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Spectrophotometric Determination of Nicorandil in Bulk and Pharmaceutical Formulation Using Phospho-Molybdenum Blue Complex

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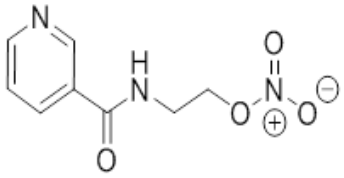
Abstract

A new spectrophotometric method is described for the determination of nicorandil in bulk and pharmaceutical dosage form. It is based on the reduction of nitrate in nicorandil to nitrite ion by using vanadium chloride, and reduction of phosphomolybdic acid to phospho-molybdenum blue complex by sodium sulfide, phospho-molybdenum blue complex is then oxidized by nitrite ion leading to a decrease in blue color intensity which found to be directly proportional to the concentration of nicorandil. Maximum absorbance was measured at 827 nm. Effect of acidity, volume of Sodium Sulfide, stability of complex, volume of vanadium chloride, time and temperature of the reaction were completely studied. The proposed method was satisfactorily applied for the determination of the drug in both bulk and pharmaceutical forms, the calibration curve was linear over the range (60–200 µg/ml) and the results were compared statistically with reference methods.

Keywords: Nicorandil; Vanadium chloride; Phosphomolybdenum complex; Spectrophotometry

Introduction

Nicorandil is a nicotinamide nitrate used as an antianginal agent. It has two modes of action. First, by opening adenosine triphosphate–dependent potassium channels, it increases transmembrane potassium conductance and relaxes peripheral and coronary arterioles. Second, through its nitrate moiety, that allows increasing intracellular concentrations of cGMP, results in a peripheral vein and coronary artery dilation. Due to its ability to dilate arteries and veins, nicorandil maximizes coronary flow and reduces myocardial work through a reduction in afterload. So, nicorandil has been used successfully in managing angina and hypertension. Growing evidence suggests that this drug provides additional benefits that reach beyond its original therapeutic indications [1]. Nicorandil is listed in Martindale, The Extra Pharmacopoeia [2]. The assay of the drug in bulk and dosage forms is not mentioned in British and United States Pharmacopoeia. The literature citation revealed few analytical procedures for the estimation of nicorandil which includes: Spectrophotometric methods, High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), LC-MS/MS and Liquid chromatography [3-14]. In the present work, a new method is described for the determination of nicorandil. It is based on the reduction of phosphomolybdic acid to phospho-molybdenum blue complex by sodium sulfide. The obtained phospho-molybdenum blue complex is oxidized by the addition of nitrite, causing a reduction in intensity of the blue colour [15]. The decrease in the absorbance of the blue colour is directly proportional to the amount of nitrite added. The absorbance of the reaction is monitored spectrophotometrically at 827 nm. The critical step for accurate determination of nitrate is its efficient reduction to nitrite. Nitrate reduction to nitrite can be achieved by NH_4Cl+Zn/dust [4] or by using different reducing metals such as cadmium which is the most commonly used one [16-18]. Although cadmium reduction method had been widely proposed in order to increase sample throughput and decrease sample volume required [19,20] the method suffers from various disadvantages as it is time-consuming, efficiency of the column varies, a continuous activation of the Cd-column is required and it is highly toxic. A spectrophotometric method using a Vanadium Solution (VCl_3) for the reduction of nitrate was described [21,22], which is less toxic than cadmium that has been commonly used for the reduction of both nitrate and nitrite at high temperatures (80-90°C) to nitric oxide, then measured by chemiluminescence [23]. At room temperatures, nitrate is reduced to nitrite [21]. In the proposed method vanadium chloride has been used for the reduction of nitrate to nitrite (Figure 1).



Nicorandil

Figure 1: Chemical structure of Nicorandil.

Materials and Methods

Apparatus

Labomed Spectro® UV-vis Double beam (Model UVD-2950) spectrophotometer with matched 1 cm quartz cell connected to windows compatible computer using UV win 5 software v5.0.5.

Materials and reagents

All the following chemicals are of analytical reagent grade.

- Nicorandil was provided by Adwia, Egypt. Adancor® tablets, labeled to contain 10 mg of nicorandil, batch No. 19262120 (Merck, Egypt).
- Phosphomolybdic acid (Sigma-Aldrich, Germany), standard solution (10 mg/ml) was prepared by dissolving 1 g of pure reagent in 100 ml bidistilled water.
- Sodium sulphide was purchased from EL-Nasr chemicals, Egypt. Standard solution (10 mg/ml) was prepared by dissolving 1 g of Na2S in 100 ml bidistilled water.
- Vanadium (III) was provided from Sigma-Aldrich, Germany. Standard solution (2 mg/ml) was prepared by dissolving 0.1 g of pure reagent in 50 ml 20% HCl.
- Concentrated HCl (34%) was purchased from EL-Nasr chemicals; Egypt. 20% HCl was prepared by diluting 20 ml C.HCl to 100 ml by distilled water.

General Procedures

Preparation of standard drug solution

A stock standard solution of nicorandil (2 mg/ml) was prepared by weighing accurately 0.1 g of pure drug and dissolving in 50 ml distilled water.

Preparation of working solution

Aliquot containing 60-200 µg/mL of Nicorandil standard solution was transferred into 10 ml volumetric flask, followed by 1 ml of standard (VCl_3), gently mixed and incubated in a water bath at 6°C for 20 min, then cooled down to room temperature in a water bath.

Preparation of phospho-molybdenum blue complex

Working solution of phospho-molybdenum blue complex was freshly prepared by transferring seven ml volume of a standard solution of phosphomolybdic acid into a test tube, followed by three ml volume of standard Na_2S solution, followed by 1.5 ml of HCl, gently mixed and left for 20 min at 25°C.One ml of phospho-molybdenum blue complex is transferred to the 10 ml volumetric flask (containing sample and VCl_3) The volume was completed with distilled water and left for exactly 15 min. Then the decrease in the absorbance was measured at 827 nm against blank (Figure 2).

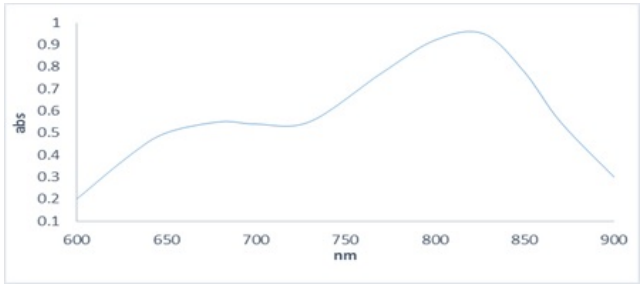


Figure 2: Absorption spectra of Nicorandil (200 µg/ml) at λ max 827 nm using phospho-molybdenum blue method.

Pharmaceutical preparation (Adancor®) tablets

Ten tablets were crushed, powdered and weighed, an accurate amount of powder equivalent to 100 mg of nicorandil were dissolved in bidistilled water, filtered into 50 ml measuring flask and the volume was completed to give a final concentration of 2000 µg/ml. The procedures were then completed as previously mentioned. The drug concentration was detected by standard addition technique.

Results and Discussion

It was found that the reduction of phosphomolybdic acid to phospho-molybdenum blue by Na_2S and the oxidation of phospho-molybdenum complex by nitrite were affected by many factors. Also, the reduction of nicorandil was affected by some factors. In the present work each of these factors was studied carefully in order to optimize the conditions for spectrophotometric determination of nicorandil.

$H3PMO12O40+Na2S \xrightarrow{Reduction}$ Phosphomolybdenum blue complex (1)

$NICORANDIL+VCl3 \xrightarrow{Reduction} NO2\text{-ion}$ (2)

Phosphomolybdenum blue $\xrightarrow{Oxidation}$ the intensity of blue colour decreases (3)

Factors Affecting Proposed Method

Effect of acidity

The effect of hydrochloric acid volume on the absorbance of the solution was studied while other factors were held constant; Figure 3 shows the absorption spectra as a function of hydrochloric acid concentration in the solution. The maximum absorbance of the solution containing 1.5 ml of 36% concentrated hydrochloric acid is obtained. Increasing the volume of HCl resulted in a decrease in absorbance.

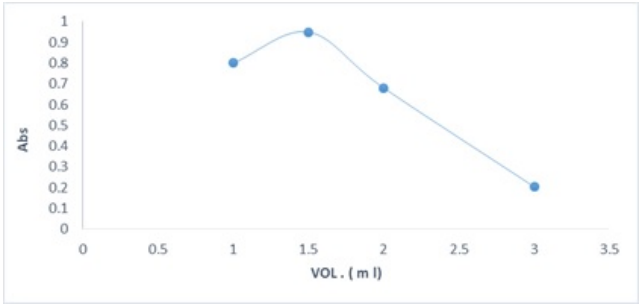


Figure 3: Effect of volume of HCl on absorbance in presence of 200 µg/ml Nicorandil.

Effect of phosphomolybdic acid volume

The effect of phosphomolybdic acid volume on the absorbance of the solution was studied, while other factors were held constant. Figure 4 shows the absorption spectra as a function of phosphomolybdic acid concentration in the solution. The maximum absorbance of the solution containing 7 ml of phosphomolybdic acid was obtained.

Beyond which any further increase caused a decrease in the absorbance.

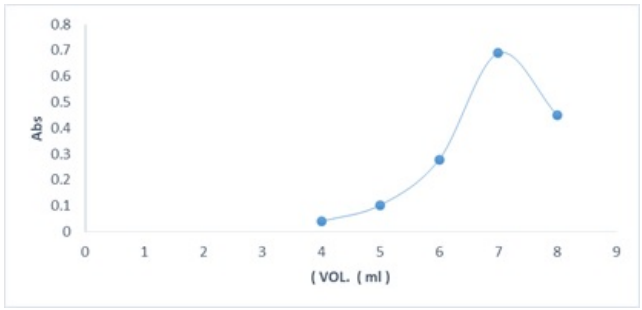


Figure 4: Effect of volume of Phospho-molybdic acid on absorbance in presence of 200 µg/ml Nicorandil.

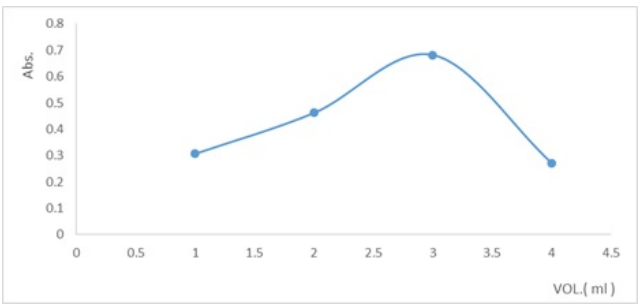


Figure 5: Effect of volume of Na_2S on absorbance in presence of 200 µg/ml Nicorandil.

Effect of sodium sulfide volume

The effect of sodium sulphide volume on the absorbance of the solution was studied while other factors were held constant. Figure 5 shows the absorption spectra as a function of sodium sulphide concentration in the solution. The maximum absorbance of the solution containing 3 ml of Na_2S was obtained. Beyond which any further increase caused a decrease in the absorbance.

Effect of vanadium chloride volume

The effect of vanadium chloride volume on the absorbance of the solution was studied while other factors were held constant. Figure 6 shows the absorption spectra as a function of vanadium chloride concentration in the solution. The maximum absorbance of the solution containing 1 ml of VCl_3 is obtained. Beyond which any further increase caused a decrease in the absorbance.

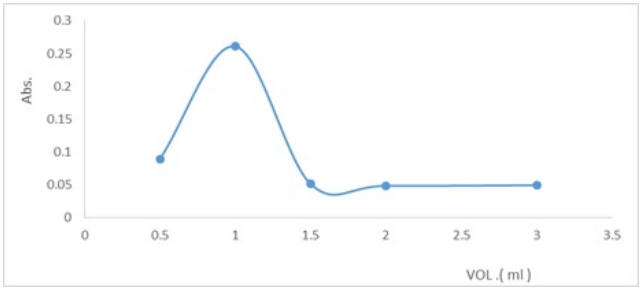


Figure 6: Effect of volume of VCl_3 on absorbance in presence of 200 µg/ml Nicorandil.

Effect of temperature required for reduction of Nicorandil

The effect of temperature on the absorbance of the solution was studied while other factors were held constant. Figure 7 shows maximum absorbance of the solution was obtained upon heating sample with VCl_3 in a water bath at 60°C beyond which any further increase in temperature caused a decrease in the absorbance. At room temperature, the reaction showed a decrease in the colour intensity of phospho-molybdenum blue complex and the colour was unstable with low absorbance. But increasing temperature resulted in a stable colour with high absorbance.

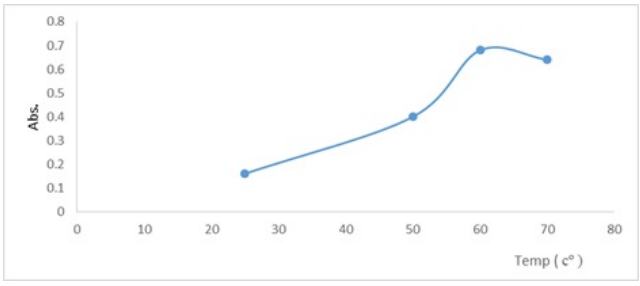


Figure 7: Effect of temperature required for reduction on absorbance in presence of 200 µg/ml Nicorandil.

Effect of time upon heating

The effect of time of heating on the absorbance of the solution was studied while other factors were held constant. Figure 8 shows maximum absorbance of the solution was obtained upon heating sample with VCl_3 in a water bath for 20 min, beyond which any further increase in time caused a decrease in absorbance.

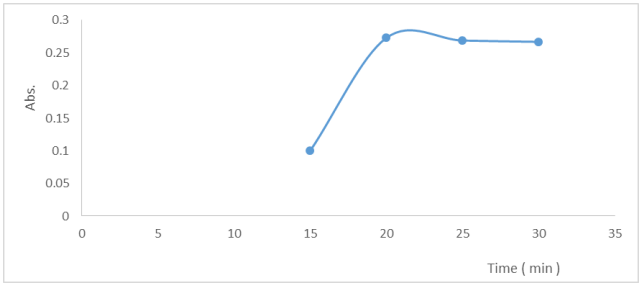


Figure 8: Effect of time of heating required on absorbance in presence of 200 µg/ml Nicorandil.

Effect of time required for formation of phospho-molybdenum blue complex

The effect of time required for formation of phospho-molybdenum blue complex on the absorbance of the solution was studied, while other factors were held constant. Figure 9 shows maximum absorbance of the solution was obtained on leaving phospho-molybdenum blue complex for 20 min after preparation. Beyond which any further increase in time caused a decrease in absorbance.

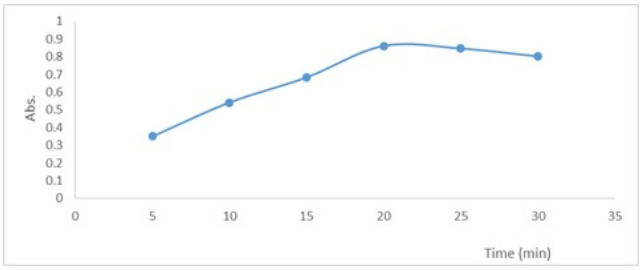


Figure 9: Effect of time required for formation of phospho-molybdenum blue complex on absorbance in presence of 200 µg/ml Nicorandil.

Effect of time after dilution

The effect of time after dilution on the absorbance of the solution was studied while other factors were held constant. Figure 10 shows maximum absorbance of the solution was obtained on leaving the reaction for 15 min after dilution.

Beyond which any further increase in time caused a decrease in absorbance. Dilution should be applied at once after addition of phospho-molybdenum complex to the drug, delaying dilution resulted in a significant decrease in absorbance.

Method validation

The proposed method had been validated for linearity, precision, accuracy, recovery, limits of detection and quantification according to the current ICH guidelines [24].

Linearity and range

The absorbance versus concentration was plotted and a linear relation was found (Figure 11). Beer's law was followed at a concentration range 60-200 µg/ml. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1.

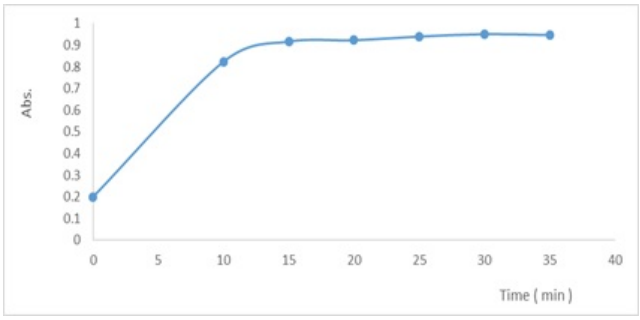


Figure 10: Effect of time required after dilution on absorbance in presence of 200 µg/ml Nicorandil.

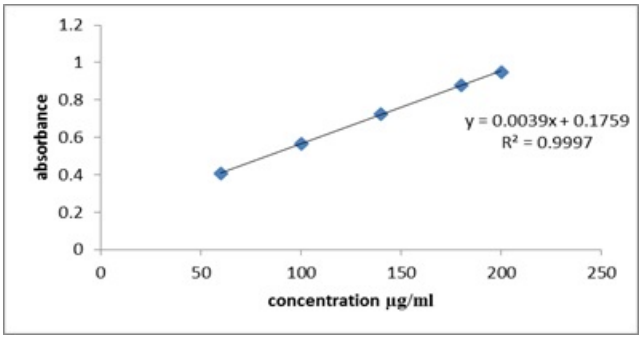


Figure 11: Calibration curve of Nicorandil authentic sample using the proposed method.

Parameters	Nicorandil
λ max, nm	827
Volume of Phosphomolybdic acid, ml	7
Volume of Na_2S, ml	3
Volume of HCl, ml	1.5
Volume of VCl_3, ml	1
Temperature required for reduction of drug, ˚C	60˚C
Time required for heating, min	20 min
Time required for formation of phospho-molybdenum blue complex, min	20 min
Time after dilution, min	15 min
Beer's law limits, µg/ml	60–200
Regression equation	Y=0.0039 x+0.1759
Correlation coefficient	0.9997
Y=a+bx, where y is the absorbance, a is the intercept, b is the slope and x is the concentration in µg/ml	

Table 1: Analytical parameters for determination of Nicorandil using phosphomolybdenum blue.

Limits of detection and limits of quantification

Limits of detection and quantitation were determined according to ICH recommendations LOD=3.3 S/K and LOQ=10 S/K, where S is the standard deviation of three replicate determination values under the same conditions as for the same analysis and K is the slope of calibration graph. Results were represented in Table 2.

Parameters	Nicorandil		
	Taken conc. (µg/ml)	Found conc. (µg/ml)	Recovery %
	60	59.25	98.76
	100	100.02	100
	140	140.28	100.2
	180	180.5	100.3
	200	198.23	99.1
Mean			99.68
SD			0.696
RSD			0.698
SE			0.31
Variance			0.48
Slope			0.00389
LOD			7.7
LOQ			25.7
S.S.			1.586
Apparent Molar absorptivity ($L.Mol^{-1}.cm^{-1}$)			1150.68
*Mean of three different experiments			

Table 2: Results of analysis for determination of Nicorandil using phospho-molybdenum blue method.

Precision of the method

The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of nicorandil (within the working limits) were analysed in five replicates during the same day and five consecutive days.

The SD and RSD values of intra-day and inter-day studies showed that precision was good as seen in Table 3.

Conc. (µg/ml)	Intraday		Interday	
	mean+SD	RSD	mean+SD	RSD
60	98.3+0.427	0.4	98.76+0.85	0.865
200	98.987+0.128	0.129	99.37+0.339	0.34

Table 3: Results of the intraday and interday precision for the determination of Nicorandil using phospho-molybdenum blue method.

Robustness

The robustness of the method was evaluated by making small changes in the volume of phosphomolybdic acid, sodium sulfide, HCl, VCl_3, time and temperature. The effect of the changes was studied on the percent recovery of drugs and it was found that all changes had a negligible influence on the results as seen in Table 4.

Parameters	(Nicorandil)
	% recovery ± SD
HCl 1.4 ml	99.65 ± 0.72
HCl 1.6 ml	99.7 ± 0.67
Phospho-molybdic acid 6.9 ml	100 ± 1.4
Phospho-molybdic acid 7.1 ml	98.8 ± 1.729
Na_2S 2.9 ml	99.7 ± 0.729
Na_2S 3.1 ml	99.65 ± 0.666
VCL_3 0.9 ml	99.5 ± 0.6
VCL_3 1.2 ml	99.9 ± 1
Heating temperature 55°C	99.475 ± 0.7
Heating temperature 65°C	99.8 ± 0.857
Time of heating 22 min	100 ± 0.6
Time of heating 18 min	99.2 ± 2
Time for Phospho-molybdic acid reduction 22 min	99.475 ± 0.7
Time for Phospho-molybdic acid reduction 18 min	99.7 ± 0.7
Time after dilution 32 min	99.765 ± 0.8
Time after dilution 28 min	99.65 ± 0.666

Table 4: Results of robustness for determination of Nicorandil using phospho-molybdenum blue method.

Analysis of pharmaceutical preparations

The applicability of the proposed method for the determination of nicorandil in drug formulations has been tested on commercially available tablets using standard addition technique; results were explained in Table 5. Excipients did not show interference indicating high specificity. The results of the proposed method were statistically compared with those of the reference method [6]. T-test and F-test was performed for comparison. Results are shown in Table 6, where calculated t and F values were less than tabulated values, and that indicates that there is no significant difference between proposed method and reference one relative to precision and accuracy.

Items	Adancor® tablet			
	Added pure drug (µg/ml)	Taken Adancor tablet (µg/ml)	Conc. Found (µg/ml)	Recovery* %
	60	0	59.97	99.96
	60	40	98.58	98.58
	60	80	141	100.8
	60	120	179.97	99.987
	60	140	198.58	99.29
Mean*	-	-	-	99.7
N	-	-	-	5
S.D.	-	-	-	0.8376
R.S.D.	-	-	-	0.8
V	-	-	-	0.7
S.E.	-	-	-	0.4

Table 5: Application using standard addition technique for the determination of nicorandil tablets using phospho-molybdenum blue method.

Parameters	Phosphomolybdenum blue method.	Reported method [6]
Mean Recovery	99.7	99.2
N	5	5
S D	0.8376	0.37
VARIANCE	0.7	0.136
F-ratio	5 (6.39)	-
Student-t test	1.2 (2.78)	-
Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39		

Table 6: Statistical analysis of results obtained by the proposed methods applied on Adancor® tablets compared with reported method.

Conclusions

The method was selective, sensitive and reproducible. The method did not suffer from instability of colours. The validation experiments provided proof that the proposed method was linear in the proposed working range as well as precise, specific and reproducible. No interference was observed from the reduced nicorandil and common excipients. The method had been successfully applied for the determination of nicorandil in formulations.

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