—Regular Articles—

Quantitation of Nicorandil in Pharmaceutical Formulations by Spectrophotometry Using N-(1-naphthyl) Ethylenediamine Dihydrochloride as Coupling Agent

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A validated and sensitive spectrophotometric method is developed for the quantitation of nicorandil in pharmaceutical formulations. The method is based on the reduction of nitroxy ethyl group of nicorandil to carbonyl derivative and nitrite ion by Zn/NH_4Cl. The nitrite ion undergoes diazotization with sulphanilamide in presence of HCl followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride (NED) to form a colored product with λ_{max} at 525 nm. Under the optimized experimental condition, Beer's law is obeyed in the concentration range of 0.4—12.0 μg/ml with molar absorptivity of 1.92×10^4 l mol^{-1} cm^{-1}. The statistical analysis of calibration data yields the linear regression equation: $A=6.304\times10^{-4}+9.13\times10^{-2}C$ with correlation coefficient of 0.9999. The limits of detection and quantification are 0.05 and 0.15 μg/ml, respectively. The results obtained by the proposed method are acceptable with average recoveries of 100.0—100.1%. The results of the proposed method are compared with those of the reference method by point and interval hypothesis tests, which showed excellent agreement and there is no significant difference in accuracy and precision of methods compared.

Key words——nicorandil; spectrophotometry; N-(1-naphthyl) ethylenediamine dihydrochloride; pharmaceutical formulation

INTRODUCTION

Nicorandil, N-[2-(nitroxy) ethyl] 3-pyridine carboxamide, is a cardiovascular drug with both nitrate-like and ATP-sensitive potassium-channel (K^+ ATP) activating properties.[1] By virtue of this dual mechanism of action, the drug acts as a balanced coronary and peripheral vasodilator and reduces both preload and after load. Initial interest in the drug focused on its activity in ischaemic heart disease. Overtime, however, the potential cardioprotective effect of the drug has received increasing attention. Nicorandil is primarily metabolized via oxidation to nicorandil-N-oxide and hydroxy nicorandil and by denitration to N-[2-hydroxy ethyl] nicotinamide. The latter is further transformed to nicotinamide, nicotine and N-methyl-nicotinamide.

The drug is listed in Martindale The Extra Pharmacopoeia.[2] Many efforts have been made to determine nicorandil concentration in biological fluids and drug formulations. The techniques used for the assay include high performance liquid chromatography,[3—8] high performance thin layer chromatography,[9] gas chromatography coupled with mass spectrometry,[10] and polarography.[11] The literature reveals that few spectrophotometric methods have also been developed to determine nicorandil in drug formulations and biological fluids. The nicorandil reacts with 4-(methylamino) phenol sulfate and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) resulting in the formation of a colored product with λ_{max} at 560 nm. Beer's law is obeyed in the concentration range of 0.4—2.2 μg/ml.[12] The quantitation of the drug has also been done based on its reaction with sulfanilic acid in presence of cyanogen bromide,[13] phenoldisulfonic acid,[14] phloroglucinol-sulphanilic acid reagent in sulfuric acid medium.[15] Nicorandil oxidizes the MBTH and DL-2,3-dihydroxyphenyl alanine and finally a colored product is formed owing to the oxidative coupling of MBTH with DL-2,3-dihydroxyphenyl alanine.[15]

In the present communication, the nitroxy ethyl group of nicorandil is reduced to carbonyl derivative of nicorandil and nitrite ion by Zn/NH_4Cl. The nitrite ion obtained undergoes diazotization reaction with sulfanilamide in presence of HCl followed by coupling with NED to give a colored product with λ_{max} at 525 nm. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[16]

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MATERIALS AND METHODS

Apparatus A Shimadzu UV–Visible Spectrophotometer (model 1601) Kyoto, Japan with matched quartz cells was used for recording the absorption spectra. Absorbance measurements were made on Spectronic 20D$^+$ spectrophotometer (Milton Roy, USA).

Reagents and Standards Reference standard sample of nicorandil was kindly provided by M/s. Zydus Medica, Ahmedabad, India and was used as received. The commercial dosage forms of nicorandil such as Nikoran (Torrent Pharmaceutical, Ahmedabad, India), Korandil (Sun Pharma. Industries Ltd., Mumbai, India) and Zynicor (Zydus Medica, Ahmedabad, India) were purchased from the local market.

- Standard solution of nicorandil (0.2 mg/ml) was prepared in distilled water
- 2.32×10^{-3} M sulfanilamide solution (Sigma Chemical Company, USA) was prepared by dissolving 0.40 g in 5 ml of 0.1 M HCl and then diluted up to 100 ml with distilled water.
- 0.05 M HCl solution
- 0.15 M ammonium chloride (Merck, India) was prepared in distilled water.
- 3.09×10^{-3} M NED solution (Koch-light Laboratories, England) was prepared by dissolving 0.08 g in 100 ml distilled water.

Preparation of Degraded Product 500 mg of nicorandil was hydrolysed with 4N HCl at 100°C for 30 min.[9] The degraded product was isolated by preparative thin layer chromatography using plate coated with silica gel G as stationary phase and chloroform: methanol: ethyl acetate (4 : 1 : 5 v/v/v) as mobile phase. The band corresponding to the degradation product was located under UV lamp. The band was scrapped and extracted with chloroform. Finally, the pure degradation product (denitrated nicorandil) was obtained after removal of chloroform under vacuum distillation.

Recommended Procedure for the Quantitation of Nicorandil Aliquots of standard nicorandil solution (0.2 mg/ml) in the concentration range of 0.4—12.0 μg/ml were pipetted into a series of 25 ml beakers. To each beaker, 3.5 ml of 0.15 M NH_4Cl and 0.1 g zinc powder were added and left for 3 min. The whole content was filtered through Whatmann No. 42 filter paper (Whatman International Limited, Kent, UK) in a 25 ml volumetric flask. To the flask, 1.5 ml of 2.32×10^{-3} M sulfanilamide was added followed by 1.2 ml of 0.05 M HCl with shaking and left still in an ice bath (0—3°C) for 1 min. Then 1.3 ml of 3.09×10^{-3} M NED was added and the volume was completed with distilled water. The absorbance of the colored product was measured at 525 nm against the reagent blank.

Quantitation of Nicorandil in Drug Formulations Five tablets were weighed and grounded. The powder equivalent to 5 mg of nicorandil was weighed accurately and dissolved in 10 ml of distilled water. The undissolved particle was then allowed to settle down and filtered through Whatmann No. 42 filter paper. The residue was washed with additional 10 ml of distilled water for recovery of drug. Then the filtrate was diluted to 25 ml with distilled water and subjected to recommended procedure for determination of nicorandil.

Validation The proposed method has been validated for linearity, precision, accuracy, recovery, limits of detection, and quantitation.

Specificity The specificity of the proposed method was examined by taking 8.0 μg/ml nicorandil in presence of varying concentration of denitrated nicorandil (10.0—1000.0 μg/ml). To check the specificity of the proposed method, synthetic mixture containing nicorandil with excipients commonly used in solid dosage forms contains nicorandil (25 mg), lactose (75 mg), starch (100 mg), talc (100 mg) and magnesium stearate (10 mg). A portion of synthetic mixture equivalent to 5 mg nicorandil was transferred to 50 ml conical flask and treated as described in ''quantitation of nicorandil in drug formulations''.

Linearity The linearity was evaluated by determining the nicorandil at eleven concentration levels: 0.4, 0.8, 1.2, 2.4, 4.8, 5.6, 7.2, 8.0, 10.4, 11.2, 12.0 μg/ml. Each concentration was analyzed for five times.

Accuracy and Precision The accuracy and precision of the proposed method were ascertained by performing five replicate analysis of nicorandil in pure form at three concentration levels: 0.8, 5.6 and 11.2 μg/ml and in pharmaceutical formulations at two concentration levels (4.8 and 12.0 μg/ml) within one day (intraday). The same analysis was repeated for five consecutive days (interday).

Recovery Studies Recovery experiments were carried out by standard addition method. For this 2.0

ml (or 4.0 ml) of reference nicorandil (0.2 mg/ml) was transferred into 100 ml volumetric flask followed by 1.0 ml of sample solution (0.2 mg/ml). The volume was completed with distilled water and the nominal value was determined by the proposed procedure.

Limits of Detection (LOD) and Quantitation (LOQ) The values of the LOD and LOQ were calculated using the expression:

$$LOD = 3.3 \times S_0/b \text{ and } LOQ = 10 \times S_0/b$$

where S_0 and b are standard deviation and slope of the calibration line, respectively.

Evaluation of Bias The bias has been evaluated by means of point and interval hypothesis tests.[17,18] In interval hypothesis the proposed method (method 2) is accepted when the true mean is within $\pm 2\%$ of that of the reference method (method 1), *i.e.*, $0.98 < \mu_2/\mu_1 < 1.02$, which can be generalized to $\theta_L < \mu_2/\mu_1 < \theta_u$ where θ_L and θ_U are lower and upper acceptance limits, respectively. The limits of this confidence interval can be calculated using the following quadratic equation:

$$\theta^2(\overline{x_1^2} - S_p^2 t^2/n_1) - 2\theta \overline{x_1}\,\overline{x_2} + (\overline{x_2^2} - S_p^2 t^2/n_2) = 0$$

RESULTS AND DISCUSSION

Zinc is one of the most frequently used reducing agent for reduction of nitrate into nitrite[19] while ammonium chloride is used to assist the reduction process.[20] The same concept has been utilized in the present communication. The nicorandil possesses nitroxy ethyl group which on treatment with Zn/NH_4Cl yields carbonyl derivative of nicorandil and nitrite ion. The nitrite thus obtained undergoes diazotization with sulfanilamide in presence of HCl and then subsequently coupling with NED to give a colored product. The related absorption spectra are depicted in Fig. 1 and the reaction sequence is shown in Scheme 1. The absorption spectrum of the reaction product has an absorption maximum at 525 nm. The reagents do not absorb radiation at 525 nm.

Optimization of Variables Various parameters responsible for the formation of the colored product were studied and optimized by conducting a series of experiments.

Effect of Zinc To examine the effect of zinc on the reduction of nicorandil to yield nitrite ion, a known amount of nicorandil (280 μg) was treated with different amount of zinc dust (0.005—0.2 g) in

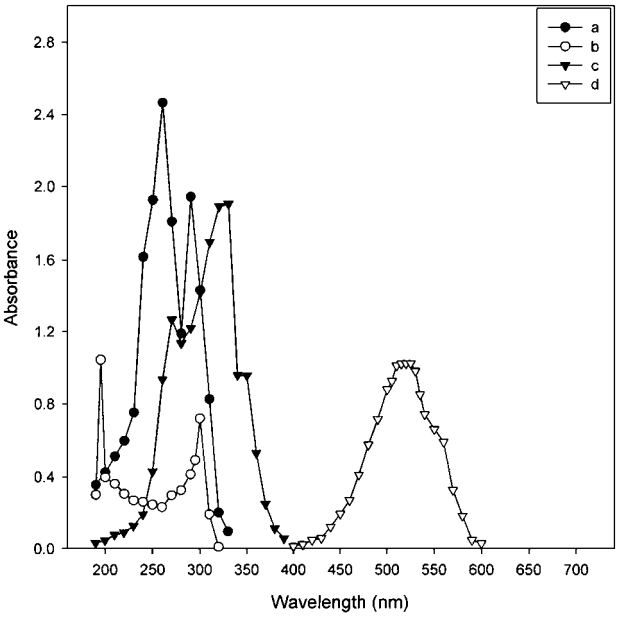
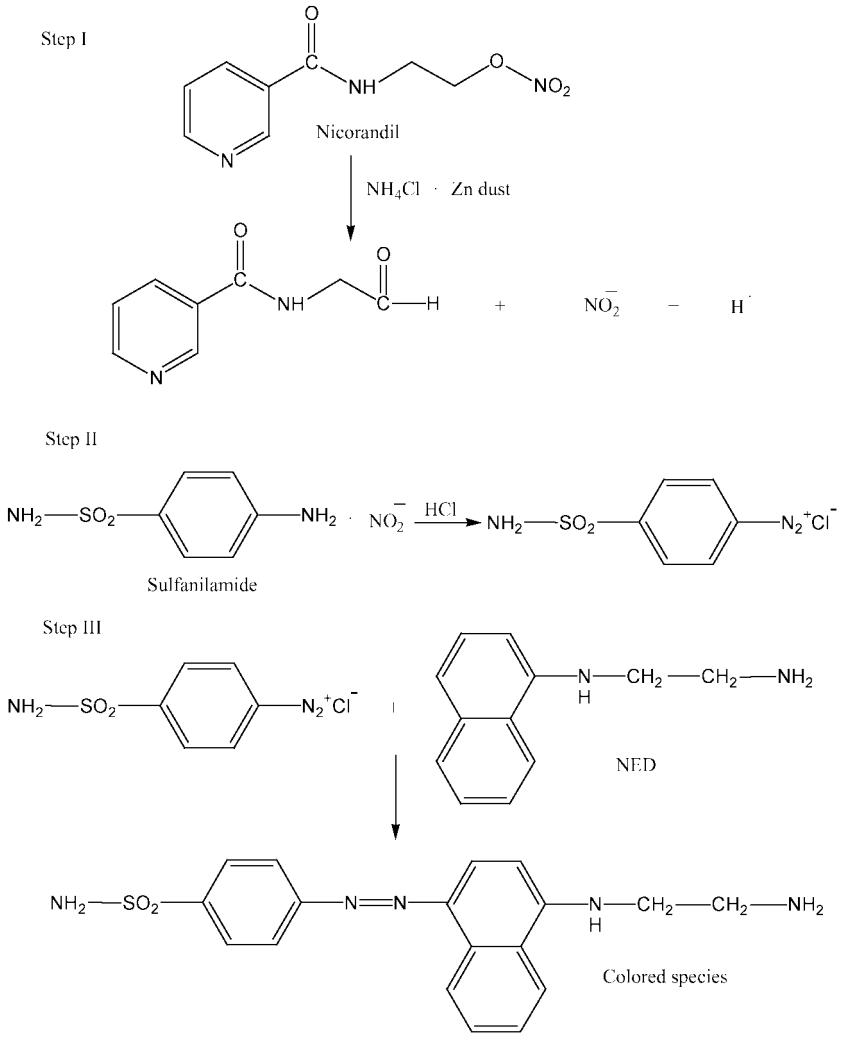


Fig. 1. Absorption Spectra of (a) 1.0 ml of 0.01% Nicorandil (10 μg/ml) in 10 ml Distilled Water (b) 1.0 ml of 0.01% Nicorandil with 3.5 ml of 0.15 M NH_4Cl and 0.2 g of Zinc Dust Followed by Filtration through Whatmann No. 42 Filter Paper (c) Blank Solution: 1.39×10^{-4} M Sulfanilamide, 2.8×10^{-3} M HCl and 1.730×10^{-4} M NED in 25 ml Volumetric Flask and Diluted to Volume with Distilled Water (d) Sample Solution: 1.4 ml of 0.02% Nicorandil with Blank Solution in 25 ml Volumetric Flask and Diluted to Volume with Distilled Water.

presence of 2.1×10^{-2} M NH_4Cl. It was observed that maximum intensity was obtained with 0.05 g of zinc, which remained constant up to 0.2 g. Therefore, 0.1 g of zinc dust was selected as an optimum quantity for the reduction process.

Effect of NH_4Cl Concentration on the Reduction Process The effect of ammonium chloride concentration on the reduction process was examined in the range of $6.0 \times 10^{-4} - 2.4 \times 10^{-2}$ M and keeping the constant amounts of nicorandil (280 μg) and zinc dust (0.1 g). It was found that the absorbance increased with increasing concentration of NH_4Cl and maximum absorbance was obtained with 1.8×10^{-2} M NH_4Cl, which remained constant up to 2.4×10^{-2} M. Therefore, 2.1×10^{-2} M NH_4Cl was selected for further investigation.

Effect of Time on the Reduction Process The effect of time on the reduction process was studied by treating 280 μg nicorandil with 0.1 g of zinc dust and 2.1×10^{-2} M NH_4Cl. A constant absorbance was obtained after 1 min and remained constant up to 3 min. Hence, a time of 2 min was selected as an optimum



Scheme 1. Reaction Sequence for the Formation of Colored Product of Nicorandil

value for the reduction process.

Effect of Reaction Time To optimize the reaction time for the color development, the drug (280 μg) was treated with 0.1 g zinc dust and 2.1×10^{-2} M NH_4Cl and left still for 2 min and then filtered through Whatmann No. 42 filter paper. To the filtrate containing nitrite ions, 1.5 ml of 2.32×10^{-3} M sulfanilamide was added followed by 1.2 ml of 0.05 M HCl and the reaction mixture was kept in an ice bath (0—3℃) for different time intervals (0.25 to 3 min). After removing the flask from the ice bath, 1.3 ml of 3.09×10^{-3} M NED was added and diluted to 25 ml with distilled water. The maximum intensity of color was obtained at 1 min and remained constant up to 2 h. Therefore, a time of 2 min was selected as an optimum time for the diazotization reaction.

Effect of Sulfanilamide Concentration The effect of the concentration of sulfanilamide on the color development was examined in the range of 9.26×10^{-6} to 1.76×10^{-4} M. It was observed that the absorbance increased with the increasing concentration of sulfanilamide and the highest absorbance was obtained with 1.02×10^{-4} M sulfanilamide and remained constant up to 1.76×10^{-4} M. Therefore, 1.39×10^{-4} M sulfanilamide was used for further studies.

Effect of HCl Concentration on Diazotization The influence of HCl concentration on the absorbance of the colored product was studied in the range of $1.0\times10^{-4}-3.0\times10^{-3}$ M. The intensity of the color increased with increasing concentration of HCl and a constant absorbance was obtained in the concentration range of 1.8×10^{-3} to 3.0×10^{-3} M. Therefore, 2.4×10^{-3} M HCl was selected as an optimum concentration of HCl required for the color development.

Effect of NED Concentration on the Color De-

velopment The effect of NED concentration on the absorbance of the product was examined over the concentration range $1.24\times10^{-5}-1.98\times10^{-4}$ M. It was found that a constant absorbance was obtained in the concentration range of $1.24\times10^{-4}-1.98\times10^{-4}$ M. Therefore, 1.61×10^{-4} M NED was selected as an optimum value in the color development.

Solution Stability The stability of the nicorandil solution was checked by recording the UV spectra of pure nicorandil and quality control sample for four days. The band for nicorandil was located at 260 nm whereas at this wavelength no band corresponding to the degradation product was observed. It was found that there was no change in the absorption spectra. The solution stability was also ascertained by applying the standard solution of nicorandil and quality control sample solution on TLC plates coated with silica gel G (Merck (India) Limited, Mumbai) and developed in chloroform: methanol: 2.61 M NH_3 system (2.5 : 1.5 : 0.4 v/v/v). The plates were air-dried and spot was detected in iodine chamber. In both the cases R_f value was found to be 0.68 confirming the stability of the solution.

Analytical Data Under the optimized and validated experimental conditions, the plot of absorbance versus the concentration of nicorandil was found to be linear over the concentration range 0.4—12.0 μg/ml with molar absorptivity of 1.92×10^4 l mol^{-1} cm^{-1}. The statistical treatment of calibration data (n =11) yielded intercept (a), slope (b), correlation coefficient (r), confidence limits of intercept ($\pm t\,S_a$) and slope ($\pm t\,S_b$) at 95% confidence level, variance, and detection and quantitation limits. The results are summarized in Table 1. The high value of correlation coefficient (r=0.9999) indicated the excellent linearity of the calibration line. The low value of variance of calibration line pointed towards the higher reproducibility of the proposed method.

Accuracy and Precision of the Proposed Method The relative standard deviations (RSD) evaluated at concentrations of 0.8, 5.6, 11.2 μg/ml in pure form for intra-day variations were 5.38, 0.85 and 0.38%, respectively; and for inter-day variations were 6.02, 0.93 and 0.48%, respectively (Table 2). The RSD evaluated at 12.0 μg/ml in pharmaceutical formulation for intra-day variations were found to vary in the range of 0.55—0.62% and for inter-day varied over the range of 0.61—1.10% (Table 3).

Specificity The specificity of the proposed method was evaluated by determining the amount of nicorandil (8.0 μg/ml) in presence of its degraded product (0.01—1.0 mg/ml). It was found that the degradation product of nicorandil did not interfere with the determination process (Table 4).

The standard drug and quality control sample solutions were stressed by light and heat (up to 50°C) for 2, and 1-day time points, respectively. It was observed that stress by such conditions did not cause degradation, as there was no change in the absorption spectra of pure drug and stressed sample solution. The specificity of the proposed method was also ascertained by determining the nicorandil in presence of various excipients commonly used in the formula-

Table 1. Optical and Regression Characteristics of the Proposed Method

Parameters	Proposed method
λ_{max}, nm	525
Stability, h	0.2
Beer's law limit, μg/ml	0.4—12.0
Molar absorptivity, 1 mol^{-1} cm^{-1}	1.92×10^4
Linear regression equation	$A=6.304\times10^{-4}+9.130\times10^{-2}C$
S_a	7.35×10^{-4}
$\pm tS_a$	1.66×10^{-3}
S_b	1.03×10^{-4}
$\pm tS_b$	2.34×10^{-4}
Correlation coefficient, r	0.9999
Variance, S_0^2	1.96×10^{-6}
Detection limit, μg/ml	0.05
Quantitation limit, μg/ml	0.15

S_a and S_b are standard deviation of intercept and slope, respectively.

Table 2. Intra-day and Inter-day Assays: Determination of Nicorandil in Pure Form

Concentration/μg/ml		Error (%)	RSD (%)	SAE[b]	C.L.[c]
Taken	Found±SD[a]				
Intra-day assay					
0.8	0.80±0.04	0.13	5.38	0.02	0.05
5.6	5.60±0.05	0.02	0.85	0.02	0.06
11.2	11.21±0.04	0.08	0.38	0.02	0.05
Inter-day assay					
0.8	0.80±0.05	0.38	6.02	0.02	0.06
5.6	5.60±0.05	0.02	0.93	0.02	0.07
11.2	11.20±0.05	0.05	0.48	0.02	0.07

a) Mean for five independent analyses, *b*) SAE: standard analytical error, *c*) C.L.: confidence limit at 95% confidence level and four degrees of freedom (t=2.776).

Table 3. Intra-day and Inter-day Assays: Determination of Nicorandil in Drug Formulations

Drug formulations	Concentration/μg/ml		Error (%)	RSD (%)	SAE[b]	C.L.[c]
	Taken	Found±SD[a]				
	Intra-day assay					
Nikoran-5 (Samarth Pharma)	12.0	12.00±0.07	0.03	0.62	0.03	0.09
	4.8	4.80±0.04	0.02	0.85	0.02	0.05
Korandil-5 (Sun Pharma)	12.0	12.00±0.07	0.03	0.58	0.03	0.09
	4.8	4.80±0.04	0.02	0.87	0.02	0.05
Zynicor-5 (Zydus Medica)	12.0	12.01±0.07	0.04	0.55	0.03	0.08
	4.8	4.81±0.04	0.08	0.86	0.02	0.05
	Inter-day assay					
Nikoran-5 (Samarth Pharma)	12.0	12.00±0.13	0.05	1.10	0.06	0.16
	4.8	4.80±0.05	0.07	1.01	0.02	0.06
Korandil-5 (Sun Pharma)	12.0	12.01±0.11	0.04	0.92	0.05	0.14
	4.8	4.81±0.05	0.14	1.0	0.02	0.06
Zynicor-5 (Zydus Medica)	12.0	12.01±0.07	0.08	0.61	0.03	0.09
	4.8	4.80±0.05	0.08	0.99	0.02	0.06

a) Mean for five independent analyses, *b*) SAE: standard analytical error, *c*) C.L.: confidence limit at 95% confidence level and four degrees of freedom (t=2.776).

Table 4. Determination of Nicorandil in Presence of Denitrated Nicorandil

Concentration (μg/ml)			Recovery (%)[a]	RSD (%)
Nicorandil taken	Denitrated nicorandil added	Nicorandil found		
8.0	10.0	8.0	100.0	1.32
8.0	100.0	8.0	100.1	0.67
8.0	1000.0	8.0	100.0	0.72

a) Mean for five independent analyses.

Table 5. Determination of Nicorandil in Presence of Excipients

Nicorandil concentration (μg/ml)		Recovery (%)[a]	RSD (%)
Taken	Found		
5.6	5.6	100.0	0.78
10.4	10.4	100.0	0.46

a) Mean for five independent analyses.

tions such as lactose, starch, magnesium stearate and talc. The results (Table 5) revealed that the excipients did not interfere. However, oxidants such as N-bromosuccinimide and potassium iodate did not interfere up to 4.0×10^{-4} M and 2.0×10^{-3} M, respectively; above these concentrations, interference was observed. Potassium persulfate, even at a very low concentration, interferes with the determination process.

The results of recovery experiment are summarized in Table 6. As can be seen from the table that the mean recoveries for Nikoran, Korandil and Zynicor commercial tablets were found to be 100.0%, in each case, with RSD 0.50—0.78%, 0.50—0.77% and 0.51—0.88%, respectively. The recovery experiments have also confirmed that the common excipients present in tablets did not interfere.

The applicability of the proposed method has been tested for the determination of nicorandil in drug formulations. The results of the proposed method were compared with those obtained by the reference method[15] using point and interval hypothesis tests.[17] The results are summarized in Table 7. As can be seen from the Table 7 that student's t– and F–values at 95% confidence level did not exceed the theoretical t– and F–values. This statistical comparison has indicated that there is no significant difference in the performance of the proposed method and the reference method. The bias based on the recovery experiments was calculated. It is evident from the table that the true bias of all samples is $<\pm2\%$, confirming the recommendations of Canadian Health Protection Branch.

CONCLUSIONS

The proposed method is based on the reduction of

Table 6. Standard Addition Method: Evaluation of the Validity of the Proposed Method for the Recovery of Nicorandil

Drug formulations[a]	Concentration, μg/ml			Recovery (%)	RSD (%)	SAE	C.L.
	Taken	Added	Found±SD[a]				
Nikoran-5 (Samarth Pharma)	3.2	4.0	7.20±0.06	100.0	0.78	0.03	0.07
	3.2	8.0	11.20±0.06	100.0	0.50	0.03	0.07
Korandil-5 (Sun Pharma)	3.2	4.0	7.20±0.06	100.0	0.77	0.03	0.69
	3.2	8.0	11.20±0.06	100.0	0.50	0.03	0.07
Zynicor-5 (Zydus Medica)	3.2	4.0	7.20±0.06	100.0	0.88	0.03	0.08
	3.2	8.0	11.21±0.06	100.0	0.51	0.03	0.07

a) Mean for five independent analyses.

Table 7. Point and Interval Hypothesis Tests: Evaluation of the Applicability of the Proposed Method with the Reference Method at 95% Confidence Level

Drug formulations[a]	Proposed method		Reference method		*t*-value	*F*-value	θ_L[c]	θ_U[c]
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)				
Nikoran-5 (Torrent Pharma)	100.0	0.41	100.1	0.56	2.707	1.372	0.987	1.011
Korandil-5 (Sun Pharma)	100.0	0.41	100.0	0.57	1.895	1.391	0.990	1.011
Zynicor-5 (Zydus Medica)	100.1	0.43	100.1	0.52	1.083	1.227	0.982	1.017

a) Mean for five independent analyses. *b*) Theoretical *t*-value ($\mu=8$) and *F*-value ($\mu=4, 4$) at 95% confidence level are 2.306 and 6.39, respectively. *c* In pharmaceutical analysis, a bias, based on recovery experiments, of ±2% is acceptable.

nitroxyethyl group of nicorandil into nitrite ion and subsequent coupling with NED, which makes the method more sensitive and rapid as compared with other existing spectrophotometric method.[15] Statistical comparison of the results with those of reference method, has proved that the proposed method is accurate, precise and reproducible. No interference was observed from the denitrated nicorandil and common excipients.

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