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REVIEW ARTICLE

A Review on recent approaches for the use of different Analytical Techniques to Analyze some Calcium Channel Blockers and their Combinations with other Antihypertensive Drugs

Saylee Ganorkar[1], Nilesh Kulkarni[2] and Rahul Khiste[1,*]

[1]*Department of Pharmaceutical Chemistry, Marathwada Mitra mandal's College of Pharmacy, Thergaon, Pune 411033, Maharashtra, India*
[2]*Department of Pharmaceutics, Progressive Education Society's PES Modern College of Pharmacy (For Ladies), Moshi, Pune 412105, Maharashtra, India*

Abstract:

Background:

Diabetes, high cholesterol, and high blood pressure all considerably raise the risk of cardiovascular disease. When all three of these characteristics occur at once, a metabolic problem is postulated. A combination of antihypertensive, hypolipidemic, and anti-diabetic medications is frequently utilised to treat cardiovascular diseases. While statins (fluvastatin, simvastatin, *etc*.) are used to lower cholesterol levels, calcium channel blockers (*e.g*. amlodipine, efonidipine, and azelnidipine, *etc*.) are used to target the smooth muscles of the heart. Diuretics (*e.g*. chlortalidone, hydrochlorothiazide, *etc*.) and angiotensin II receptor antagonist (blockers) are also used to manage high blood pressure.

Objective:

The study aimed to review liquid chromatography and related high-performance (HPLC) techniques that have been developed and used for evaluating the above drugs, together with an overview of the research work published in various scientific and drugs-linked journals.

Results:

A basic critical investigation of the detailed published information has been completed and the current status of HPLC and related techniques as a percent measure of calcium channel blockers has been examined.

Conclusion:

This survey has explored several matrices, including pharmacological products and organic samples, as well as methods for examining direct calcium blockers in them. It also discusses the current state of calcium channel blocker stability investigations. Additionally, it offers scientific approaches for the concurrent estimate of angiotensin II receptor antagonism, diuretics, statins, and beta-blockers with calcium channel blockers.

Keywords: HPLC, Azelnidipine, Efonidipine, Cilnidipine, Calcium channel blockers (CCBs), Diabetes.

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1. INTRODUCTION

Hypertension is a regular, ongoing, age-related problem, which frequently involves weakening cardiovascular and renal entanglements. Pulse is normally noted in blend with other cardiovascular factors. Hypertension is associated with other cardiovascular factors, for example, stomach weight, dyslipidemia, diabetes, hyperinsulinemia, and hyperuricemia, which are typical fundamental reasons. Hypertension progressively depends on computerized procedures of circulatory strain estimation. Antihypertensive medication treatment decreases the complications of hypertension. Historically, doctors have prescribed calcium channel blockers to manage hypertension and prevent angina. A common therapy option for hypertension is a group of medications known as dihydropyridine calcium channel blockers, which also include amlodipine, felodipine, and lacidipine.

Dihydropyridine calcium channel blockers (CCBs) act by loosening up vascular smooth muscle, widening veins and thus diminishing fringe obstruction. Benzothiazepines (such as

* Address correspondence to this author at the Department of Pharmaceutical Chemistry, Marathwada Mitra mandal's College of Pharmacy, Thergaon, Pune 411033, Maharashtra, India; E-mail: rahulkhiste@yahoo.com

diltiazem and clenazem), phenylalkylamines (such as verapamil and gallopamil), and dihydropyridines are the three subgroups of CCBs based on the synthetic design (*e.g*., nifedipine, nicardipine, felodipine, amlodipine, aranidipine, azelnidipine, cilnidipine, efonidipine, manidipine, and nilvadipine). There are a variety of activities associated with these agents due to the differences in chemical structure. Dihydropyridines do not have a negative chronotropic effect due to reflex tachycardia brought on by fringe vasodilation, unlike benzothiazepines and phenylalkylamines, which have negative inotropic or chronotropic effects.

Every year, more prescription drugs are being released into the market. From the time a medicine is introduced to the market to the time it is considered in pharmacopeias, there is frequently a delay. This is due to potential vulnerabilities in the continuous and widespread use of these drugs, reports of new toxic substance levels (resulting in their withdrawal from the market), advancement of patient opposition, and the presentation of better medications by competitors. In these circumstances, principles and scientific methods for these medications may not be accessible in the pharmacopeias. There is an extension, however, to create new logical strategies for such medications. Analytical techniques' improvement and approval assume significant parts in the revelation, advancement, and also in assembling of drugs. Drug items planned with more than one medication, normally alluded to 'mix' items, are planned to meet beforehand neglected patient needs by providing the remedial impacts of at least two medications in a single dosage form. These mix items can introduce overwhelming difficulties to the analytical chemist, which can result in the turn of events and also the approval of the scientific techniques. The authority test strategies that come out from these cycles are utilized by quality control research facilities to guarantee the character, virtue, strength, and execution of medication. ID and measurement of debasements are a pivotal assignment in drug process advancement to ensure quality and safety. Debasements in drugs are undesirable synthetic substances that remain with the dynamic drug fixings (APIs), or created during security testing, or during plan or after maturing of the two APIs to prescriptions. Even small amounts of these undesirable synthetic substances may have an impact on the drugs' efficacy and safety. Different analytical strategies are utilized for the assurance of related compounds in drugs. There is an incredible requirement for the improvement of new logical strategies for the quality assessment of newly arising drugs.

Analytical chemistry is a branch of science that uses advanced technologies to determine the above problems using scientific methods. We are capable of achieving both subjective and quantitative results. Analytical instruments play an important role in producing high-quality and trustworthy scientific data.

Analytical techniques include chromatographic, electrochemical, hyphenated mass spectrometric methods, *etc*., or a combination of these. The need for approval of the analytical strategy improvement arose because of worldwide rivalry and keeping up with the standard of items in high business and for market esteem as well as moral reasons [1].

Various instrumental methods used for developing analytical methods and validating the same are as follows:

2. UV-Vis SPECTROPHOTOMETRIC METHODS

A very practical technique for analysing medicines in pharmaceutical formulations is spectrophotometric methods analysis, which is simple, affordable, and quick.

2.1. Analysis by Spectrophotometry

In the fields of pharmaceutical and biological research, derivative UV spectroscopy is a commonly used technique for quality control, quantitative analysis, and product characterization management. Derivative spectroscopy typically produces considerably better fingerprints than the conventional absorbance spectrum. Derivative spectrophotometry's increased sensitivity is mostly attributable to the proper electrical or numerical signal amplification that reduces noise. The primary spectrum's bands' shapes, particularly the bands' half-width, are important determinants of sensitivity. The derivative of the curve's primary peak is determined by the lowering and ascending portions of the curve's slope, and the intensity of the output spectrum has an inverse relationship with the absorption. In comparison to traditional spectrometry, derivative UV spectra may improve sensitivity and selectivity, while also making it possible to locate precisely the bands' λmax values, extremely thin bands, and points of inflection.

2.2. HPLC Techniques

Over 30 years have passed since the introduction of high-performance liquid chromatography, which is now an established method. An appropriate system and 1.7 m size particles of adsorbents in columns form extremely effective liquid chromatography (UPLC) equipment, which enables the analysis of various chemicals with noticeably higher resolution, shorter run times, and increased sensitivity [2].

2.3. Methods of Thin Layer Chromatography

Thin layer techniques lost popularity as a result of HPLC technology advancement in the 1970s, which limited their ability to record and store separation data. High-performance thin-layer chromatography was created as a result of the current resurgence in interest in this method (HPTLC). The HPLC and HPTLC techniques are interconnected. The primary distinctions pertain to the kinetics of the resolution process and the stationary phase's spatial orientation for thin-layer chromatography (TLC) to advance, tinier particles, densitometers as detectors, automatic dose apparatus, and derived sample findings. Establishing TLC in pharmaceutical analysis is a significant breakthrough in quality control. TLC is quickly replacing traditional analytical methods owing to its cost advantages, increased sampling throughput, and minimal sample preparation requirements. Contrary to LC, the main TLC's benefit is that multiple samples can be run concurrently utilising a modest amount of material of solvent system, shortening analysis times, and lowering per-analysis costs. When compared to TLC, the HPTLC determination limit is substantially lower, making it possible to achieve higher

efficiency. Approximately 600 equivalent theoretical plates are used in ordinary layer chromatography, while 6000 equivalent theoretical plates are used in high-performance liquid chromatography [2, 3].

2.4. Hyphenated Techniques

Multi-dimensional chromatography is the new dimension in the field of hyphenated techniques that provides some really exceptional advantages in pharmaceutical analysis. For analysing a wide range of sample types, several setups, including coupling GC, HPLC, and CE systems together in various configurations, have been examined. As illustrations, size exclusion chromatography can be paired with RP-HPLC, CE, and GC coupled with LC. Combining RP-HPLC with CE methods in a two-dimensional mode can provide exceptionally high peak capacities and extremely high resolving power, which is particularly advantageous for complex mixtures. These techniques are both capable of high-resolution separation with orthogonal separation mechanisms [4 - 6].

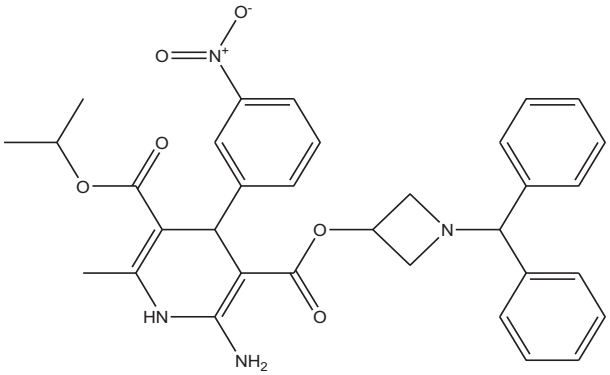

Fig. (1). Chemical structure of azelnidipine.

The substantial advantage of hyphenated speciation techniques is the ability to detect species other than the preconceived compounds. Hyphenated techniques offer the following advantages (Fig. **1**):

- Reduced time for analysis
- Increased automation
- Increased sampling output
- Superior duplicability
- A reduction in contamination due to the closed system
- Enhanced combined selectivity and increased level of information [6].

2.5. Azelnidipine

Azelnidipine, a calcium channel blocker with extended action, is exceptionally lipid dissolvable and specific for the vasculature, and is relied upon to increasingly affect cerebral blood flow (CBF). Its molecular formula is C33H34N4O6. It is insoluble in water, but is soluble in methanol. Its chemical formula is 3-*O*-(1-benzhydrylazetidin-3-yl) 5-*O*-propan-2-yl amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [7].

By combining tandem mass spectrometry using chiral liquid chromatography and positive ion air pressure chemical ionisation, K. Kawabata and colleagues were able to identify azelnidipine, and (R)- (-) and (S)- (+)-enantiomers were found in human plasma. A 96-well plate format for solid-phase extraction was used to prepare plasma samples for analysis. As an internal control, [2H6]-azelnidipine, a stable isotope-labeled variant of the medication, was introduced into the samples. On a chiral column, under isocratic flow conditions, the azelnidipine enantiomers were separated utilising 1-α-acid glycoprotein as a chiral column selector. During the study using mass spectrometric data and various monitoring modes of reaction, azelnidipine transitions for (R)- (-) and (S)- (+)-azelnidipine, as well as for [2H6]-azelnidipine, were detected from m/z 583 to 167. The chromatographic run time was 5.0 min/injection, and weighted (1/x2) quadratic regression demonstrated that the standard linearity curve was linear over the range that was examined (0.05-20 ng/mL). According to the assay results of the quality control samples, (R)-(-) azelnidipine and (S)-(+)-azelnidipine had intra- and inter-assay precision (coefficients of variation) of 1.2-8.2% and 2.4-5.8%, respectively. The accuracy for (R)-(-) azelnidipine was found to be 101.2-117.0%, whereas that for (S)-(+)-azelnidipine was 100.0-107.0%. According to the total recovery research, the effectiveness rates for (R)-(-)-azelnidipine and (S)-(+)-azelnidipine were in the corresponding ranges of 71.4-79.7% and 71.7-84.2%, respectively. The lower limit of quantification for both enantiomers using 1.0 mL of plasma was found to be 0.05 ng/mL.

All of the analytes exhibited respectable short-, long-, auto-, and stock-solution stability [8, 9] (Table **1**).

2.6. Efonidipine Hydrochloride Ethanolate

Recent research has focused on a new drug called R (-) efonidipine whose ability is to particularly impede peripheral organs that receive low-voltage (LVA or T-type) Ca2+ signals. In this study, we have investigated the effects of efonidipine R (-) on T-type and high-voltage-initiated (HVA) Ca2+ directing in mammalian-focused sensory system (CNS) neurons [14]. Chemically, it is 2-(N-benzylanilino) ethyl 5-(5,5-dimethyl-2-oxo-1,3,2λ5-dioxaphosphinan-2-yl)- 2,6-dimethyl-4-(3-nitrophenyl)- 1,4-dihydropyridine-3-carboxylate; ethanol; hydrochloride ethanoate (Fig. **2**).

A. S. Rajput and associates [14] developed solid dispersions (SDs) of EFO for the first time utilising hot-melt extrusion (HME). The present study's goal was to develop an easy-to-use RP-HPLC assay to evaluate EFO in the produced SDs. The RP-HPLC separation was accomplished using an Agilent Eclipsed XDB-C18 column (4.6 250 mm) loaded with 5 µ m particles. The ideal mobile phase composition was 1.2 ml/min flow rate, acetonitrile of HPLC grade, and 0.020 mol/L KH2PO4 (pH 2.5) buffer that were combined in a ratio of 85:15 v/v. The new and enhanced method was validated utilizing the predetermined criteria, including robustness, accuracy, linearity, and system applicability. The linearity results revealed an excellent linear correlation between the efonidipine concentration and the area of the obtained peak with an excellent correlation coefficient of 0.9998, which indicated the peak area as perfectly proportional to the

efonidipine concentration in a certain range. A study on the method's moderate precision and repeatability was conducted. The findings showed reliable results for efonidipine with an %RSD value of less than 2%. Assay outcomes were satisfactory, with an RSD of less than 2% for solid dispersion formulations.

Table 1. Analytical techniques for estimation of azelnidipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column-** Gradient [Hydrosphere C18 (100 mm 4.6 mm i.d., particle size 3 micrometre)] **Mobile phase** - A – 15 mM buffer of phosphate: methanol: acetonitrile (8:1:1 V/V/V) and B-acetonitrile **Flow rate** -1 ml /min **Column temp** - 40 0C **RT –** 7.9 min	**λ max** 220 nm	API	-	-	-	[8]
LC-MS	**Column -** Cadenza CD-C18 (particle size: 3 micrometre, 100 mm 2 mm i.d.) **Mobile phase-** A buffer of -5 mM acetate and 5 mM ammonium hydrogen carbonate, adjusted to a pH of 6.0; B, acetonitrile **Flow rate** - 0.3 ml/min	Product ion scan mode using collision Energy at 14–15%		-	-	-	
NMR	All spectra were recorded at 250 °C on an isolated sample that had been dissolved in dimethylsulfoxide-d6	Double quantum filter correlation spectroscopy (DQF-COSY)		-	-	-	
HPLC	**Column -** For the separation, a 5 m long, 250 mm 4.6 mm i.d. Hanbon Lichrospher 5-C18 column was employed. **Mobile phase** - Methanol and formic acid in a solution of 20 mM ammonium acetate (25:75:0.5, v/v) **Flow rate-** 1 ml/min **Column temperature** – 250 °C **Retention time -** 7.9 min	**λ Max** 220 nm	Tablet	0.05-40 ng/ml	-	0.05ng/ml	[7]
LC-MS	**Auxiliary gas and drying gas -** Nitrogen **Fragmentation technique -** Electrospray ionization **Drying gas temperature-**3500 °C	Positive ion mode		-	-	-	
HPLC	**Column-** Intersil ODS C-18; 5 micrometre; i.d.2.1 x150mm **Mobile phase -** Methanol, water, and acetic acid (800:200:0.2) **Flow rate** - 0.2ml/min. **Temperature of autosampler -** 60 °C.	**λ Max** 220 nm	Human plasma	0.5-200ng/ml	-	0.5ng/ml	[9]
LC-MS	**The fragmentation technique used was ESI and CID** **Sheath gas and auxiliary gas -** Nitrogen (80 p.s.i.) (3 L/min). **Heated capillary temperature -** 2400 °C. **Collision gas –** Argon at pressure 1.4m Torr **Quantification technique –** Selected reaction monitoring (SRM)	Positive ion mode		-	-	-	
HPLC	**Column –** (250 mm x 4.6 mm i.d., 5) C18 **Mobile phase –** Acetonitrile: 0.5% triethyl amine (70:30 v/v) orthophosphoric acid adjusted to a pH of 3.5 **Flow rate –**1.0 ml/min **Injection volume -** 50 microlitre **Retention time-** 4.9 min	Detection wavelength of 254 nm	Tablet	5-15mcg/ml	5mcg/ml	11mcg/ml	[10]

(Table 1) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPTLC	**Chromatographic conditions** Stock standard solution (300ng-800ng) was applied in the form of a bandwidth 8mm on pre-coated silica gel 60 F254 (20cm × 10cm × 0.2mm thickness) plates using Camag Linomat 5 applicator. **Constant application rate** - 120nL/sec **Mobile phase -** (6.5:3.5:0.1 v/v/v) chloroform, ethyl acetate, and methanol	Absorbance mode - 255 nm	Tablet	300-800ng/band	58.035ng/band	175.86ng/band	[11]
UV spectroscopy	**Diluent-** (80:20 V/V) methanol with water **Concentration range –** 2-12µg/ml	Detection wavelength 257.0 for zero-order derivative spectroscopy and 242.6 for first-order spectroscopy	Tablets	2-10µg/ml	0.75µg/ml	2.15µg/ml	[12]
HPLC	**Column -** C18 G column preceded by an ODS guard column (10µm, 10mm×5mm) **Mobile phase –** Acetonitrile: methanol: SPD buffer (50:40:10 v/v/v). **Injection volume -**20µl **Acid hydrolysis -**0.1 N HCl **Alkali hydrolysis -**0.1N NaOH **Oxidative degradation -**3% H2O2 **Thermal hydrolysis –** 60-700C	Detection wavelength of 257.0 nm		2-10µg/ml	0.8215µg/ml	2.4815µg/ml	
UV spectroscopy	Concentration range used for calibration curve was 2-12µg/ml.	Detection wavelength of 257nm	Tablet	2-14µg/ml	0.37µg/ml	1.12µg/ml	[13]

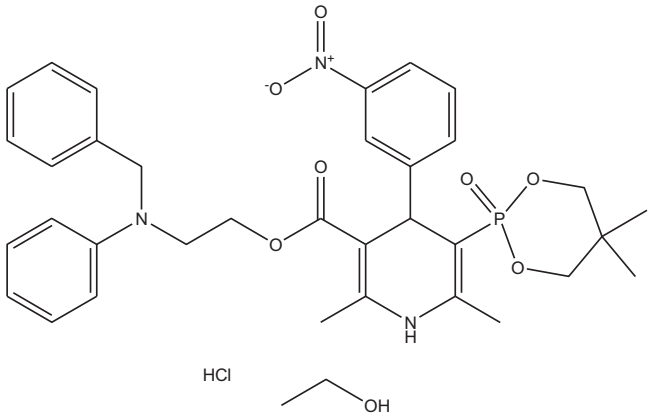

Fig. (2). Chemical structure of efonidipine hydrochloride ethanolate.

Table 2. Analytical techniques for estimation of efonidipine hydrochloride ethanolate in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** Phenomenex Kinetex®C 18 (150X4.6mm i.d. 5 µm). **Mobile phase** - Acetonitrile: 25mM phosphate buffer having pH 4.9 in proportion 45:55 **Flow rate** -1.0ml/min	Detection wavelength of 253 nm	Efonidipine and telmisartan FDC tablets prepared in lab	5-30 µg/ml for efonidipine and 10-60 µg/ml for telmisartan	0.15, 0.07 µg/ml for efonidipine and telmisartan	0.45, 0.23 µg/ml for efonidipine and telmisartan	[15]
HPLC	**Column** - Agilent Eclipsed XDB-C18 (4.6 × 250 mm) packed with 5 µm particles. **Mobile phase** – ACN: pH 2.5 phosphate buffer (85:25) **Flow rate** - 1.2ml/min **Injection volume** - 50 µL	Detection wavelength of 252.0nm	Solid dispersion	2.5-100 µg/ml	0.34µg/ml	1.04µg/ml	[14]

(Table 2) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
UV spectrometry (absorbance correction method and first-order derivative spectroscopy)	**Solvent –** Methanol **Sample holder -** 1 cm cuvette made of quartz **Concentration range:** For efonidipine - 8-20µg/ml For telmisartan - 16-40µg/ml	Efonidipine wavelength of 326nm; telmisartan wavelength of 272 nm	Efonidipine and telmisartan tablets prepared in the lab	8-20µg/ml for efonidipine and 16-40 µg/ml for telmisartan	0.678µg/ml for efonidipine and 0.270µg/ml for telmisartan	2.056 µg/ml for efonidipine and 0.818 µg/ml for telmisartan	[16]
HPLC	**Column -** Thermo Hypersil BDS **Mobile phase -**Ammonium acetate buffer (pH 5.8) and acetonitrile (gradient elution) Flow rate - 1ml/min Injection volume - 20µls	Detection wavelength of 254nm	Tablets	20-120µg/ml	0.41µg/ml	1.24µg/ml	[17]
LC-Q-TOF- MS	**Column -** Phenomenex Luna column ODS (250X50mm) 10 µm **Mobile phase -** Ammonium acetate buffer and acetonitrile (gradient flow) **Flow rate -** 50 ml/min	Detection wavelength of 254nm					
NMR	**13C NMR** The sample was prepared in DMSO d6 Internal standard - TMS	Quaternary carbon is a negative group, with the methyl and methine groups having positive peaks					

In essence, excellent specificity for the desired medication was demonstrated by the established RP-HPLC technique [1] (Table **2**).

2.7. Cilnidipine

A dihydropyridine calcium antagonist is cilnidipine. It was jointly developed and supported in 1995 by the Japanese companies Fuji Viscera Pharmaceutical Company and Ajinomoto. Contrary to other calcium antagonists, cilnidipine has the ability to follow up on the N-type calcium channel that is currently present at the thoughtful nerve end as opposed to the L-type calcium channel that is followed up on by the majority of calcium antagonists. In addition to some countries in the European Union, this drug is supported in China, Japan, Korea, China, and India. The L-type calcium channels of veins are affected by cilnidipine, which blocks the incoming calcium and stifles vein withdrawal to lessen circulatory strain. Additionally, cilnidipine works on the N-type calcium channel at the end of the thoughtful nerve, which inhibits norepinephrine release and reduces the increase in heart rate [18] (Table **3**). Chemically, it is 3-*O*-(2-methoxyethyl) 5-*O*-[(*E*)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Fig. **3**).

S. S. Panda and co-authors [23] used the RP-HPLC technique for the recently approved metoprolol succinate, telmisartan, and cilnidipine combination therapy. For the purpose of testing the aforementioned substances, this technique integrated quality-by-design and eco-scale analysis methodology. A previous study showed the current approach to have a fantastic green score. Later, risk analysis and method optimization were developed utilizing the quality-by-design-oriented method development. The Box-Behnken design was utilized during the optimization process, and the resultant response surfaces for resolution between three drug peaks were carefully mapped.

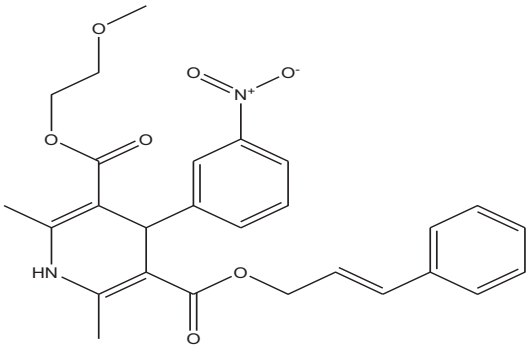

Fig. (3). Chemical structure of cilnidipine.

The ideal separation circumstances using methanol as the mobile phase were as follows: the C18 column acting as the stationary phase, a 240 nm diode array for detection, and a 0.01 M KH2PO4 buffer with a pH of 3.0 (70:30, v/v) run at a rate of 1.0 mL/min. With a resolution of more than 2.0, the three-drug peaks were all clearly distinguished from one another. For the targeted analytes, the analytical technique was linear (2.5-80 g/mL), yielding findings consistent with regulatory standards for accuracy (>99%) and precision (1%). The limits of detection and quantification, solution stability, and the maximum system suitability were all deemed to be satisfactory and good. The analytes from commercial dose forms were quantified using the currently available technology.

The development of a green analytical technique makes it possible to measure metoprolol, telmisartan, and cilnidipine all at once in a pharmaceutical formulation [23] (Table **3**).

Table 3. Analytical techniques for estimation of cilnidipine in various matrices.

Method	Method conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
UV spectrometry	Solvent - Methanol for drug and chloroform for dosage form **Linearity -** 1-9µg/ml	Detection wavelength of 430nm	Tablets	1-9 µg/ml	0.7122 µg/ml	2.1582 µg/ml	[19]
HPLC	**Column-** ODS (250mmX4.6mm, id5µm) **Mobile phase -** 0.1% OPA buffer: acetonitrile (42:58) **Flow rate -** 1ml/min **Retention time -** 2.317 for olmesartan and 3.763 for cilnidipine **Injection volume -** 10µl **Column temperature** – 300 °C	Detection wavelength - 240nm	Combined tablets of olmesartan and cilnidipine	25-150µg/ml for cilnidipine and 50-300µg/ml	0.43 µg/ml for olmesartan and 1.17 µg/ml for cilnidipine	1.31 µg/ml for olmesartan and 3.53 µg/ml for cilnidipine	[20]
HPLC	**Column -** ODS (250mmX4.6mm, id5µm) **Mobile phase –** Methanol: water (85:15) with pH adjusted to 3 with OPA **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time -**3.962 for valsartan and 7.064 for cilnidipine	Detection wavelength - 245nm	Combined tablets of cilnidipine and valsartan (10:80mg**)**	1-5 µg/ml for cilnidipine and 8-40 µg/ml for valsartan	-	-	[21]
HPLC	**Column –** ODS (150mmx4.5mm, id 5µm) **Mobile phase -** Potassium dihydrogen: acetonitrile: methanol and the phosphate buffer (pH 3) (35:05:60) **Flow rate -** 1ml/min **Injection volume -** 20µl **Retention time -** 2.65 min for fimasartan, 5.51 min for cilnidipine	Detection wavelength -240nm with diode array detector	Combined tablets of fimasartan and cilnidipine	2.5-15µg/ml for cilnidipine and 15-90µg/ml for fimasartan	0.57µg/ml for cilnidipine and 0.97µg/ml for fimasartan	1.75µg/ml for cilnidipine and 2.95µg/ml for fimasartan	[22]
HPLC	**Column -** ODS (150mmx4.5mm, id 5µm) **Mobile phase –** 0.01M KH2PO4 buffer for methanol (70:30) **Flow rate -**1ml/min **Injection volume -** 20µl **Retention time -** 3.2min for metoprolol, 7.6min for telmisartan, 12min for cilnidipine	Detection wavelength **-**240 nm with diode array detector	Combined tablets of cilnidipine, metoprolol succinate, and telmisartan (10+50+40mg)	2.5-80 µg/ml for cilnidipine, 2.5-80 µg/ml for metoprolol, 2.5-80 µg/ml for telmisartan	1.0 µg/ml for cilnidipine, metoprolol, and telmisartan	2.5 µg/ml for cilnidipine, metoprolol, and telmisartan	[23]

(Table 3) contd.....

Method	Method conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** Inertsil ODS 3V (250mmx4.6mm, id 5µm) **Mobile phase-** Potassium dihydrogen phosphate buffer with OPA-adjusted pH of 2.5 A. Acetonitrile (gradient program) **Flow rate –** 1ml/min **Injection volume –** 20µl **Retention time -** 3.872 for chlorthalidone and 7.668 for cilnidipine	Detection wavelength - 240nm with PDA detector	Combined tablets of cilnidipine and chlorthalidone	160-480 µg/ml for cilnidipine, 200-600 µg/ml for chlorthalidone	0.40 µg/ml for cilnidipine and 0.50 µg/ml for chlorthalidone	1.20 µg/ml for cilnidipine and 1.50 µg/ml for chlorthalidone	[24]
HPLC	**Column -** ODS (250mmx4.6mm, id5µm) **Mobile phase -** ACN: 0.01M sodium phosphate monohydrate dibasic buffer pH 3 (68:32) **Flow rate -** 1 ml/min **Injection volume -** 20µl By utilizing 0.1N HCl for acid hydrolysis and 0.1N NaOH for alkali, a forced degradation study was carried out. 3% H2O2 was utilized during hydrolysis for oxidation.	Detection wavelength - 245 nm with PDA detector. FDS showed no interference with assay method	Combined tablets of cilnidipine and telmisartan	10-40 µg/ml for cilnidipine and 40-160 µg/ml for telmisartan	0.01 ppm and 0.09 ppm for cilnidipine and telmisartan, respectively	0.4 ppm and 0.06 ppm for cilnidipine and telmisartan, respectively	[25]
HPLC	**Column -** Cosmosil (250mmX4.6mm, id5µm) **Mobile phase -** Buffered potassium dihydrogen: methanol (50:50) **Flow rate -** 1ml/min **Injection volume -** µl **Retention time -** 4.8165	Detection wavelength - 241nm with UV/Vis detector	Cilnidipine single-content tablets	100-500 mcg/ml for cilnidipine	0.00471 ppm	0.01427 ppm	[26]
HPLC	**Column:** ODS (250mmX4.6mm, id5µm) **Mobile phase**: Acetonitrile: methanol (50:50) **Flow rate:** 1.0ml/min **Injection volume:** 20µl **Retention time:** 3.067min	Detection wavelength - 242nm with PDA detector	Cilnidipine single-content tablets	2-10 µg/L for cilnidipine	0.003 µg/L	0.009 µg/L	[27]
UV spectroscopy	**Solvent -** Mobile phase ACN: MeOH (50:50) **Linearity range -** 1-10µg/ml	Detection wavelength -242nm		2-10 µg/L for cilnidipine	0.150951 µg/L	0.457429 µg/L	
UV (Dual wavelength method)	**Solvent -** Methanol **Linearity range** For cilnidipine - 2-10µg/ml For chlorthalidone - 2.5-12.5µg/ml	Detection wavelength**:** For cilnidipine 271.83nm, 278.34nm For chlortahlidone233.83nm, 250.0nm	Cilnidipine and chlorthalidone combination tablets	2-10 µg/ml for cilnidipine and 2.5-12.5 µg/ml for chlorthalidone	0.4174 µg/ml and 0.068 µg/ml for cilnidipine and chlorthalidone	1.264 µg/ml and 0.206 µg/ml for cilnidipine and chlorthalidone	[28]

2.8. Amlodipine Besylate

Amlodipine besylate has a molecular weight of 567.1, and is a white, transparent powder. It is poorly soluble in water and ethanol. For oral administration, the white tablets of amlodipine besylate come in three strengths: 2.5, 5, and 10 mg.

Chemically, it is 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]- 4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate.

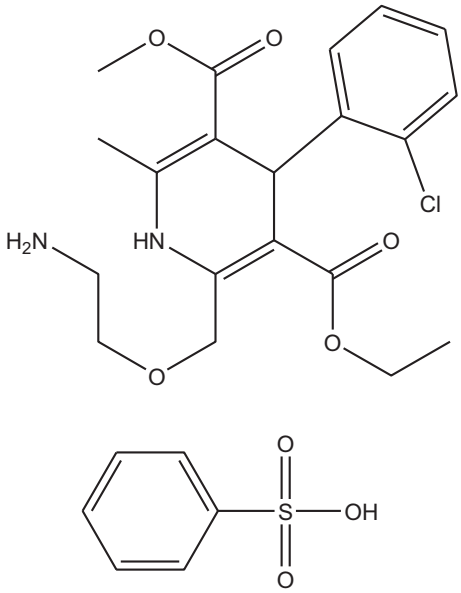

Fig. (4). Chemical structure of amlodipine besylate.

For the simultaneous measurement of formulation of aspirin, atenolol, and amlodipine besylate, V. K. Bhusari and S. R. Daneshwar developed an HPLC approach [29, 30]. This procedure uses HPLC to separate the three analytes, amlodipine besylate, atenolol, and aspirin, on a Thermo Hypersil BDS-C18 (250 mm x 4.6 mm, 5.0) from Germany with isocratic elution and a straightforward mobile phase containing methanol: 10 mM phosphate buffer with pH 7.0 adjusted with orthophosphoric acid (70:30), flowing at a rate of 1 mL/min and using UV detection at 235 nm with retention times of2.58 min for amlodipine besylate, 3.40 min for atenolol, and 4.23 min for aspirin (Fig. **4**). Without the excipients of formulation interfering, this method has been used successfully. The calibration plots' linearity analysis data revealed fair linearity for the concentration ranges of 2–12 g/mL for aspirin, 4–24 g/mL for atenolol, and 2–12 g/mL for amlodipine besylate. The average values of the regression coefficient, slope, and intercept were determined to be 0.9993, 1.02, 15182, 0.48, and 64910 for aspirin, respectively. For amlodipine besylate, atenolol, and amlodipine besylate, respectively, they were 0.9994, 0.91, 21326, 1.02, and 42960. Precision, robustness, and recovery were the three validation parameters that were used in the procedure. For amlodipine besylate, atenolol, and aspirin, the limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.5 g/mL and 1 g/mL, respectively.

The approach has been found to be repeatable and selective for the quantification of amlodipine besylate, atenolol, and aspirin, according to the statistical analysis [30] (Table **4**).

2.9. Isradipine

The dihydropyridine calcium antagonist isradipine showed a strong selectivity for calcium channels that are voltage-operated. This yellow drug isradipine is translucent in powder form. It is soluble in methanol, openly dissolvable in (CH3)2CO, and insoluble in water. Its molecular formula is C12H21N3O5.

Isradipine is chemically called methyl 1-methylethyl (4RS)- 4-(2, 1, 3-benzoxadiazol-4-yl)- 2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate. The United States Pharmacopeia and the British Pharmacopoeia both recognize isradipine as an authoritative medicine. Its formula weight is 371.4 D. Isradipine has a very strong affinity for and remarkable particularity for calcium channels. When administered orally or intravenously, it is an effective hypertensive medication and is also used in the management of intraoperative emergencies, and hypertensive issues in pregnancy. Intravenous isradipine is also viable in controlling hypertension after a coronary medical procedure [49] (Fig. **5**).

Table 4. Analytical techniques for estimation of amlodipine besylate in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC`	**Column -** (150 x 4.6 mm, 5) C-8 Inertsil ODS **Mobile phase -** Buffer phosphate and ACN (40:60) **Run time -** 10 min **Flow rate -** 1ml/min **Retention time** For Aliskiren 3.98 min and for amlodipine 5.14 min	Detection wavelength-237nm PDA detector	Tablets	30-225 µg/ml for Aliskiren and 2-15 µg/ml for amlodipine	0.1614 µg/ml and 0.1336 µg/ml for Aliskiren and amlodipine	0.4890 µg/ml and 0.4049 µg/ml for Aliskiren and amlodipine	[29]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column-** Thermo Hypersil BDS–C18 (250 mm × 4.6 mm, 5.0 µ) **Mobile phase -** pH 7.0 adjustment for 10mM phosphate buffer using OPA:methanol (30:70) **Run time -** 15min **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time** For amlodipine besylate -2.58min, atenolol-4.23min, and aspirin-3.40min	Detection wavelength-235nm with UV detector	Combined tablets of amlodipine besylate, atenolol, and aspirin	-	-	-	[30]
UV spectroscopy	**Solvent-** Methanol, distilled water	Detection wavelength-245nm and 365nm	Combined tablets of atorvastatin and amlodipine besylate	-	-	-	[31]
HPLC	**Column-** SunFire C8 column (4.6 × 150 mm, 5 µm) **Mobile phase** Methanol combined with potassium dihydrogen phosphate (80:25) **Run time -** 20min **Injection volume -** 50µl **Flow rate -** 0.7ml/min **Retention time -** 5.1 for amlodipine and 10.5 for lisinopril **Column temperature -** 35ºC	Detection wavelength - 212nm with PDA detector	Combined tablets of amlodipine besylate and lisinopril	40-100 µg/ml for amlodipine and 30-70 µg/ml for lisinopril	-	-	[32]
HPTLC	**Sample bandwidth -** 6mm **Injection volume -** 100µl **Application rate -** 150nl/min **Mobile phase –** Ethyl acetate, toluene triethylamine, methanol (4:1:1:0.4) **Precoated silica gel aluminium plate was used for sample spotting**	Detection wavelength-254nm	Combined tablets of amlodipine besylate and metoprolol	400-1400 ng/spot for amlodipine and 3800-13300 ng/spot for metoprolol succinate, respectively	39.99 and 121.20 ng/spot for amlodipine and metoprolol succinate, respectively	234031 and 710.03 for amlodipine and metoprolol succinate, respectively	[33]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column -** ODS (250mmx4.6mm, id5µm) **Mobile phase -** Phosphate buffer pH 4: acetonitrile (42:58) **Run time -** 8min **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time** For amlodipine besylate 4.348min, and for telmisartan 5.342min	Detection wavelength – 236nm with UV detector	Combined tablets of amlodipine and telmisartan	2.5-15 µg/ml for amlodipine and 20-120 µg/ml for telmisartan	0.04791 µg/ml for amlodipine and 0.4496 µg/ml for telmisartan	0.1452 µg/ml for amlodipine and 1.3625 µg/ml for telmisartan	[34]
HPLC	**Column -** C18 (250X4.5mm,5µ) **Mobile phase -** Acetonitrile and water **Run time -** 15min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** 1.9 min for amlodipine and for hydrochlorthiazide 3.2 min	Detection wavelength-235nm	Combined tablets of amlodipine and hydrochlorthiazide	80-120 µg/ml and 200-300 µg/ml for amlodipine and hydrochlorothiazide	0.80mcg/ml for amlodipine and 3.4 mcg/ml for hydrochlorothiazide	2.43 µg/ml for amlodipine and 10.31 µg/ml for hydrochlorthiazide	[35]
HPLC	**Column-**ODS (250*4.5mm,5µ) **Mobile phase -** Acetonitrile: acetate buffer (pH 5 adjusted with OPA) (60:40) **Run time -** 15min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** For HCT - 2.56min, AMB - 3.58min, TEL - 7.10min	Detection wavelength-333nm with UV detector	Combined tablets of amlodipine, hydrochlorthiazide, and telmisartan	20-100 µg/ml for amlodipine, hydrochlorthiazide and telmisartan	9.45 µg/ml,7.60 µg/ml, and 5.2 µg/ml for amlodipine, telmisartan, and hydrochlorthiazide, respectively	31.18 µg/ml, 25.08 µg/ml, and 17.16 µg/ml for amlodipine, telmisartan, and hydrochlorthiazide, respectively	[36]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column -** Shodex C18 column **Mobile phase -** Phosphate buffer: ACN (55:45); pH of the buffer was adjusted to 5.3 using OPA **Run time -** 15min **Injection volume -** 50µl **Flow rate -** 1ml/min **Retention time -** 4.43min and 5.70min for AMB and BZH, respectively	Detection wavelength-237nm with UV detector	Combined tablets of amlodipine and benazepril	1-6 µg/ml for amlodipine and benazepril	0.074 µg/ml for amlodipine and 0.053 µg/ml for benazepril	0.223 µg/ml for amlodipine and 0.161 µg/ml for benazepril	[37]
UV (Correlation equation method)	**Solvent -** 0.01 N HCl	Detection wavelength-237 nm and 366nm		2-24 µg/ml for amlodipine and benazepril	0.291 µg/ml for amlodipine and 0.070 µg/ml for benazepril	0.883 µg/ml for amlodipine and 0.212 µg/ml for benazepril	
HPLC (Multimedia dissolution study of amlodipine, enalapril, and bisoprolol combined tablets for determination of amlodipine release)	**Column -** C 18 (4.6*50 mm, 5µm) **Mobile phase -** Phosphate buffer: methanol (65:35) **Run time -** <2.5 min **Injection volume -** 100µl **Flow rate -** 1.0ml/min **Retention time -**1.89 min for amlodipine **Dissolution medium:** pH buffers of 1.2, 4.5, and 6.8 Paddle II of the USP apparatus **RPM-50 Time (min)-** 5, 15, 30, 45, 60, 90, 120, and 180 **Sample withdrawal volume -**1ml	Detection wavelength-240nm with UV detector	Combined tablets of amlodipine, enalapril, and bisoprolol	-	-	-	[38]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPTLC	**Mobile phase -** n-butanol: acetic acid: water (5:1:0.1) **Sample band width -**5mm **Injection volume -**100µl **Application rate -**0.1µl/sec **Precoated silica gel aluminium plate was used for sample spotting**	Detection wavelength-254nm in reflectance absorbance mode	Combined tablets of amlodipine and olmesartan	100-1000 ng/spot for amlodipine and 400-4000 ng/spot for olmesartan	-	-	[39]
HPLC	**Column -** ODS (4.6*250mm,5µ) **Mobile phase -** Acetonitrile: acetate buffer (55:45) **Run time -** 10min **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time -** 5.8min for AMB and 7.2min for OLM	Detection wavelength - 254 nm with UV detector		-	-	-	
HPTLC	**Mobile phase -** Chloroform, toluene, methanol, and acetic acid (6: 2.5: 1.5: 0.5) **Sample band width-** 6mm **Injection volume -** 100µl **Application rate -** 0.1µl/sec **Precoated silica gel aluminium plate was used for sample spotting**	Detection wavelength-244nm in absorbance mode	Combined tablets of irbesartan and amlodipine	400-900ng per band for amlodipine besylate, 50-500 ng per spot	12.7 ng/band for amlodipine and 14.4 ng/band for irbesartan	42.0 ng/band for amlodipine and 47.4 ng/band for irbesartan	[40]
HPLC	**Column -** (150 x 4.6 mm, 5) Luna C18 **Mobile phase -** Acetonitrile: acetate buffer pH 5 (50:50) **Run time -** 10min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** 3.4 min	Detection wavelength -232nm using UV visible detector	Single-content tablets of amlodipine	3-7 µg/ml for amlodipine	0.04 µg/ml for amlodipine	0.14 µg/ml for amlodipine	[41]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column -** ODS (250X4.6mm,5µ) **Mobile phase -** ACN: potassium dihydrogen phosphate pH 3.5 (45:55) **Run time -** 8min **Injection volume -** 20µl **Column temp. -** 400C **Flow rate -** 1.5ml/min **Retention time -** 1.7min for HCT, and 2.2 min and 6.0min for AMB and VAL, respectively	Detection wavelength -230nm	Combined tablets of hydrochlorthiazide, valsartan, and amlodipine	0.2-80 µg/ml for amlodipine and hydrochlorthiazide 0.25-100 µg/ml for valsartan	0.011 µg/ml for amlodipine, and 0.010 µg/ml for hydrochlorthiazide and valsartan	0.032 µg/ml for amlodipine, 0.020 µg/ml for hydrochlorthiazide, and 0.019 µg/ml for valsartan	[42]
HPLC	**Column -** Flowrosil ODS (250X4.6mm,5µ) **Mobile phase -** ACN: water (80:20) **Run time -** 10min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** 1.98min for AML and 3.15 for CEL	Detection wavelength -250nm using UV detector	Combined tablets of amlodipine and celecoxib	2-12 µg/ml for amlodipine and 50-300 µg/ml for celecoxib	0.09 µg/ml for amlodipine and 0.24 µg/ml for celecoxib	0.29 µg/ml for amlodipine and 0.75 µg/ml for celecoxib	[43]
HPLC	**Column -** Lichrospher ODS (250*4mm,5µ) **Mobile phase -** Acetonitrile: 3.0 pH phosphate buffer (40:60) **Run time -** 15 min **Injection volume -** 20µl **Flow rate -** 0.8ml/min **Retention time -** For AMB 7.47min and for NBH 10.25 min	Detection wavelength -268 nm using PDA detector	Combined tablets of amlodipine and nebivolol	30-70 µg/ml for amlodipine and nebivolol	0.062 µg/ml for amlodipine and 0.010 µg/ml for nebivolol	0.188 µg/ml for amlodipine and 0.31 µg/ml for nebivolol	[44]
HPLC	**Column -** Kromasil ODS (250*4.6mm, 5µ) **Mobile phase -** 0.02M phosphate buffer: acetonitrile (70:30) **Run time -** 10min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** 2.57min for amlodipine, and 4.49min for metoprolol	Detection wavelength -221nm using UV detector	Combined tablets of amlodipine and metoprolol	10-110 µg/ml for amlodipine and metoprolol	0.029 µg/ml for amlodipine and 0.025 µg/ml for metoprolol	0.090 µg/ml for amlodipine and 0.075 µg/ml for metoprolol	[45]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column-** Octadecylsilane (250X4.6mm, 5µ) **Mobile phase -** 0.1% each of acetonitrile, formic acid, and methanol (5:50:45) **Run time -** 10min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** For amlodipine 6.32 min and for chlorthalidone 5.32min	Detection wavelength -266nm using PDA detector	Combined tablets of amlodipine and chlorthalidone	2.5-7.5 µg/ml for amlodipine and 10-30 µg/ml for chlorthalidone	7.0846 µg/ml for amlodipine and 2.3173 µg/ml for chlorthalidone	0.00021 µg/ml for amlodipine and 7.7246 µg/ml for chlorthalidone	[46]
UPLCMS	**Column** - Acquity Beh C18 (2.1*50mm, 1.7µm) **Mobile phase-** Gradient flow, A- Acetonitrile, B- 1% ammonium acetate, with pH 2.5 adjusted with trifluoracetic acid **Run time -** 8min **Injection volume -** 2 µl **Flow rate** - 0.4ml/min **Retention time -** 3.7min for amlodipine, 2.5 min for hydrochlorthiazide, 3.9min for losartan Parent peak m/z obtained for amlodipine was 409.02m/z, for hydrochlorthiazide, it was 297.97m/z, and for losartan, it was 422.91m/z	Detection wavelength -PDA detector and ELSD	Combined tablets of amlodipine, hydrochlorthiazide and losartan	50-300 ng/ml for amlodipine, 125-750 ng/ml for hydrochlorthiazide, and 500-3000 ng/ml for losartan	0.1 ng/ml for amlodipine, 0.6 ng/ml for hydrochlorthiazide, and 2.0 ng/ml for losartan	1.0 ng/ml for amlodipine, 1.0 ng/ml for hydrochlorthiazide, and 5.0 ng/ml for losartan	[47]

(Table 4) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column -** Eclipse XDB plus C18 (25*4.6mm, 5µ) **Mobile phase -** Acetonitrile with methanol (70:30) **Run time -** 5min **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time -** 2.533min for amlodipine and 1.083 min for valsartan	Detection wavelength -224.1nm using a UV detector	Combined tablets of Amlodipine and valsartan	10-60 µg/ml for amlodipine and valsartan	0.97 µg/ml for amlodipine and 0.54 µg/ml for valsartan	0.29 µg/ml for Amlodipine and 1.63 µg/ml for Valsartan	[48]
UV spectroscopy	**Solvent -** Methanol	Detection wavelength - For zero order derivative spectroscopy -234.6nm, 231.3nm, 239.6nm		10-50 µg/ml for amlodipine and valsartan	For first order, 0.044 µg/ml for amlodipine and 0.261 µg/ml for valsartan For second order, 0.105 µg/ml for amlodipine and 0.402 µg/ml for valsartan For third order, 0.674 µg/ml for amlodipine and 0.468 µg/ml for valsartan	For first order, 0.133 µg/ml for amlodipine and 0.7916 µg/ml for valsartan For second order, 0.320 µg/ml for amlodipine and 0.1219 µg/ml for valsartan For third order, 2.044 µg/ml for amlodipine and 1.421 µg/ml for valsartan	

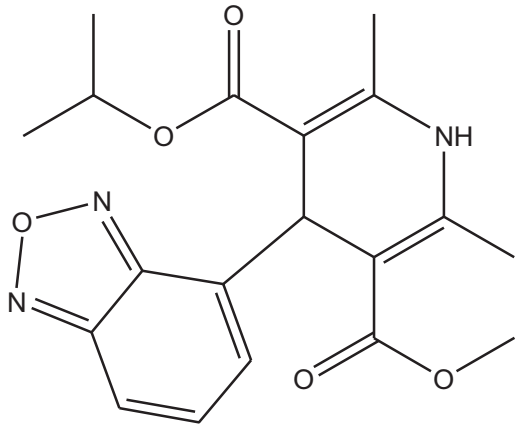

Fig. (5). Chemical structure of isradipine.

Isradipine, an inhibitor of calcium from the dihydropyridine class, and five of its degradation products can be found in urine and plasma thanks to a method created by C. Jean and R. Laplanche [50]. Toluene was used to extract the neutral analytes, and GC with a wide-bore silica capillary column was used for analysis. The acidic analytes were separated and measured individually using the same column after being extracted twice and esterified with diazomethane. The limits of detection for isradipine alone were 0.04 ng/ml and 0.7 ng/ml for the drug's oxidised metabolite, and utilizing chemical ionisation and negative-ion mass spectrometry, the detection was carried out. The range of the plasma metabolite detection limits was 0.15 to 2 ng/ml.

The technique worked well in both traditional pharmacokinetic investigations and in a multicentre population pharmacokinetics study [50] (Table **5**).

Table 5. Analytical techniques for estimation of isradipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
GCMS	**Column -** Hewlett Packard (10m*0.53mm, 2.65µm) **Carrier gas -** Methane **Column temperature -**2300 °C **Injector temperature -**3500 °C **Flow rate of gas -** 10ml/min **Retention time -** 1.3 min for isradipine and for metabolite 2.5min Parent peak for isradipine at 311m/z	Chemical ionization and negative ion peak detection was used for GC-MS analysis	Drug and its metabolites were extracted from human plasma	-	0.04ng/ml for isradipine	-	[50]

(Table 5) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** Octadecyl silane **Mobile phase -** Methanol: water (60: 40) **Run time -** 15min **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time -** 12.8min for isradipine	Detection wavelength - 325nm using UV detector	Bulk, tablets, and capsules	10-60 µg/ml for isradipine	-	-	[51]
HPLC	**Column -** C8 column and C18 column **Mobile phase -** Water: NaH2PO4: tetrabutylhydrogen sulphate: methanol: tetrahydrofuran (55.4: 0.017: 43.6: 1) **Run time -** 40min **Injection volume -** 20µl **Flow rate -** 1ml/min **Retention time -** 37min	Detection wavelength-240nm with PDA detector	Cell culture	-	0.11nmol/106 cells for isradipine	0.35 nmol/106 cells for isradipine	[49]

2.10. Felodipine

Voltage-subordinate calcium channels are blocked by felodipine, a calcium antagonist of the dihydropyridine class that reversibly competes with other calcium channel blockers as well as nitrendipine for dihydropyridine-restricting locations. Felodipine varies in colour from white to bright yellow, and resembles glass. It is practically insoluble water, but is quite soluble in (CH3)2CO, anhydrous ethanol, methanol, and methylene chloride. Its chemical formula is C18H19Cl2NO4. Felodipine is chemically named 3-ethyl 5-methyl 4-(2, 3-dichlorophenyl) 2, 6-dimethyl-, 1, 4-dihydropyridine-3,5-dicarboxylate, with a formula weight of 384.25D [52].

An innovative RP-HPLC technology was developed by P. Kallepalli and M.M. Annapurna [53] for the quantification of doses of felodipine in oral forms approved by the FDA, and the technique was validated. Phosphate buffer pH 7.0 (20:80, v/v) and acetonitrile were employed as the mobile phase, and the rate of flow was 1.2 ml/min (UV detection at 234 nm). By subjecting felodipine to various stress conditions, stressed degradation test was carried out and was validated according to ICH criteria. In oxidative circumstances, felodipine was severely destroyed (Fig. **6**).

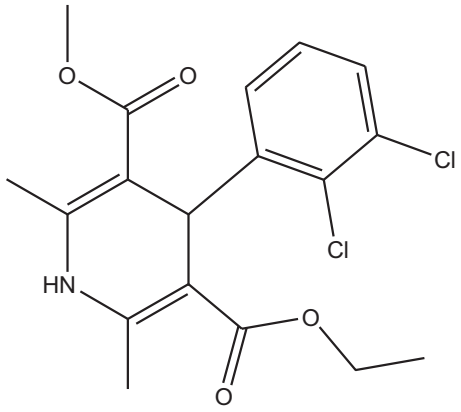

Fig. (6). Chemical structure of felodipine.

This destructive approach can be used to determine the presence of felodipine in pharmaceutical dosage forms and is straightforward and affordable [53] (Table **6**).

Table 6. Analytical techniques for estimation of felodipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** C8 (DD250*4.6mm, 5µm) **Mobile phase -** pH 4.5 phosphate buffer: acetonitrile (75:25) **Run time -** 1ml/min **Injection volume -** 20µl **Flow rate -** 1.0ml/min **Retention time -** 5.81min	Detection wavelength-360nm using UV detector	Rabbit plasma	0.25 -20.0 µg/ml For felodipine	0.055 µg/ml for felodipine	0.201 µg/ml for felodipine	[52]
HPLC	**Column -** Zorbax C18 column (250*4.6mm,5µm **Mobile phase -** pH 7.0 Phosphate buffer: acetonitrile (20:80) **Run time -** 10min **Injection volume -** 20µl **Flow rate -** 1.2ml/min **Retention time -** 2.512min	Detection wavelength -234nm using UV detector	Tablets	0.1-150 µg/ml for felodipine	0.0279 µg/ml for felodipine	0.0852 µg/ml for felodipine	[53]

(Table 6) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column-** Adsorbosil C18 (250*4.6mm,5µm) **Mobile phase -** Methanol: acetonitrile: H2O (50:30:20) **Run time -** 6min **Injection volume -** 10 µl **Flow rate** - 1.5ml/min **Retention time -** 2.2 min and 2.9 min for ramipril and felodipine, respectively	Detection wavelength -200-400nm using UV detector	Combined tablets of ramipril and felodipine	50-150 µg/ml for felodipine and ramipril	0.029 µg/ml for felodipine and 0.062 µg/ml for ramipril	0.078 µg/ml for felodipine and 0.187 µg/ml for ramipril	[54]
UV spectroscopy	**Solvent -** Methanol	Detection wavelength: For first derivative spectrophotometry - 250nm, Second derivative spectrophotometry - 260nm		-	-	-	
Spectrofluorometry	**Solvent -** Methanol	Detection wavelength: emission wavelength - 675nm and 580nm for felodipine and ramipril, respectively		-	-	-	
HPLC	**Column -** Lichrocart C18 (250*4.0mm, 5µm) **Mobile phase -** pH3.5 potassium dihydrogen phosphate in methanol (25:75) **Run time -**10min **Injection volume** - 20 µl **Flow rate** - 1.5ml/min **Retention time -** 5.96min	Detection wavelength -238nm using UV detector	Tablets	1-7 µg/ml for felodipine	150ng/ml for felodipine	500ng/ml for felodipine	[55]
LCMS	**Column-** Princeton Spher (150*4.6mm,5µm) **Mobile phase -** acetonitrile: buffered ammonium acetate (80:20) **Run time -** 6min **Injection volume** - 10 µl **Flow rate** - 0.8ml/min **Retention time -** 4.539min **MS conditions: Operation mode** – ESI **Nebulizer gas flow** -1.51ml/min **Drying gas** – 10ml/min **Detector voltage -**1.3kv	Detection wavelength -38.01nm using PDA detector	Human plasma	0.8 -13 ng/ml	0.10 ng/ml for felodipine	0.50 ng/ml for felodipine	[56]
Spectrofluorometry	**Solvent -** Methanol	Detection wavelength**:** emission spectra were obtained at 260nm and 375 nm for metoprolol and felodipine, respectively	Combined tablets of metoprolol and felodipine	02-20 µg/ml for felodipine and 0.5-100 µg/ml for metoprolol	0.02 µg/ml for felodipine and 0.11 µg/ml for metoprolol	0.06 µg/ml for felodipine and 0.32 µg/ml for metoprolol	[57]

2.11. Verapamil

One of the main calcium channel antagonists used to treat hypertension is verapamil, a phenylalkylamine calcium divert blocker, which was first introduced to medicine in the middle of the 1960s. It is a part of a different class of blockers of calcium channels than the dihydropyridine group, which includes medications, like diazepam and flunarizine, but is synthetically unrelated to other prescriptions for cardioactive substances. Verapamil is a racemic combination that contains S- and R-enantiomers in equal proportions, each of which has unique pharmacological properties. The S-enantiomer has around a 20-overlap increase in strength over the R-enantiomer, but is metabolized at a faster rate. Its chemical formula is 2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphe nyl) ethyl-methylamino]- 2-propan-2-ylpentanenitrile. It is soluble in water, ethanol, and methanol (Fig. **7**).

Table 7. Analytical techniques for estimation of verapamil in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** Supelcosil (150*4.6,5µm) **Mobile phase -** Acetonitrile: 0.05M buffer with phosphate pH 3 (40:60) **Run time -** 10min **Injection volume** - 50µl **Flow rate** - 1.0ml/min **Retention time -** 8.0min	Detection wavelength – 204nm (excitation) and 314nm (emission) using fluorescence detector	Human plasma	5-250ng/ml	0.924 ng/ml	1.232 ng/ml	[58]

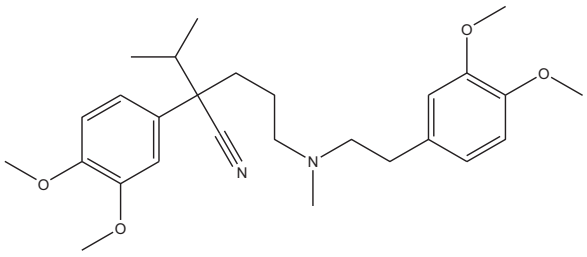

Fig. (7). Chemical structure of verapamil.

Verapamil (V), and Norverapamil (N), one of its metabolites, were quickly extracted from human plasma using Wieslaw Sawicki's straightforward and accurate analytical approach, which made the application of an HPLC isocratic system with fluorescence detection mode feasible for V and N; the derivation recovery was 92.12% and 89.58%, respectively. Propranolol was the internal standard used in HPLC. The average recovery rate was 82.50%. Detection limits and determination for V were found to be 0.924 ng/ml and 3.080 ng/ml, respectively. This translated to a plasma concentration of 1.232 ng/ml. For N, the detection limit was 0.030 ng/ml, while the determination limit was 1.001 ng/ml, translating to a plasma concentration of 0.4 ng/ml (Table **7**).

The validation parameters showed the proposed method's precision to be very high. One chromatogram separation using the approach only took about 8 minutes. The verapamil is administered directly by mouth or by floating medication formulations as an alternative to tablets in pharmacokinetic and bioavailability investigations [58].

2.12. Diltiazem

Diltiazem is an oral and parenteral drug other than a dihydropyridine calcium channel blocker. It is utilized in numerous clinical situations like hypertension, arrhythmia and angina. FDA supports its use for signs that include atrial arrhythmia, hypertension, paroxysmal supraventricular tachycardia, and persistent stable angina. Diltiazem is soluble in water, methanol, and formic acid. Its chemical formula is [(2*S*,3*S*)-5-[2-(dimethylamino) ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate [59] (Fig. **8**).

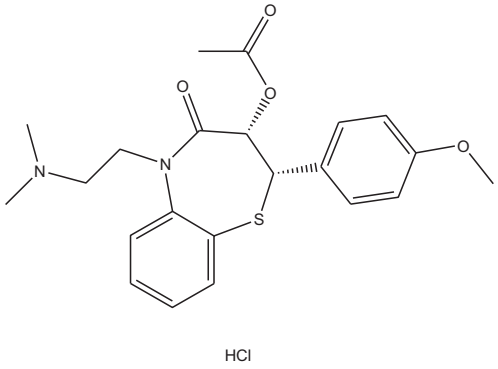

Fig. (8). Chemical structure of diltiazem.

M. A. Souza, C. E. Pereira, and co-authors have developed an HPLC method having stability for the purpose of determining the amount of diltiazem hydrochloride (DTZ) in tablets and custom-made capsules. The method used a Merck Millipore Purospher Star® C18 HPLC column (150 x 4.6 mm i.d., 5 m particle size). 0.05% (v/v) trifluoroacetic acid aqueous solution and a 0.05% (v/v) trifluoroacetic acid methanolic solution were mixed at a 44:56 v/v ratio to make up the mobile phase. The flow rate was set to 1 mL.min-1 with a 14-minute duration. The detection of DTZ and degradation products (DP) was carried out at 240 nm using a diode array detector. The proposed approach was linear, accurate, exact, robust, and suitable for stability studies and regular quality assessment tests of DTZ in tablets and compounded capsules [60] (Table **8**).

Table 8. Analytical techniques for estimation of diltiazem in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
GC	**Column -** 30mmX0.25mm with crosslink fused silica capillary column **Mobile phase -** Argon: methane (95:5) **Detector temperature** –3000 °C **Injector temperature** -2800 °C **Oven temperature** -2650 °C **Flow rate** - 37ml/min **Retention time -** 7.5min	Detection wavelength – Electron capture detector	Human plasma	-	-	-	[59]

(Table 8) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** Purospher starC18 (150*4.6mm, 5µ) **Mobile phase -** 0.05% trifluoracetic acid: 0.05% methanolic trifluoracetic acid (44:56) **Run time -**14min **Injection volume** - 20µl **Flow rate -** 1.0ml/min **Retention time -** 4.46min	Detection wavelength – 240nm using a diode array detector	Tablets and compounded capsules	50% to 150%	-	-	[60]
HPLC	**Column -** C18 analytical column **Mobile phase -** Phosphoric acid solution with ethanol (pH 2.5) **Run time -** 10min **Injection volume** - 20µl **Column temperature - 5**00 °C **Flow rate** - 2.0ml/min **Retention time -** 4.2min for desacetyl diltiazem, 5.8 min for diltiazem	Detection wavelength – 240nm using a PDA detector	Tablets	0.5-50 µg/ml	-	0.5 µg/ml	[61]
HPTLC	**Mobile phase -** ethyl acetate: methanol: strong ammonia (80:10:10) **Sample band width -**6.0mm **Application rate -**15µl/sec **RF value -** 0.54 **Precoated silica gel aluminium plate was used for sample spotting**	Detection wavelength – 238nm using absorbance mode	Tablets and bulk	-	-	-	[62]
HPLC (RS method)	**Column -** BDS C18 (150*4.6mm,5µ) **Mobile phase -** A- 0.2% triethylamine pH 4.5 B- Acetonitrile (A: B) (3:2) **Run time -** 90min **Injection volume -** 10µl **Flow rate** - 1.0ml/min **Retention time -**31.261min for DTZ, 9.025 min for diltiazem sulphoxide impurity, and 18.048 min and 27.244min for diltiazem HCl imp - F and diltiazem impurity – A, respectively; 29.081min for impurity - D, 35.551 min for impurity - E, and 47.166 min for impurity - B	Detection wavelength – 240nm using UV detector	Tablets	-	-	-	[63]

2.13. Nifedipine

Nifedipine is an original calcium channel antagonist that is used to treat hypertension and angina pectoris. Treatment with nifedipine is associated with low serum chemical levels and has been connected to a few cases of clinically evident intense liver injury. Its formula weight is 346.3 D. Nifedipine-expanded delivery tablets are indicated for ingestion in constant stable angina (exertion-related angina, unstable angina) without evidence of vasospasm in patients who stay indicative regardless of enough dosages of beta antagonists or potentially natural nitrates. Chemically, it is dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [64].

In order to create dependable, efficient, and effective analytical techniques for NIF and its degradation products, S Choiri *et al*. used extremely effective liquid chromatography and the quality by design methodology. The analytical condition of NIF and its degradants was optimised using a 22-complete factorial analysis method with a curve as the centre point. Flow rate (FR) and mobile phase ratio are variables based on system appropriateness characteristics. Changes in the solvent phase ratio had a big impact on time retention. Additionally, a higher FR reduces the tailing factor. Additionally, there has been found an improvement in NIF and its degradants' number of theoretical plates and resolution as a result of the interaction of the two elements (Fig. **9**).

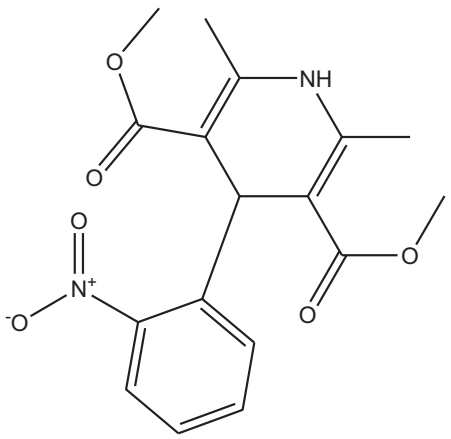

Fig. (9). Chemical structure of nifedipine.

During an analysis period of less than 10 minutes, the developed analytical method for NIF and its degradants demonstrated good linearity, precision, accuracy, and efficiency at concentrations between 1 and 16 g/mL [66] (Table **9**).

Table 9. nalytical techniques for estimation of nifedipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPLC	**Column -** Nova pak 100mm*8mm,4µm **Mobile phase-** methanol: water (65:35) **Run time -** 20min **Injection volume** -20µl **Column temperature -** 250C **Flow rate** - 1.1ml/min **Retention time -** min	Detection wavelength –240nm using UV detector	Human plasma	5-250ng/ml	5ng/ml	1ng/ml	[64]
HPTLC	**Mobile phase -** Acetic acid, acetonitrile, methanol, and n-butanol (0.1, 6, 2, 2) **Sample band width-**6.0mm **RF value -** 0.25, 0.65 for valsartan and nifedipine, respectively. **Precoated silica gel aluminium plate was used for sample spotting**	Detection wavelength –230nm	Combined tablets of valsartan and nifedipine	120-320 ng/band for nifedipine and 900-2400ng/band for valsartan	35.01ng/band for nifedipine and 287.62 ng/band for valsartan	106.1 ng/band for nifedipine and 871.60ng/band for valsartan	[65]
HPLC (based on the QbD Approach)	**Column -** Lichrospher C18 (250*4.6mm,5µm) **Mobile phase -** Various ratios of acetonitrile and methanol were used as per DoE. **Run time -** 10min **Injection volume** -20µl **Column temperature -** 400 °C **Flow rate** - 1.5ml/min	Detection wavelength - UV detector	Nifedipine bulk and its degradants	1-16 µg/ml for nifedipine	8.9 ng/ml for nifedipine	27.0ng/ml for nifedipine	[66]
LCMSMS	**Column -**C18 (50*2.1mm, 3µm) **Mobile phase -** Water: methanol (80:20) containing 1% formic acid **Run time -** 2.5min **Injection volume** -200µl **Flow rate** - 200µl/min **Retention time -** 1.93 min for NIF and 1.84 min for DNIF	Detection wavelength**:** ESI technique was used for the detection of nifedipine and dihydronifedipine	Nifedipine and dihydronifedipine bulk	0.5-100 ng/ml for nifedipine and dihydronifedipine	0.05 ng/ml for nifedipine and 0.1 ng/ml for dihydronifedipine	-	[67]

2.14. Lacidipine

According to a physico-synthetic perspective, lacidipine is somewhat soluble in water, while it is more soluble in ethanol, methanol, and (CH3)2CO. It is light and temperature sensitive. Lacidipine, however, is stable under UV light at 240nm. Lacidipine is contra-indicated in atherosclerotic and cell reinforcement impacts. It has a long-term activity due to its high lipophilicity. This dynamic change is useful in treatment. The chemical conversion of angiotensin, as well as its effects on chemical receptors and particle channels, are all inhibited by lacidipine or a metabolite of it. Chemically, lacidipine is 3, 5-diethyl4-{2-[(1E)-3-(tert-butoxy)-3-oxoprop-1-en-1-yl] phenyl}-2,6-dimethyl-1,4-dihyd ropyridine-3,5dicarboxylate (Fig. **10**). It has a molecular formula of C16H33NO6 and a formula weight of 455.54 D [68].

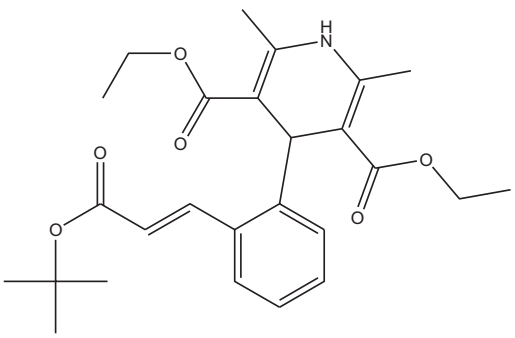

Fig. (10). Chemical structure of lacidipine.

Table 10. Analytical techniques for estimation of lacidipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs
HPTLC	**Mobile phase -** Toluene: ethyl acetate (6.5:3.5) **Sample band width-** 6.0mm **RF value -** 0.45 for lacidipine **Precoated silica gel aluminium plate was used for sample spotting**	Detection wavelength - 287nm	Urine samples	10-80ng/ml for lacidipine	5 ng/ml for lacidipine	8 ng/ml for lacidipine	[68]
HPLC	**Column -** Xbridge C18 (150*4.6mm, 5µm) **Mobile phase-**Ammonium acetate: acetonitrile (5:95) **Run time -** 15min **Injection volume** - 20µl **Column temperature -** 250 ° C **Flow rate** - 1.0ml/min **Retention time -** 8.309min	Detection wavelength -240nm using PDA detector	Bulk and tablet	50-250 µg/ml for lacidipine	1.0 µg/ml for lacidipine	7.3 µg/ml for lacidipine	[69]
SFC-MS/MS	**Column -** SB column (3mmX100mm, 1.8µm) **Mobile phase -** Carbon dioxide: methanol (92:8) **Run time -** 1.5min **Injection volume** - 2.0µl **Column temperature -** 500 °C **Flow rate** - 0.2ml/min **Retention time -** 1.07 min	Detection wavelength: Tandem quadrupole detector was used	Beagle dog plasma	0.10-100 ng/ml	-	0.10ng/ml	[70]
UV-visible spectroscopy	The sample was prepared in methanol and blue color was developed by reacting lacidipine, ferric chloride, potassium ferrocyanide, and hydrochloric acid	Detection wavelength-740nm	Tablets	0-10 µg/ml for lacidipine			[71]

A technique known as SFC-MS/MS, or supercritical fluid chromatography, was invented by Y. Geng and colleagues, and used to evaluate lacidipine levels in beagle dog plasma whilst administering nimodipine employed as an internal reference. Tert-butyl methyl ether was used in a straightforward liquid-liquid extraction procedure. On an Acquity UPC2 set to 50 °C, a C18 HSS SB column (3 mm 100 mm, 1.8 m) was used to evaluate the pharmaceuticals. For each sample, a total of 1.5 minutes were allotted for the analysis, with the mobile phase, *i.e.*, a carbon dioxide to methanol ratio of 92:8, and a flow rate of 2 ml/min. With a flow velocity of 2% formic acid set at 0.2 ml/min and a total analysis period of 1.5 minutes for every sample, methanol was employed as the compensatory solvent. Ion transitions for lacidipine and nimodipine, respectively, at m/z 473.32354.10 and 419.00343.10, were evaluated using the multiple reaction-monitoring mode. The linearity range was found to be 0.10–100 ng/ml (r2 = 0.9980). The accuracy ranged from 0.83% to 3.27% at all quality control levels, with intra- and inter-day precision determined to be less than 15%. The devised approach was successfully used when studying the pharmacokinetics of lacidipine in beagle dogs [69] (Table **10**).

2.15. Nicardipine Hydrochloride

The systematic name of nicardipine hydrochloride is 2-[benzyl (methyl) amino] ethyl methyl 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl) pyridine-3, 5- dicarboxylate hydrochloride [72]. The medication is soluble in methanol, ethanol, chloroform, and water, while it is insoluble in 0.1 N sodium hydroxide. Nicardipine is a calcium channel blocker. It inhibits the development of calcium into the smooth muscle cells encompassing the veins of the body. Since calcium causes the withdrawal of muscles, hindering calcium passage into the muscle cells, it loosens up the blood vessel muscles and causes the supply routes to expand, eventually diminishing the circulatory strain [73] (Fig. **11**).

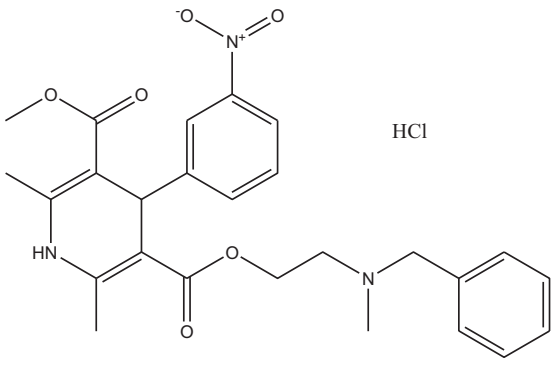

Fig. (11). Chemical structure of nicardipine hydrochloride.

Table 11. Analytical techniques used for estimation of nicardipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column -** Kromacil C18 (250X4.6mm) **Mobile phase -** pH 3.0 potassium dihydrogen phosphate with methanol (70:30) **Run time -** 10 min **Injection volume** - 20µl **Column temperature -** 250 °C **Flow rate** - 1.0ml/min **Retention time -** 3.71min	Detection wavelength**:** 236nm using UV detector	Bulk	10-60 µg/ml for nicardipine	430ng/ml for nicardipine	130 ng/ml for nicardipine	[72]
LC-MS	**Column -** C18 (150X4.6,5µm) **Mobile phase -** 70% methanol:acetic acid and 0.01M triethylamine pH 4.0 **Run time** - 10 min **Injection volume** -20µl **Column temperature -** 250 °C **Flow rate** - 1.0ml/min **Retention time -** 8.42 min	Detection wavelength**:** 353nm ESI technique was used	Capsules and human plasma	0.5-40 µg/ml for nicardipine	0.11 µg/ml for nicardipine	0.036 µg/ml for nicardipine	[73]
HPLC MS/MS	**Column -** C18 (250X4.6mm, 5µm) **Mobile phase -** Methanol, water, and formic acid (320:180:0.4) **Run time -** 5min **Injection volume** -20µl **Column temperature -** 250 °C **Flow rate** - 0.6ml/min **Retention time -** 4.0min	Detection wavelength**:** ESI technique was used with positive ion mode	Tablet	0.05-20.0ng/ml for nicardipine	-	0.05ng/ml for nicardipine	[74]
HPLC	**Column -** C18 (250*4.6mm, 5µm) **Mobile phase -** Triethylamine Phosphoric acid buffer pH3.5: acetonitrile **Run time** - 40 min **Injection volume** -20µl **Column temperature -** 250 °C **Flow rate** - 1.0ml/min **Retention time -** 4.392min	Detection wavelength: 353nm using UV detector	Bulk and tablets	10-15 µg/ml for nicardipine	-	-	[75]
UV-visible spectroscopy	**Solvent -** Acetonitrile:water (50:50)	Detection wavelength - 235nm	Bulk and tablet formulation	5-25 µg/ml for nicardipine	-	-	[76]

By using a stability-indicating high-performance liquid chromatographic (HPLC) assay method developed by Ubale *et al*., the amount of nicardipine hydrochloride in bulk API and the degradation impurities generated by forced degradation studies were measured. To separate and quantify the drug from the degradation products, an isocratic reversed-phase HPLC method was developed using a Waters Symmetry shield C18 (250 x 4.6) 5um column and a solvent system composed of a combination of triethylamine phosphoric acid buffer (pH-3.5), orthophosphoric acid, and acetonitrile (35:65, v/v), and 353 nm was chosen as the analytical wavelength. The deteriorated products were clearly distinguished from the primary peak.

The linearity, accuracy (recovery), precision, robustness, selectivity, system suitability, and forced degradation investigations all confirmed the stability of the method [74, 75] (Table **11**).

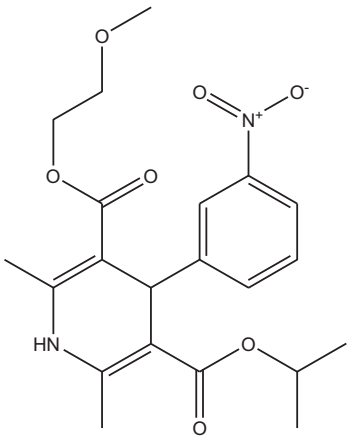

Fig. (12). Chemical structure of nimodipine.

2.16. Nimodipine

The 1,4-dihydropyridine nimodipine (Nim) is another derivative with calcium channel-blocking activity. It was identified as having neuronal effects and cerebral vasodilatory effects in animals and its viability was proposed for the treatment of cerebral haemorrhage or worldwide ischemia, as well as epilepsy [77]. Chemically, nimodipine is 3-*O*-(2-methoxyethyl) 5-*O*-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl) -1,4-dihydropyridine-3,5-dicarboxylate with empirical formula C21H26N2O7. It is soluble in methanol, dioxane, ethanol, and DMSO [78].

A simple, linear, error-free, specific, robust, and selective RP-HPLC technique for the determination of nimodipine impurity in bulk and in formulations has been devised by Shaikh *et al*. Methanol, acetonitrile, and water were used as the mobile phase in the following proportions: 35v: 40v: 25v. 0.8 ml/min was the specified flow rate. An analytical wavelength of 234 nm, a C18 column with 250 x 4.6 mm dimensions, and a UV-vis detector were utilised (Fig. **12**). Finally, based on bulk nimodipine and its tablet formulation, nimodipine impurities were determined.

According to the results, there were 0.0876% and 0.0219% impurities in the tablet and bulk drug, respectively. As a result, it was determined that the impurity of nimodipine was within ICH limits, *i.e*., no more than 0.1% [79] (Table **12**).

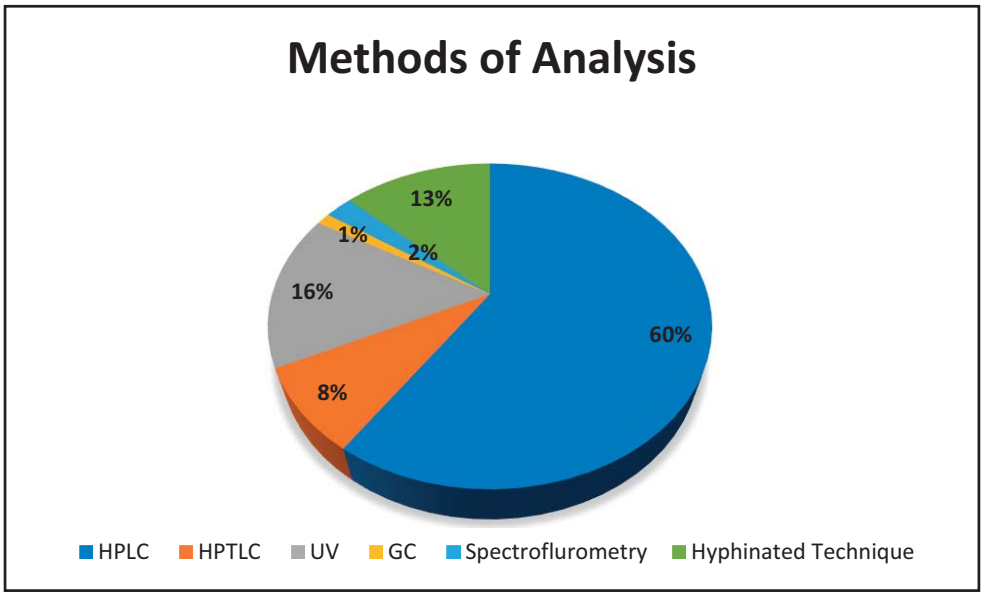

Fig. (13). Data based on a review of the scientific literature. Frequency of usage of the techniques employed in the stability-indicating approaches (n = 84) applied to the determination of calcium channel blockers and their various combinations.

Table 12. Analytical techniques used for estimation of nimodipine in various matrices.

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
LC-MS	**Column -** Zorbax C18 (150*4.6mm,5µm) **Mobile phase -** Methanol: water: formic acid (80:20:1) **Run time** - 5min **Injection volume** - 20µl **Column temperature -** 200 °C **Flow rate** - 0.75ml/min **Retention time -** 3.6min	Detection wavelength**:** ESI technique was used.	Human plasma	0.24-80 µg/ml for nimodipine	-	0.24ng/ml for nimodipine	[77]
Dissolution study by HPLC (4.5 pH acetate buffer). Samples were withdrawn at time points 5, 10, 20, 30, 45, and 60 min	**Column -** Zorbax C18 (150*4.6mm,5µm) **Mobile phase -** Water, formic acid, and methanol (20:1:80) **Run time** - 5 min **Injection volume** - 20µl **Column temperature -** 200 °C **Flow rate** - 0.75ml/min **Retention time -** 3.6min	Detection wavelength: ESI technique was used.	Tablets and human plasma	-	-	-	[78]

(Table 12) contd.....

Method	Method Conditions	Detection	Matrix	Linearity	LOD	LOQ	Refs.
HPLC	**Column-** ARP-C18 (250*4.6,5µm) **Mobile phase -** Water: acetonitrile: methanol (25:40:35) **Run time** - 15min **Injection volume** - 20µl **Column temperature -** 250 °C **Flow rate** - 0.8ml/min **Retention time -** 8.5min	Detection wavelength**:** 234nm using a UV detector	Tablets and bulk material	2-12 µg/ml for nimodipine	0.2177 µg/ml for nimodipine	0.6597 µg/ml for nimodipine	[79]
HPLC	**Column -** C8 (150*4.6mm, 5µm) **Mobile phase -** Acetonitrile: water (65.5:35.5) **Run time** - 15 min **Injection volume** -10µl **Column temperature -** 400 °C **Flow rate** - 0.9ml/min **Retention time -** 7.0min	Detection wavelength: 236nm using UV detector	Tablets	7.5-37.5 µg/ml for nimodipine	0.09 µg/ml for nimodipine	0.28 µg/ml for nimodipine	[80]
UV-visible spectrophotometry	**The method was based on a diazotization reaction.**	Detection wavelength: 555nm	Tablets	0-10 µg/ml for nimodipine	-	-	[81]

CONCLUSION

Over the past 25 years, numerous analytical techniques for CCB detection in biological samples and pharmaceutical formulations have been published. In the area of analytical techniques, LC methods have achieved the most advancements. Currently, HPLC with conventional UV detectors is used to determine the purity of a vast majority of drugs because it provides the necessary sensitivity for determinations at trace levels and high automation. These techniques provide adequate quantitative results for the analysis of samples that contain large concentrations of drug, such as those found in pharmaceutical formulations, and raw materials, as well as in stability studies. Sample pre-treatment is necessary prior to analysis in the case of biosamples containing small amounts of medicines in order to attain the necessary drug concentration. Other detecting techniques used include fluorimetry and diode arrays. The method of choice nowadays for analysing pharmaceuticals in biosamples, including CCBs, is the combination of HPLC with MS/MS, which delivers LOD stated in ng/mL. The determination of CCBs in pharmaceutical formulations and biosamples is also carried out using UPLC methods. Less frequently used chromatographic techniques for CCB determination include TLC and GC. While TLC is used to analyse individual CCB and in admixtures with other medicines, primarily in bulk form and in pharmaceutical formulations, GC is utilized to determine residual solvents. At the moment, chromatographic or similar techniques, of which HPLC is the most significant, are generally used to determine the presence of almost all organic contaminants.

It is now possible to increase the detection of active ingredients and contents in bulk materials by replacing all non-specific test techniques with extremely precise and accurate ones (mostly HPLC). This article provides rapid access to data on the development of various CCBs' analytical properties in bulk and in mixed dosage forms. As a result, it will reduce the time needed for the preliminary literature review (Fig. **13**).

L8IST OF ABBREVIATIONS

API = Application Programming Interface
CBF = Cerebral Blood Flow
CCB = Calcium Channel Blocker
CE = Capillary Electrophoresis
CNS = Central Nervous System
D = Daltons
EFO = Efonidipine
GC = Gas Chromatography
HV = High Voltage
HME = Hot Melt Extrusion
HPLC = High Performance Liquid Chromatography
LOD = Limit of Detection
LOQ = Limit of Quantitation
LV = Low Voltage
N = Metabolites
NIF = Nifedipine
Nim = Nimodipine
NMR = Nuclear Magnetic Resonance
RP = Reverse Phase
SD = Solid Dispersion
SFC = Supracritical Fluid Chromatography
TLC = Thin Layer Chromatography
UPLC = Ultra Performance Liquid Chromatography
V = Verapamil

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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