https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(September 2024); 370-376

Research Article

Development and Validation of Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Citicoline and Nimodipine in Bulk and Tablets

Chitra Manickam[1], Praveenraj Kumarsz[*], Kalaiselvi Ponnusamy[2], Subash Vadivel[3], Senthilkumar Natesan[4]

*[*1,2,3,4]Department of Pharmaceutical Analysis, JKKMMRF's - Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam, Namakkal. Tamil Nadu – 638 183, Affiliated with The Tamil Nadu Dr. MGR Medical University, Chennai, India*

Abstract

A stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) approach was developed and validated for the simultaneous measurement of Citicoline and Nimodipine concentrations in tablet dosage forms. Chromatographic separation was accomplished by utilizing a C18 column filled with a mobile phase composed of methanol and phosphate buffer (pH 3.0) in a 60:40 proportion, delivered at a flow rate of 1.0 mL/min. Citicoline and Nimodipine exhibited excellent resolution, with retention durations of 3.006 minutes and 5.739 minutes, respectively. The method proved to be remarkably linear for both drugs over the concentration range of 100-350 µg/ml, with correlation values (r) of 0.9994 for Citicoline and 0.9997 for Nimodipine. Both Citicoline and Nimodipine had limits of detection (LOD) of 2.4714 µg/ml and 1.4600 µg/ml, respectively. The lower and upper limits of quantification were found to be 7.4893 µg/ml and 4.4242 µg/ml, respectively. The method exhibited robust performance with low %RSD values, ensuring precision and accuracy. Stability studies revealed that both drugs were stable under various stress conditions, with degradation percentages well within acceptable limits. The method that was developed was effectively used on commercial tablet formulations, therefore demonstrating its appropriateness for common quality control and stability testing in pharmaceutical analysis.

Keywords: Stability-indicating RP-HPLC, Citicoline, Nimodipine, Simultaneous estimation, Tablet dosage forms, Method validation

Author for correspondence: Email: *jp26011969@gmail.com*

Received: 10/07/2024 Accepted: 09/08/2024

DOI: https://doi.org/10.53555/AJBR.v27i3.1779

INTRODUCTION

The pharmaceutical industry demands rigorous analytical methods to ensure drug quality, safety, and efficacy. RP-HPLC is a critically important technique commonly used to simultaneously determine many active pharmaceutical ingredients (APIs) in bulk and dose forms [1]. Citicoline, chemically known as (2R,3S,4R,5R)-2-(4-Amino-2-oxopyrimidin-1-yl)-5-(hydroxymethyl) oxolan-3-yl] oxyphosphonic acid, is a nootropic agent that enhances brain function and is widely used in the treatment of cognitive impairments, particularly in stroke and Alzheimer's disease [2,3]. Citicoline's ability to elevate levels of neurotransmitters like acetylcholine and enhance neuroplasticity underpins its therapeutic efficacy [4]. Nimodipine is a calcium channel blocker that is mainly used to prevent cerebral vasospasm after subarachnoid hemorrhage. It is also known by its IUPAC designation, isopropyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate. Its

lipophilic nature allows it to cross the blood-brain barrier, making it an essential drug in neurocritical care [5,6]. The chemical structure of citicoline and mmodipine as shown in figure 1. The combination of Citicoline and Nimodipine in tablet dosage forms presents a promising therapeutic approach for neuroprotective strategies, particularly in managing cerebrovascular disorders [7]. However, their co-formulation also poses analytical challenges, necessitating the development of robust and reliable methods for their simultaneous quantification.

Several analytical techniques have been documented for the separate quantification of Citicoline and Nimodipine. Citicoline has been quantified using techniques such as UV-spectrophotometry, HPLC [8], and LC-MS/MS [9] in various dosage forms and biological matrices. Similarly, Nimodipine has been analyzed through RP-HPLC [10], UV-spectrophotometry [11], and other chromatographic methods in pharmaceutical preparations. A stability-indicating RP-HPLC approach for the concurrent measurement of citicoline and nicodipine has received little attention despite these developments. To ensure the stability of the formulation throughout its shelf life, stability-indicating methods are essential for determining the degradation profile of the medications under various stress conditions, such as heat, light, pH, and oxidative stress.

This study aims to create and validate a stability-indicating RP-HPLC method that can quickly separate, identify, and quantify Nimodipine and Citicoline in both bulk and tablet dosage forms.

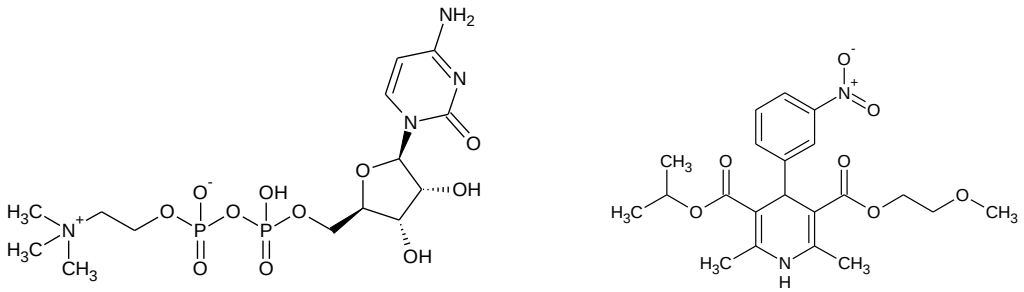

Figure 1: structure of Citicoline and Nimodipine

Materials and methods

The drug samples used in this study were Nimodipine and Citicoline, which were obtained by Madras Pharmaceutical Limited, Chennai. The formulation employed was Nimodilat plus tablet, procured from a local pharmacy. The solvents used included Methanol, Water HPLC analytical grade from Qualigens; Acetonitrile and Orthophosphoric Acid, analytical grade from Merck. These chemicals and solvents were selected based on their purity and suitability for the analytical procedures involved.

HPLC instrumentation and condition

An RP-HPLC analysis was performed using a gradient separation technique using a C18 column as the stationary phase. Acetonitrile and phosphate buffer (pH 6.2) were mixed in an optimum mobile phase preparation at a ratio of 55:45% v/v, starting with an initial condition of 60:40% v/v. The temperature of the column was kept at 30°C and the flow rate was adjusted to 1.0 ml/min. The particular wavelength of 225 nm was chosen for the detection because of the strong absorption observed in the UV spectra of Nimodipine and Citicoline at this particular wavelength. In each run, a sample volume of 20 microliters was injected. Both the bulk and tablet pharmaceutical formulations of Nimodipine and Citicoline were tested using a validation approach to guarantee accurate separation and quantification.

Preparation of standard stock solution

The 30 mg and 100 mg samples of Nimodipine and Citicoline, respectively (Nimodilat plus tablet), were carefully measured and transferred to a 100 ml calibrated standard flask. The volume was prepared by dissolving in methanol of HPLC-grade quality. The fundamental concentrations of the stock solution were 300 micrograms per millilitre for Nimodipine and 1000 micrograms per millilitre for Citicoline. A further series of dilutions were prepared using the mobile phase.

Mobile phase preparation

The solvent reservoirs A and B contained approximately 550 ml of Acetonitrile and 450 ml of phosphate buffer with a pH of 6.2, respectively. These reservoirs were combined and subjected to degassing in an ultrasonic water bath for 10 minutes.

Preparation of standard stock solution

The precise weight of approximately 30 mg of Nimodipine and 100 mg of Citicoline was determined and then put into a calibrated standard flask with a volume of 100 ml. The volume was prepared by dissolving in methanol of HPLC-grade quality. The initial concentrations of the stock solution were precisely 300 µg/ml for Nimodipine and 1000 µg/ml for Citicoline. A subsequent round of dilutions was made utilizing the mobile phase.

System suitability studies

ICH recommendations and USP standards conducted the system suitability investigations. Calculations were performed to determine parameters such as tailing factor, capacity factor, asymmetry factor, and number of theoretical plates.

Linearity

Dispenses of Nimodipine stock solution (300 µg/ml) and Citicoline stock solution (1000 µg/ml) varying from 1 to 6 ml were added to six 10 ml volumetric flasks and filled with the mobile phase up to the designated mark. Water-based solutions with nimodipine concentrations between 30 and 180 µg/ml and citicoline concentrations between 100 and 600 µg/ml. After injecting a volume of 20µl from this solution, the resultant chromatogram was recorded at a wavelength of 225 nm. By applying Beer's law, we discovered that the concentration range mentioned earlier was linear. The process was carried out three times in an iterative fashion. A calibration curve was subsequently built by graphing concentration against peak regions.

LOD and LOQ

An investigation of linearity was performed in triplicate. Utilizing the calibration curve method, the LOD and LOQ were determined. An average of the slope and intercept was used to establish the Limit of Detail (LOD) and Limit of Quantification (LOQ).

Quantification of Formulation

The ten tablets of NIMODLAT -PLUS, containing 30mg of Nimodipine and 100mg of Citicoline, were administered and their mean weight was computed. The accurate weight of a tablet powder comprising 30 mg of Nimodipine and 100 mg of Citicoline was established. Also, half of a 100 ml volumetric flask was filled with methanol after the contents were transferred. Subsequently, a 10-minute sonication process was performed on the solution. The solution was then passed through Whatman's No. 41 filter paper, which has an aperture of 0.45 microns. After being decanted to a level of 3.0 ml, the mobile phase was used to adjust the volume to 10 ml. After quantifying the solutions, the final concentrations of Nimodipine and Citicoline were found to be 90 µg/ml and 300 µg/ml, respectively.

Precision

To evaluate the reproducibility of the method, six consecutive assays of the formulation were conducted using the same concentrations. Quantification of the drug content in the formulations was performed. The relative standard deviation (RSD) value was computed.

Recovery studies

A recovery investigation was conducted using the conventional sequential addition technique. To perform the recovery experiment, a predetermined concentration (50, 100, 150%) of Nimodipine and Citicoline working standard was added to the pre-analyzed formulations. %RSD was computed.

Ruggedness

To assess the level of consistency in test findings obtained from the suggested analyte approach, the drug sample was analyzed using many analyzers.

Forced Degradation studies

All drug compounds and their corresponding products were subjected to forced degradation using hydrolytic, oxidative, photolytic, and thermolytic methods. Solid-state photodegradation of pharmaceutical compounds and pharmaceutical products was conducted. To attain the concentrations stated on the tablet formulation label in the original commercial formulation—90 µg/ml for Nimodipine and 300 µg/ml for Citicoline—the solutions were diluted with mobile phase after degradation. Next, 20 microliters of processed solutions were introduced into the HPLC system and the resulting chromatograms were documented.

Hydrolysis by acid

First, 1 ml of 0.1 N HCl was added to a standard stock solution that included 90 µg/ml of Nimodipine and 300 µg/ml of Citicoline. Two hours were then spent letting the solution sit at room temperature before adding the mobile phase to bring the volume down to the specified level. The chromatograms were subsequently recorded.

Hydrolysis of bases

Standard stock solutions of Nimodipine and Citicoline were produced at concentrations of approximately 90 µg/ml and 300 µg/ml, respectively. Subsequently, 1 ml of 0.1 N NaOH was added to each solution. Following that, the solution was maintained at ambient temperature for a duration of 2 hours, and the volume was adjusted to the indicated level by adding the mobile phase. This was followed by the recording of the chromatograms.

Decomposition by oxidation

The oxidative degradation investigation involved the storage of solutions of the medicinal ingredient in a 1% hydrogen peroxide solution at room temperature for 2 hours. 20 microliters of sample solutions were injected and the resulting chromatograms were documented.

Degradation by photolysis

To conduct the photolytic degradation investigation, the APIs were solidified and exposed to UV light for 24 hours. Finally, the solid samples were transformed into the concentration indicated above for the analytes. The apparatus was injected with sample solutions of twenty microlitres, and chromatograms were subsequently recorded.

Results

The official compendia for evaluating Citicoline and Nimodipine in dosage forms by RP-HPLC do not currently provide any stability indication for the procedure. The current proposed approach was extensively evaluated with the existing approaches documented in the literature [12-17] and is presented in Table 1.

Table 1: Comparison of the performance characteristics of the present method with the published methods

Parameters	Drugs	Current Method	Reported Method
Retention Time	Citicoline	3.006 min	4.1 min
	Nimodipine	5.739 min	7.3 min
Tailing Factor	Citicoline	1.2	1.3
	Nimodipine	1.4	1.5
Theoretical Plates	Citicoline	124655	8000
	Nimodipine	16229	9500
Linearity Range (µg/ml)	Citicoline	100-350	50-300
	Nimodipine	100-350	20-120
Correlation Coefficient (r)	Citicoline	0.9994	0.998
	Nimodipine	0.9997	0.997
LOD (µg/ml)	Citicoline	2.4714	2.0
	Nimodipine	1.4600	1.5
LOQ (µg/ml)	Citicoline	7.4893	6.5
	Nimodipine	4.4242	4.5

METHOD VALIDATION (ICH Q2A, Q2B Guidelines)

System suitability test offers the additional guarantee that the approach is producing accurate and exact results on a particular occasion. Upon evaluating the outcomes of each system compatibility test against the defined acceptance criteria, the technique is deemed acceptable in that specific instance. The acceptance criteria for system applicability specify that the asymmetry factor should not exceed 2.0, the theoretical plates should not be fewer than 2000, and It is important that the relative standard deviation of the peak area is under 2.0%. All measured variation parameters met the specified acceptance standards. The system suitability evaluation of the devised test method concludes with the results presented in Table 2.

Table 2: Optimization C riteria for System Suitability in RP-HPLC

Parameters	Citicoline	Nimodipine
Retention time	3.006	5.739
Tailing factor	1.2	1.4
Theoretical plates	124655	16229
Capacity factor	0.93	0.529
Resolution	0.00	3.49

Linearity and Calibration:

Both Citicoline and Nimodipine showed exceptional linearity within the concentration range of 100-350 µg/ml using this approach. An analysis revealed correlation coefficients (r) of 0.9994 for Citicoline and 0.9997 for Nimodipine, suggesting a robust linear association between the concentration and peak area. The regression equations were Y = 152.92X + 78118 for Citicoline and Y = 112.25X + 13102 for Nimodipine, with slopes (m) of 152.92 and 112.25, respectively, and intercepts (c) of 78118 and 13102, respectively. Citicoline had a LOD of 2.4714 µg/ml and Nimodipine had an LOD of 8.4893 µg/ml. Our research determined that 4.4242 µg/ml was the LOQ for Nimodipine. Figure 2 shows Chromatograms illustrating the linearity of Citicoline and Nimodipine. Figure 3 displays linearity curves for Citicoline and Nimodipine. Table 3 shows as Linearity of Citicoline and Nimodipine by the RP-HPLC method.

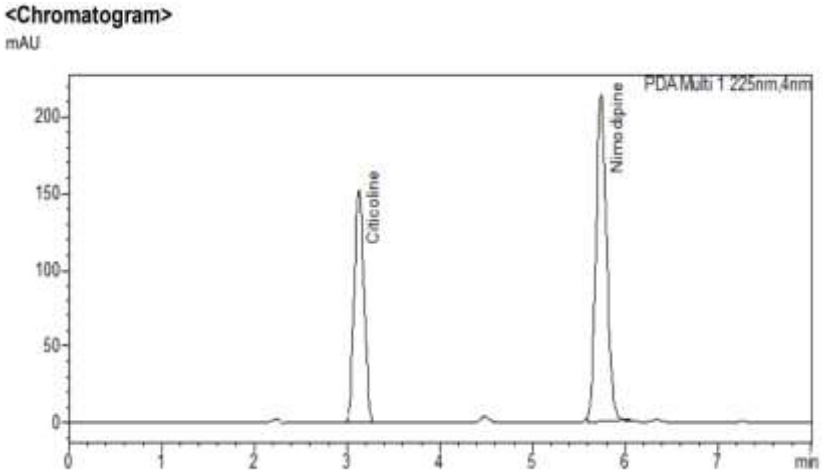

Figure 2: Chromatograms illustrating the linearity of Citicoline and Nimodipine

Table 3: Linearity for Citicoline and Nimodipine by the RP-HPLC method

Parameters	Citicoline	Nimodipine
Beers Law limit (µg/ml)	100-350	30-180
Correlation Coefficient (r)	0.9994	0.9997
Regression equation (Y=mx+c)	Y= 152.92X+ 78118	Y=112.25+13102
Slope(m)	152.92	112.25
Intercept(c)	78118	13102
LOD (µg/ml)	2.4714	1.4600
LOQ (µg/ml)	7.4893	4. 4242

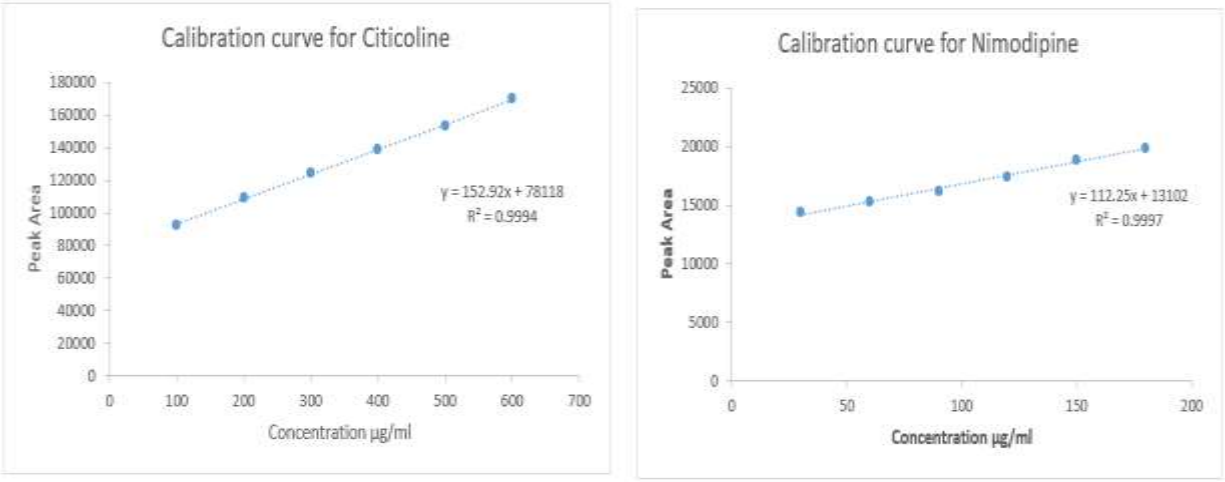

Figure 3: linearity curves for Citicoline and Nimodipine

Quantification of Tablet Formulation:

An analysis of the tablet formulation (Nimodilat Plus comprising 100 mg Citicoline and 30 mg Nimodipine) was conducted using the established approach. The percentage label claim obtained for Citicoline was 100.20% and for Nimodipine, it was 100.53%. The results exhibited robustness over several assessments, as evidenced by the % RSD values falling well within acceptable levels, therefore confirming the accuracy of the technique. The detailed results are shown in Table 4.

Table 4: Quantification of formulation (Nimodilat plus tablets)

Drug	Sample No	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average (%)	SD	% RSD
CITI	1 2 3	100 100 100	100.35 100.16 100.10	100.35 100.16 100.10	100.20	0.1305	0.1302
NIMO	1 2 3	30 30 30	29.69 30.66 30.15	98.96 102.2 100.5	100.53	1.6206	1.6117

Precision:

The accuracy of the approach was verified by iterative examination of the formulation, producing consistent outcomes. Relative standard deviation (RSD) values of 1.4741 for Citicoline and 0.7324 for Nimodipine showed that the approach was successful and repeatable.

Accuracy (Recovery Studies):

The precision of the approach was verified by recovery experiments, in which predetermined quantities of Citicoline and Nimodipine were introduced into the pre-analyzed formulation at varying concentration states. The observed percentage recovery ranged from 100.12% to 100.22% for Citicoline and from 100.29% to 100.73% for Nimodipine. The low relative standard deviation (RSD) figures validated the accuracy of the approach and its independence from any influence caused by excipients in the formulation. A summary of the results may be found in Table 5.

Table 5: Recovery Analysis of Formulation

Drug	Percentage	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	SD	% RSD
CITI	50	300	150.0	450.0	150.25	100.16	0.0503	0.0502
	100	300	300.0	600.0	300.66	100.22		
	150	300	450.0	750.0	450.58	100.12		
					Mean	100.16		
NIMO	50	90	45.0	135.0	45.33	100.73	0.2200	0.2189
	100	90	90.0	180.0	90.45	100.50		
	150	90	135.0	225.0	135.40	100.29		
					Mean	100.50		

Ruggedness:

The method's robustness was evaluated by several analysts under identical HPLC experimental settings. The % RSD values for Analyst I were 0.2154 and 0.9284 for Citicoline and Nimodipine, respectively, and for Analyst II, they were 0.2125 and 0.9314, respectively.

Stability Studies:

According to stability studies, the analytes are stable. A combination of photolytic techniques, hydrochloric acid (0.1N), sodium hydroxide (0.1N), and hydrogen peroxide (0.1%) was used in the decomposition studies. The results showed that the percentages of degradation for Citicoline and Nimodipine were 6.84% and 5.83 % for 0.1N HCl and 0.1N NaOH, respectively, and 8.38% and 9.71% for 0.1% H_2O_2. The degradation percentage was found to be for all stress conditions below 20% (ICH guidelines within the limit). Hence conclude that the analytes were stable under the above stress conditions. The report is shown in Table 6.

Table 6: Forced degradation study of Citicoline and Nimodipine

Degradation Condition	% Assay		% Degradation	
	Citicoline	Nimodipine	Citicoline	Nimodipine
0.1N HCl Acidic / 2hr	94.17	92.12	5.83	7.88
0.1N NaOH Basic / 2hr	97.00	94.23	3.00	5.77
1% H2O2 Peroxide/2hr	91.62	90.29	8.38	9.71
Photo/ UV light/ 24hr	93.11	93.16	6.89	6.84

Discussion

To evaluate Citicoline and Nimodipine in tablet pharmaceutical formulations simultaneously, this work developed and validated a stability-indicating RP-HPLC technique. Table 1 presents a comprehensive comparison between the created method and previously documented methods in the literature. The results show notable enhancements in several analytical parameters, including retention duration, tailing factor, theoretical plates, and linearity range, thus establishing the superiority of the present approach. The system suitability parameters, as shown in Table 3, confirm that the developed method is robust and meets the stringent criteria set by regulatory guidelines (ICH Q2A, Q2B). The method presented was effectively used to measure the concentration of Citicoline and Nimodipine in commercially available tablet formulations, namely Nimodilat Plus. The percentage label claim for Citicoline was 100.20%, and for Nimodipine, it was 100.53%, with % RSD values well within acceptable limits, demonstrating the method's precision and accuracy. Recovery testing confirmed the method's accuracy, which exhibited recovery rates ranging from 100.12% to 100.22% for Citicoline and from 100.29% to 100.73% for Nimodipine. The low relative standard deviation (RSD) figures provide additional evidence of the reproducibility of the approach, suggesting that it is not affected by contamination from excipients in the formulation. The results of the forced degradation experiments demonstrated the stability of Citicoline and Nimodipine under different stress conditions, encompassing acidic, basic, oxidative, and photolytic destruction. The degradation percentage for both medications was determined to be under the allowed thresholds (<20%) according to ICH standards, verifying the effectiveness of the proposed method in signalling stability.

Conclusion

To measure Citicoline and Nimodipine in tablet dosage forms simultaneously, this study created and validated a stability-indicating RP-HPLC technique. The method demonstrated superior analytical performance compared to previously reported methods, offering significant advantages in terms of shorter retention times, improved peak symmetry, enhanced column efficiency, and broader linearity ranges. The method's robustness, precision, and accuracy were confirmed through rigorous validation and application to commercial tablet formulations. Additionally, the stability studies under various stress conditions confirmed the method's capability to accurately monitor degradation, ensuring its reliability as a stability-indicating method.

Reference

Aguilar MI. Reversed-phase high-performance liquid chromatography. HPLC of peptides and proteins: Methods and protocols. 2004:9-22.

Jasielski P, Pięgel F, Piwek M, Rocka A, Petit V, Rejdak K. Application of citicoline in neurological disorders: a systematic review. Nutrients. 2020 Oct 12;12(10):3113.

Jamshidovich AS. NEUROPROTECTIVE EFFECT OF CITICOLINE. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE. 2024 Jan 12;4(1):1-4.

Bermejo PE, Dorado R, Zea-Sevilla MA. Role of citicoline in patients with mild cognitive impairment. Neuroscience Insights. 2023 Feb;18:26331055231152496.

Carlson AP, Hänggi D, Macdonald RL, Shuttleworth CW. Nimodipine reappraised: an old drug with a future. Current neuropharmacology. 2020 Jan 1;18(1):65-82.

Mahmoud SH, Ji X, Isse FA. Nimodipine pharmacokinetic variability in various patient populations. Drugs in R&D. 2020 Dec;20(4):307-18.

Fakharaldeen Z, Al-Mudhafar A, Radhi A, Hadi N. POTENTIAL PROTECTIVE EFFECTS OF NIMODIPINE FROM CEREBRAI ISCHEMIA REPERFUSION INJURY IN RATS. Wiadomości Lekarskie monthly journal. 2022 Jan 1;75(12):3094-101.

Omar MA, Ahmed AB, Abdelwahab NS, Abdelrahman MM, Derayea SM. Spectrofluorimetric approach for determination of citicoline in the presence of co-formulated piracetam through fluorescence quenching of eosin Y. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020 Aug 5;236:118337.

Sarkar AK, Ghosh D, Haldar D, Sarkar P, Gupta B, Dastidar SG, Pal TK. A rapid LC–ESI-MS/MS method for the quantitation of choline, an active metabolite of citicoline: Application to in vivo pharmacokinetic and bioequivalence study in Indian healthy male volunteers. Journal of pharmaceutical and biomedical analysis. 2012 Dec 1;71:144-7.

Sotoudegan F, Amini M, Faizi M, Aboofazeli R. Development of an RP-HPLC-UV method for simultaneous detection of nimodipine and its metabolite in cerebrospinal fluid of rat. Iranian Journal of Pharmaceutical Research: IJPR. 2017;16(2):471.

Jadhav RS, Jagdish VB. Development and Validation of Analytical method for estimation of Nimodipine content by UV spectroscopic method. World Journal of Pharmaceutical Research. 2018 Jan 7;7(5):1075-84.

Bindaiya S, Argal A. Development and validation of RP-HPLC method for determination of citicoline monosodium in pharmaceutical preparations. Int. J. Pharmaceut. Chem. 2012;2:85-8.

Chaudhary M, Kumar P, Thapliyal D. Analytical method development and validation for determination of Montelukast by UV-spectroscopy in API and in pharmaceutical dosage forms. Int. J. Pharm. Bio. Sci. 2018;4:482-7.

Malgundkar MS, Mulla S. Validated Hptlc Method for Simulteneous Determination of Citicoline sodium and Piracetam in Combined Dosage Form.

Derbouz S, Guermouche MH, Guermouche S. Stability-Indicating HILIC Method for the Determination of Citicoline and Characterization of its Degradation Products by LC–MS/TOF, 1H and 13C NMR. Chromatographia. 2017 Feb;80: 265-74.

Almughamisi E. *Stability Indicating Method Development and Validation for the Determination of Nymalize (Nimodipine) Raw Material and Its Impurities/Degradent Using Reversed-Phase High Performance Liquid Chromatography* (Master's thesis, Northeastern Illinois University).

Jadhav RS, Jagdish VB. Development and Validation of Analytical method for estimation of Nimodipine content by UV spectroscopic method. World Journal of Pharmaceutical Research. 2018 Jan 7;7(5):1075-84.

Narayan S, Choudhary M. A review on stability studies of pharmaceutical products. International Journal of Applied Pharmaceutical and Biological Research. 2017;2(3):67-75.

González-González O, Ramirez IO, Ramirez BI, O'Connell P, Ballesteros MP, Torrado JJ, Serrano DR. Drug stability: ICH versus accelerated predictive stability studies. Pharmaceutics. 2022 Oct 28;14(11):2324.