epidural Anesthesia in Pregnant Women with a Total AV Block and Preeclampsia: A Case Report. J Med Chem Sci.;7(3):492–9.
2. Fahad S, Alghassab M, Omar A, Alshuqair A, Abdulsayed W, Bamsoud A, et al. (2024). Nutritional and Biochemical Aspects of Obesity: A Comprehensive Study to Understand Obesity Mechanism. J Med Chem Sci.; 7:1982–2002.
3. Srivastava, B., Sharma, S., & Pathak, A. (2013). Development and validation of RP-HPLC method for the simultaneous estimation of Atenolol and Hydrochlorothiazide in pharmaceutical dosage forms. *Int. J. Pharm. Pharm. Sci.*, 5(4):673–677.
4. Patil, P., & Sane, R. (2018). Development and validation of stability-indicating RP-HPLC method for simultaneous estimation of Lisinopril and Hydrochlorothiazide in bulk and tablet dosage form. *J. Pharm. Biomed. Sci.*, 8(3):66–72.
5. Bansal, A., & DeStefano, A. (2007). Key elements of bioanalytical method validation for small molecules. *AAPS J.*, 9(1):E109–E114.
6. Rane, V. P., Patil, K. R., Sangshetti, J. N., & Shinde, D. B. (2009). Stability-indicating LC method for the simultaneous determination of losartan potassium and hydrochlorothiazide in pharmaceutical dosage form. *J. Chromatogr. Sci.*, 47(7):599–604.
7. Patel, R. B., Patel, M. R., & Patel, B. G. (2012). Simultaneous RP-HPLC estimation of amlodipine and atenolol in combined dosage forms. *Int. J. Pharm. Sci. Res.*, 3(7):2278–2283.
8. Shinde SA, Deshmukh AD, More UK. (2023). Neonatal Sickle Cell Disease Assessment in Rural Indian Community: Demand for Point of Care Testing (POCT). J Med Chem Sci.;7(1):1–8.
9. Chandramore KR, Sonawane SS, Ahire RS, Reddy H, Ahire SB, Jadhav PB, et al. (2025). Development and Validation of Stability Indicating LC Method for Selexipag : In-Silico Toxicity Study and Characterization of its Degradation Products. Chem Methodol.;9:427–47.
10. Balamurugan, K., & Ganesh, M. (2016). Analytical method development and validation for estimation of lisinopril in pharmaceutical dosage form by RP-HPLC. *Int. J. Pharma Bio Sci.*, 7(2):74–80.
11. Rote, A. R., & Bari, S. B. (2010). Simultaneous densitometric analysis of amlodipine besylate and atorvastatin calcium in tablets by HPTLC. *J. Chromatogr. Sci.*, 48(7):566–569.
12. Patel J, Tandel J, Chhalotiya U, Patel K. (2021). Stability indicating planar chromatographic method for estimation of minoxidil and finasteride combination used in the treatment of hair loss. J Med Chem Sci.;4(1):17–28.
13. Nagda D, Captain A. (2025). Validated Green Densitometric Method for Estimation of Naringin Biomarker in Hydroalcoholic Extract of Leaves of Ceiba Pentandra (L.) Gaertn. Asian J Green Chem.; 9:427–42.
14. Bhatia, N. M., & Deshmukh, P. T. (2013). Stability-indicating HPTLC method for simultaneous estimation of metoprolol succinate and hydrochlorothiazide in pharmaceutical formulation. *J. Planar Chromatogr. – Mod. TLC*, 26(5):410–415.
15. Puranik, M., Wadher, S. J., & Taksande, J. B. (2011). HPTLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in bulk and tablet formulation. *Asian J. Res. Chem.*, 4(3):453–456.
16. Devarajan, P. V., & Gore, S. P. (2006). UV Spectrophotometric method for the estimation of amlodipine besylate in tablets. *Indian Drugs*, 43(8):655–658.
17. Tabrizi, A. R. (2003). Spectrophotometric determination of atenolol in pharmaceutical formulations using bromocresol green. *Il Farmaco*, 58(11):1181–1186.
18. Salman SS. (2024). Green Synthesis, Analysis, and Characterization of Nano-silver- Based Conyza Canadensis (SYN: Erigeron Canadensis) Extract. Chem Methodol.;8:856–73.

19. Ashindortiang OI, Anyama CA, Ayi AA. (2022). Phytosynthesis, Characterization and Antimicrobial Studies of Silver Nanoparticles Using Aqueous Extracts of *Olax Subscorpioidea*. Adv J Chem Sect A.;5(3):215–25.
20. Al-Azzam, K. M., Alzoubi, K. H., & AbuRuz, S. M. (2005). Development and validation of a spectrophotometric method for the determination of 5-fluorouracil in pharmaceutical formulations. *Il Farmaco*, 60(6–7):555–558.
21. Sethi, P. D. (2008). *High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations*. CBS Publishers & Distributors Pvt. Ltd., New Delhi, pp. 1–15.
22. Beckett, A. H., & Stenlake, J. B. (2001). *Practical Pharmaceutical Chemistry*, Vol. 2. CBS Publishers & Distributors Pvt. Ltd.
23. Meher, V.K., Gautam, G.K., Patro, S.K. & Dash, S.K. (2022). Method development and validation for Torsemide in tablet dosage form by RP-HPLC. *J. Pharm. Negative Results*, 13(Special Issue 04).
24. Kakde, R., Chaudhary, N., Barsagade, A. & Kale, D. (2011). Stability-indicating HPTLC method for analysis of Torsemide in pharmaceutical preparations. *Acta Chromatographica*, 23(1):145–155.
25. Singh, P., Patel, A., Patel, S. & Patel, B. (2019). Development and validation of RP-HPLC method for the determination of Torsemide in bulk dosage form and pharmaceutical formulation. *World J. Pharm. Res.*, 8(9):1381–1386.
26. Ghodke, A.Y., Poul, B.N. & Sorde, M.B. (2014). Analytical method development and validation for quantitative estimation of Torsemide in bulk and pharmaceutical dosage form by RP-HPLC. *Int. J. Pharm. Chem. Anal.*, 1(1): October.
27. Zaazaa, H.E., Abbas, S.S., Essam, H.M. & El-Bardicy, M.G. (2016). Development and validation of stability-indicating methods for determination of Torsemide. *Bull. Chem. Soc. Ethiop.*, 30(1).
28. Dubey, N., Patil, G., Jain, D.K. & Chaturvedi, S. (n.d.). Simultaneous estimation of Torsemide and Spironolactone in combined dosage form using reverse phase liquid chromatography. *Int. J. Biomed. Pharm. Sci.*, 7(1):38–41.
29. Rekulapally, V.K. & Rao, V.U. (2014). Stability indicating RP-HPLC method development and validation for simultaneous estimation of Aliskiren, Amlodipine, and Hydrochlorothiazide in tablet dosage form. *Int. J. Pharm. Pharm. Sci.*, 6(1):2014.
30. Swamy, G.K., Rao, J.V.L.N.S., Kumar, J.M.R., Kumar, U.A., Bikshapathi, D.V.R.N. & Kumar, D.V. (2011). Analytical method development and validation of Aliskiren in bulk and tablet dosage form by RP-HPLC method. *J. Pharm. Res.*, 4(3):865–867.
31. Ghosh, S., Anusha, B., Santhoshi, S., Banji, D., Kumar, Y.C., Raghavendra, P. & Roy, S. (2013). Method development and validation of Aliskiren hemifumarate and Valsartan in bulk drug by RP-HPLC method. *Asian J. Res. Chem.*, 6(1):19.
32. Wrasse-Sangoi, M., Secretti, L.T., Diefenbach, I.F. & Rolim, C.M.B. (2010). Development and validation of a UV spectrophotometric method for the determination of Aliskiren in tablets. *Quim. Nova*, 33(6):1330–1334.
33. Mohammed, N.S. (2017). Method development and validation of Atenolol using two HPLC systems. *Int. J. Pharm. Sci. Res.*, 8(6):1000–1006.
34. Kori, S., Goyal, J., Sharma, S., Pahade, N.K. & Tandekar, M. (n.d.). Method development and validation of Atenolol drug by spectrophotometric and HPLC technique in forensic application. *Int. J. Sci. Res. (IJSR)*, ISSN (Online):2319–7064.
35. Haque, M.A., Naznin, A., Kabir, A.N.M.H., Hossain, M.K. & Islam, S.M.A. (2010). Development and validation of RP-HPLC method for the simultaneous estimation of Atenolol and Amlodipine in tablet dosage form. *Dhaka Univ. J. Pharm. Sci.*, 9(2):131–138.
36. Kavitha, J. & Muralidharan, S. (2010). Development and validation of new method for Atenolol, Hydrochlorothiazide, and Losartan Potassium by RP-HPLC: its application to routine quality control analysis. *Int. J. ChemTech Res.*, 2(2):880–884.
37. Kumar, P.V., Lokeswara, G., Haribaskar, V. & Gobinath, M. (2015). Development and validation of RP-HPLC method for simultaneous estimation of Metoprolol Succinate and Cilnidipine in combined tablet dosage form. *Int. J. Pharm.*, 5(4):1196–1202.
38. Aqil, M., Ali, A., Ahad, A., Sultana, Y., Najmi, A.K. & Saha, N. (2007). A validated HPLC method for estimation of Metoprolol in human plasma. *Acta Chromatographica*, 19:130–141.
39. Phale, M.D. & Hamrapurkar, P.D. (2010). Optimization and establishment of a validated stability-indicating HPLC method for study of the stress degradation behavior of Metoprolol Succinate. *J. AOAC Int.*, 93(3):882–890.
40. Shashank, S., Veerma, R., Divya, V. & Anurag, V. (2021). Analytical method development and validation of Metoprolol Succinate by high performance liquid chromatography and ultraviolet spectroscopy technique. *Res. J. Pharm. Technol.*, 14(2):936–941.
41. Verma, N., Ghosh, A.K. & Chattopadhyay, P. (2011). UV-spectrophotometric determination of Metoprolol Succinate. *Res. J. Pharm. Technol.*, 4(2):271–272.
42. Dhale, C., Joshi, S. & Shete, S. (2014). Development and validation of spectrophotometric method for determination of Metoprolol Tartrate. *J. Appl. Pharm.*, 6(3):280–285.
43. Fathima, S.F., Azam, S.G.U. & Bee, S.S. (2023). Assay method development and validation of Minoxidil film-coated tablets. *Eurasian J. Anal. Chem.*, 18(1):01–13.
44. Patel, N. & Meshram, D. (2015). Development and validation of analytical method for simultaneous determination of Minoxidil and Finasteride in pharmaceutical dosage form by RP-HPLC method. *Int. J. Pharm. Sci. Res.*, 6(11):4882–4885.

45. Vijayamma, G., Salomi, P., Venkatesh, P., Hepcy Kala Rani, D., Gowramma, A. & Mounika, P. (2015). Analytical method development and validation of Minoxidil in pharmaceutical dosage forms by UV spectrophotometry. *Int. J. Innov. Pharm. Res.*, 6(1):464–467.
46. Rudrapal, M., Surya Kiran, B.V.V.S., Sridhar, N. & Raghavendra, M. (2016). Development and validation of RP-HPLC method for simultaneous estimation of Minoxidil and Aminexil in topical formulation. *Asian J. Chem.*, 28(1):157–160.
47. Raju, V.B. & Rao, A.L. (2012). Development, estimation, and validation of Lisinopril in bulk and its pharmaceutical formulation by HPLC method. *E-J. Chem.*, 9(1):340–344.
48. Chavan, V. & Phasate, P. (n.d.). Development and validation of a UV spectrophotometric method for the determination of Lisinopril both in bulk and marketed dosage formulations. *Int. J. Pharm. Sci. Res. (IJPSR)*.
49. Chander, V., Mohan, M., Seth, R., Singh, P., Singh, R. & Gupta, S. (2012). Development and validation of RP-HPLC method for the estimation of Lisinopril in tablet dosage form. *Anal. Chem. Lett.*, 2(5):309–313.
50. Piponski, M., Bakovska Stoimenova, T., Trendovska Serafimovska, G. & Stefova, M. (2019). Development and validation of a fast, simple, cost-effective, and robust RP-HPLC method for simultaneous determination of Lisinopril and Amlodipine in tablets. *Anal. Chem. Lett.*, 9(3):385–402.
51. Pawar, V. & More, H. (2023). HPLC method validation for quantification of Lisinopril. *Int. J. Pharm. Qual. Assur.*, 14(2):298–302.
52. Patil, D.A., Rangari, S.S., Nikumbh, P.P., Chaudhari, S.S. & Bhurat, M.R. (2022). Analytical method development for the determination and quantification of Ramipril in pharmaceutical formulation by HPLC. *Int. J. Res. Trends Innov.*, 7(9), ISSN:2456-3315.
53. Jawla, S., Jeyalakshmi, K., Krishnamurthy, T. & Kumar, Y. (2010). Development and validation of a simultaneous HPLC method for estimation of Telmisartan and Ramipril in pharmaceutical formulations. *Int. J. PharmTech Res.*, 2(2):1625–1633.
54. Taha, E.A., Fouad, M.M., Attia, A.K. & Youse, Z.M. (2019). RP-HPLC method development and validation for simultaneous estimation of Ramipril and Felodipine. *Eur. J. Chem.*, 10(2):113–117.
55. Gupta, A., Majee, C., Salahuddin & Jindal, R. (2019). Analytical method development and validation of Hydrochlorothiazide in tablet formulation by using RP-HPLC. *Eur. J. Biomed. Pharm. Sci.*, 6(7):281–285.
56. Hossen, M.A., Haque, M.A., Dewan, I., Kabir, A.N.M.H., Hossain, M.K. & Islam, S.M.A. (2011). Development and validation of RP-HPLC method for the simultaneous estimation of Hydrochlorothiazide and Losartan Potassium in tablet dosage form. *Dhaka Univ. J. Pharm. Sci.*, 10(1):35–42.
57. Eswarudu, M.M., Chary, T.N., Junapudi, S. & Sushma, M. (2012). RP-HPLC method development and validation for simultaneous estimation of Irbesartan and Hydrochlorothiazide in pharmaceutical dosage form. *Asian J. Res. Chem.*, 5(4).
58. Raja, A. & Dakshnamurthy, S. (2010). Method development and validation of Hydrochlorothiazide in tablet dosage form by UV spectroscopy. *Int. J. Res. Pharm. Sci.*, 1(3).
59. [51]. Ahmad, S., Mujawar, T., Pawara, J., Sable, V., Patil, K. & Tare, H. (2023). Analytical method development and validation for the simultaneous estimation of Olmesartan and Hydrochlorothiazide by RP-HPLC in bulk and tablet dosage forms. *Int. J. Pharm. Qual. Assur.*, 14(1).
60. Tejaswi, J.K.D., Rajan, R.G. & Nagaraju, K. (2016). Analytical method development and validation of Hydrochlorothiazide and Irbesartan in combined dosage form by RP-HPLC. *World J. Pharm. Res.*, 5(8):938–948.
61. Mohammed, N.S. & Ahmed, J. (2016). Development and validation of RP-HPLC method for the determination of Hydrochlorothiazide in bulk drug and pharmaceutical dosage form. *Chromatogr. Res. Int.*, 2016, Article ID:1693024.
62. Shah, K.V., Chauhan, S.P. & Suhagia, B.N. (2014). Analytical methodologies for the determination of Hydralazine: A review. *Res. Rev. J. Pharm. Anal.*, 3(2).
63. Monika, M., Swathi, B. & Faheemuddin, M. (2017). Method development and validation of Hydralazine Hydrochloride by using UV and RP-HPLC. *Indo Am. J. Pharm. Res.*, 7(11).
64. Anerao, A., Dighe, V., Pagire, R., Sonavane, S. & Pradhan, N. (2015). Development and validation of related substances method by HPLC for analysis of Hydralazine Hydrochloride. *World J. Pharm. Pharm. Sci.*, 4(11):1944–1965.
65. Singhal, R., Cauhan, C., Sharma, P. & Sachdeva, M. (2022). HPLC method development and validation of Spironolactone in tablet dosage forms in presence of impurities and degradants. *Int. J. Pharm. Sci. Res.*, 13(12).
66. Maulik, D., Dadhania, K. & Faldu, S. (2012). Development and validation of RP-HPLC method for simultaneous estimation of Furosemide and Spironolactone in their combined tablet dosage form. *J. Pharm. Sci. Bio-Res.*, 2(3):144–147.
67. Ram, V.R., Dave, P.N. & Joshi, H.S. (2012). Development and validation of a stability-indicating HPLC assay method for simultaneous determination of Spironolactone and Furosemide in tablet formulation. *J. Chromatogr. Sci.*, 50:721–726.
68. Sandall, J.M., Millership, J.S., Collier, P.S. & Mcelnay, J.C. (2006). Development and validation of an HPLC method for the determination of spironolactone and its metabolites in paediatric plasma samples. *J. Chromatogr. B*, 839:36–44.
69. Spandana, V. & Siddartha. (n.d.). Analytical method development and validation of Azelnipidine and Telmisartan by RP-HPLC method. *Res. Rev. J. Pharm. Nanotechnol.*

70. Ahmad, S., Sonawane, N. & Tatiya, A.U. (2022). Analytical method development and validation for the simultaneous estimation of azelnidipine and telmisartan by RP-HPLC in bulk and tablet dosage forms. *Res. J. Pharm. Technol.*, 15(12):5736–5740.
71. Sharma, S., Soni, V., Rahar, S. & Bhatia, N. (2016). Development and validation of method for the estimation of telmisartan as active pharmaceutical ingredient in tablet dosage form and prepared spherical agglomerates by RP-HPLC. *J. Pharm. Technol. Res. Manag.* DOI: 10.15415/jptrm.2016.41005.
72. Basaveswara Rao, M.V., Nagendrakumar, A.V.D., Sivanadh, M. & Venkata Rao, G. (2012). Validated RP-HPLC method for the estimation of telmisartan in tablet formulation. *Bull. Pharm. Res.*, 2(2):50–55.
73. Srikanth, M.V., Janaki Ram, B., Sunil, S.A., Sreenivasa Rao, N. & Ramana Murthy, K.V. (2012). Development and validation of HPLC method for estimation of propranolol HCl in human plasma. *J. Sci. Ind. Res.*, 71:120–123.
74. Mahesh, M., Thandra, S., Muneer, S., Sai Kiran, B. & Mamatha, H. (2019). Method development and validation of RP-HPLC method for the simultaneous estimation of propranolol and hydralazine in pharmaceutical dosage form. *Asian J. Pharm. Anal.*, 9(1):1–10.
75. Shabir, G.A. (2011). Development and validation of RP-HPLC method for the determination of methamphetamine and propranolol in tablet dosage form. *Indian J. Pharm. Sci.*, 73(4):391–397.
76. Imam, S.S., Ahad, A., Aqil, M., Sultana, Y. & Ali, A. (2013). A validated RP-HPLC method for simultaneous determination of propranolol and valsartan in bulk drug and gel formulation. *J. Pharm. Bioall. Sci.*, 5:61–65.
77. Yadav, P. & Majee, C. (2015). Method development and validation for quantification of propranolol HCl in pharmaceutical dosage form by RP-UPLC. *Int. J. Pharm. Sci. Res.*, 7(1):197–203.
78. Sultana, N., Arayne, M.S., Shafi, N., Siddiqui, F.A. & Hussain, A. (2011). Development and validation of new assay method for the simultaneous analysis of diltiazem, metformin, pioglitazone, and rosiglitazone by RP-HPLC and its applications in pharmaceuticals and human serum. *J. Chromatogr. Sci.*, 49(11–12):1057–1064.
79. Patil, B.R., Bhusnure, O.G., Paul, B.N., Ghodke, A.Y. & Mulaje, S.S. (2014). Analytical method development and validation for the estimation of diltiazem hydrochloride in bulk and pharmaceutical dosage form by RP-HPLC. *Int. J. Drug Regul. Aff.*, 2(2):78–84.
80. Palliwar, V.A., Porwala, P.K. & Upmanyu, N. (2012). Validated gradient stability indicating HPLC method for determining diltiazem hydrochloride and related substances in bulk drug and novel tablet formulation. *J. Pharm. Anal.*, 2(3):226–237.
81. Arafat, M. (2014). Simple HPLC validated method for the determination of diltiazem hydrochloride in human plasma. *Int. J. Pharm. Pharm. Sci.*, 6(9):213–216.
82. Sadeghi, F., Navidpour, L., Bayat, S. & Afshar, M. (2013). Validation and uncertainty estimation of an ecofriendly and stability-indicating HPLC method for determination of diltiazem in pharmaceutical preparations. *J. Anal. Methods Chem.*, 2013, Article ID: 353814.