EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF NIMODIPINE THROUGH ION-PAIR COMPLEX FORMATION WITH BROMOTHYMOL BLUE

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Abstract

A new spectrophotometric method was established for the determination of nimodipine (NMD) in pharmaceutical formulations. This method is based on the formation of a yellow ion-pair complex between the reduced nimodipine and bromothymol blue (BTB) in an acidic medium. Chloroform was used as an optimal solvent for the extraction of ion-pair complex and measured the absorbance of this complex at wavelengths of 414.5 nm. The effect of optimum condition via extracting solvent, volume of dye, shaking time, reaction ratio was studied. Beer's law is obeyed in the concentration ranges of 4.0-50.0 μg/mL with a molar absorptivity of 1.50×10^4 $L.mol^{-1}.cm^{-1}$ and limit of detection (LOD) of 1.87 μg/mL. The accuracy and precision of the method were evaluated on an intra-day and inter-day basis. The relative error (%RE) and the relative standard deviation (%RSD) were < 5.0% while the recovery (%) was 99.05-103.25. The proposed method was applied successfully to determine nimodipine in pharmaceutical preparation (Nomitop, Bayer).

Keywords: *Nimodipine; extraction-spectrophotometry; ion-pair complex; bromothymol blue.*

1. Introduction

Nimodipine, which is shown in Fig. 1a, is a dihydropyridine calcium channel blocker and has a chemical name of isopropyl-2-methoxyethyl-1,4-dihydroxy-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate that works by relaxing the muscles of blood vessels, especially in the brain. NMD is used in the treatment of senile dementia and the prevention of vasospasm. At ambient conditions, Nimodipin is stable in acidic and neutral environments while unstable in alkali conditions [1, 2].

Currently, several analytical methods have been reported for the determination of NMD in different subjects such as bulk, pharmaceutical formulations, and biological fluids. These methods include HPLC [3], LC-MS/MS [4], polarography [5], spectrofluorimetry [6], and spectrophotometry [7]. However, these methods need long chromatographic run time, expensive instruments, and were difficult to implement

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under the basic conditions of some laboratories. The spectrophotometric method used for the determination of NMD is based on the use of sensitive and selective organic reagents such as phloroglucinol [1], 4-dimethylaminobenzaldehyde [8], tetrabutylammonium hydroxide (TBAH) [9], vanillin [10], *N*-(1-naphthyl)ethylenediamine dihydrochloride [11], metol-dichromate [12]. However, the disadvantages of these reagents when applied to determine NMD are low sensitivity, need for heating, and low stability of color complexes.

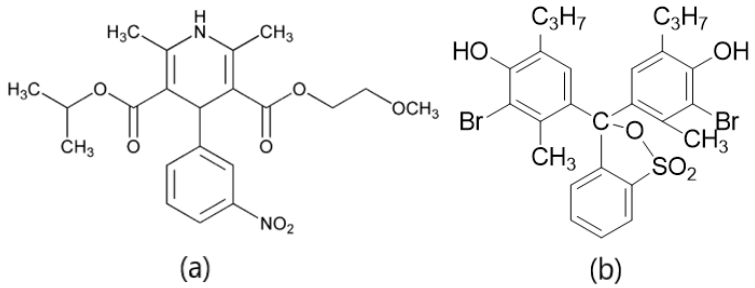

Fig. 1. Chemical structure of nimodipine (a) and bromothymol blue (b)

Extractive spectrophotometric methods are popular because of their sensitivity in drug analysis and ion-pair extraction spectrophotometry, which has received considerable interest for the quantitative determination of many pharmaceutical compounds. Some reports on the use of acidic anionic dyes such as BTB (Fig. 1b), which are complexing and ion-pair forming agents complex with cationic salts and serve as the basis for quantitative analysis of drugs [13-17]. To our knowledge, up to now, there has been no reports of using BTB reagent to determine NMD based on the ion-pair extractive spectrophotometric method. In this study, we determined NMD in Nimotop preparation based on the extractive spectrophotometric process using BTB as an anionic dye to form an ion-pair complex with the cationic form of reduced NMD under acidic conditions. The proposed method is simple, sensitive, accurate, and readily applied to the analysis of selected drugs in pharmaceutical formulations.

2. Experiments

2.1. Apparatus

All absorbance measurements were conducted on a UV-Vis spectrophotometer (Biochrom Model SP-60 double beam, Biochrom Ltd., UK) with 1.0 cm matched quartz cells. pH of the solution was controlled with a pH meter WTW 710 (Germany), calibrated daily at pH of 4, 7, and 10 using buffer solutions before measurement.

2.2. Chemical and Reagent

All chemicals used were of analytical grade and double distilled water was used for dilution of reagents and samples. Standard NMD powder form (99.78% of purity) was purchased from the National Institute of Drug Quality Control (48 Hai Ba Trung, Hanoi, Vietnam). The pharmaceutical grade Nimotop (Bayer) was purchased from local hospitals. Bromothymol blue (BTB- 99.7% of purity) was purchased from Xilong Chemical (China). Other organic solvents were purchased from Xilong Chemical.

2.3. Preparation of stock and working solutions

1 mg/mL NMD reducing stock solution was prepared by accurately weighing 25 mg of NMD and dissolving it in 10 mL of methanol. The solution was then stirred for 60 min with a magnetic stirrer and added with 4.0 g of zinc powder and 5 mL of concentrated HCl. After that, the solution was stirred for 90 min with a magnetic stirrer to perform the complete reduction of NMD. The solution was subsequently filtered and made up to 25 mL with methanol. A 100 µg/mL working solution was prepared by diluting the stock solution 10 times with methanol. A stock solution of 0.025% BTB was prepared by dissolving the appropriate weight of BTB in 5 mL of methanol and diluted to 100 mL with double distilled water.

2.4. Preparation of analytical solutions from pharmaceutical preparations

Each tablet contains 30 mg of NMD. At first, 5 tablets were weighted and averaged, ground, and mixed well. After that, a power equivalent to 0.025 g of NMD was weighted and added with 10 mL of methanol, followed by stirring for 60 min with a magnetic stirrer. After that, 4.0 g of zinc powder and 5 mL of concentrated HCl were added to the solution, which was then stirred for 90 min with a magnetic stirrer to perform the complete reduction of NMD. The solution was then filtered and made up to 25 mL with methanol. A 100 µg/mL NMD solution was prepared by diluting the stock solution 10 times with methanol.

2.5. Construction of the calibration curve

A volume of 100 µg/mL NMD (0.2-2.5 mL) corresponding to a concentration of 4.0-50 µg/mL, and 2.0 mL of 0.025% BTB, and 0.20 mL of buffer KCl-HCl (pH = 2.0) were added into a 50 mL separating funnel. The formed ion-associates were extracted with 5.0 mL chloroform after shaking for 1.5 min. The reaction mixture was allowed to separate into two phases. The absorbance of the extracts was measured at the recommended maximum wavelength of 414.5 nm against the corresponding reagent blank which was similarly prepared. All measurements were made at room temperature (25±2^{o}C).

3. Results and discussion

3.1. Principle of the method

The method is based on the reduction of the nitro group of NMD by a mixture of Zn and concentrated HCl to form a reduced form of NMD with a positive charge (1). The BTB reagent performs the lactoid ring-opening, forming the quinoid intermediate structure and the BTB anion (2). An ion-pair complex (3) is formed between the NMD cation and the BTB anion and is extracted into the chloroform and stable for at least 24 h. UV-visible spectrophotometry was used to measure the absorbance of this complex. This method is of advantage in accurately determining the NMD content under the presence of other components in the drug products. Besides, this method can be extended for other compounds containing nitrogen, which can receive protons in the acidic environment to form cations then ion complexes with anion dyes. A possible reaction mechanism is proposed in Fig. 2.

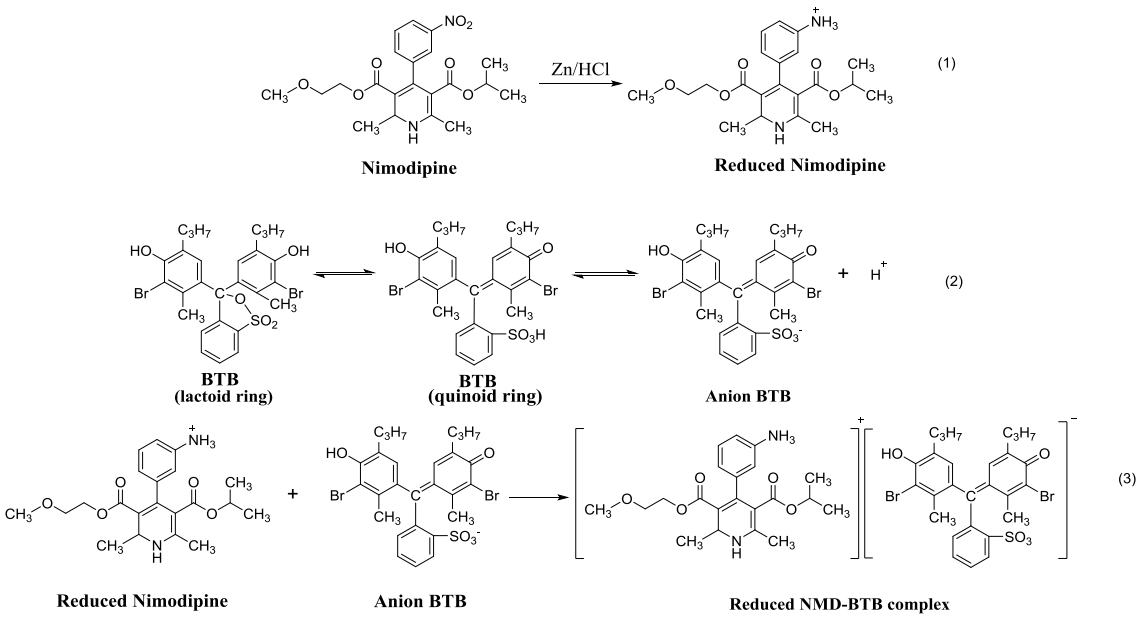

Fig. 2. The proposed reaction mechanism forms an ion-pair complex

3.2. Optimum reaction conditions for complex formation

3.2.1. Effect of extracting solvent

The effect of some organic solvents such as chloroform, dichloromethane, benzene, toluene, dichloroethane, and carbon tetrachloride was tested for efficient extraction of the color complex from the aqueous phase (Fig. 3). Chloroform is considered as one of the most suitable solvents for extraction color complex because of its maximal absorption intensity as well as its selective extraction ability in comparison

to other solvents, which is significantly lower extractability for reagent blank and simultaneously for quantitative extraction of ion-pair complexes with higher recovery efficiency. This result is consistent with our previous publications [13-14].

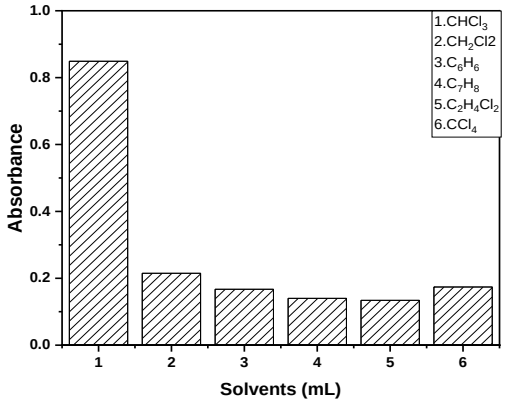

Fig. 3. Effect of extracting solvent (25 µg/mL NMD)

3.2.2. Effect of dye concentration

The effect of dye concentration was studied by adding different volumes of 0.025% BTB from 0.5 to 3.0 mL with a fixed concentration of NMD (20 µg/mL). Fig. 4 shows that the maximum absorbance of the complex was achieved with 2.0 mL of 0.025% BTB. The increase of BTB volume (e.g. > 2 mL) could compete with extraction equilibrium of the ion complex into chloroform, which decreases the absorbance of the complex. Therefore, 2.0 mL of 0.025% BTB is the optimum volume of dye and it is kept constant for the next studies.

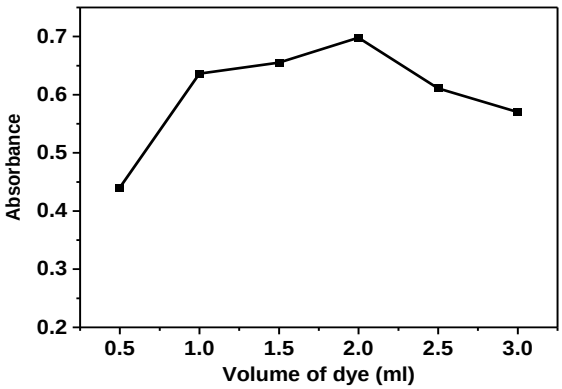

Fig. 4. Effect of dye concentration on the absorbance of 20 µg/mL NMD

3.2.3. Effect of shaking time

The effect of shaking time was investigated by conducting a series of experiments with time-varying from 0 to 5.0 min (Fig. 5). The maximum absorbance was achieved at 1.5 min. Therefore, 1.5 min was used as an optimum shaking time through the experiments.

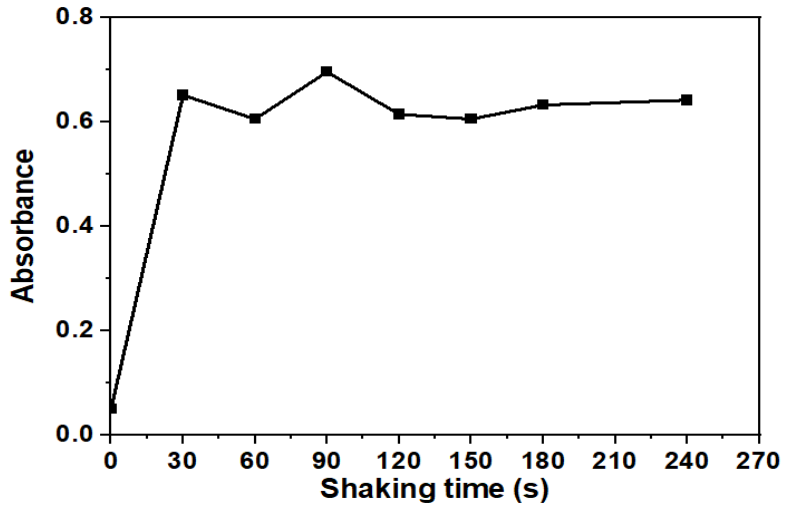

Fig. 5. Effect of shaking time on the ion-pair complex (20 µg/mL NMD)

3.2.4. The stoichiometric ratio of the ion-associate

The reaction ratio between reduced NMD and BTB determined by Job's method of continuous variation of equimolar solutions was employed a $2.4x10^{-4}$ (mol/L) standard solution of NMD and $2.4x10^{-4}$ (mol/L) anion BTB. The total volume of NMD and BTB was kept at 2.5 mL. The absorbance was measured at 414.5 nm. Fig. 6 shows that the reaction rate between NMD and BTB was found at 1:1, which was also reported by Shah, Jasmin [15], N. Rahman [16], and H. E. Abdellatef [17]. The ionic complex is formed based on electrostatic attraction between reduced NMD cations and BTB reagent anions to induce electrical neutralization and extraction into chloroform solvent. The extraction equilibrium can be represented as follows:

$$NMD^{+}_{(aq)} + D^{-}_{(aq)} \rightleftharpoons NMD^{+}D^{-}_{(aq)} \rightleftharpoons NMD^{+}D^{-}_{(org)}$$

where NMD^{+} expresses the reduced and protonated NMD and D^{-} denotes the anion of the dye. The notation of (aq) and (org) describe the aqueous and solvent phases, respectively.

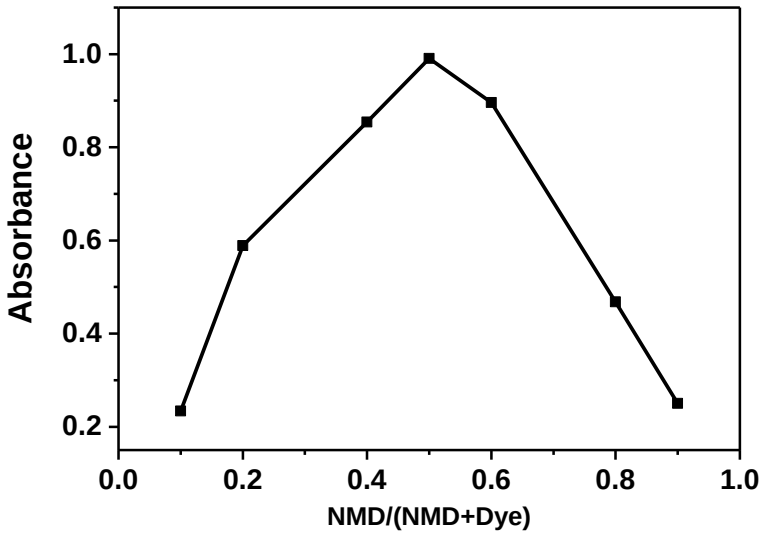

Fig. 6. Job's method of continuous variation graph for the reaction of reduced NMD with anion BTB in chloroform [NMD] = [BTB] = 2.4x10^{-4} (mol/L).

3.3. Method validation

The method was validated according to ICH guidelines [18], in terms of linearity and range, the limit of detection (LOD), the limit of quantitation (LOQ), accuracy, and precision.

3.3.1. Linearity and sensitivity

A linear relationship between the measured absorbance and the concentration range (4-50 μg/mL) is shown in Fig. 7. The standard curve equation is y = 0.036x - 0.042 and the correlation coefficient (R^2) at 0.99 was achieved, where y = absorbance, x = concentration of NMD in μg/mL. The LOD and LOQ of the method were determined by LOD = 3.3 (SD/b) and LOQ = 10 (SD/b), respectively, where SD is the standard deviation of blank absorbance values with the number of replicate experiments being 8 times, and b is the slope of the calibration curve equation. The results of determining LOD and LOQ are 1.87 μg/mL and 5.66 μg/mL, respectively. The values LDO and LOQ for NMD determination in this study are lower than those in the report of M. A. El Hamd with 2.076 and 6.923 μg/mL, respectively [10]. The molar absorptivity (ε) of ion-pair complex was calculated and found to be 1.5x10^4 L.mol^{-1}.cm^{-1}.

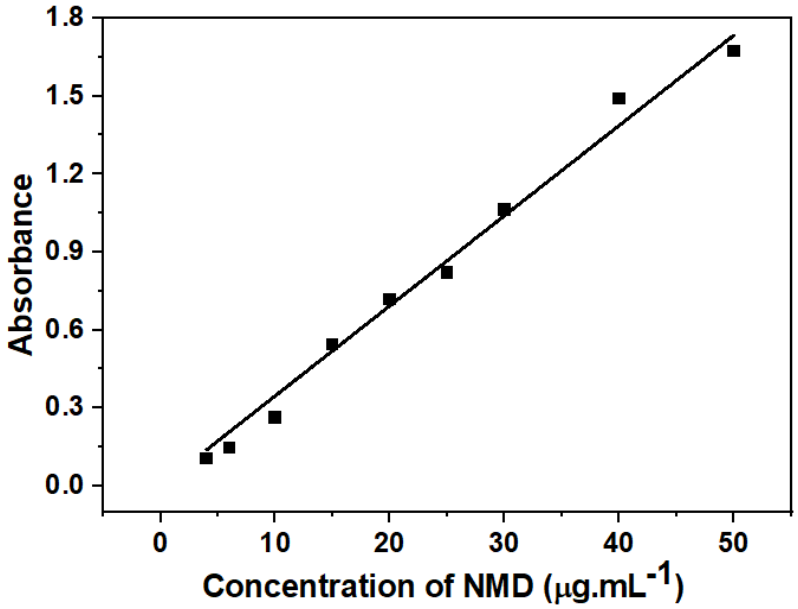

Fig. 7. Standard curve of ion-pair reduced NMD with BTB at λ_{max} 414.5 nm

3.3.2. Accuracy and precision

The accuracy and precision of the method were determined by preparing solutions of three different concentrations of NMD (10, 20, and 40 μg/mL) in inter-day and intra-day and analyzing them in six replicates. The accuracy and precision of the method were evaluated as recovery (%), percentage relative standard deviation (RSD%), and percentage relative error (RE%). The R (%), RSD (%), and RE (%) were calculated by using the equations:

$$R\ (\%) = C_f \times 100/C_a \quad (1)$$

$$RSD\ (\%) = SD \times 100/X \quad (2)$$

$$RE\ (\%) = (C_f - C_a) \times 100/C_a \quad (3)$$

where C_a is the standard concentration of NMD added, C_f is the concentration of NMD analyzed by the proposed method, SD is the standard deviation, X is the average value of the measurement. The inter-day and intra-day precision and accuracy results are shown in Tab. 1. The recovery (R%) of NMD was in the range of 99.05-103.25% with relative standard deviation (%RSD) and relative error (%RE) < 5%. These results of accuracy and precision showed that the proposed methods have good repeatability and reproducibility.

Tab. 1. The intra-day and inter-day precision and accuracy data for nimodipine obtained by the proposed methods (n = 6)

Intra-day					Inter-day			
C_a (µg/mL)	C_f (µg/mL)	R (%)	RSD (%)	RE (%)	C_f (µg/mL)	R (%)	RSD (%)	RE (%)
10.00	10.29	102.92	2.37	2.92	10.13	101.3	2.63	1.3
20.00	19.86	99.28	3.68	-0.72	19.81	99.05	2.19	-0.95
40.00	40.02	102.71	1.59	2.71	41.3	103.25	1.59	3.25

3.4. Application of the process to analyze NMD in pharmaceutical preparations

From the optimized conditions, the results of method validation were evaluated. The proposed methods have been successfully applied to the determination of NMD in commercial tablets (Nimotop). The results obtained are shown in Tab. 2. The relative standard deviation values are below 2% indicating the precision of the method. The validations of the proposed methods were further confirmed by recovery studies. The recovery (R%) is 100.23 indicating the high accuracy of the method.

Tab. 2. The results of analysis from pharmaceutical preparation

Grade name	Content on the package (mg/tablet)	Reagents	Amount found (mg)	R (%)	SD	RSD (%)
Nimotop (Bayer)	30	BTB	30.62	100.23	0.042	0.137

3.5. Comparison with other methods

The proposed method compares with other reported methods. The results are presented in Tab. 3. The results show that the extractive spectrophotometric method with BTB reagent for NMD determination has several advantages of wide linear range, high sensitivity, no heating requirement, stable ion complex, and inexpensive reagents.

Tab. 3. Comparison with other spectrophotometric methods

	Reagents	λ_{max} (nm)	Linear range (µg/mL)	Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	Remarks	References
1	BTB	414.5	4.0-50.0	$1.5x10^4$	High sensitive and has wider linear range High colour stability	Proposed method
2	Phloroglucinol	410	0.0-25.0	$1.23x10^4$	Narrow linear range Less stable colour	[1]
3	p-Dimethylaminocinnamaldehyde	510	0.5-4.0	-	Requires heating condition	[8]
4	Tetrabutylammonium hydroxide (TBAH)	458	5.0-40.0	$0.939x10^4$	Narrow linear range	[9]
5	Vanillin	500	10.0-60.0	$0.572x10^4$	Less sensitive	[10]
6	*N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDA)	550	0.0-40.0	$0.37x10^4$	Less sensitive	[11]
7	Metol-dichromate	520	0.0-70.0	$3.15x10^3$	Less sensitive	[12]

4. Conclusion

In the proposed method, the ion-pair complex of reduced nimodipine with bromothymol blue was studied under the optimized extraction conditions. The proposed method was developed and validated for the determination of nimodipine in pharmaceutical preparation (Nimotop). The results showed a linear range following Beer's law of 4.0-50.0 µg/mL, LOD and LOQ of 1.87 µg/mL and 5.66 µg/mL, respectively, and molar absorptivity of $1.5x10^4$ $L.mol^{-1}.cm^{-1}$. The proposed method is simple, highly sensitive, accurate, and has no heating requirement. Furthermore, this method does not require elaborated procedures, making it applicable for the analysis of nimodipine (pure form and pharmaceutical formulations) in routine quality control pharmaceutical laboratories.

References

[1] H. N. Deepakumari and H. D. Revanasiddappa, "A Sensitive Spectrophotometric Estimation of Nimodipine in Tablets and Injection Using Phloroglucinol," *ISRN Spectrosc.*, Vol. 2013, pp. 1-7, 2013.

[2] Manoela K. Riekes, Gabriela S. Rauber, Gislaine Kuminek, Monika P. Tagliari, Simone G. Cardoso, Hellen K. Stulzer, "Determination of Nimodipine in the Presence of its Degradation Products and Overall Kinetics through a Stability-Indicating LC Method," *Journal of Chromatographic Science*, Vol. 51, No. 6, pp. 511-516, 2013.

[3] Y. Dan-Bo, Z. Jia-Bi, L. Rui-Qin, H. Zhi-Qiang, and S. Jin-Qiu, "Liquid chromatographic method for determination of free and niosome-entrapped nimodipine in mouse plasma and different tissues," *Anal. Lett.*, Vol. 41, No. 4, pp. 533-542, 2008.

[4] Isse, Fadumo Ahmed, Tyson Le, and Sherif Hanafy Mahmoud, "Enantioselective assay of nimodipine in human plasma using liquid chromatography-tandem mass spectrometry," *Biomedical Chromatography*, Vol. 35, No. 2, e4971, 2021.

[5] J. A. Squella, J. C. Sturm, R. Lenac, and L. J. Nuñez-Vergara, "Polarographic and Spectrophotometric Determination of Nimodipine in Tablets," *Anal. Lett.*, Vol. 25, No. 2, pp. 281-92, 1992.

[6] H. M. Abdel-Wadood, N. A. Mohamed, and A. M. Mahmoud, "Validated spectrofluorometric methods for determination of amlodipine besylate in tablets," *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, Vol. 70, No. 3, pp. 564-570, 2008.

[7] Revanasiddappa, Hosakere, "Spectrophotometric methods for the determination of nimodipine in pure and in pharmaceutical preparations," *Jordan Journal of Chemistry*, Vol. 6, No. 4, pp. 413-422, 2011.

[8] Nirmala Bharathi, S., M. S. Prakash, M. Nagarajan, and K. Asok Kumar, "Spectrophotometric determination of nimodipine and its formulations," *Indian drugs*, 36, No. 10, pp. 661-662, 1999.

[9] M. A. El Hamd, S. M. Derayeab, O. H. Abdelmageedc, and H. F. Askald, "Colorimetric Method for Determination of some 1,4-Dihydropyridine Drugs in their Tablets and Capsules," *J. Adv. Chem.*, Vol. 4, No. 1, pp. 278-287, 2008.

[10] M. A. El Hamd, S. M. Derayea, O. H. Abdelmageed, and H. F. Askal, "A Novel Spectrophotometric Method for Determination of Five 1,4-Dihydropyridine Drugs in Their Tablets and Capsules Using Vanillin Reagent," *Am. J. Anal. Chem.*, Vol. 04, No. 03, pp. 148-157, 2013.

[11] Chowdary, K. P. R., and G. Devala Rao, "A new spectrophotometric method for the determination of nimodipine," *Indian drugs*, Vol. 32, No. 11, pp. 548-550, 1995.

[12] H. D. Revanasiddappa, S. M. Mallegowda, H. N. Deepakumari, and K. B. Vinay, "Spectrophotometric determination of nitrazepam and nimodipine in pure and the tablet dosage forms," *Asian Journal of Biochemical and Pharmaceutical Research*, Vol. 1, pp. 70-77, 2011.

[13] Nguyen Trung Dung, Le Hoc Bau, Le Quang Thao, and Nguyen Quang Dat, "Extractive spectrophotometric methods for determination of ciprofloxacin in pharmaceutical formulations using sulfonephthalein acid dyes," *Vietnam Journal of Chemistry*, Vol. 55(6), pp. 767-764, 2017.

[14] Nguyen, Trung Dung, et al., "Determination of fluoroquinolones in pharmaceutical formulations by extractive spectrophotometric methods using ion-pair complex formation with bromothymol blue," *Journal of Analytical Methods in Chemistry* 2018, 2018.

[15] Shah, Jasmin, M. Rasul Jan, and Suraya Manzoor, "Extractive spectrophotometric methods for determination of clarithromycin in pharmaceutical formulations using bromothymol blue and cresol red," *Journal of the Chinese Chemical Society*, 55.5 (2008), pp. 1107-1112.

[16] N. Rahman and S. N. Hejaz-Azmi, "Extractive spectrophotometric methods for determination of diltiazem HCl in pharmaceutical formulations using bromothymol blue, bromophenol blue and bromocresol green," *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 24, No. 1, pp. 33-41, 2000.

[17] H. E. Abdellatef, "Extractive-spectrophotometric determination of disopyramide and irbesartan in their pharmaceutical formulation," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol. 66, No. 4, pp. 1248-1254, 2007.

[18] ICH Topic Q2 (R1). Validation of analytical procedures: Text and methodology (CPMP/ICH/281/95); accessed June 30, 2010.

CHIẾT TRẮC QUANG XÁC ĐỊNH NIMODIPIN BẰNG SỰ HÌNH THÀNH PHỨC CẶP ION VỚI BROMTHYMOL XANH

Nguyễn Trung Dũng, Lê Thị Hoa, Nguyễn Thu Hà, Hoàng Thị Tuệ Minh

Tóm tắt: *Phương pháp quang phổ mới đã được thiết lập để xác định nimodipin trong các chế phẩm dược. Phương pháp này dựa trên sự hình thành phức cặp ion màu vàng giữa dạng khử của nimodipin và thuốc thử bromothymol xanh (BTB) trong môi trường axit. Chloroform được sử dụng như một dung môi tối ưu để chiết phức cặp ion và độ hấp thụ quang được đo ở bước sóng 414,5 nm. Ảnh hưởng của một số thông số như dung môi chiết, thể tích thuốc nhuộm, thời gian lắc chiết, tỉ lệ phản ứng đã được nghiên cứu. Khoảng nồng độ nimodipin tuân theo định luật Bia là 4,0-50,0 µg/mL, với độ hấp thụ mol của phức là* $1{,}50\times10^{4}$ $L.mol^{-1}.cm^{-1}$ *và giới hạn phát hiện (LOD) là 1,87 µg/mL. Độ chính xác và độ chụm của phương pháp được đánh giá trên cơ sở nghiên cứu trong và giữa các ngày, phần trăm sai số tương đối (%RE) và độ lệch chuẩn tương đối (%RSD) bé hơn 5,0%, độ thu hồi (%) là 99,05-103,25. Phương pháp đề xuất đã được áp dụng thành công để xác định nimodipin trong chế phẩm dược Nomitop (Bayer, Đức).*

Từ khóa: Nimodipin; chiết trắc quang; phức liên hợp ion; bromothymol xanh.

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