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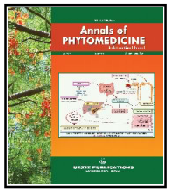

Review Article : Open Access

An overview: Analytical method development and validation of citicoline and nimodipine

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Abstract

Citicoline is one type of nootropic. It is used to treat neurological conditions such as brain stroke, trauma, parkinson's disease and brain insufficiency. By selectively inhibiting L-type calcium channels found in the cerebral blood vessel smooth muscle cells, imidapine functions as a calcium channel blocker. Many chromatographic and spectroscopic techniques, including UV, GC plasma samples by LC-MS, UPLC, HPTLC, stability-indicating HPLC and HPLC, have been extensively used for citicoline. The review covers the optimization of chromatographic conditions, such as detection techniques, mobile phase composition, and column selection. The review explores the variety of analytical methods applied to nimodipine, including capillary electrophoresis (CE), ultraviolet spectroscopy, liquid chromatography (LC) and gas chromatography-mass spectrometry (GC-MS). The review also looks at new developments in hyphenated techniques for measuring citicoline and nimodipine simultaneously in complex biological samples. Examples of these techniques are GC-MS (gas chromatography-mass spectrometry) and LC-MS (liquid chromatography-mass spectrometry). There is a discussion of the benefits and drawbacks of these methods, providing insights into how well they work in clinical and pharmaceutical research. The overall goal of this review is to provide researchers and analysts working on the creation and verification of analytical techniques for nimodipine and citicoline with a thorough reference. The insights provided herein contribute to the advancement of analytical methodologies, ensuring the accurate and reliable quantification of these compounds in various matrices, thereby facilitating their pharmacokinetic studies and therapeutic monitoring.

1. Introduction

WHO reports indicate that the prevalence of Parkinson disease (PD) has doubled over the previous 25 years (Adibhatla *et al.,* 2002). Degenerative neurological conditions Parkinson's disease (PD) is characterised by a broad range of non-motor complications include pain, mental health issues, impaired cognitive function, and other sensory disturbances in addition to motor symptoms like tremor, stiffness, slow movement, and unbalanced walking (Fioravanti and Yanagi, 2005). Due to various factors involved in this metabolic syndrome 75% of the population require combined therapy. One such ideal combination tablet introduced was citicoline (CLE) and nimodipine (NDE) into the market by Dr. Lazar's laboratory under the brand nimodilat plus contains (100 mg of CLE and 30 mg of NDE). The chemical compound citicoline, also referred to as cytidine diphosphate-choline (CDP-choline), is necessary for the appropriate function and structure of cell membranes, especially those found in the brain (Reddy *et al.,* 2013). The potential neuroprotective and cognitive-enhancing qualities of citicoline have made it a topic of interest in the fields of neuroscience and brain health (Adibhatla *et al*., 2001).

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Citicoline has been studied for its potential benefits in conditions such as age-related cognitive decline, stroke, and neurodegenerative disorders (Secades and Lorenzo, 2006). Its ability to support brain metabolism, enhance neurotransmitter release, and provide antioxidant effects has led to its exploration as a potential therapeutic agent for cognitive disorders and neurological conditions (Jahnavi Bandla and Ashok Gorja, 2022).

In addition to its role in neurotransmitter synthesis and brain cell membrane formation, citicoline has been investigated for its impact on cerebral blood flow, myelination, and anti-inflammatory properties (Secades, 2011). These diverse mechanisms of action contribute to its potential neuroprotective effects (Saver *et al*., 2008)

Citicoline supplements are available in various forms, and individuals often use them to support cognitive function, enhance memory, and promote overall brain health (Rao *et al*., 2000). Before using any dietary supplement or medication, People should always consult a healthcare professional, particularly if they are currently taking other medications or have underlying medical conditions (Adibhatla *et al*., 2003). Ongoing research continues to shed light on the full range of benefits and potential applications of citicoline in promoting brain health and function (López-Coviella *et al*., 1995).

A medicine called nimodipine is a member of the calcium channel blocker drug class (Barmpalexis *et al.,* 2011). Its main application is to avoid problems after a subarachnoid hemorrhage (SAH), a kind of bleeding into the area around the brain (Kale *et al.,* 2008). Nimodipine is intended to increase blood flow in this region and is well-known

for its particular effects on blood vessels in the brain (Janjua and Mayer, 2003).

2. Mechanism of action

2.1 Citicoline

Citicoline, (Figure 1) sometimes referred to as CDP-choline or cytidine diphosphate-choline, is a chemical that is required for the phospholipid synthesis process, particularly phosphatidylcholine, a crucial component of cell membranes. Studies have looked into the potential advantages of citicoline for improving cognitive function and neuroprotection (Secades, 2002).

Citicoline's mode of action entails several biochemical processes, including a choline donor is citicoline. Acetylcholine, a neurotransmitter involved in memory, learning, and other cognitive processes, is produced through the conversion of choline. For nerve cells to communicate with one another, acetylcholine is essential (Mounika and Hymavathi, 2021). It contributes to the production of phosphatidylcholine, a significant phospholipid that serves as the structural foundation for cell membranes, especially those found in the brain. This process is not only required to maintain the fluidity and integrity of the cell membrane, but it has also been demonstrated to improve the release of neurotransmitters like dopamine, norepinephrine, and serotonin (Porciatti *et al*., 1998). These neurotransmitters play important roles in mood regulation, attention, and other cognitive functions. It may support brain metabolism by increasing cerebral blood flow and glucose utilization. This can contribute to improved energy production and overall brain function (Sudha *et al*., 2023) Mainly it has been shown to support the formation of myelin, the sheath surrounding nerve fibers for protection. Myelin is crucial for efficient nerve signal transmission and is important for overall neural function (Plataras *et al*., 2000).

2.2 Nimodipine

The main indication for the calcium channel blocker nimodipine (Figure 2) is subarachnoid hemorrhage (SAH), which is a kind of bleeding in the area surrounding the brain. Nimodipine works by selectively blocking calcium channels, especially those in cerebral blood vessels. This is how it works. It works by preventing calcium ions (Ca^{2+}) from entering specific voltage-gated calcium channels, primarily the L-type calcium channels. These channels are present in smooth muscle cells, such as those in the blood vessels of the brain. Nimodipine lowers the intracellular calcium concentration by inhibiting calcium channels in blood vessel smooth muscle cells (Shanmugasundaram *et al.,* 2023) vasodilation results from this, widening the blood vessels. The dilation primarily occurs in the cerebral arteries, increasing blood flow to the brain. Its vasodilatory effects are specific to cerebral blood vessels. This selective action helps improve cerebral blood flow without significantly affecting blood flow in other areas of the body. This increased blood flow is particularly important in the context of subarachnoid hemorrhage, where maintaining adequate cerebral perfusion is crucial for preventing further damage to brain tissue. It has been suggested to have neuroprotective properties, possibly beyond its vasodilatory effects. In order to stop calcium-mediated cellular damage, it may lessen the release of excitatory neurotransmitters like glutamate and inhibit the influx of calcium into neurons (Afroz Patan *et al.,* 2023). This neuroprotective aspect is relevant in the treatment of subarachnoid hemorrhage, where protecting the brain from secondary injury is a therapeutic goal (Vergouwen *et al.,* 2006). Drug profile of citicholine and nimodipine were shown in Table 1. Pharmacokinetic parameters of citicholine and nimodipine were shown in Table 2. Marketed formulation brand name and its manufacturer name for citicoline, nimodipine individually and its combination, pharmaceutical dosage forms lists were shown in Table 3.

3. Drug profile

Table 1: Drug profile of citicoline and nimodipine

Contents	Citicoline	Nimodipine
IUPAC name	3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphate phosphoryl]2-(trimethylazaniumyl)ethyl	3-(2-methoxyethyl) 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Appearance	White, hygroscopic powder	It is a yellow crystalline powder
Molecular formula	$C_{14}H_{26}N_4O_{11}P_2$	$C_{21}H_{26}N_2O_7$
Molecular weight	488.324 g/mol	418.4 g/mol
Melting point	242.0°C	125°C
Boiling point	851.4ºC at 760 mmHg	534.8 ± 50.0°C at 760 mmHg
Solubility	It dissolves easily in water.	It dissolves in organic solvents
Storage	Stored at -20°C	Store in a cool, dry place and protect from light

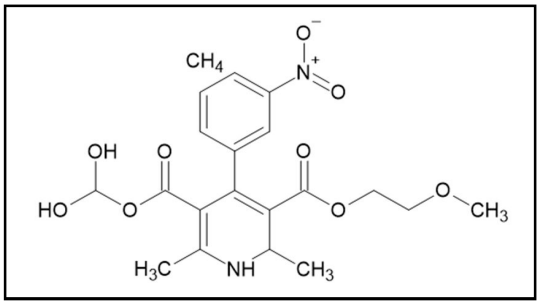

Figure 1: Structure of nimodipine.

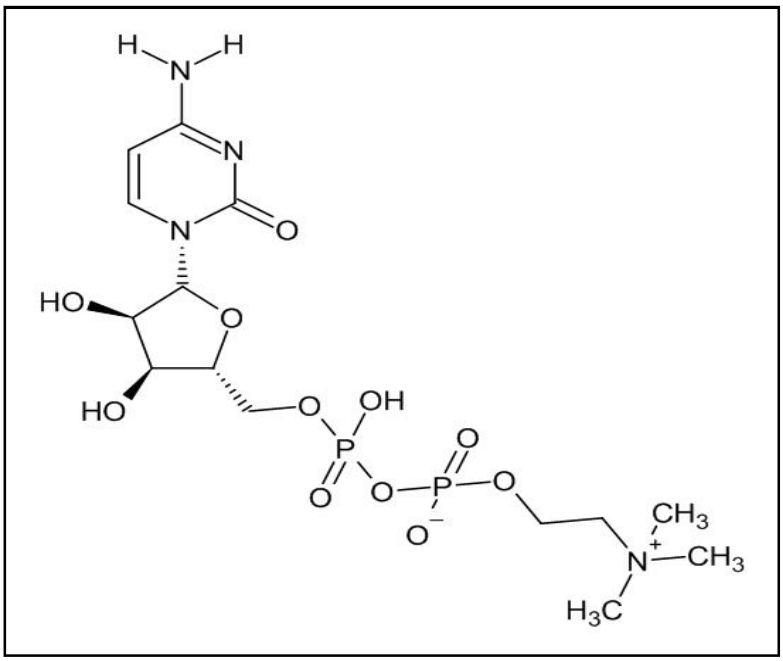

Figure 2: Structure of citicoline.

3.1 Pharmacokinetic parameters of citicoline and nimodipine

Table 2: Pharmacokinetic parameters of citicoline and nimodipine

Parameters	Citicoline	Nimodipine
Absorption	It is rapidly absorbed, and in the intestinal wall, it hydrolyzes to produce cytidine and choline.	It is quickly absorbed when taken orally. Nimodipine has a 13% bioavailability because of its high first-pass metabolism.
Distribution	It has minimal plasma protein binding, allowing for a relatively high degree of distribution in the body.	The range of nimodipine's volume of distribution (Vd) is 0.94 l/kg to 2.46 l/kg.
Metabolism	It is metabolised in different tissues to produce other cellular components, such as phospholipids.	Both CYP3A4 and CYP3A5 actively metabolise it. Nimodipine glucuronide and nimodipine oxidation products are major metabolites.
Excretion	The principal organ for the elimination of citicoline and its metabolites is urine.	Urine is the main organ through which nimodipine and its metabolites are primarily eliminated.

Table 3: List of marketed formulations

Analyte	Marketed formulation	Manufacturing companies
Citicoline	Citicoz inj, Citiwise tab, Citicoz syp, Cogitom oral drops, CDP coline cap	Sag Health Science Pvt. Ltd, New Delhi, Psycho Care Health Pvt. Ltd, Punjab Sag Health Science Pvt Ltd, New Delhi, Livealth Biopharma Pvt. Ltd, Maharastra Duble Wood Supplements, USA
Nimodipine	Nimocer tablet, Nimodip inj, Nimotide tablet Modipin inj	Micro Labs Limited, Bangalore, India USV Private Limited, Govandi East, Mumbai USV Private Limited, Govandi East, Mumbai Cadila Pharmaceuticals, Ahmebad, India
Citicoline and nimodipine	Nimodlat-plus tablets	Dr. Lazar y cia. S.A.Q.el Industria Argentina

4. Method development and validation of citicoline

Meenu Chaudhary *et al.* (2018) have developed a method for estimating citicoline in bulk that is precise, cost-effective, and accurate. The calibration curve method produced a wavelength of 270 nm for citicoline in pure water. Between 10 and 60 µg/ml, the concentration range of citicoline exhibits a linear calibration curve and a 0.999 correlation coefficient. It is determined that the regression equation $y = 0.016x + 0.012$ is satisfactory. There is a 2% limit on the precision of the results. In order to facilitate the application of the analysis in the pharmaceutical dosage form and to verify linearity, accuracy, and precision for the intended use, the analysis results have been validated.

Sofiane Derbouz *et al.* (2017) developed a stability-indicating HILIC technique to examine citicoline when its breakdown products are

present. The mobile phase used was 70% acetonitrile in 0.02 M (v/v) formate buffer, pH-adjusted to 3.0., along with an Atlantis HILIC Si column (50 × 4.6 mm, 3 µm particle size). The thermostat compartment in the column was set at 30ºC. Heat, UV light, hydrolysis, acid, base, and oxidation were all applied to citicoline. Mass detectors and photodiode arrays were used to monitor the column effluents at 270 nm. Three degradation products emerged contingent on the type of stress: one after an oxidative stress, one after a NaOH reaction, and one after an acidic or photolytic action. The recommended method worked well for figuring out the dosage forms of citicoline.

Kempegowda *et al*. (2017) reported that substances that act as drugs and have therapeutic effects are known as physiologically active components of pharmaceuticals. The two main organic volatile impurities (OVI) used in the production process, methanol and isopropyl alcohol (IPA), are mixed in a process solvent. The developed method demonstrated linearity in solutions with concentrations ranging from 1 to 150%, including methanol and IPA, with a correlation coefficient of 0.999. The GC-headspace approach was developed to recognize and manage these solvents. The United States Pharmacopoeia and the ICH guidelines were followed in the validation process. For OVI (methanol and IPA), the % RSD for LOD and LOQ were, respectively, 22.3 and 45.2 and 104.9 and 125.1.

Alagar *et al*. (2015) created and approved a procedure to ascertain the piracetam and citicoline pharmaceutical dosage forms. The chromatographic separation employed a 45:55% v/v water:acetonitrile ratio as the mobile phase, which was moving at 0.3 ml /min. The pH was adjusted to 2.8. The shield C18 (2.1 mm × 100 mm and 1.7 µm) column was utilized for chromatographic separation. 225 nm was chosen as the detection wavelength. The procedure demonstrated linearity within the 5 -25 µg/ml concentration range (0.999). At 2%, the precision between days and within days were both within the allowable range. Additionally, the mean recovery of citicoline and piracetam, which ranged from 99.04 to 101.58%, proved the accuracy of the method. Due to the shorter retention time (3 min) of the analysis, the suggested method has a high throughput. The method complied with Food and Drug Administration and ICH regulations. The approach can be successfully implemented for regular usage in industry laboratories for quality control, according to the results.

Panda *et al*. (2013) published a technique for using spectrophotometry to determine the amount of citicoline present in tablets. The basis for this spectrophotometric technique is the notion that in an acidic and basic medium, citicoline exhibits two distinct forms with distinct absorption spectra. Two solvents, HCl (0.1 M) and NaOH (0.1 M), were employed. It was discovered that the maxima and minima of the citicoline difference spectrum are 239 nm and 283 nm, respectively. The spectrum maxima and minima were used to compute amplitude. The drug exhibits linearity between 1-50 µg/ml (R^2 = 0.999). The suggested procedure has been validated.

Shailendra Bindaiya and Ameeta Argal (2012) revealed the creation and approval of a reverse-phase HPLC method that is quick, accurate, affordable, and specific for measuring the quantity of citicoline monosodium utilized in the production of pharmaceuticals. A mobile phase comprising tetra butyl ammonium hydrogen sulfate buffer (pH 6) : methanol (95:05, v/v) at a flow rate of 1.0 ml/min and a C18 column (250 mm × 4.6 mm, 5 µm particle size) were used to carry out the isomeric separation process. The wavelength used for detection was 270 nm. Citicoline monosodium has been quantified using the recommended method, yielding a coefficient of correlation (R^2) of 0.9999 and a linearity range of 20-100 µg/ml. At room temperature, the column was allowed to elute in 6.3 ± 0.5 min. The recovery percentage varied from 97.85 to 99.75%, and the precision percentage RSD was found to be 0.187 ± 0.18. As a result of the assay's validation for linearity, accuracy, precision, robustness, and solution stability, regular industry analysis can use it.

Sachan *et al*. (2011) created a technique for measuring citicoline with a UV spectrophotometer that has two beams. The maximum absorption of citicoline is found to occur at 272 nm. Data was gathered and compared with deionized water. A range of 5-50 µg/ml for concentrations and a correlation coefficient of 0.9998, was observed by Beer Lambert's law. To ensure quality control, the active ingredient is quantified in the pharmaceutical specialty as well.

Chen *et al*. (2011) created the citicoline uridine metabolite found in human plasma. Using this methodology, the pharmacokinetic and bioequivalency studies of citicoline tablets and capsules were then carried out. Kinetex C18 Phenomenex (100 mm × 4.6 mm, 2.6 µm) column was used to perform the separation. The components of the mobile phase are methanol (98:2% v/v) and potassium dihydrogen phosphate (0.05 M). The flow rate of the mixture is 0.8 ml/min. There was a linear relationship between the calibration curve and the uridine concentration and range (0.05-2 µg/ml). The relative bioavailability and basic pharmacokinetic characteristics (t = 1/2 T_{max} C_{max} AUC) of the citicoline tablet and capsule were noted. The capsules and tablets underwent a bioequivalence analysis.

Bindaiya *et al*. (2010) proposed a high-performance reversed-phase liquid chromatography (RP-LC) technique to measure the amount of human plasma that contains citicoline monosodium. In a room-temperature nucleosil C18 analytical column, Tetra butyl ammonium hydrogen sulphate pH 5.0, 0.005 M buffer and 95:05% v/v of methanol were used as the mobile phase in an isocratically eluted sample of the active drug. It was demonstrated that the drug maintained its potency for 6.64 min when a photodiode array (PDA) at 270 nm was used. When using citicoline monosodium in spiked plasma, the process was discovered to be linear in the range of 150 - 900 ng/ml of drug concentration. Pharmacokinetic studies and CTM in human plasma can benefit from the successful application of the suggested methodology. Under a variety of test circumstances, the drug remained stable in human plasma, and the process was confirmed.

5. Method development and validation of nimodipine

Sagar Suman Panda *et al.* (2020) outlined the use of an innovative, simple liquid chromatographic technique for measuring nimodipine in formulations and spiked serum samples was developed and validated using a quality-by-design methodology. Retention time, theoretical plates, tailing factor, and other pertinent critical analytical parameters were defined using this method. In order to create a dependable method and additional control techniques, key procedure parameters, such as pH, methanol percentage, and mobile phase flow rate, were identified and optimized using the Box-Behnken experimental design. A ShimPack GWS C18 column was used for separation with methanol : water (80:20 % v/v) being used at a flow rate of 1.0 ml/min. The mixture pH was kept at 3.5 by the addition of o-phosphoric acid. The presence of the photodiode array was detected at 355 nm. The method dependability was confirmed by

the results for the validation parameters: precision (< 1 %), accuracy (> 98 %), and linearity (0.5-80 µg/ml). To guarantee the chromatographic method's specificity, the drug was violently broken down. Given that nimodipine has demonstrated sensitivity to applied conditions of stress involving acid, alkali, and photolysis, the proper safety measures must be taken. The technique was also used to assess the target analyte's suitability for estimation in biological fluids that had been spiked. Overall, the procedure proved to be dependable and suitable for both routine and bioanalytical uses.

Weiping Wang *et al.* (2019) determined to ascertain the concentration of nimodipine in human plasma, supercritical fluid chromatography, and tandem mass spectrometry were combined to create an easy-to-use, sensitive, and effective technique. In a single step, the analytes were extracted from the plasma using an acetone-based protein precipitation method. The benchmark used internally was nittrendipine. Using a gradient elution program with a 1.5 ml/min flow rate and an Acquity uplc 2 tm beh 2 ep column, chromatographic separation was performed. An electrospray ionization source running in positive ion mode on a triple quadrupole tandem mass spectrometer was used to make the detection. The drug transitions between m/z 361.4 - 315.2 for nitrendipine and m/z 419.3 - 301.3 for nimodipine were observed using multiple reactions. In the concentration range of 0.5-800 ng/ml, the linearity ($r^2 > 0.996$) was determined to be satisfactory. For every quality control level, the precision and accuracy results within and between days were less than 9.1 %. The area under concentration-time curves (0-720 min) for the test and reference formulations were observed, along with peak concentrations of 279.28 ± 211.46 and 265.13 ± 149.26 ng/ml, 25608.00 ± 17553.665, and 28553.67 ± 20207.92 ng/ml respectively. Following oral treatment, nimodipine's pharmacokinetic profiles in beagle dogs were effective, it was determined that the linearity ($r^2 > 0.996$) was satisfactory. revealed through the application of the validated method. Additionally, additional bioequivalency research could be conducted using the analytical method.

Jagdish and Rajesh (2018) developed the drug formulation for nimodipine has been successfully determined using a straightforward, quick, accurate, and precise UV spectrophotometer method. Nimodipine had a correlation coefficient of 0.9996 and a linear range of 5 µg/ml to 25 µg/ml. At 239 nm, the substance's absorption maxima were discovered. By measuring the accuracy and precision of the previously suggested method, it was validated. Less than two standard deviations, 0.522% and 0.355%, respectively, are present, the analytical method showed good intraprecision (repeatability). It was discovered that the percentage recovery at 50%, 100%, and 150% was 49.9%, 99.1%, and 149.6%, respectively. The developed method was verified concerning the parameter specificity.

Simanchal Panda *et al*. (2017) Pharmaceuticals for various chronic illnesses are primarily administered orally for a considerable amount of time. A calcium channel blocker used to treat hypertension, imidipine is poorly soluble in water. Treatment failure, high intra- and inter-subject variability, low bioavailability, and inadequate dose proportionality are frequently associated with the oral administration of this drug. Increasing the solubility of nimodipine will increase its bioavailability because of its dissolution-dependent pattern of bioavailability. To increase solubility, self micro-emulsifying drug delivery systems (SMEDDS) were developed. An attempt was made to release nimodipine directly into the intestine because its window of absorption in the stomach is limited. This was accomplished by covering the hard gelatin capsule with a pH-sensitive polymeric solution and encasing the medication in the SMEDDS formulation (SMEDDS CAP). The selection of oleic acid as the lipid and cremophor RH 40 and PEG 400 as the emulgents for the SMEDDS CAP formulation was guided by equilibrium solubility studies. To identify the microemulsion area and the lipid and emulgent amounts as the critical factor variables, ternary phase diagrams were built. 32 full factorial designs were used to systematically optimize the SMEDDS. The optimized SMEDDS's noncoalescent nature was demonstrated by their nanometer size range and high negative zeta potential value. The globules in the reconstituted SMEDDS showed uniform size and shape, according to TEM studies. The stability of the optimized formulation was determined using thermodynamic studies, cloud point measurement, and accelerated stability studies. The *in vivo* pharmacokinetics of the optimal formulation OS 6 CAP demonstrated a greater AUC and a longer plasma half-life compared with the commercial formulation. In comparison to the commercial formulation, the relative bioavailability increased 4.01 times. Thus, the research demonstrated that the enteric coated SMEDDS CAP system was effectively created, providing a markedly enhanced bioavailability possibility for the poorly soluble medication nimodipine.

Lubna Shaikh *et al*. (2015) developed an RP-HPLC method that is straightforward, linear, precise, accurate, robust, and selective for determining the presence of nimodipine impurities in formulation and bulk. Methanol, acetonitrile, and water were added to the mobile phase at the following ratios: 35:40:25% v/v. The mobile phase had a flow rate of 0.8 ml/min. The HPLC equipment is a Liquid Chromatography LC 20 AD prominence system by Shimadzu Japan SPD 20-A. The 250 x 4.6 mm C18 column and the UV-VIS detector were used at a wavelength of 234 nm. Ultimately, both the tablet and bulk forms of imidapine had their impurity content measured. Impurities were found to be present in the bulk and tablet at 0.0219 % and 0.0876 %, respectively. Consequently, it was discovered that the nimodipine impurity was within the allowed limit.

Manoela Riekes *et al*. (2014) explained modification I, the metastable racemate, and modification II, the stable conglomerate, of the nimodipine polymorphs were evaluated and their intrinsic dissolution process explained. To achieve this, a 50:50 v/v hydroalcoholic solution of ethanol and water was chosen as the dissolving medium, and it was kept at 37 ± 0.5°C. For validation, the lower rotation speed was selected, and tests were conducted at 50, 75, and 100 rpm. Under all tested conditions, the substance that was first recognized as polymorph mod I showed greater intrinsic dissolution rates; however, no statistically significant differences between the two polymorphs were discovered. Differential scanning calorimetry and X-ray powder diffraction were used to identify the partial solution-mediated phase transformation that produced this result, which was from mod I to mod II. Furthermore, for the Mod II polymorph, trustworthy intrinsic dissolution rate data was obtained. After validation, it was found that the dissolution method was exact, accurate, logical, linear, stable, and linear.

Ying Zhao *et al*. (2010) determined the liquid chromatographic–electrospray ionisation mass spectrometric (LC-ESI-MS) method is a simple, accurate, and selective method that can be used to determine the concentration of nimodipine in human plasma. Nimodipine is

extracted liquid-to-liquid from plasma samples in the negative ion selected ion monitoring mode (SIM) with the aid of ESI mass spectrometric detection, anhydrous diethyl ether, and simple reversed-phase chromatography. The [M-] target ions for nimodipine (internal standard, IS) and nitrendipine are found at m/z 417 and 359, respectively. Saturated sodium bicarbonate ($NaHCO_3$) solution (200 µl) is added to the plasma to enhance the extraction recovery. An analytical run was completed in 3.5 min. A quantification limit of 0.5 ng/ml was established. The calibration regression line had a correlation coefficient of at least 0.9995. Acceptable levels of accuracy and precision were found both within and between batches. The analyte passed several stability tests and was found to be stable. The efficacy of the approach has been shown by the bioequivalency study.

Famei Li *et al.* (2008) outlined How nimodipine pharmacokinetics in human plasma were measured and analysed using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS), a quick and accurate technique. The sample pretreatment process included extracting 0.5 ml of plasma using diethyl ether and nitrendipine as the internal standard. Using an ACQUITY UPLCTM BEH C18 column (50 mm × 2.1 mm, i.d., 1.7 µm) and mobile phases of acetonitrile and water both containing 0.1 % formic acid, the separation was carried out at a gradient flow rate of 0.35 ml/min. Three quadrupole tandem mass spectrometers were used for the detection, with the multiple reaction monitoring (MRM) mode and an electrospray ionisation (ESI) sources. The concentration range of 0.20-100 ng/ml was covered by the linear ($r^2 \leq 0.99$) standard curves, with 0.20 ng/ml serving as the limit of quantification (LLOQ). The accuracy range (relative error R.E.) was 2.2-7.7% at all three quality control (QC) levels, and the intra- and inter-day precision (R.S.D.) values were below 14% using nimodipine oral tablets in a pharmacokinetic study with volunteers who were healthy men, the method presented here proved to be more successful than earlier methods.

Dafang Zhong *et al*. (2004) revealed that Nimodipine in human plasma may be found using the extremely sensitive and specialised method called tandem mass spectrometry–liquid chromatography (LC-MS-MS). Nitrendipine, the internal standard, and the analyte were extracted from plasma samples using n-hexane : dichloromethane : isopropanol (300:150:4 % v/v/v). A C18 column was then used for chromatography. The ratio of formic acid, methanol, and water in the mobile phase was 80:20:1 or v/v/v. An atmospheric pressure chemical ionisation (APCI) source, a selected reaction monitoring (SRM) mode and a triple quadrupole tandem mass spectrometer were employed in the detection procedure. An endpoint of 0.24 ng/ml is reached by using this technique. Between 0.24 and 80 ng/ml of concentration, linear calibration curves were generated. Relative error (RE) of the precision was less than 4.4%, and the accuracy ranged from 0.0 to 5.8%. Eighteen healthy volunteers were given nimodipine tablets, and their pharmacokinetic profiles were evaluated using this validated method. Summary for citicoline and nimodipine reported methods were shown in Table 4.

Table 4 : Summary for citicoline and nimodipine reported methods

Analyte	Analytical technique	Conditions applied	Reference
Citicoline	UV method	270 nm, water as solvent	Meenu Chaudhary *et al.* (2018)
	Stability-indicating HILIC method	Atlantis HILIC C_{18} column (50 × 4.6 mm, 3 µm particle size)	Sofiane Derbouz *et al*. (2017)
	GC - method	Methanol and IPA as solvent	Kempegowda *et al*. (2017)
Piracetam and citicoline	HPLC	Stationary phase; C_{18} column (2.1 mm ×100 mm and 1.7 µm) Mobile phase: 45:55% v/v water: acetonitrile	Alagar *et al*. (2015)
	UV	Absorption spectrum using different solvents HCl (0.1 M) and NaOH (0.1 M),	Panda *et al*. (2013)
	RP-HPLC	Mobile phase: tetra butyl ammonium hydrogen sulfate buffer (pH 6) : methanol (95:05, v/v)	Shailendra Bindaiya and Ameeta Argal (2012)
Citicoline uridine	Metabolite found in human plasma by RP-HPLC	Stationary phase: C_{18} Phenomenex (100 mm × 4.6 mm, 2.6 µm).Mobile phase: methanol (98:2% v/v) and potassium dihydrogen phosphate (0.05 M)	Chen *et al*. (2011)
Nimodipine	RP-HPLC with CCD	Methanol:water (80:20, v/v)	Sagar Suman Panda *et al.* (2020)
	SFC-MS	Acetone-based protein precipitation method. Acquity uplc 2tm beh 2 ep column, electrospray ionization	Weiping Wang *et al.* (2019)
UV	239 nm, 5 µg/ml to 25 µg/ml	Jagdish and Rajesh (2018)	

5. Conclusion

In conclusion, the analytical techniques discussed in this review provide a robust foundation for evaluating the citicoline and nimodipine combination. The diverse array of methodologies offers researchers a comprehensive toolkit for characterizing these drugs individually and in combination. Understanding the pharmacological effects and potential synergies between citicoline and nimodipine is crucial for optimizing therapeutic outcomes. This review serves as a valuable resource for researchers, clinicians, and pharmaceutical

professionals engaged in the exploration of this drug combination, fostering a deeper comprehension of its analytical nuances and pharmacological implications. Ongoing research in this area is encouraged to further refine our understanding of the complex interplay between citicoline and nimodipine and also this review provides a comprehensive synthesis of existing approaches, readers are equipped with the necessary tools to navigate the complexities of drug analysis. This knowledge is essential for ensuring the accuracy and reliability of estimations, ultimately contributing to the enhancement of pharmaceutical research, development, and quality control. As advancements in analytical technology continue, ongoing exploration and updates to this knowledge base will further refine the methodologies available for the estimation of citicoline and nimodipine.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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