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A Kinetic Spectrophotometric Method for Determination of Amlodipine and Nifedipine in Pharmaceutical Preparations

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A simple, accurate, sensitive and economical procedure for the estimation of amlodipine besylate and nifedipine, both in pure and dosage forms, has been developed. The method is based on the reduction of iron(III) by the studied drugs and subsequent interaction of iron(II) with ferricyanide to form Prussian blue. The reaction develops through a slow kinetics and completes in about 10 min. Both initial slope and fixed time methods were used to derive calibration graphs. The resulted calibration equations were linear in the concentration ranges of 1.0-20.0 and 3.0-19.0 μg ml^{-1} for AML and NIF, and the detection limits were 0.10 and 0.19 μg ml^{-1}, respectively. Seven replicate analyses of solutions containing three different levels of each drug resulted in very low relative error of prediction (less than 1.6%) and relative standard deviation (less than 4%) confirming accuracy and precision of the proposed method. The proposed method was applied to the determination of these drugs in pharmaceutical formulations and excellent recoveries were obtained.

Keywords: Nifedipine, Amlodipine, Kinetic, Spectrophotometry, Iron (III), Ferricyanide

INTRODUCTION

Amlodipine, (2-[(2-amino-ethoxy)-methyl]-4-2-chloro-phenyl-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid-3-ethyl-5-methyl ester, AML) and nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid, NIF) are belonging to the dihydropyridine (DHP) family, which are calcium channel blockers with cardiovascular activity. They are mainly used as antianginal, anthihyper-tensive and antiarrythmic agents [1-3]. AML and NIF, similar to the other DHP derivatives, are photosensitive and are converted to inactive and even toxic products when they are irradiated by ultraviolet or visible lights [4-9]. Therefore, determination of these drugs in pharmaceutical formulations is very important.

Detailed survey of literature for AML revealed several chromatographic methods for its determination in pharmaceuticals and biological fluids [10-15]. Assay of the drugs have also been achieved by different spectroscopic methods [16-23], but the methods lack the desired sensitivity or involve tedious extraction step or use of organic solvents. Similarly, survey of literature for NIF revealed methods based on GC [24-26], different liquid chromatographic methods [27-32], and stripping voltammetry [33,34]. There are also some equilibrium visible spectrophotometric methods for NIF that are based on oxidation or reduction of the drug, where reactions products are colored [35-38]. Surprisingly, according to the best of our knowledge, no kinetic spectrophotometric method has been reported for the quantitation of the DHP derivatives.

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Kinetic spectrophotometric methods are simple and sensitive analytical methods which have the advantages of high selectivity, possibility of no interference from the colored and/or turbidity background of the samples, possibility of no interference from the other active compounds present in the commercial product, if they are resisting the chemical reaction conditions established for the proposed kinetic method. In addition, kinetic methods may come with higher sensitivity in comparison with equilibrium methods [39].

This article describes the development of a simple kinetic spectrophotometric method for the assay of amlodipine and nifedipine, in pure and dosage forms, based on the reduction of iron(III) by the studied drugs in acidic medium and subsequent interaction of iron(II) with ferrocyanide to form a Prussian blue color [23]. The proposed kinetic method was fast and resulted in dynamic ranges and detection limits better or comparable with the existing spectrophotometric methods based on oxidation of drugs [22-23,37-38]. The method was applied to the analysis of pharmaceutical preparations containing AML or NIF, successfully.

EXPERIMENTAL

Chemicals

All reagents used were of analytical reagent grade and used as received. Doubly distilled water was used to prepare all solutions unless specified otherwise. Pure AML and NIF were provided by Fluka and were used as received. Stock standard solutions of the drugs (200.0 mg ml^{-1}) were prepared by dissolving appropriate amounts of the drugs in a little portion of ethanol (95%) and diluting to 100.0 ml with water in a volumetric flask. The stock solutions were diluted with water to get working standards of 50.0 μg ml^{-1} solution of the drugs.

A 0.2% (w/v) solution each of hexahydrate $FeCl_3$ (Merck KGaA, 64271 Darmstadt, Germany) and $K_3Fe(CN)_6$ (SIGMA-ALDRICH, CHEMIE GmbH, Germany) was prepared in aqueous solution of pH 3.

Instrumentation

A UVIKON XL model 106 digital spectrophotometer (Bio-Tek Instrument, Italy) with 10.0 mm matched glass cells was used for absorbance measurements. The pH measurements were performed with a Metrohm 780 pH-meter employing a combined glass electrode.

General Procedure

Into a series of 10.0 ml volumetric flasks, different aliquots (0.2-4.0 ml) of standard AML solution (50.0 μg ml^{-1}) or (1.4-4.0 ml) of standard NIF solution (50.0 μg ml^{-1}) were transferred using a micro burette. Then, 1.5 ml each of $FeCl_3$ (0.2%) and ferricyanide (0.2%) were added to the flasks and diluted to mark with water and mixed well. The absorbance of the resulting solution was continuously measured in the time duration of 10 min at 690 nm and 740 nm against reagent blank for AML and NIF, respectively. Using the resulted kinetic curves, both initial slope and fixed time methods were used to construct the calibration graphs. In the case of initial slope method, the differences between the absorbance readings at different time windows were used as analytical signal. While, in the fixed time method, the absorbance measured at a fixed time after reaction proceeded was used to drive the calibration curve. The time of absorbance measurement was optimized to obtain the best sensitivity and the widest dynamic range.

Analysis of Pharmaceutical Preparations

For analysis of pharmaxeutcal products the procedure conducted by Basavaiah *et al*. [23] was employed. The tablets commercial formulations containing AML or NIF were prepared, respectively from Arya Pharmaceutical Company and Zahravi Company both from Iran. Twenty tablets were weighed accurately and ground into a fine powder. An amount of the powder equivalent to 20.0 mg of AML or NIF was weighed into a 100.0 ml volumetric flask and then dissolved in little portions of 95% ethanol by shaking for 20 min. Then, the volume was made up to the mark with water, mixed well and filtered using quantitative filter paper. The first 10.0 ml portion of the filtrate was rejected. The filtrate (200.0 mg ml^{-1}) was diluted with water to get about 50.0 mg ml^{-1} of the drugs, and the recommended procedure was followed.

RESULTS AND DISCUSSION

AML and NIF reduce iron(III) to iron(II), the latter reacting with ferricyanide to form an intense Prussian blue color [23]. Figure 1 shows the absorption spectra of the

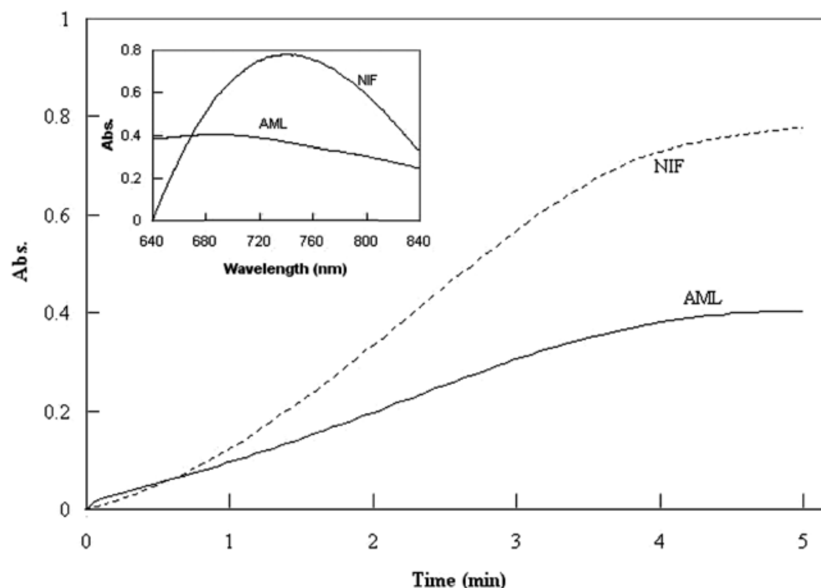

Fig. 1. (A) Absorption spectra (inset) and absorbance-time kinetic curves of 10 μg ml^{-1} and amlodipine and nifedipine in the presence of 0.05% (w/v) $FeCl_3$ and 0.03% (w/v) ferricyanide.

reaction products of AML and NIF with iron(III) and ferricyanide after 10.0 min of the reaction proceeded against the reagent blank. As is observed, the greenish-blue products from AML and NIF exhibit absorption maxima at 690 and 740 nm, respectively. Neither iron(III) nor ferricyanide solution absorbs at these wavelengths. It is interesting to note that the resulted absorbance spectra are not absorbed light at the same wavelength and this indicates that the reaction is not a simple Ox/Red reaction. This can be attributed to the interaction of Fe(II)-ferricyanide with the reduced form of the drugs (*i.e.*, pyridinium moiety). The resulting kinetic curves (*i.e.*, changes in absorbance spectra of the reactions products as a function of time; Fig. 1) indicate that the reactions are developed gradually with a slow kinetics. This behavior allows us to develop a kinetic-based analytical method for assay of the drugs in pharmaceutical preparations. To obtain more sensitive results, the effects of some experimental parameters were optimized.

Optimum Experimental Conditions

Firstly, the effect of changing in the added volumes of 0.2% $FeCl_3$ to 10.0 ml solutions of 10 μg ml^{-1} of drugs, containing a constant amount of ferrocyanide (1.5 ml of 0.2 %), on the rate of color development was studied (Fig. 2). As seen, in the case of both drugs the absorbance (measured 5.0 min after addition of reagents) increased with increasing concentration of iron(III) solution and is reached a plateau at 0.05% (w/v) of iron(III). Similar observations (Fig. 3) were obtained when varying volumes of 0.2% ferricyanide solution were added to 10.0 ml solutions containing fixed amounts of drug (10.0 μg ml^{-1}) and Fe^{3+} (2.5 ml of iron 0.2%). According to the plots shown in Fig. 3, the optimum value for ferrocyanide is 0.04% (w/v) of its aqueous solution. These optimum concentrations are more than 10-fold of the most concentrated solutions of the drugs. This ensures that, even for the most concentrated solutions of the analytes, there are enough amounts of $Fe^{3+}$and ferricyanide available for complete reactions.

Since Fe(III) is hydrolyzed in aqueous solution of pH > 3, the Fe(III) solution was prepared at an optimal pH of 3. In the course of the study of pH effect, the pH of corresponding solutions were adjusted with concentrated HCl or NaOH solutions. It was observed that both alkaline and acidic media lowered the reaction's color development.

After fixing all experimental parameters, some more experiments were performed to ascertain the influence of the order of addition of reactants. It was found that the order iron(III), ferricyanide and drug for AML and drug, iron(III)

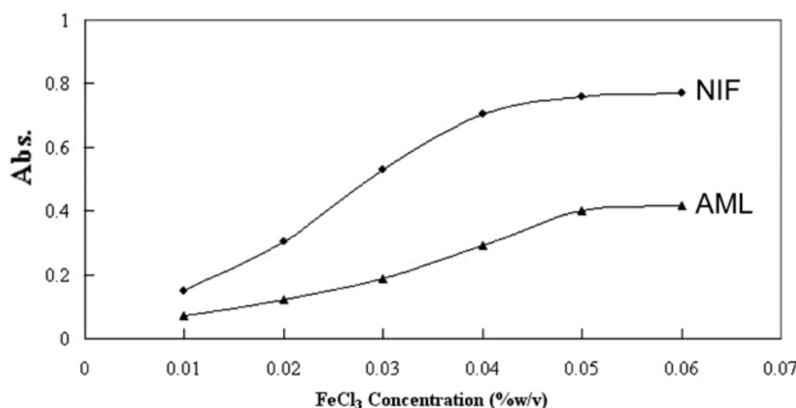

Fig. 2. Effect of concentration of $FeCl_3$ on the absorbance of the reaction products of 10 µg ml^{-1} amlodipine and nifedipine in the presence of 0.03% (w/v) ferricyanide.

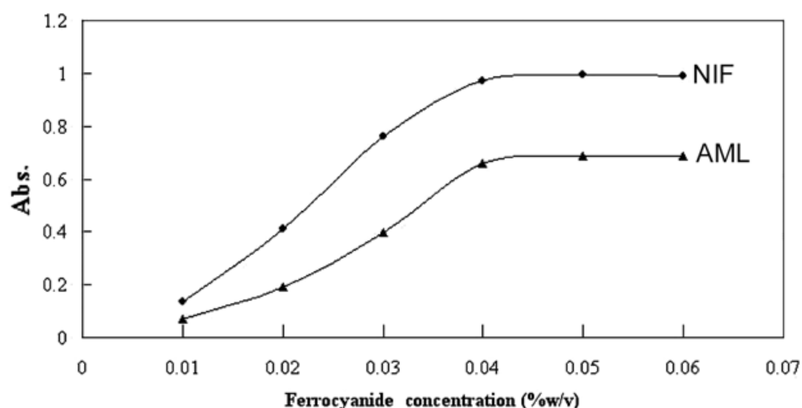

Fig. 3. Effect of ferricyanide concentration on the absorbance of the reaction products of 10 µg ml^{-1} amlodipine and nifedipine in the presence of 0.05% (w/v) $FeCl_3$.

and ferricyanide for NIF results in maximum absorbencies. Hence the above orders of additions were followed throughout the investigation.

Analytical Appraisal

To achieve an appropriate linear range, both initial slope and fixed time methods were employed. In the initial slope method, we calculated the slope of the absorbance-time curve at two limited time intervals for each concentration. For the case of both drugs, the calibration graphs were obtained for different time intervals and the best resulting graph was selected. Also, in the fixed time method, some different calibration curves were obtained for each drug based on the absorbance measurements at different fixed time intervals. In order to select the best calibration graph. Table 1 lists the dynamic ranges and correlation coefficient of the calibration curves represented better quality. As is obvious from Table 1, the fixed time method produced better results. The best dynamic range has been reached when the calibration graphs were obtained at 5.0 min and 4.0 min for AML and NIF, respectively. In this case, and under the experimental conditions described, the Beer's law was obeyed over the respective concentration ranges of 1.0-20.0 and 3.0-19.0 µg ml^{-1} for AML and NIF with regression equations of

$$A = 0.0312 + 0.0025\ C \qquad \text{for AML}$$

Table 1. Linear Calibration Curves Obtained by Initial Slope and Fixed Time Methods for Amlodipine (AML) and Nifedipine (NIF)

Drug	Initial slope method			Fixed time method		
	Time interval (min)	Linear range ($\mu g\ ml^{-1}$)	R^2	Measuring time (min)	Linear range ($\mu g\ ml^{-1}$)	R^2
AML	2-4	4.0-11.0	0.993	2	2.0-19.0	0.990
	3-5	2.0-15.0	0.997	3	1.0-20.0	0.991
NIF	1-3	8.0-19.0	0.993	3	10.0-19.0	0.999
	2-4	6.0-14.0	0.991	4	3.0-19.0	0.994

Table 2. Results for Accuracy and Precision of the Method

Drug studied	Taken value ($\mu g\ ml^{-1}$)	Found value[a] ($\mu g\ ml^{-1}$)	RE (%)	SD ($\mu g\ ml^{-1}$)	RSD (%)	$\bar{x} - \mu$	$\pm ts/\sqrt{n}$ [b]
AML	3.0	3.04	1.33	0.13	4.38	0.04	0.123
	10.0	10.16	1.6	0.25	2.48	0.16	0.229
	18.0	18.02	0.11	0.18	0.99	0.02	0.165
NIF	9.0	8.96	0.44	0.25	2.84	0.04	0.23
	14.0	13.87	0.93	0.23	1.66	0.13	0.21
	18.0	17.79	1.19	0.67	3.76	0.215	0.62

[a]Average of seven determinations. [b]t is tabulated value (2.447) at 95% confidence level, n = 7 and s is standard deviation (SD).

$$A = 0.0538 + 0.0031\ C \qquad \text{for NIF}$$

where C is concentration in $\mu g\ ml^{-1}$. The calculated molar absorptivities at 690 and 740 nm are 1.27×10^4 and 2.00×10^4 $l\ mol^{-1}\ cm$ for AML and NIF, respectively. Therefore, the higher value of calibration curve's slope of NIF can be attributed to higher molar absorptivity of the reaction product for this drug (Fig. 1). However, wider dynamic ranges have been obtained for AML.

Accuracy and precision were established by performing seven replicate determinations containing different amounts within the respective linear dynamic range of each drug. The resulting values of the percent of relative errors (RE), standard deviation (SD), relative standard deviations (RSD), absolute deviation ($\bar{x}$-μ) and confidence limits at 95% confidence level ($\pm ts/\sqrt{n}$), obtained from 7 replicate measurements, are included in Table 2. The accuracy of the method is evident from the very low relative errors lying between 0.11% and 1.60 for AML, and 0.44% and 1.19% for NIF. The low RSD values, which are less than 4% for three different levels studied for both drugs, indicate the high reproducibility of the method. The statistical significance of the results obtained for bulk drug is also confirmed by the data reported in two last columns of Table 2. Obviously, for all analyzed samples the absolute deviations are laying into the corresponding confidence limits, which indicate the absence of systematic error and that the observed deviation between the measured and taken values can be attributed to noise.

The detection limits were calculated by seven replicate measurements on corresponding blank solutions and taking the standard deviation of measurements. The signal of detection limits were chosen as three times of the standard deviation of blank measurements. The detection limit for each drug was then calculated using resulted calibration equations. The calculated detection limits for AML and NIF were 0.10 and 0.19 $\mu g\ ml^{-1}$, respectively.

Table 3. Comparison of the Proposed Methods with the Existing Spectrophotometric Methods for the Determination of AML and NIF

	Reagent	Linear range (μg ml^{-1})	Detection limit (μg m^{-1})	Time (min)	Ref.
AML	Fe(III)-ferricyanide	5-15	0.13	10	[23]
	Fe(III)-1,10-phenanthroline	2-10	0.08	20	[22]
	Fe(III)-2,2′-bipyridyl	4-14	0.12	20	[22]
	Ammonium molybdate	15-59	0.35	25	[22]
	Fe(III)-ferricyanide-Kinetic	1-20	0.10	3	This work
NIF	Fe(III)-1,10-phenanthroline	0.5-14	0.06	5[a]	[38]
	Potassium hydroxide	5-50	0.16	-[b]	[37]
	Ammonium molybdate	2.5-45	0.06	20	[37]
	Fe(III)-ferricyanide-Kinetic	3-19	0.19	4	This work

[a]With heating at 100 °C. [b]Time not reported but the reaction was conducted in dimethyl sulphoxide.

Table 4. Results of Determination of AML and NIF in Dosage Form

Drug	Declared value (mg)	Spiked value (mg)	Found (mg)[c]	Recovery (%)
AML[a]	5[a]	0.0	5.11	102
	5[a]	1.5	6.31	97
	5[a]	2.5	7.36	98
	5[a]	3.5	8.72	103
NIF[b]	10[b]	0.0	9.80	98
	10[b]	3.0	13.21	102
	10[b]	5.0	15.06	100
	10[b]	7.0	17.44	103

[a]ARYA Pharmaceutical Co. [b]Zahravi Co. [c]Average of five determinations.

In Table 3 are compared the linear range, detection limit and procedure time of the previously reported methods using different oxidizing agents [22-23,37-38] with those of the proposed methods for AML and NIF. As it is seen, the proposed method for AML not only represented wider in comparison with the other existing methods. The detection dynamic range but also can be performed in much lower time limit of the proposed method is also comparable with that of previous methods. In the case of NIF, although the proposed method does not represent significant improvement for dynamic range and detection limit, it can be preferred with respect to lower measuring time or better experimental conditions. For example, the method used Fe(III)-1,10-phenanthroline as reagent [38], needs 5 min heating at 100 ºC and that used potassium hydroxide [37] was performed in dimethyl solfuxide which is not preferred for its environmental hazards.

Application

The proposed method was applied to the determination of AML and NIF in proprietary drugs purchased from local stores and containing other inactive ingredients. The results, shown in Table 4, suggest that the method is suitable for the determination of AML and NIF and that the excipients in the

dosage forms do not interfere. Also, very accurate results were obtained for spiked values of the drugs into the tablets. In addition to accuracy, the method is simple and economical for the determination of AML and NIF both in pure form and in formulations. However, since the proposed method is based on the oxidation of drugs, the presence of other oxidizing agents can be considered as interference.

CONCLUSIONS

A kinetic spectrophotometeric method based on the oxidation of drugs by Fe(III) and subsequent color formation between Fe(II) and ferrocyanide was developed for the determination of amlodipine and nifedipine in dosage form. Among the analytical kinetic methods used, *i.e.*, fixed time and initial slope methods, the former produced better results with wider dynamic ranges and lower detection limits for both drugs. The resulted calibration equations were linear in the concentration ranges of 1.0-20.0 and 3.0-19.0 $\mu g\ ml^{-1}$ for AML and NIF, and the detection limits were 0.10 and 0.19 $\mu g\ ml^{-1}$, respectively. The methods also represented very high accuracy and precision so that the respective relative standard deviation and relative error of prediction for drugs were lower than 4% and 1.3%. The proposed method was applied successfully to the analysis of drugs in tablets.

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