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Spectrophotometric determination of some 1,4-dihydropyridine drugs in their pharmaceutical preparations and spiked human plasma

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ABSTRACT

A selective and validated spectrophotometric method was developed for the determination of some 1,4-dihydropyridines (1,4-DHPs) nifedipine, nicardipine, and nimodipine in pure form and pharmaceutical preparations. The proposed method is based on the condensation reaction of 1,4-DHP with *p*-anisaldehyde in acidic medium and measuring the absorbance at 460 nm. The absorbance-concentration plot was rectilinear over the concentration range of 5-60 µg/mL with a minimum detection limit of 0.72-2.08 µg/mL. The factors affecting the absorbance of the formed products were carefully studied and optimized. The proposed method was applied for the determination of these 1,4-DHPs in their pharmaceutical preparations and spiked human plasma. The results obtained were compared with those obtained using the reported methods. Also proposal of the reaction pathway was postulated.

Keywords: Spectrophotometric method; nifedipine; nimodipine; nicardipine; *p*-Anisaldehyde.

INTRODUCTION

1,4-dihydropyridines (1,4-DHPs) drugs are used for treatment of some cardiovascular diseases such as hypertension, angina and some forms of cardiac arrhythmias. The therapeutic importance and successful clinical uses of these drugs have promoted the development of many analytical methods for their determination in bulk, in their pharmaceutical formulations and in biological fluids. Analytical techniques such as; titrimetric methods [1, 2], spectrometric methods (spectrophotometry [3-32] or spectrofluorimetry [33-37], electrochemical methods [38-45], liquid chromatographic methods [46-54] and gas chromatographic methods [55-62] were reported for their determination. The inherent simplicity of spectrophotometric methods, economic advantages and availability of their instruments in most quality control laboratories permit development of a simple and selective method for determine these drugs.

MATERIALS AND METHODS

Instruments

Absorbance measurements were made on Shimadzu model 1601PC, UV-Visible Spectrophotometer (Shimadzu, Tokyo, Japan) with two matched 1 cm quartz cells.

Chemicals

All chemicals and reagents used were of analytical reagent grade and some of them were used as such without any further purification. An aldehydic reagent *p*-anisaldehyde (0.5%, w/v) (El Gomhouria Co, Cairo, Egypt) was prepared hydrochloric acid (HCl) (35.5%, w/v). The reference standards of pure drugs (NIF, atenolol, NIC, and NIM) were

generously supplied by their respective manufacturers in Egypt and their standard solutions (0.5 mg/mL) were prepared by dissolving 50 mg of NIF, NIC and NIM in 3.0 mL methanol then the resulting solution were completed to 100 mL by HCl. The working standard solutions were prepared by further dilution with diluted HCl (17.75%, v/v) to obtain the concentration ranges more than 60 µg/mL.

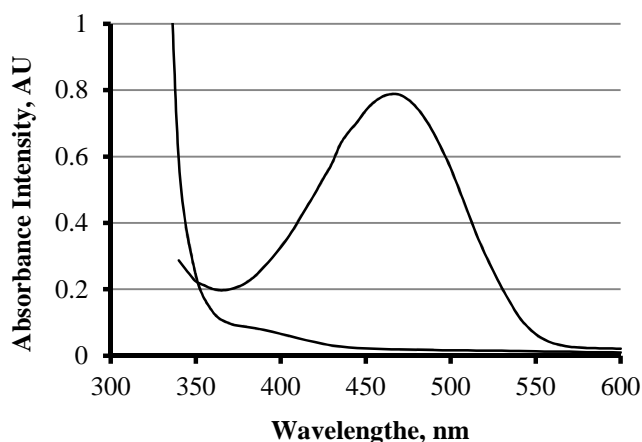


Figure (1) The chemical structures of the studied 1,4-DHP drugs

Preparation of pharmaceutical dosage form samples

Twenty tablets or capsules were weighed and finely powdered and mixed thoroughly and a quantity of the powder equivalent to 25 mg of the active ingredients of the drugs was dissolved in 3 mL methanol. The contents were swirled and sonicated for 5 min then filtered through a Whitman No.42 filter paper previously moistened with methanol. The collected filtrate was transferred quantitatively into a 50-mL calibrated flask, the resultant solution was completed to mark with HCl (35.5% v/v) to obtain a stock solution of 0.5 mg/mL and then subjected to subsequent dilution.

Procedure for calibration curves

An aliquot of 1.0 mL of the sample or standard solution was transferred into a 10-mL calibrated flask. 1.0 mL of *p*-anisaldehyde reagent was added, and putted for 20 min into a water bath previously adjusted to 80 °C. After the reaction has been completed, the absorbance was measured at 460 nm after dilution with aqueous HCl (17.75%, v/v) against blanks which treated similarly.

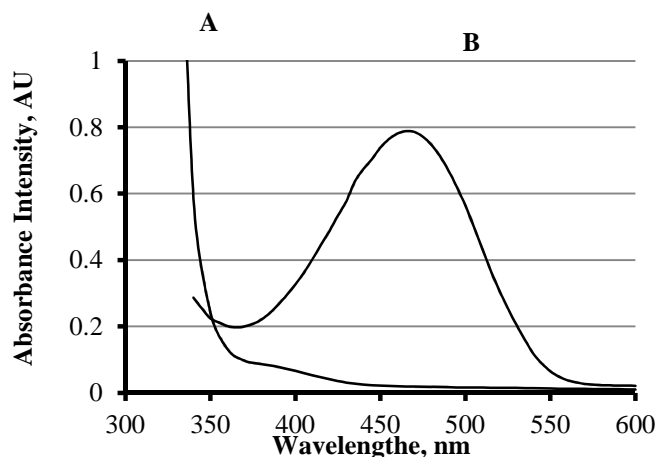


Figure 1: Absorbance spectra of *p*-anisaldehyde alone (A), and absorbance spectra of the reaction product between it and NIF (30 µg/mL), as a representative example (B)

2.5. Procedure for the spiked plasma

Standard working solutions (1 mL, each of NIF, NIC, and NIM) were added into 1 mL blank human plasma sample, vortex-mixed for 10 sec and followed with 2 mL of acetonitrile for deproteinization. The mixtures were centrifuged at 4000 rpm for 10 min at 10 °C. The organic phase was then transferred to a clean tube and evaporated to dryness.

The residue was dissolved in 100 μL methanol, completed to 1 mL by diluted HCl(17.75%, v/v), then treated by the proposed method and the resulted chromogen was measured at the specific wavelength.

RESULTS AND DISCUSSION

The proposed method involved treatment of the investigated drugs directly with *p-anisaldehyde* reagent in the presence of HCl. The colored products which measured at 460 nm for all the studied drugs were formed after elevating the reaction temperature for 20 min at 80°C, **Figure 1**.

Optimization of the Parameters

A series of experiments were conducted to establish the optimum experimental conditions for the proposed method.

Reagent Concentration

The results were revealed that the product's colour development was reagent concentration dependence and the absorbance values were increased as the concentration of *p-anisaldehyde* was increased. 5 mg/mL, w/v in diluted HCl of *p-anisaldehyde* was selected for further experiment studies, **Figure 3**.

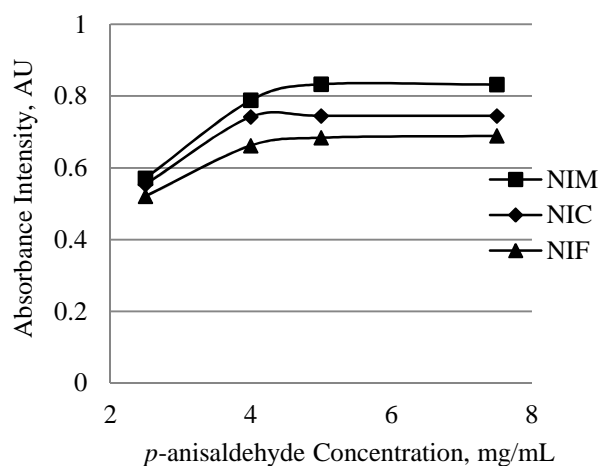


Figure 3: Effect of *p-anisaldehyde* concentration in the absorbance spectra of NIF, NIC, and NIM

Acid type and its concentration

The reaction between 1,4-DHP drugs and *p-anisaldehyde* reagent was found to proceed only in acidic medium. Different acids were tested. HCl was selected for the further studies as it resulted in increase of the absorbance intensities accompanied by hyperchromic shift other than tested acids.

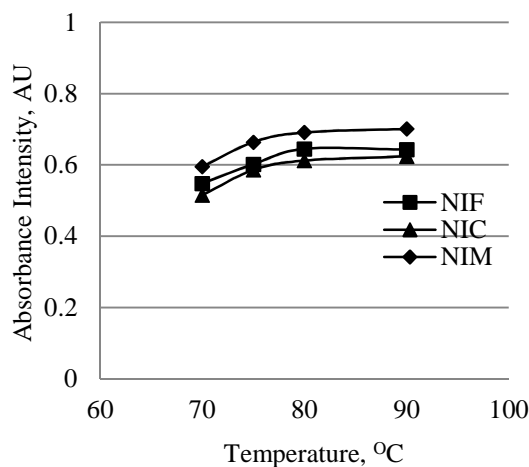


Figure 4: Effect of reaction temperature in the absorbance spectra of NIF, NIC, and NIM

Temperature and reaction time

The effect of temperature (25°C - 100°C) was studied for different time periods (5 - 35 min). Unfortunately, the reaction did not proceed up to 60°C. The reaction products' absorbance was increased by increasing the temperature up to 80°C for 20 min, **Figures 4**.

Reaction Stoichiometry

Job's method of continuous variation [63] was employed to establish the stoichiometry of the reaction; Master equimolar solutions 5×10^{-4} M of NIF, NIC, NIM, and *p*-anisaldehyde were prepared. Series of 10-mL portions of the master solutions were made up comprising different complimentary proportions (0.00:0.10, 0.10: 0.90, ... , 0.90:0.10, 0.10:0.00) in 10-mL volumetric flasks, mixed well then subjected to the recommended procedure. The stoichiometry of the reaction between the investigated drugs and *p*-anisaldehyde revealed a 1:1 ratio for all drugs, **Figure 5**.

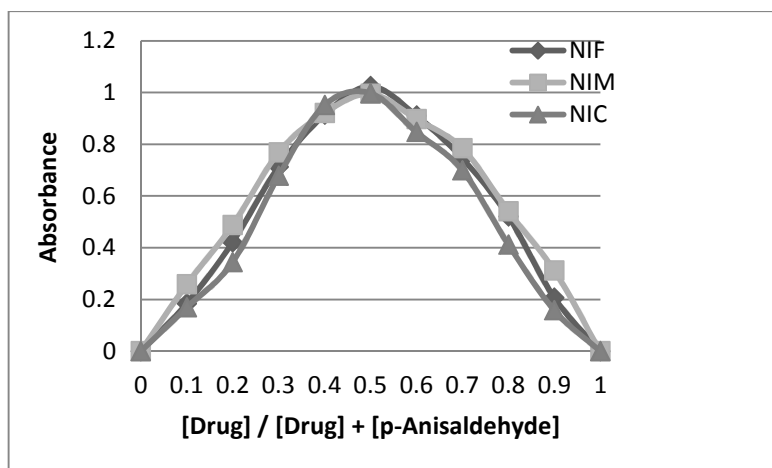
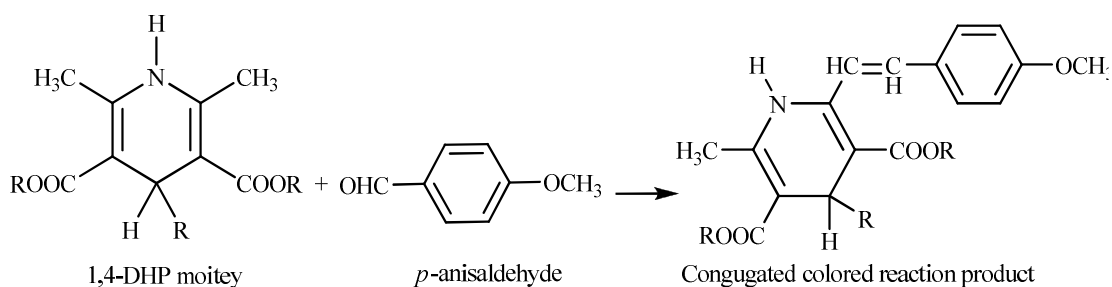


Figure 5: Molar ratios for the reaction of NIF, NIC, NIM, and *p*-anisaldehyde (5×10^{-4} M)

The reaction mechanism was thought as a reaction between active methyl group in the cited drugs and the aldehydic group of *p*-anisaldehyde [64, 65], through the nucleophilic addition of double bond of carbonium ion in the 1,4-DHP to the reagent which undergoes deprotonation and finally, followed by loss of a molecule of water under acidic conditions, to yield the conjugated coloured product, as shown in **Scheme 1**.



Scheme 1: Suggested reaction mechanism between 1,4-DHP and *p*-anisaldehyde under the optimum conditions

Validation of Proposed Methods**Linearity Range, detection and quantification limits**

Under the optimum reaction conditions, the calibration curves for the investigated drugs; NIF, NIC, and NIM with *p*-anisaldehyde were constructed by analyzing a different drug concentrations. The good linearity between different drugs concentration and the absorptions was indicated by the high correlation (correlation coefficient, $r=0.999$). The regression equations for the results were derived using the least square method: $Y = a + bx$. The limits of detection (LOD) and limits of quantitation (LOQ) were determined according to the IUPAC definitions [66] using the formula, $kSDa/b$; where $k = 3.3$ for LOD and 10 for LOQ, SDa is the standard deviation of the blank, and b is the slope of the calibration curves, **Table 1** summarizes the obtained results.

Table 1: Quantitative parameters and statistical data for NIF, NIC, and NIM analyzed by *p*-anisaldehyde

Drug	Concentration range, $\mu\text{g/mL}$	Intercept $\pm\text{SD}^1$	Slope $\pm\text{SD}$	r^2	$\epsilon \times 10^4$ /mol \times cm	LOD, $\mu\text{g/mL}$	LOQ, $\mu\text{g/mL}$
NIF	5-30	0.03 \pm 0.01	0.03 \pm 0.01	0.999	1.07	0.72	2.41
NIC	5-35	0.04 \pm 0.01	0.02 \pm 0.01	0.999	1.04	0.75	2.50
NIM	10-60	0.02 \pm 0.01	0.01 \pm 0.03	0.998	0.57	2.08	6.29

¹SD= standard deviation, n= 5; ²r= Correlation coefficient.**Accuracy and precision**

Intra- and inter-day assay accuracy and precision were assessed; five replicate measurements at one concentration level [67]. Relative standard deviations (RSD) did not exceed 10%, indicating the good repeatability of the proposed method.

Table 2: Assay of five replicate samples of NIF, NIC, and NIM by *p*-anisaldehyde

Drug	Concentration, $\mu\text{g/mL}$	Accuracy ¹ %, n=5	Precision as RSD%, n=5	
			Intra-day	Inter-day
NIF	20	91.11 \pm 0.01	1.30	4.3
NIC	25	96.14 \pm 0.03	1.17	4.1
NIM	40	100.30 \pm 0.02	0.56	3.0

¹Accuracy % = (Found concentration/Nominal concentration) \times 100**Robustness and ruggedness**

Method robustness was examined by evaluating the influence of small variation in some experimental parameters such as the concentration of analytical reagent and reaction times on the method's suitability and sensitivity. In these experiments, one parameter was changed where the others were kept unchanged and the recovery percentages were calculated at each time. It was found that none of these variables significantly affect the proposed methods, **Table 3**. Method ruggedness was tested by applying the proposed methods to the assay of the investigated drugs using the same operational conditions but using different elapsed time. The results obtained from day-to-day variations were found to be reproducible as RSD did not exceed 2% , **Table 4**.

Table 3: Robustness of the proposed method for analysis of NIF, NIC, and NIM

Variation	% Recovery $\pm\text{SD}^a$		
	NIF, 20 $\mu\text{g/mL}$	NIC, 20 $\mu\text{g/mL}$	NIM, 40 $\mu\text{g/mL}$
No variation	99.17 \pm 0.64	99.10 \pm 0.61	99.23 \pm 1.45
<i>p</i> -anisaldehyde concentration			
0.45 %	98.98 \pm 0.71	99.07 \pm 0.89	97.67 \pm 0.89
0.55 %	98.94 \pm 0.52	99.01 \pm 0.72	98.71 \pm 0.62
Reaction time			
18 min	98.93 \pm 0.81	99.10 \pm 0.40	98.46 \pm 0.89
22 min	99.01 \pm 0.59	98.86 \pm 0.88	97.61 \pm 0.62
Temperature			
85 °C	98.78 \pm 0.97	98.60 \pm 0.92	99.03 \pm 0.65
95 °C	98.70 \pm 0.71	98.80 \pm 0.70	99.05 \pm 0.83

^an=5.**Table 4: Ruggedness of the proposed for analysis NIF, NIC, and NIM**

Drug	Recovery% $\pm\text{SD}^a$		
	Day-to-day variation		
	Day-1	Day-2	Day-3
NIF	98.74 \pm 0.95	98.55 \pm 1.05	100.79 \pm 0.94
NIC	99.30 \pm 0.91	98.79 \pm 0.82	98.75 \pm 0.82
NIM	98.28 \pm 1.31	98.47 \pm 0.39	98.15 \pm 1.42

^an=5.**Application of the proposed method**

Good satisfactory results which obtained by the proposed method for the investigated drugs in bulk forms also were extended to the cited drugs analysis in their tablets and capsules forms (**Table 5**). The results were compared with those obtained by the official [14] and other reported method [68] with respect to the accuracy (*t*-test) and precision (*F*-value). No significant differences were found between the calculated and theoretical values of both the proposed

and the official or reported methods at 95% confidence level which indicated similar accuracy by comparing *t*-test and *F*-value to the reported one.

Table 5: Analysis of 1,4-DHP containing dosage forms by the proposed and official or reported methods

Dosage forms	Recovery%±SD (n=5)		<i>t</i> -test ^b	<i>F</i> -value ^b
	Proposed method	Reported method ^a		
Epilate [®] capsules	98.5±0.2	99.5±0.1	1.5	2.9
Epilate Retard [®] tablets	99.4±0.2	98.5±0.1	1.2	2.1
Tenolat SR [®] capsules ^c	99.5±0.1	100.5±0.7	1.6	1.2
Pelcard SR [®] capsules	98.4±0.1	99.7±0.1	1.0	1.6
Nimotop [®] tablets	99.3±0.1	98.6±0.2	1.1	1.5

^aReported methods, [14] and [68]; ^bTheoretical values for *t*- and *F*-values at 95% confidence limit (*n* = 5) were 2.78 and 6.39, respectively; ^cCapsules contain binary drugs.

Analysis of spiked human plasma

The high sensitivity of the proposed method however, allows the determination of nifedipine, nifedipine and nimodipine in spiked human plasma. A dose of 20 mg nifedipine, 50 mg nifedipine and 30 mg daily nimodipine are orally administered. The anticipated concentration in biological fluids will be about 5, 15 and 30 µg ml⁻¹ for nifedipine, nifedipine and nimodipine, respectively, which lies within the working concentration range of the proposed method. The application of the method for plasma was performed adopting the extraction procedure described. The method was successfully applied without prior extraction. The results obtained in Table 6 are satisfactorily accurate and precise.

Table 6: Analysis of spiked human plasma

DRUG	Spiked human plasma		
	Amount taken (µg ml ⁻¹)	Amount found (µg ml ⁻¹)	% Recovery*
NIF	5.00	4.84	96.8 ± 1.16
NIC	15.00	14.73	98.2 ± 0.84
NIM	30.00	29.33	97.8 ± 1.24

*Values are the mean of three determinations ± SD.

CONCLUSION

Compared with other reported methods, the proposed method has the advantages of simplicity, selectivity, reproducibility and it satisfies the need for a rapid procedure for the determination of all members of 1,4-DHP drugs which containing active methyl group using different reagents contain aldehydic groups.

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