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A New Spectrophotometric Method for the Determination of Nadolol

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A new spectrophotometric method has been developed for the assay of nadolol in pure form and in tablets. The assay procedure is based on a derivatization methodology employing 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) as a diazo coupling reagent. The azo dye formed between nadolol and CDNBD absorbed visible light at the wavelength maximum of 416 nm (λ_{max}) demonstrating a bathochromic shift from the absorption maximum of nadolol. Optimization studies established an optimal reaction time of 10 min at 60 ^{o}C. The assays were linear over 1.25-10 μg ml^{-1} of nadolol, and the reaction occurred by a 3:1 reagent/drug stoichiometric ratio. The method is found to be selective and has a lower detection limit of 0.29 μg ml^{-1}. Recovery studies over three days gave mean recovery of 101.4% (RSD 3.0%). This new method has been successfully applied in the determination of nadolol and nadolol/bendroflumethiazide tablets with accuracy and precision similar to the official (USP) HPLC procedure ($p > 0.05$). The new procedure has the advantages of high sensitivity, lower limit of detection and could find application as an in-process quality control method for nadolol.

Keywords: Nadolol, Diazo coupling, Phenol ether, CDNBD, Spectrophotometry

INTRODUCTION

Nadolol, 5-[3[(1,1dimethylethyl)amino]-2-hydroxylpropoxyl]-1,2,3,4-naphthalene diol [1], is a long acting antagonist with equal affinity for both β_1 and β_2- adrenergic receptors. It is devoid of both membrane-stabilizing and intrinsic sympathomimetic activity, with a distinguishing characteristic of relatively long half-life compared to other adrenoceptor antagonists [2].

Nadolol is used in the management of hypertension, angina pectoris and cardiac arrhythmias, hyperthyroidism and migraine. Like other β-adrenergic blockers, however, it causes a mild increase in plasma volume and antagonizes the thyroxine-mediated stimulation of β-adrenergic receptors. Nadolol is marketed as tablets containing nadolol alone or in combination with bendroflumethiazide.Nadolol has been assayed by non-aqueous titration [3,4], HPLC analysis in biological fluids [5,6], fluorimetry [7] as well as analysis of the enantiomers in human plasma [8]. The dosage form has been assayed using HPLC [4,9,10,11] and colorimetry using 2,4-dinitrophenylhydrazine after oxidation with periodic acid [12], extractive method in chloroform with bromophenol blue [13] and charge transfer complexation with 4-chloro-7-nitro-2,1,3-benzoxadiazole [14]. The majority of the methods based on colorimetry suffer from the disadvantage of poor detection limits, high calibration range and extensive extraction procedures.

In continuation of our work on the development of relatively simple colorimetric methods for the assessment of organic compounds of pharmaceutical importance [15,16], we report a new spectroscopic method for the determination of nadolol in bulk and dosage form using the newly developed 4-

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carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) [17, 18,19].

EXPERIMENTAL

Chemicals and Reagents

We purchased Corgard® tablets, 80 mg nadolol (Sanofi~Synthelabo), Corgaretic™ 40 tablets, 40 mg nadolol (Sanofi~Synthelabo), nadolol CRS (Sigma-Aldrich Inc., USA), ethanol, ethylacetate, glacial acetic acid, concentrated sulfuric acid, sodium nitrite (all analytical reagents from BDH, Poole, England), pre-coated thin layer chromatography plates GF_{254}, 0.2 mm (Merck, Germany), but the 4-amino-3,5-dinitrobenzoic acid (ADBA) was synthesized in our laboratory.

Equipment

We used a UV-Vis spectrophotometer (Unicam Aurora, Helios Scan Software v 1.1, Pye Unicam, England), analytical balance H80 (Mettler, UK), ultrasonic bath (Langford Electronics, UK), vortex mixer (Griffins and George Ltd., UK), and CECIL 1000 series HPLC with variable wavelength detector (CECIL Instruments Cambridge, England).

Preparation of CDNBD Solution and Nadolol Stock solution

An optimized process was used for the preparation of the CDNBD reagent solution. It was made routinely from an assay kit comprised of the following: 0.125% solution of ADBA in concentrated sulfuric acid (Solution A), 5% solution of dried sodium nitrite in concentrated sulfuric acid (Solution B), 85% orthophosphoric acid (Solution C) and powdered urea (Solute D).

Solutions A and B (10 ml of each) were mixed by magnetic stirrer in a suitable beaker maintained in an ice-bath. Ice-cold solution C (20 ml) was added slowly, with vigorous mixing. The reaction mixture was covered with aluminum foil and maintained at 0 ^{o}C for 60 min after which Solute D (2 g) was added slowly. The mixture was further stirred magnetically until the ensuing frothing subsided [19]. Nadolol stock solution was made by dissolving nadolol powder (10 mg) in 10 ml glacial acetic acid to give a stock solution of 1 mg ml^{-1}.

Optimization Studies

Temperature and reaction time were optimized using the method of steepest ascent [20]. An aliquot of the nadolol stock solution (100 µl) was added to the reagent solution (500 µl) in a test tube and the reaction mixture was vortexed for 10 sec followed by incubation at 30 ^{o}C and then 50 ^{o}C, for 5 and 20 min, respectively. Similar experiments were performed at 60 ^{o}C and 80 ^{o}C. Each determination was performed in duplicate. The reaction was terminated by the addition of ice-cold water (3 ml) to the reaction mixture kept in an ice bath. Brine solution (1 ml) was then added and the aqueous solution was extracted with ethylacetate (5 ml) and kept in a vial wrapped with aluminum foil. A blank reagent solution was similarly prepared, replacing nadolol stock solution with glacial acetic acid. The absorption spectrum of the reaction mixture extract was determined against the absorption of the blank reagent extract, using the UV-Vis spectrophotometer at the optimal absorption wavelength of 416 nm.

The optimal reaction time was determined by adding nadolol stock solution (100 µl) to the reagent solution (500 µl) in eight test tubes. The coupling reaction was carried out by incubation at 60 ^{o}C for 0, 2, 5, 10, 20, 25, and 30 min. Ethylacetate extracts of the reaction mixture was prepared as usual; after each reaction time the absorbance was spectrophotometrically measured at 416 nm. The optimal reaction time was then determined as the time corresponding to the maximal absorption of the samples. All determinations were performed in duplicate.

Stoichiometric Ratio of Drug-Reagent Adduct Formation

Equimolar solutions (0.918 mM) of the reagent and the drug stock solution were prepared using the procedure described above. In eleven different test tubes, 0, 0.2, 0.25, 0.33, 0.4, 0.50, 0.6, 0.67, 0.75, 0.8, and 1.0 ml of the reagent solution was added. Each tube was then made up to 1.0 ml with the drug stock solution. Blank determinations were similarly carried out using glacial acetic acid in place of the drug stock solution. The mixtures were vortexed for 10 sec and kept at 60 ^{o}C for 10 min. Brine solution (1 ml) was added to each tube, followed by extraction with 5 ml ethylacetate. The absorbance was measured at 416 nm against the blank and the absorbance values obtained were plotted against the mole

fraction of the reagent solution. Each determination was carried out in duplicate.

Assay of Dosage Forms

The amount of powdered tablets containing 10 mg of nadolol were weighed and dispersed in glacial acetic acid (6 ml) and mixed in an ultrasonicator for 5 min. The solution was filtered through a cotton wool plug into a 10 ml volumetric flask. The volume was then made up with more glacial acetic acid, rinsing the filter aid in the process. The drug stock solution (40 µl) was added to the reagent solution as usual. The mixture was vortexed for 10 sec and kept at 60 ^{o}C for 10 min, followed by extraction as described above. The absorbance was measured at 416 nm. The amount of nadolol in the tablets was then determined by interpolation from calibration lines. The sample analysis was repeated using the USP [4] HPLC procedures.

The assay results using the two methods (USP method and the spectrophotometric method reported here) were compared using student's t-test. The confidence interval was also determined for the CDNBD assay results.

Assessment of Method Selectivity

Two approaches were adopted for investigating the selectivity of this reported method. In the first procedure, standard solutions of nadolol stock (40 µl) were spiked into tablet excipients (namely gelatin, starch, magnesium stearate, lactose and a mixture of these substances). The recovery was determined and then compared with the recovery from reference samples alone. In the second procedure, powdered samples of the two brands of tablets investigated and the nadolol reference sample were kept in a 100 ^{o}C oven for 5 h. Afterwards, a methanolic solution of the tablets and the reference were analyzed for possible degradation products. Three separate chromatographic systems were adopted.

Validation of Methods

Calibration lines using standard solutions of 0, 1.25, 2.5, 5.0, 7.5, and 10 µg ml^{-1} nadolol were carried out using the optimal analytical conditions as described above. Linear regression analysis was used to calculate the slope, intercept and the coefficient of determination (r^2) of each calibration line. The assay precision and accuracy were determined as documented by USP [20]. The limit of detection was computed as previously described as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank [21].

RESULTS AND DISCUSSION

Nadolol coupled readily with CDNBD to give a pink color after about 1 min and later became more intensely pinkish-orange at higher temperatures. Nadolol does not possess strong UV absorption. The majority of the methods that have been described are chromatographic techniques, while few colorimetric procedures are available. The drug is officially listed in the USP 2000, with HPLC being the method of analysis.

This will be the first described analytical procedure for nadolol based on azo dye formation. The UV-Vis spectra of nadolol, nadolol-CDNBD adduct and the reagent are presented in Fig. 1. The azo adduct is found to give a strong and intense new absorption maximum at 416 nm which was selected as the analytical wavelength. This new λ_{max} also clearly demonstrates that the azo-dye formation brings the two rings of nadolol into conjugation, forming an adduct that absorbs at 416 nm. Nadolol, as previously reported, has no light absorption at values lower than 240 nm and higher than 290 nm. Its two main peaks are 270 and 278 nm with no alkaline shift. The absorptivity at 416 nm, when the adduct was incubated at 60 ^{o}C, was found to be profoundly higher than when incubation was carried out at room temperature. Thus, it seems that elevated temperature is required to hasten the coupling reaction. The adduct of nadolol with CDNBD exhibited a strong polar character and could not be successfully extracted with ethyl acetate. In all cases, a salting-out with brine solution was optimized and adopted prior to the extraction from aqueous solution.

The optimization procedure for the coupling reaction temperature was performed two times (5 and 20 min) at 30, 50, 60, and 80 ^{o}C, as presented in Fig. 2. At 5 min, the absorbance rose gradually from 30 ^{o}C and peaked at 60 ^{o}C declining at 80 ^{o}C. Therefore 60 ^{o}C was selected as optimum temperature for the azo dye formation. A similar pattern was produced for the adduct at 20 min, whereby the absorbance gradually rose to a maximum at 60 ^{o}C. The values obtained at

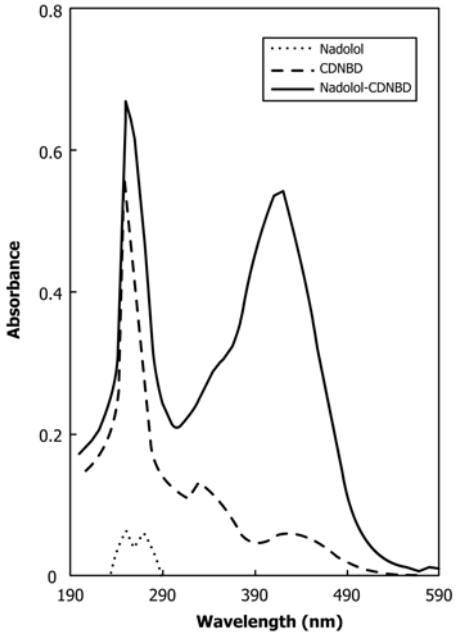


Fig. 1. Absorption spectra of nadolol, nadolol-CDNBD azo adduct and CDNBD in ethyl acetate using 10 µg ml^{-1} of nadolol and 0.5 ml of CDNBD reagent at 60 ^oC for adduct and blank reagent.

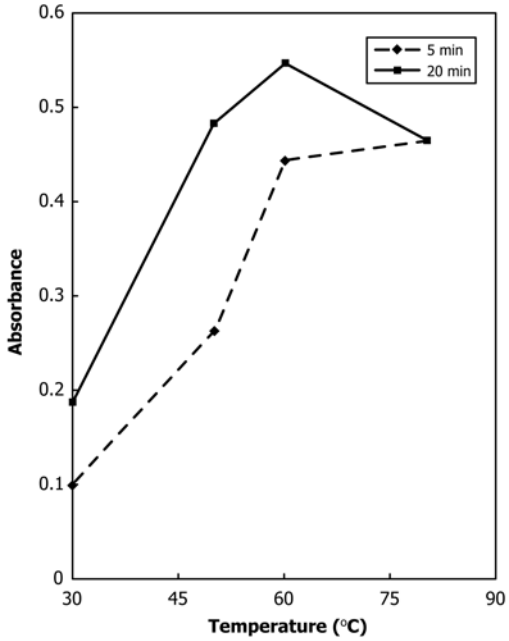


Fig. 2. Optimization of coupling reaction temperature using 8 µg ml^{-1} of nadolol and absorbance measured at 416 nm.

20 min at all four temperatures were higher than corresponding values at 5 min (viz: 0.466 AU at 80 ^oC, 20 min compared to 0.464AU at 80 ^oC, 5 min).

Optimization of the coupling reaction time was thereafter carried out at 60 ^oC for 0, 2, 5, 10, 20, 25, and 30 min, as presented in Fig. 3. The absorbance value gradually increased from the beginning of the reaction and peaked at 10 min. There was a gradual decline of the absorbance readings beyond 10 min. Ten minutes was therefore selected as the optimum time. Thus, for the coupling of CDNBD to nadolol, the optimum conditions used were 60 ^oC and 10 min.

Results of the stoichiometric ratio determination for the formation of the nadolol-CDNBD adduct are shown in Fig. 4. An anomalous result was produced whereby the drug combined with CDNBD at a mole ratio of 1:3, i.e. maximum absorbance was only obtained when three moles of CDNBD to one mole of nadolol was used. While this may not appear structurally feasible, there is the likelihood of other reactions taking place apart from coupling. TLC revealed that only one product was formed, thus the likelihood of multiple products is eliminated, and only one product was formed with the rate of reaction being hastened by a high concentration of reagent and temperature elevation. The series of reactions that may have occurred to generate the eventual azo adduct is presented in Scheme 1. The first stage is the coupling reaction, closely followed by the cleavage of the ether linkage as reported for the coupling of phenol ethers with diazonium ions [22]. The third stage, the dehydration of the 1,2-diol, is feasible due to the mixture of H_3PO_4 and H_2SO_4 in the coupling medium, a reagent mixture commonly utilized for the mild dehydration of diols [23]. Thus it is most likely that the nadolol molecule has 5,8-dihydro-1-naphthol as the residue the precursor molecule used in its synthesis. The above reaction sequence taking place consecutively or simultaneously may have led to the 3:1 mole ratio (reagent: drug) observed.

In order to accommodate the 3:1 mole ratio obtained, various aliquots of a 1 mg ml^{-1} stock solution of nadolol in glacial acetic acid was utilized subsequently. The regression line equation for the analysis of the nadolol azo adduct was found to be $Y = 0.01798X + 0.103$ ($r^2 = 0.9962$), where Y is

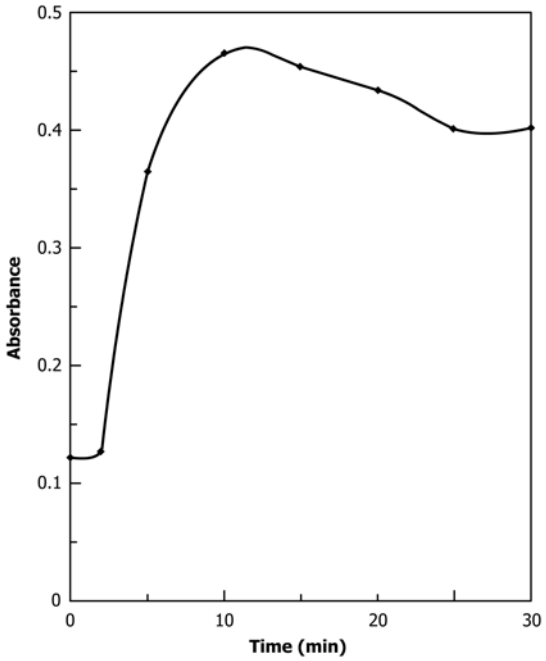


Fig. 3. Optimization of coupling reaction time at 60 °C.

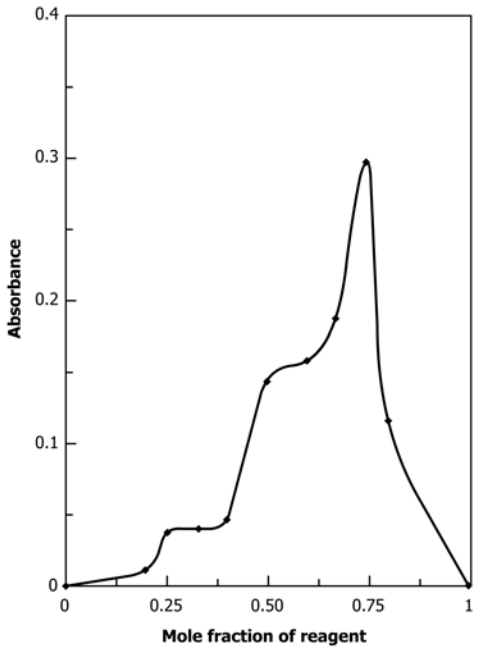


Fig. 4. Stoichiometric ratio determination for nadolol-CDNBD adduct formation at 60 °C for 10 min.

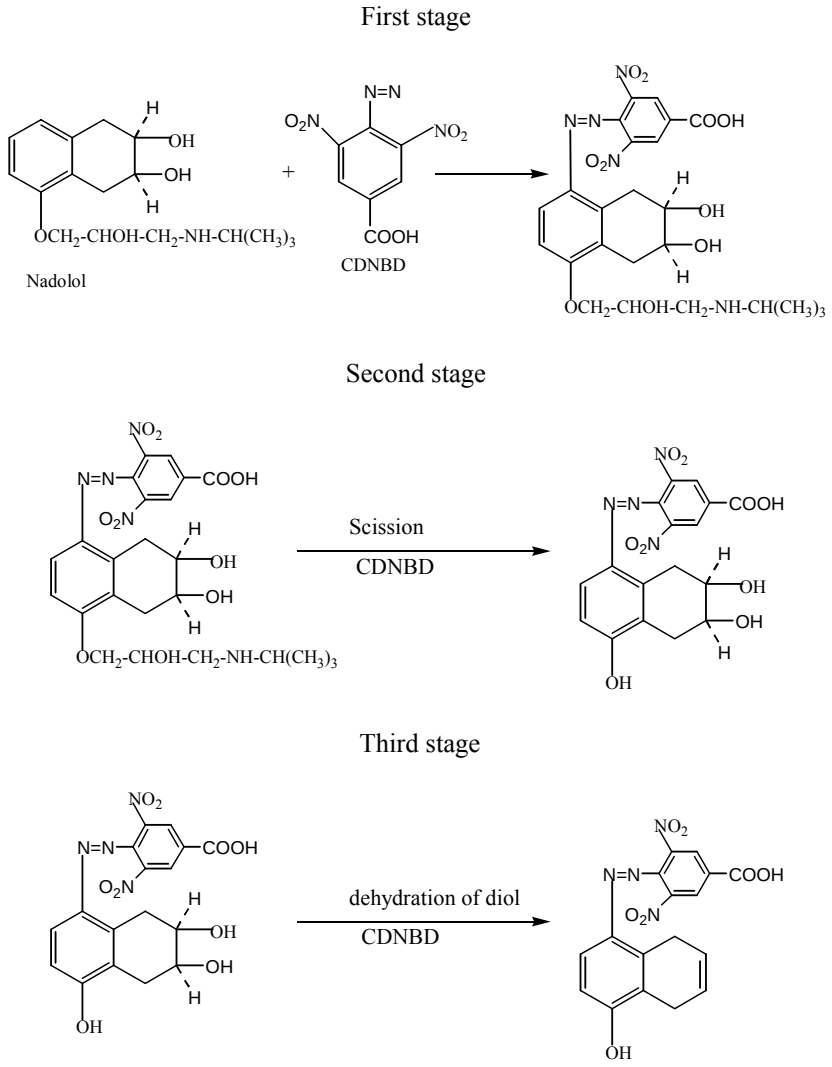
the absorbance and X is the concentration in μg ml^{-1}. The limit of detection is 0.29 μg ml^{-1} (calculated from the expression, $Y = Y_B + 3S_B$, where Y = LOD signal; Y_B = Blank signal and S_B is given by $S_{Y/X} = \{\Sigma (y_i - y)^2/(N-2)\}^{1/2}$ [21]).

The 95% confidence limit for the slope and intercept are respectively 0.01798 ± 0.00053 and 0.103 ± 0.003. The seemingly high intercept might be due to the fact that the reagent has a minor peak at around 430 nm. The molar absorptivity of the nadolol-CDNBD adduct is given as 1.319×10^4.

The accuracy and precision for the assay of nadolol using CDNBD was carried out at the three different concentrations of 4, 6, and 8 μg ml^{-1} (Table 1). As shown in the last column in Table 1, the best precision and accuracy were obtained for the 8 μg ml^{-1} concentration of analyte and this was therefore selected for subsequent analysis. The overall recovery of nadolol from the sample matrix was found to be 101.4 ± 3.0%. This recovery is found to compare favorably with some HPLC methods previously reported for nadolol. The HPLC method of Patel *et al.* [9] gave recoveries of 100.4 and 100.6% while that of Perlman *et al.* [11] using phenyl column gave recoveries of 99.4% (RSD 0.4%) for nadolol.

When compared to other previously described colorimetric methods, the new method described in this work is found to provide the advantages of high sensitivity, good accuracy and comparable reproducibility. The colorimetric method of Ivashkiv [12] using 2,4-dinitrophenylhydrazine to generate hydrazone of oxidized nadolol only absorbed maximally at 352 nm with a calibration range of 0-200μg. The recovery ranged between 99 and 100.66% for different analyte concentrations. In the CDNBD method, λ_{max} of the azo adduct was 416 nm and a micro-analyte range of 1.25-10 μg ml^{-1} was used with comparable accuracy. Similarly, the dye technique of Ozden and Gumus [13] using bromophenol blue was sensitive only in the range 60-600 μg nadolol. The advantage of CDNBD over the charge-transfer technique of Amin *et al,* [14] using 4-chloro-7-nitro-2,1,3-benzodiazole, is also observed. In this procedure, the linear range was 0.4-60 μg ml^{-1} with a recovery of 99.3 ± 1.1%. The determination of nadolol using CDNBD is therefore not only a novel approach but also a sensitive and more convenient application.

The results of the analysis of both brands of nadolol tablets using the new method and the USP 2000 HPLC method are presented in Table 2. Statistical analysis using Microsoft



Scheme 1

Excel software gave p-values for both the F-ratio and t-test that are greater than 0.05, hence there is no significant difference in the content of the nadolol between the two methods. In addition, the accuracy was not significantly affected when the tablet samples were spiked with reference samples.

In the assessment of method selectivity, the percentage recovery obtained from the use of the 8 μg ml^{-1} analyte concentration was 101.21%. The following percentage recoveries were obtained in the presence of excipients: 100.2% (lactose), 102.1% (starch), 100.63% (gelatin), 101.6% (magnesium stearate) and 101.31% (mixture of excipients). The closeness of the recoveries suggests a lack of interference from tablet excipients and thereby establishes some degree of selectivity. The second approach using TLC for tablets kept at an elevated temperature shows the absence of any degradation product (Table 3) and that Nadolol appears to be thermally stable. The presence of any degradation product may invalidate the result or make prior separation necessary. Routine TLC investigation of tablets to be analyzed before performing the full assay will guarantee some measure of specificity of the method.

Table 1. Accuracy and Reproducibility of the Spectrophotometric Method

Concentration ($\mu g\ ml^{-1}$)	Day 1		Day 2		Day 3		Statistics	
	Mean	RSD (%)[a]	Mean	RSD (%)[a]	Mean	RSD (%)[a]	Mean	RSD (%)[a]
4	97.6	2.6	105.6	1.3	97.6	2.6	100.3	4.6
6	96.0	2.1	98.7	2.2	102.0	3.7	99.1	2.7
8	101.9	1.3	100.5	0.9	103.0	3.6	101.8	1.2

[a]n = 9, Regression equation: $y = 0.01798x + 0.103$ ($r^2 = 0.9962$). Between-day statistics = 101.4 ± 3.0% (Mean ± s.e.m.), CV (of s.e.m.) = 3.0%.

Table 2. Analysis of Nadolol by CDNBD and USP HPLC Methods [4]

Tablet brand	CDNBD method (%)	%Recovery in the presence of additional analyte	95% C.I. (of 8 ug ml^{-1})	USP 2000 method (%)	p-value[a]	
					F-test	t-test
Corgard®	105.7 ± 3.0	105.6 ± 2.7	8.5 ± 0.2	102.0 ± 2.6	0.44	0.08
Corgaretic®	100.6 ± 1.4	101.3 ± 1.0	8.1 ± 0.1	99.4 ± 2.8	0.08	0.49

[a]Statistical analysis between the results obtained from the proposed method and official HPLC method. USP requires content of nadolol to be 90-110%. a, n = 5; b, n = 4.

Table 3. R_f Values for the Thin Layer Chromatographic Analysis of Nadolol Tablets and Nadolol/CDNBD Reaction Mixture on Silica Gel GF_{254}

Mobile phase	Tablet brands			Reaction mixture	
	Nadolol CRS	Corgard®	Corgaretic®	ADDUCT	CDNBD
EtOAc: MeOH (9:1)	0.21	0.21	0.21	0.37	0.76
$CHCl_3$: MeOH (8:2)	0.32	0.32	0.32	0.47	0.86
MeOH: Conc. NH_3 (100:1.5)[a]	0.25	0.25	0.25	0.40	0.60

[a]Plate was coated with 0.1 M methanolic KOH before spotting samples on it. Visualization: UV-254 nm and iodine vapor.

CONCLUSIONS

The new approach for the determination of nadolol as described in this work is found to be simple, sensitive and accurate for the assay of nadolol in bulk and dosage forms. With an accuracy equivalent to that of the official (USP) assay, this method could find application as a simple routine analytical technique as an in-process assay for nadolol.

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