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Research Article

Simple Spectrophotometric Method for the Determination of Nebivolol Hydrochloride Using Redox Reaction

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Abstract

A rapid, simple, precise, accurate, and sensitive spectrophotometric methods for the determination of nebivolol hydrochloride (NVH) in bulk sample and in pharmaceutical dosage forms is described. The methods are based on oxidation of the drug by ceric ammonium sulphate and/or N-bromosuccinimide in acidic medium and determination of the unreacted oxidant by measuring the decrease in absorbance for two different dyes; amaranth (AM) and methyl red (MR), at a suitable λ_{max}. Regression analysis of Beer's plots showed good correlation in the concentration ranges 1.0–14 μg/ml. The apparent molar absorbtivity, Sandell sensitivity, detection and quantitation limits were calculated. For more accurate results, Ringbom optimum concentration ranges were 2.5–11 μg/ml. The validity of the proposed method was tested by analyzing in pure and in dosage forms containing NVH. Statistical analysis of the results reflects that the proposed procedures are precise, accurate and easily applicable for the determination of NVH in pure form and in pharmaceutical preparations compared with the Official method.

Keywords: nebivolol hydrochloride, spectrophotometry, Oxidation reaction, N-Bromosuccinimide, ceric ammonium sulphate, Pharmaceutical analysis.

Introduction

Nebivolol (NVH) is chemically 1-(6-flourochroman-2-yl)-{[2-(6-fluoro-chroman-2-yl)-2-hydroxy-ethyl] amino}ethanol or 2,2'-azanediylbis (1-(6-flouro-chroman-2-yl) ethanol (Figure 1) [1–4], a new antihypertensive drug, is a racemate of two enantiomers with four chiral centres. The mechanism action of nebivolol is a competitive and highly selective β1–receptor antagonist and does not show an intrinsic sympathomimetic activity. Nebivolol is endowed with peripheral vasodilating properties mediated by the modulation of the endogenous production of nitric oxide and thus lowers peripheral resistance. The SRRR- enantiomer (d-nebivolol) is a potent and cardioselective β1–adrenergic blocker. The RSSS- enantiomer (l-nebivolol) has a favourable hemodynamic profile, in that normal energy supply during exercise is nor affected [5].

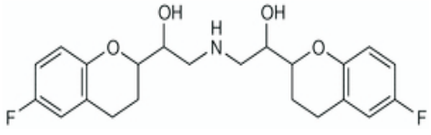

Figure 1: Structure of nebivolol

Nebivolol Hydrochloride is official in Indian Pharmacopoeia 2010 [6] and BP 2009 [7]. Literature survey reveals that few analytical methods were reported which include UV spectrophotometry [8–14], liquid-chromatography with tandem mass spectrometry [15], RP-HPLC and HPTLC methods [16] and derivative spectrometric determination [17,18], liquid chromatography coupled with electro spray ionization tandem mass spectrometry [19], Stability indicating RP-HPLC estimation [20,21], LC-MS [22,23], and visible spectrophotometry [24–28] techniques were reported.

The present investigation has been undertaken to develop visible spectrophotometric methods in which, the colored species obtained by the oxidation of the unreacted oxidant with the studied dyes with their absorption maximum and Beer's law is obeyed in the concentration range of 1.0–14 µg/ml. MR and AM are well known for their high absorptivity and they will have been utilized for estimation of excess oxidant. Where modern and expensive apparatus such as GLC, HPLC and HPTLC are not

Materials and Methods

Apparatus

All the absorption spectral measurement were made using JASCO v-530 (UV–VIS) spectrophotometer (Japan), with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10 mm matched quartz cells. pH-meter Orion-Research Model 601 A/Digital Ionalyzer was used for adjusting the pH of solution.

Reagents and standard solution

All chemicals used were of analytical or pharmacopoeia grade purity and water was bidistilled. Standard MVH was obtained from El-Obour Modern Pharmaceutical Industries Company, Egypt, its potency was 99.99 ± 0.39%. Stock MVH solution (100 μg/ml) was prepared by dissolving 0.01 g in water and adjusted to 100 ml with bidistilled water. Working solutions of lower concentration were prepared by serial dilutions.

Aqueous solutions of 2.0 × 10–3 M of AM and/or MR (Sigma Aldrich), were prepared by dissolving an appropriate weight in 100 ml bidistilled water.

A stock solution 1.0 M of H2SO4 was prepared by adding 5.4 ml of concentrated acid (Merck, Darmstadt, Germany, 98%, Sp. Gr. 1.84) to bidistilled water, cooled to room temperature, transfer to 100 ml with measuring flask, diluted to the mark and standardized as recorded [29].

A stock solution of 100 µg/ml NBS (Sigma-Aldrish) was freshly prepared by dissolving about 0.01 g of NBS in least amount of warm bidistilled water in a 100 ml measuring flask and then diluted to the mark with bidistilled water and standardized [30]. The solution was kept in an amber colored bottle and was diluted appropriately to get

100 µg/ml NBS for use in all methods. The NBS solution was stored in a refrigerator when not in use. A 1.0% w/v KBr solution was also prepared by dissolving 1.0 g of KBr in 100 ml water.

A stock solution of 2×10^{-3} M cerium(IV) ammonium sulphate (CAS) (E-Merk, Darmstadt, Germany) was freshly prepared by dissolving appropriate weight of CAS in the least amount of H2SO4 (2.0 M) then completed to the mark in a 100 ml calibrated flask with the same acid and kept in a dark bottle and a refrigerator when not in use.

Recommended procedure using CAS

Different aliquots (0.1-1.2 ml) of a standard 100 µg/ml NVH solution using AM and MR methods, respectively, were transferred into a series of 10 mL calibrated flasks followed by adding 2.0 mL of 1.0 M H_2SO_4 and 2.0 mL of (2 × 10-3 M) CAS solution. The flasks were stoppered and the contents were mixed well and the flasks were kept aside for 5.0 min at 60 ±1.0 oC with occasional shaking. Finally, 2.2 ml of 2×10^{-3} M AM and/or 1.0 mL of 2 × 10-3 < MR, dye solution was added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The decrease in color intensity of dyes were measured after 2.0 min against reagent blank solution treated similarly omitting the drug, at their corresponding λ_{max} 574, and 546 nm, respectively. The concentration of unknown was determined in each case from calibration graph which obtained by plotting the concentration of NVH against absorbance.

Recommended procedures using NBS

Different aliquots (0.1-1.4 ml), of a standard 100 µg/ml NVH solution using amaranth, and methyl red methods, respectively, were transferred into a series of 10 ml calibrated flasks by means of a micro burette. To each flask 1.25 ml each of 5.0 M HCl; 1.0 ml of NBS solution (100 µg/ml) and 1.0 ml of 1.0% (w/v) KBr were added successively. The flasks were stoppered, content mixed and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 0.7 and 1.0 ml of 2 × 10-3 M amaranth and methyl red solution, respectively, were added to each flask and mixed well and then the volume was diluted to the mark with water. The absorbance of each solution was measured at 556, and 560 nm for amaranth, and methyl red methods, respectively, after 2.0 min against a reagent blank.

Procedure for pharmaceutical formulations (tablets)

The contents of twenty tablets of each drug were weighed accurately and ground into a fine powder. An accurate weight of the powdered tablets equivalent to 20 mg NVH was dissolved in bidistilled water with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with bidistilled water for NVH in a 100 ml measuring flask to give 200 µg/ml stock solution of NVH for analysis by spectrophotometric methods. A convenient aliquot was then subjected to analysis by the spectrophotometric procedures described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

Results and Discussion

Absorption spectra

Many dyes are irreversibly destroyed to colorless species by oxidizing agents in acid medium [31]. The proposed spectrophotometric methods are based on the reaction between NVH and measured excess of NBS and/or CAS, and subsequent determination of the latter by reacting it with a fixed amount of amaranth, and methyl red dye and measuring the absorbance at their λ_{max}. These methods make use of the bleaching action of NBS and/or CAS on the dyes, the decolorization being caused by the oxidative destruction of the dyes. NVH when added in increasing concentrations to a fixed concentration of NBS and/or CAS consumes the latter and there will be a concomitant decrease in the concentration of oxidant. When a fixed concentration of either dye is added to decreasing concentrations of NBS and/or CAS, a concomitant increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentrations of NVH.

Chemistry of the reactions

NBS is a strong oxidizing or brominating agent and perhaps the most important positive bromine containing organic compound used to determine many pharmaceutical compounds [32–36]. It is also used for the specific purpose of brominating alkenes at the allylic position [37]. Because of its high oxidation potential and excellent solution stability, cerium(IV) ammonium sulphate, has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds [38–42].

The analytical reactions involved two steps; the first one was concerned with the oxidation of the investigated drugs with a known excess amount of oxidant in acid medium. The second step involved the determination of the excess residual of oxidant via its reaction with a fixed amount of both amaranth, or methyl red dyes and measuring the absorbance at the respective λ_{max}. The tentative reaction scheme of spectrophotometric methods is shown in the following equations. In all methods, the absorbance increased linearly with increasing concentration of drugs. The latter methods make use of the bleaching action of NBS and/or CAS on dyes, the discoloration being caused by the oxidative destruction of the dye.

$$\text{NVH} + \text{known excess of Ce(IV)} + \text{H+} \longrightarrow \text{oxidation product of NVH} + \text{Ce(III)}$$

$$\text{Unreacted Ce(IV)} + \text{known excess MR} \longrightarrow \text{oxidation product of dye} + \text{MR}$$

Selection of acid type and concentration

The reaction between NVH with NBS and/or CAS were performed in different acid media CH3-COOH, HCl, HNO_3, and H_2SO_4 solutions. Better results were suitable in hydrochloric acid medium using NBS, whereas H_2SO_4 medium was the optimum on using CAS. The effect of HCl and /or H_2SO_4 concentration on the redox reaction between NVH and NBS and/or CAS were studied by varying the concentration of acid keeping the concentrations of NBS and/or CAS and drug fixed. The reaction was found to be rapid yielding a constant absorbance with maximum sensitivity and stability when the HCl concentration was 5.0 M and maintained in the range of 0.25—3.0 ml of HCl (5.0 M) in a total volume of 10 ml. The results indicated that, at 1.0-1.5 ml of HCl (5.0 M), there were almost same absorbance values were obtained in the presence of NVH, the absorbance values obtained were constant and were almost the same as those of the reagent blank. At the acid volumes less than 1.0 ml, reaction led to go slower and incomplete. Therefore, 1.25 ml of HCl (5.0 M) was used though out the study for both drug.

The effect of H_2SO_4 concentration on the redox reaction between NVH and CAS was studied by varying the concentration of acid keeping the concentrations of CAS and drug fixed. The reaction was found to be rapid yielding a constant absorbance with maximum sensitivity and stability when the H_2SO_4 concentration was 1.0 M and maintained in the range of 0.5—3.0 ml of H_2SO_4 (1.0 M) in a total volume of 10 ml. The results indicated that, at 2.0-2.5 ml of H_2SO_4 (1.0 M), there were almost same absorbance values were obtained in the presence of NVH, the absorbance values obtained were constant and were almost the same as those of the reagent blank. At the acid volumes less than 1.5 ml, reaction led to go slower and incomplete. Therefore, 2.0 ml of 1.0 M H_2SO_4 was used though out the study for both drug.

Effect of NBS and/or CAS concentration

To investigate the optimum concentration of NBS and/or CAS, different concentrations were treated in the range of 0.25—3.0 ml with a fixed concentration dyes in HCl and/or H_2SO_4 medium and the absorbance was measured at the optimum wavelength. It was found that maximum color intensity of the products was achieved with 1.0

m; of NBS (100 µg/ml), whereas for CAS the higher absorbance is obtained with 2.0 ml of 2 × 10^{-3} M CAS were selected for all further studies due to high concordant results obtained (Figure 2).

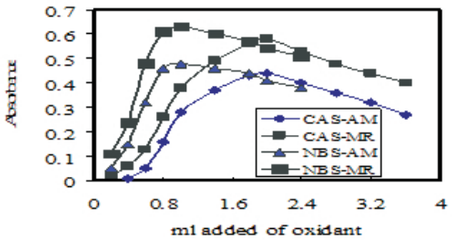

Figure 2: Effect of volume of NBS (100 µg/ml) and CAS 2 × 10^{-3} M on the oxidation product of NVH using AM and MR dyes in HCl and H_2SO_4 media

Effect of KBr concentration

The effect of KBr concentration was studied in the range of 0.5-2.5 ml. 1.0 ml of 1.0% (w/v) KBr was chosen as an optimum volume to accelerate the oxidation process on using NBS, whereas it has no effect on using CAS.

Effect of dye concentration

The effect of amaranth, or methyl red concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of NBS and/or CAS. The effect of dye concentration was studied in the range of 0.25-3.0 ml of each dye (2 × 10−3 M). It was found that maximum color intensity of the oxidation products was achieved with 1.0 ml of 2 × 10^{-3} M of MR for both NBS and CAS, whereas 0.7 and 2.2 ml of 2 × 10^{-3} M AM were used for NBS and CAS, respectively (Figure 3).

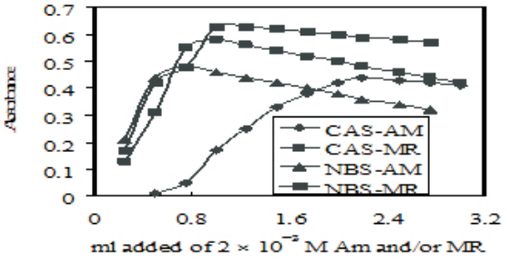

Figure 3: Effect of volume of dyes (2 × 10^{-3} M) of the oxidation product of NVH using NBS and/or CAS and dyes at the other optimum conditions

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 25 to 60 °C in water bath. It was found that raising the temperature does not accelerate the oxidation process and does not give reproducible results on using NBS as oxidant, so maximum color intensity was obtained at room temperature (25 ± 2 °C). On using CAS the redox reaction increase by increasing temperature upto 60 ± 1.0 oC. The effect of mixing time required completing oxidation of the studied drugs and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0-20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min at room temperature (25±2 °C) for using NBS and (60 ± 1.0 oC) using CAS. The time required for complete oxidation of the drug is not critical and any delay up to 15 min in the determination of unreacted NBS and/or CAS had no effect on the absorbance. A 2.0 min standing time was found necessary for the complete bleaching of the dye color by the residual NBS and/or CAS was found necessary for complete reduction of residual NBS and/or CAS by the examined dyes and the absorbance of the unreacted dye was stable for at least 6.0 h.

Effect of sequence of addition

The optimum sequence of addition was drug–acid–oxidant and then dye. Other sequences gave lower absorbance values under the same experimental conditions.

Method validation

The proposed methods have been validated for linearity, sensitivity, accuracy, precision, selectivity and recovery. Linearity and sensitivity Under the optimum conditions a linear correlation was found between absorbance at λmax and the concentration of NVH in the ranges of 1.0−14 µg mL−1. The calibration graph is described by the equation:

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL−1, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in Table 1. For accurate determination, Ringbom concentration range [43] was calculated by plotting log concentration of drug in µg mL−1 against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of NVH and represented in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [44] and illustrated in Table 1. The high molar absorptivity and lower Sandell sensitivity values reflect the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis [45] between the results achieved from the proposed methods and that of the reported method.

Regarding the calculated Student's t-test and variance ratio F-test (Table 1), there is no significant difference between the proposed and reported method [6,7] regarding accuracy and precision. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas [44, 45]:

$LOD = 3.3\,\sigma/s$ and $LOQ = 10\,\sigma/s$ (2)

Where σ is the standard deviation of two reagent blank determinations, and s is the

slope of the calibration curve.

Accuracy and precision

In order to evaluate the precision of the proposed methods, solutions containing four different concentrations of NVH were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 2. Lower values of the relative standard deviation (RSD%) and percentage relative error (RE%) indicate the precision and accuracy of the proposed methods. The assay procedure was repeated six times, and RSD % values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). For the same concentrations of drugs inter- and intra-day accuracy of the methods was also evaluated. The percentage recovery values with respect to found concentrations of each drug were evaluated to ascertain the accuracy of the methods. The recovery values close to 100 % as compiled in Table 2 shows that the proposed methods are very accurate.

Table 1: Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of NVH

Parameters	NBS		CAS	
	AM	MR	AM	MR
Beer's law limits, μg/ml	1.0−12	1.0−12	1.0−14	1.0−14
Ringboom limits, μg/ml	2.5−10	2.5−10	2.5−11	2.0−11.5
Molar absorptivity, x 10^4 L/mol cm	2.0956	1.7502	1.8077	1.9595
Sandell sensitivity, ng/cm^2	26.80	32.08	29.54	30.58
Regression equation[a]				
Intercept (a)	0.0056	0.0014	0.004	- 0.009
SD of intercept (S_a)	0.009	0.016	0.005	0.006
Slope (b)	0.0358	0.0307	0.0454	0.0306
SD of slope (S_b)	0.018	0.027	0.007	0.008
Correlation coefficient,(r)	0.9993	0.9999	0.9996	0.9997
Mean ± SD	100.81±1.06	100.42 ± 0.89	100.36±1.40	100.01±1.33
RSD%	1.05	0.89	1.40	1.33
RE%	1.10	0.93	1.47	1.40
Limit of detection, μg/ml	0.59	0.56	0.55	0.58
Limit of quantification, μg/ml	1.97	1.87	1.83	1.93
Calculated *t*-value [b]	1.03	0.34	0.90	0.52
Calculated *F*-value [b]	3.58	2.53	1.18	1.06

[a] A = a + bC, where C is the concentration in μg/ml, A is the absorbance units, *a* is the intercept, *b* is the slope.
[b] The theoretical values of *t* and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

Table 2: Results of intra-day and inter-day accuracy and precision study for NVH obtained by the proposed methods

Method	Taken (µg/ml)	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
			Intra-day using Ce(IV)		
AM	2.0	99.60	1.07	-0.40	1.992 ± 0.022
	4.0	99.30	1.42	-0.70	3.972 ± 0.059
	8.0	100.70	1.65	0.70	8.056 ± 0.14
MR	3.0	99.00	0.76	-1.0	2.97 ± 0.024
	6.0	99.40	1.28	-0.60	5.964 ± 0.08
	9.0	99.80	1.93	-0.20	8.982 ± 0.182
			Intra-day using NBS		
AM	4.0	99.20	0.83	-0.80	3.968 ± 0.035
	8.0	100.30	1.14	0.30	8.024 ± 0.096
	12	99.60	1.55	-0.40	11.952 ± 0.194
MR	5.0	99.00	0.90	-1.0	4.95 ± 0.047
	10	99.30	1.20	-0.70	9.93 ± 0.125
	15	99.50	1.70	-0.50	14.93 ± 0.266
			Inter-day using Ce(IV)		
AM	2.0	99.30	0.95	-0.70	1.986 ± 0.02
	4.0	100.60	1.38	0.60	4.024 ± 0.058
	8.0	99.40	1.85	-0.60	7.952 ± 0.154
MR	3.0	99.20	0.82	-0.80	2.976 ± 0.026
	6.0	100.40	1.16	0.40	6.024 ± 0.073
	9.0	100.10	1.40	0.10	9.009 ± 0.132
			Inter-day using NBS		
AM	4.0	99.40	0.51	-0.60	3.976 ± 0.021
	8.0	99.60	0.97	-0.40	7.968 ± 0.081
	12	99.10	1.58	-0.90	11.892 ± 0.197
MR	5.0	99.10	0.65	-0.90	4.955 ± 0.034
	10	99.70	0.90	-0.30	9.97 ±0.094
	15	99.40	1.20	-0.60	4.91 ± 0.188

[a] RSD %, percentage relative standard deviation; RE %, percentage relative error.
[b] Mean ± standard error.

Robustness and ruggedness

For the evaluation of method robustness, volume of HCl and/or H2SO4 was slightly altered (± 0.2 mL) and the reaction time (after adding NBS and/or CAS, time varied was 5.0 ± 2.0 min) were slightly varied deliberately in the proposed methods. The analysis was performed with altered conditions by taking three different concentrations of drugs and the methods were found to remain unaffected as shown by the RSD values in the ranges of 0.85−2.15% for NVH. Methods ruggedness was expressed as the RSD of the same procedure applied by two different analysts as well as using three different instruments (spectrophotometers). The inter-analysts RSD were in the ranges 0.80−2.35% NVH, whereas the inter-instruments RSD ranged from 0.75−2.45% NVH suggesting that the developed methods were rugged. The results are shown in Table 3.

Table 3: Results of method robustness and ruggedness (all values in RSD%) studies for NVH

Methods	Nominal amount concentration (µg mL^{-1})	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a,b]			
		Acid Volume (n=3)	Reaction Time (n=3)	Different analysts (n=3)	Different instruments (n=3)
			NBS[a]		
AM	2.0	1.14	1.06	0.90	0.80
	4.0	1.70	1.95	1.70	1.80
	8.0	2.20	2.30	2.25	2.45
MR	3.0	1.15	0.92	1.25	0.85
	6.0	1.60	1.80	1.94	1.70
	9.0	2.30	2.15	2.50	2.10
			CAS[b]		
AM	2.0	1.10	0.90	0.85	0.94
	4.0	2.10	1.85	1.70	1.90
	8.0	2.50	2.30	1.85	2.05
MR	4.0	1.02	0.78	1.10	0.90
	8.0	1.50	1.42	1.30	1.50
	12	2.20	1.90	2.10	2.40

[a] Volume of (5.0 mol L−1) HCl is (1.25 ± 0.2 mL) and reaction time is (5.0 ± 2.0 min) (after adding NBS) were used.
[b] Volume of (1.0 mol L−1 HCl is (2.0 ± 0.2 mL) and reaction time is (5.0 ± 2.0 min) (after adding CAS) were used.

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure drugs (50, 100 and 150% of the level present in the tablet) to a fixed amount of drugs in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated. The results of this study presented in Table 4 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Table 4: Results of recovery experiments by standard addition method for the determination of NVH, in tablets using the proposed methods

Samples	Taken drug in Tablet (µg/ml)	Pure drug Added (µg/ml)	AM		MR	
			found (µg/ml)	Recovery a (%) ± SD	found (µg/ml)	Recovery a (%) ± SD
Neblet tablets using NBS	4.0	2.0	5.976	99.60 ± 0.40	5.952	99.20 ± 0.65
		4.0	7.976	99.70 ± 0.72	7.96	99.50 ± 0.87
		6.0	10.02	100.20 ± 0.86	9.90	99.00 ± 1.08
		8.0	8.015	100.20 ± 0.39	7.960	99.40 ± 0.54
Neblet tablets using CAS	2.0	2.0	1.992	99.60 ± 0.57	2.004	100.20 ± 0.98
		4.0	6.01	100.17 ± 0.82	5.92	98.67 ± 1.13
		6.0	8.15	101.88 ±0.67	8.10	101.25 ± 0.78
		8.0	10.15	101.50 ± 1.04	9.99	99.90 ± 0.79
		10	12.12	101.00 ± 0.79	11.84	98.67 ± 1.22

[a] Average of six determinations.

Application of pharmaceutical formulations (tablets)

The proposed methods were applied to determine NVH in pharmaceutical formulations (tablets). The results in Table 5 showed that the methods are successful for the determination of NVH and that the excipients in the dosage forms do not interfere. A statistical comparison of the results obtained from the assay of NVH by the proposed methods and the reported methods [6,7] for the same batch of material is presented in Table 5. The results agree well with the label claim and also were in agreement with the results obtained by the reported methods [6.7]. When the results were statistically compared with those of the reported methods by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [45]. Hence, no significant difference between the proposed methods and the reported methods at the 95 % confidence level with respect to accuracy and precision.

Table 5: Results of analysis of tablets by the proposed methods for the determination of NVH, and statistical comparison with the reference methods

Samples	Recovery a (%) ± SD		Reported methods [7]
	Proposed Method using Ce(IV)		
	AM	MR	
	NBS		
Neblet tablets	99.45 ± 0.80	99.92 ± 0.64	101.36 ± 0.47
t-value [b]	1.02	1.23	
F-value [b]	1.56	1.85	
Concor tablets	100.21 ± 0.50	99.90 ± 0.67	98.64 ± 0.38
t-value [b]	0.82	0.75	
F-value [b]	1.79	3.1	
	CAS		
Neblet tablets	98.95 ± 0.60	99.50 ± 0.72	98.76±0.54
t-value [b]	1.03	0.64	
F-value [b]	2.12	1.77	

Conclusion

Two new, useful simple, rapid and cost-effective spectrophotometric methods have been developed for determination of NVH in bulk drugs and in its tablets using NBS and/or CAS as oxidizing agent and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous methods reported for NVH. The assay methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of NVH in pure and dosage forms.

Conflicts of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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