Spectrophotometric Determination Of Nitrazepam And Dapson Using Vanillin Reagent in Pharmaceutical Preparations

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الملخص

تم وصف طريقة طيفية بسيطة وحساسة وانتقائية لتقدير الدابسون والنايترازيبام في الحالة النقية وفي مستحضـراتهما الصيدلانية بواسطة كاشف الفانيلين. تعتمـد الطريقـة على التفاعل التكثيفي بين الدابسون أو النايترازيبام المختزل مع الفانيلين والحصول على ناتج أصفر اللون. إن قاعدة شيف الناتجة تمتلك أقصى امتصاص عند الطول الموجي 405 نانوميتر لكلا الدوائين. لقـــــد اتبعــــــت التراكيــــــز قـــــانون بيــــــر للمــــــديات 1-20 و 30-1 مايكروغرام/مللتر بامتصاصية مولارية $8.69×10^3$ و $1.332×10^4$ لتر/مول.سم لكل من الدابسون والنايترازيبام على التوالي. كمـا وجد حد الكشف والحد الكمي 0.087 و 0.299 مـايكروغرام/مللتر للدابسـون على التوالي في حين وجد 0.069 و 0.231 مـايكروغرام/مللتر للنايترازيبام على التوالي. أظهرت الطريقة دقة وتوافق جيدين وأمكن تطبيقها في تقدير الدوائين أعـلاه في مستحضـراتهما الصيدلانية كأقراص، كمـا وجد أن الطريقـة لا تعـاني من تداخل مواد السواغ المضـافة لتلك المستحضـرات. قورنت الطريقـة بنجاح مـع الطريقة القياسية في دستور الأدوية البريطاني بتطبيق اختبار-t واختبار-F .

Abstract

A simple, sensitive and selective spectrophotometric method is described for the determination of dapson and nitrazepam in bulk drug and in dosage forms using vanillin as the chromogenic agent. The method is based on the condensation reaction between dapson (DN) and reduced nitrazepam (RNZ) with vanillin to produce an intense yellow colored product. The resulting Schiff's bases show an absorption maximum at

405 nm for both drugs. Beer's law was obeyed in the concentration range 1-30 and 1-20 µg/ml with a molar absorptivity of 8.69×10^3 and 1.332×10^4 L mol^{-1} cm^{-1} for DN and RNZ respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.087 and 0.299 µg/ml for DN, respectively and 0.069 and 0.231 µg/ml for RNZ, respectively. The method shows good accuracy and precision, and was applied successfully to the determination of DN and RNZ in tablets. No interference was observed from common additives found in pharmaceutical preparations. The results obtained by the proposed method were validated statistically by comparing the results with those of official methods by applying the Student's t-test and F-test.

Key words: Schiff's base, Vanillin, Dapson, Nitrazepam.

Introduction

Nitrazepam (NZ) chemically known as 7-nitro-5-phenyl-1,3-dihydro-2*h*-1,4-benzodiazepine-2-one (Figure 1) is a hypotic agent that belongs to the benzodiapine class. NZ used in the treatment of insomnioe which has sedative and motor impairing properties [1], as well as anxiolytic, amnestic, anticovulsant and skeletal muscle relaxant properties, and it has been used in the treatment of stress related disorders [2].

Dapsone (DN) chemically known as 4,4-diaminodiphenyl sulfone (Figure 1) is antileprotic drug used into the treatment of paubacillary leprosy [3,4].

MWt= 281.3 g/ml

Nitrazepam

MWt = 248.302 g/ml

Dapsone
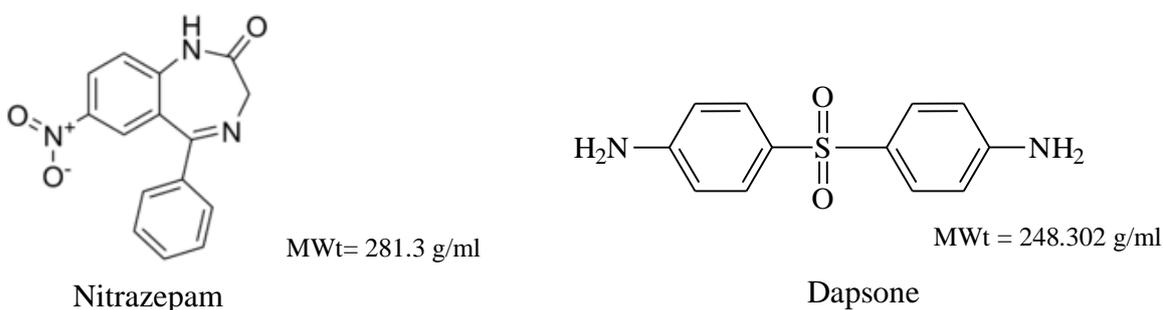
Figure 1 : Chemical structures of DN and NZ

Various analytical techniques have reported for the determination of DN and NZ drugs such as chromatography [5-8], voltammetry [9-10], fluorimetry [11] and chemiluminescence [12].

Spectrophotometric methods are still considered to be a very convenient and cost-effective technique and hence widely used for the determination of these drugs in pure and in pharmaceutical preparations, various spectrophotometric methods have been reported for determination

of NZ, after reduction by Zn/HCl, and DN such as coupling of the diazotized NZ and DN with different reagents namely acetyl acetone, diphenylamine and citrazinic acid [13], resorcinol [14], N-(1-napthyl) ethylenediamine dihydrochloride [15], orcinol [16], benzoylacetone [17], phloroglucinol [18], N,N-diethyl-p-phenylenediamine sulphate [19] and m-aminophenol [20]. Metol was used as π-acceptor to form charge transfer complex with reduced NZ as n-donor [21]. p-Dimethylaminocinnamaldehyde forming Schiff's bases complex with DN in acidic medium [22]. 9-Chloroacridine[23] and sodium 1,2-naphthoquinone-4-sulfonic [24] as chromogenic reagents were used for determination of dapsone.

The present investigation method is development of simple and sensitive spectrophotometric method for the estimation of DN and NZ in both pure and in pharmaceutical preparations as tablet based on the condensation reaction between DN and NZ with vanillin as the chromogenic agent in acidic medium to form Schiff's base products.

Experimental
Apparatus
Shimadzu UV-1800 PC UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell, Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements. All calculations in the computing process were performed in Microsoft Excel for Windows.

Chemicals
All reagents were of analytical-reagent grade which were provided by BDH and Fluka companies.

Working standard solution DN (100 µg.ml^{-1}) is prepare by dissolving 0.01g of pure material in few mls of ethanol and diluted to the mark in a 100 ml volumetric flask with distilled water. The solution was kept in refrigerator.

Working standard solution of RNZ (100 µg.ml^{-1}) Accurately 50 mg of NZ was weighed into a separate 100 ml beaker and dissolved NZ in 20ml absolute ethanol with heating. To this 5 ml conc. hydrochloric acid and 1g of zinc dust were added and shaken thoroughly for about 15 min and then diluted up to the mark with water in a 100 ml standard flasks (500 µg ml^{-1}), and filter through Whatmann no.41 filter paper, then 20 ml from this solution was neutralized with 20% sodium carbonate solution to pH 6 and filtered using sintered glass crucible No.2, then diluted with water in 100 ml standard flask to obtain 100 µg ml^{-1}.

Vanillin Reagent (10%) is prepared by dissolving 10g of 4-hydroxy-3-methoxy benzaldehyde in 100 ml absolute ethanol in a volumetric flask.

Sulphuric acid solution (0.1M) is prepared by diluting of a suitable volume of conc. H_2SO_4 to 100 ml with distilled water in a volumetric flask.

Nitric acid solution(0.1 M) is prepared by diluting of a suitable volume of conc. HNO_3 to 100 ml with distilled water in a volumetric flask.

Recommended procedure

Aliquots of the working solution of DN (1-20 µg.ml^{-1}) and RNZ (1-30 µg.ml^{-1}) were transferred into separate series of 5 ml calibrated flasks. Then, 2 ml of 10% vanillin and 1.5 ml of 0.1 M H_2SO_4 for DN and 1ml of 0.1 M HNO_3 for RNZ were added. The solutions were diluted to the mark with distilled water and kept at room temperature (20ºC) for 15 and 5 min for DA and RNZ, respectively. The absorbance was measured at 405 nm against reagent blank.

Procedure for DN tablets:

Ten tablets were powdered and mixed thoroughly. An amount of powder equivalent to 10 mg of DN was weighed into a 100 ml calibrated flask and dissolved in 10 ml water-ethanol (50:50 v/v) mixture and was shaken for 10 min, then filtered through Whatman no. 41 filter paper and the volume was made up to the mark with distilled water to obtain 100 µg/ml DN and subjected to analysis following the procedure described above.

Procedure for NZ tablets:

Ten tablets (5 mg each) were emptied, pulverized. A quantity of the powder equivalent to 25 mg of NZ was weighed accurately and dissolved in 10 ml ethanol into a separate 50 ml calibrated flask. Then, 5 ml of conc. hydrochloric acid and 1g of zinc dust were added and shaken thoroughly for about 30 min. The volume was diluted to the mark with distilled water, mixed well and filtered using a Whatmann no.41 filter paper and 20 ml from this solution was neutralize with 20% sodium carbonate solution to pH 6 and filtered using sintered glass crucible No.2, then diluted with water in 100 ml calibrated flask to obtain100 µg.ml^{-1}

RESULTS AND DISCUSSION:
Chemistry

The Schiff's base linkages are form by a condensation reaction of aromatic amine with an aldehyde group at the terminus of an organic compound [25]. Based on this reaction many pharmaceutical compounds containing aromatic amine group have been determined [26-30] and the same reaction was adopted for the assay of DN and NZ (after reduction), since they contain primary aromatic amine group. The proposed method

utilized vanillin which contains an aldehyde group. The condensation reaction requires the presence of an acid for the protonation of the carbonyl oxygen and thereby leaving the carbonyl carbon fully positive charge. The NH$_2$ group in DN and RNZ donates a lone pair of electrons to the carbon present in the carbonyl group of vanillin. Internal rearrangement thereafter results in the formation of imine (Schiff's base) and then giving water and proton as by-products [25]. The tentative reaction scheme is shown in Scheme 1.
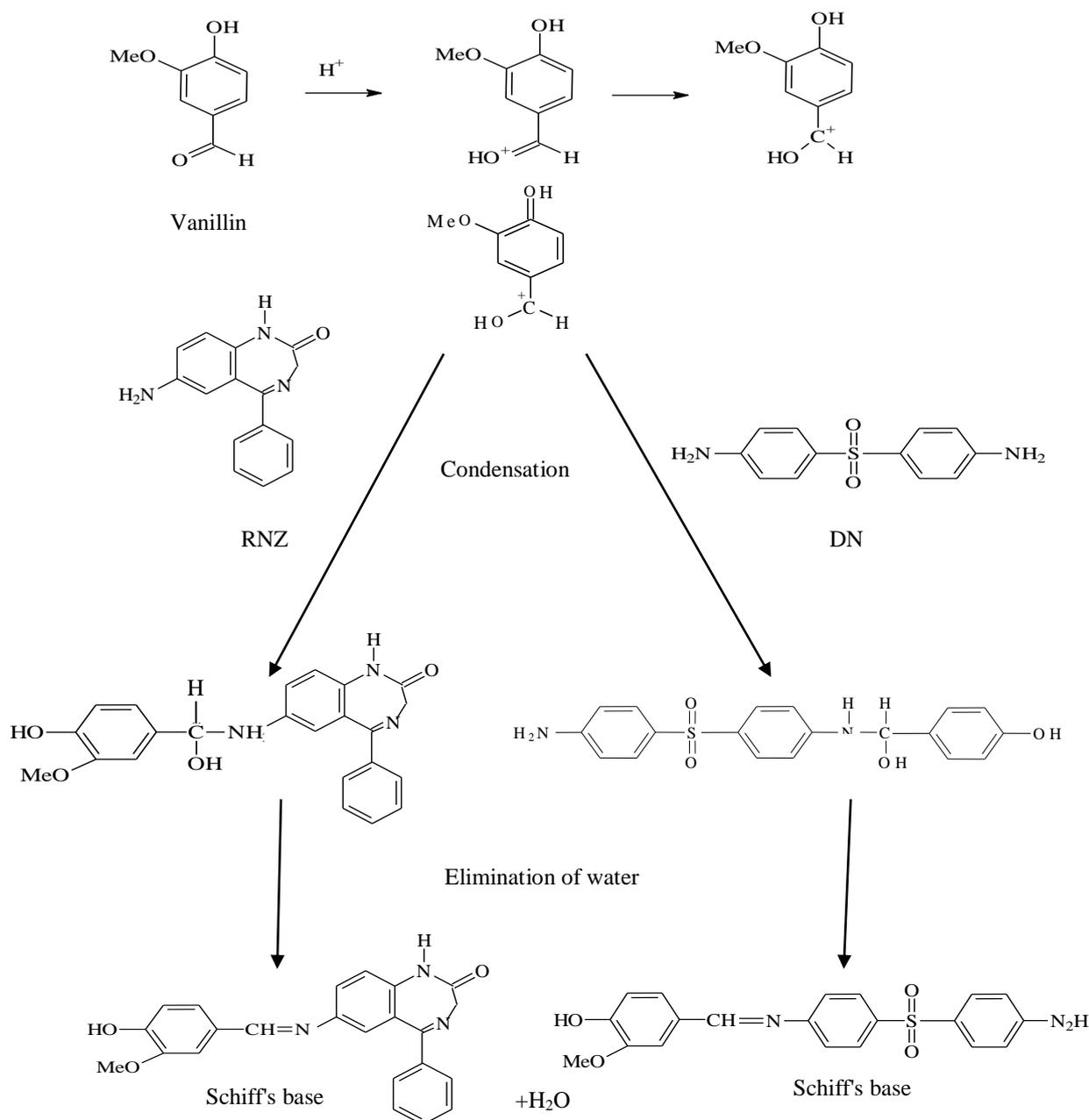
Scheme1. Probable mechanism of the Schiff bases formation reaction between DN and RNZ with vanillin reagent

Spectral characteristics

The proposed method involves the reaction of DN and RNZ with vanillin reagent in acidic medium and forming a yellow colored Schiff's base products having maximum absorption at 405 nm. This wavelength was used for all subsequent measurements. The absorption spectra of the reaction product are shown in Figure 2. The corresponding reagent blank have no absorbance at this wavelength.
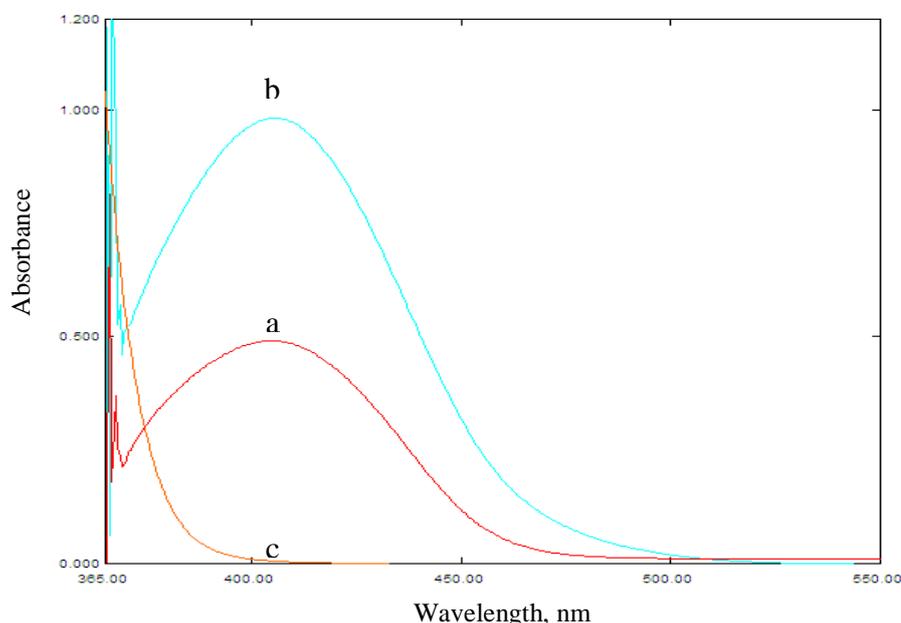
Figure 2: Absorption spectra of (a) DN (14 μg.ml^{-1}), (b) RNZ (18 μg.ml^{-1}) products with vanillin (10%) against reagent blank and (c) Reagent blank against distilled water under the optimum conditions

Optimization of conditions

The optimum conditions for the color development of the products were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of colored species. The following experiments were conducted for this purpose and conditions so obtained were incorporated in general procedure.

Effect of solvents

Vanillin is insoluble in water but soluble in organic solvent, therefore; methanol, ethanol, acetonitrile, acetone and 1,4-dioxane were used as solvent for vanillin and for final dilution have been examined in order to achieve maximum sensitivity and products stability, As shown in Table1, It was found that on using methanol as solvent for the vanillin, and dilution with water, for both drugs, were gave maximum color intensity. So; this system of solvents has been recommended in this method.

Table 1: Effect of solvents on absorbance of drugs-vanillin products

DN or RNZ Dissolved in	Vanillin Dissolved in	Dilution by	DN		RNZ	
			λ_{max}(nm)	Absorbance	λ_{max}(nm)	Absorbance
Water	Methanol	Water	405	0.194	405	0.132
Water	Methanol	Methanol	401	0.090	405	0.128
Water	Ethanol	Water	405	0.149	404.5	0.110
Water	Ethanol	Ethanol	403	0.042	408	0.085
Water	Water	Water	403.5	0.068	402	0.095
Water	Acetone	Acetone	403.5	0.022	403	0.068
Water	Acetonitrile	Water	402	0.129	402	0.107
Water	Acetonitrile	Acetonitrile	369	0.059	401	0.105
Water	1,4-Dioxane	Water	404.5	0.079	402	0.111
Water	1,4-Dioxane	1,4-Dioxane	410	0.024	399	0.106

Effect of vanillin concentration

The effect of vanillin on the sensitivity of its reaction with DN and RNZ was studied by using 10% vanillin and it was observed that when 2 ml was used, the absorbance readings were nearly constant with high sensitivity for both drugs; below and above this volume there was a decrease in absorbance (Fig. 3). Hence, 2 ml of 10% vanillin was used as optimum in a total volume of 5 ml.
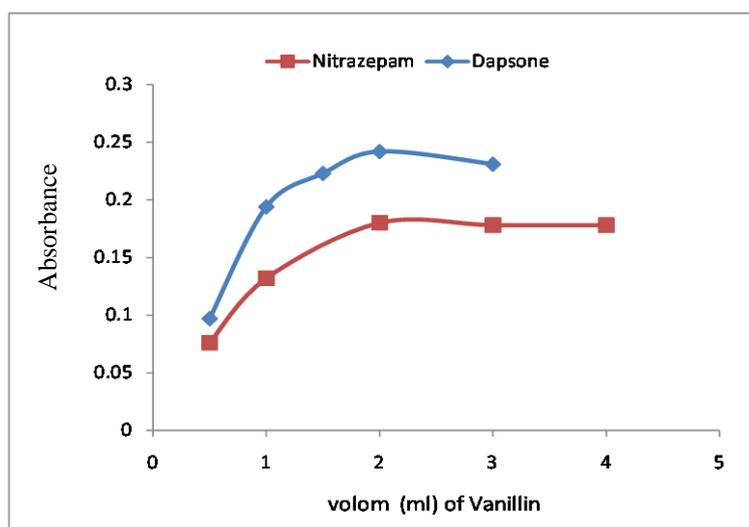

Figure 3: Effect of vanillin reagent concentration on the absorption

Effect of pH and acids

The condensation reaction of vanillin with DN and RNZ are formed with low sensitivity in the absence of acid with pH 6.13, but gave high sensitivity in acidic medium with pH 0.77 in the presence of 0.1 M HCl, therefore; the effect of different acids, nitric acid, phosphoric acid, sulphuric acid acetic acid and formic acid with 0.1M concentration were examined. As seen in Fig. 4, the maximum absorbance value was obtained in the presence of sulphuric acid and nitric acid for DN and RNZ respectively. The amount of these acids are studied and found 1.5-2.0 ml

of 0.1M sulphuric acid and nitric acid gave high coloured products for DN and RNZ respectively, and beyond this amounts, the absorbance would be decreased, (Fig. 5). However 1.5 ml for DN and 1ml for RNZ were chosen as optimum amounts in this method.
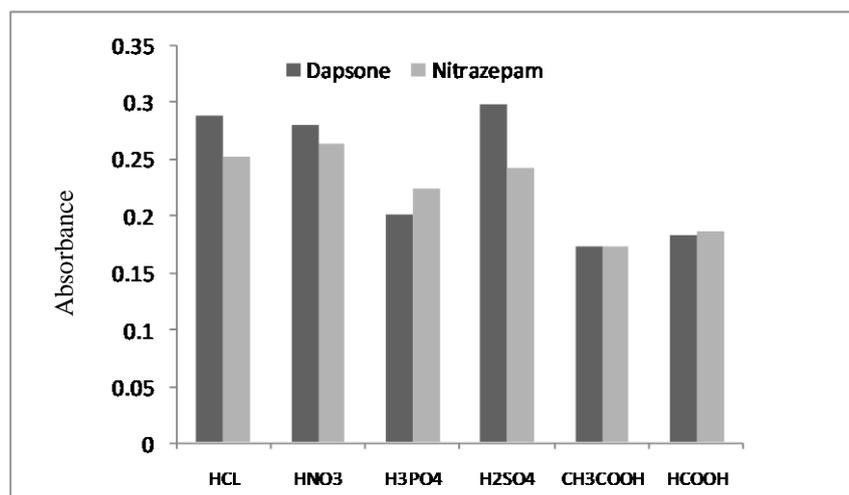

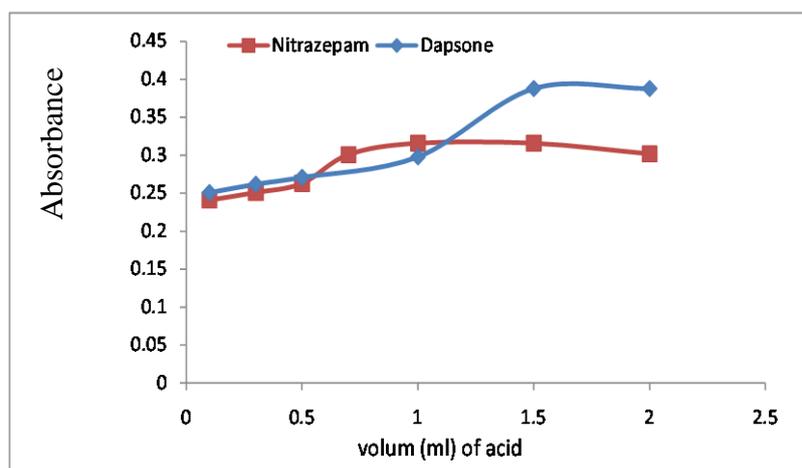**Figure 4. Effect of acids on the absorption of vanillin products with DN and RNZ**

Figure 5. Effect of H_2SO_4 and HNO_3 on the absorption of vanillin products with DN and RNZ respectively

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature(23˚C) and at 40˚C. The absorbance was measured at 5 and 10 minutes intervals against reagent blank treated similarly. it was found that the color products show maximum absorbance after 15 and 5 min at room temperature and were stable for further 25 and 35 min for DN and RNZ respectively, (Fig.6). At 40°C, the absorbance decreases, indicating dissociation. Hence, 20 and 10 min at room temperature are recommended for the proposed method.
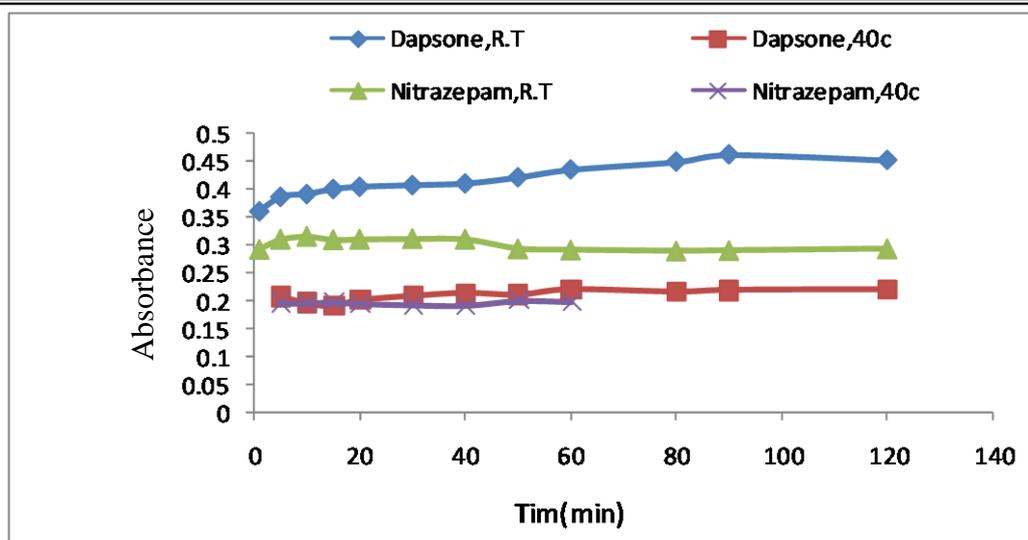
Figure 6. Effect of temperature on the absorbance of 10 μg.ml⁻¹ DN and 6 μg.ml⁻¹ RNZ with vanillin

Order of addition

The order of addition of reactants on the color development was examined After optimizing all other experimental variables. Maximum sensitivity was achieved when drug and vanillin were added before adding the acid. Hence, the method was performed in the order: drug + vanillin + acid.

Quantification

In order to investigate the range in which the colored products adhere to Beer's law, the absorbance of the products were measured at λ_{max} value after developing the color by following the suggested procedure for a series of solutions containing increasing amounts of DN or RNZ (Figure 7). The Beer's law limits and molar absorptivity values were evaluated and given in Table 2, which are indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for drugs determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of five replicates of each three different concentrations for DN and RNZ indicated that the method is precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following equations:

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

where σ is the standard deviation of five reagent blank determinations and b is the slope of the calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range.
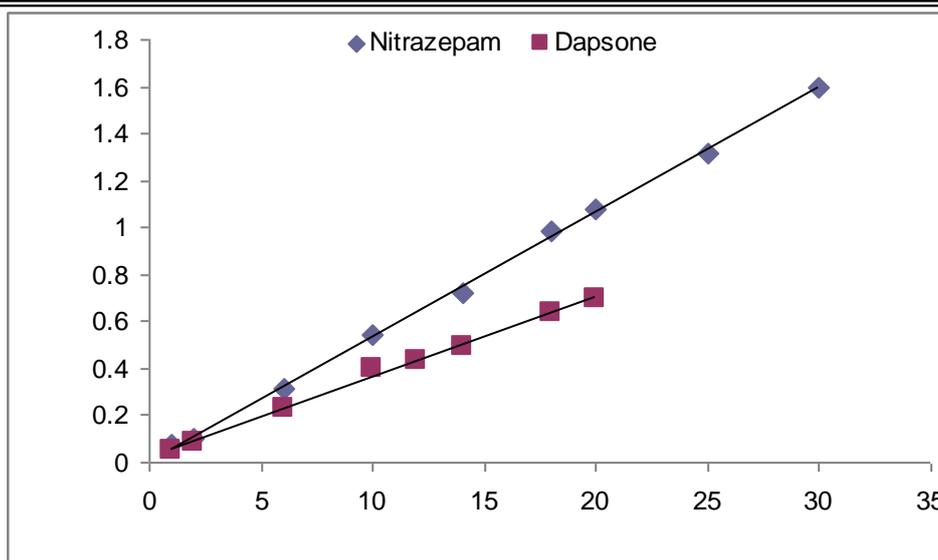
Figure 7: Calibration graph for determination of dapson and nitrazepam drugs

Accuracy and precision

The accuracy and precision of the method were evaluated by performing five replicate analyses on pure drug solution at three different concentration levels (within the working range). The average recoveries (%), an indicator of accuracy were 100.31 % and 98.94 % and relative standard deviation (RSD %), an expression of precision were ≤ 1.57 and ≤ 0.67 for DN and NZ respectively, indicating high accuracy and repeatability of the method. The results of the study are given in Table 2.

Table 2 : Summary of optical characteristics and statistical data for the proposed method

Parameter	DN	RNZ
λ_{max} (nm)	405	405
Linear range ($\mu g.ml^{-1}$)	1 – 20	1-30
Molar absorptivity ($l.mol^{-1}. cm^{-1}$)	8.69×10^3	1.332×10^4
LOD ($\mu g.ml^{-1}$)	0.087	0.069
LOQ ($\mu g.ml^{-1}$)	0.299	0.231
Average recovery (%)*	100.31	98.94
Correlation coefficient	0.996	0.999
Regression equation (Y)**		
Slope, a	0.035	0.053
Intercept, b	0.023	0.004
RSD**	≤ 1.57	≤ 0.67

* Average of five determinations
** $Y = a X + b$, where X is the concentration of DN and RNZ in $\mu g\ ml^{-1}$.

Interference

The extent of interference by some excipients which often accompany pharmaceutical preparations were studied by measuring the absorbance of solutions containing 10 µg ml^{-1} of each DN and RNZ separately, and various amounts of diverse species, up to 20 fold excess, in a final volume of 5 ml. It was found that the studied excipients did not interfere seriously, (Table 3).

**Table 3: Effect of excipients for assay of DN and RNZ**

Foreign Compound	Recovery %							
	10 µg.ml^{-1} of DN per µg.ml^{-1} foreign added				10 µg.ml^{-1} of RNZ per µg.ml^{-1} foreign added			
	20	50	100	200	20	50	100	200
Glucose	100.49	102.23	103.73	103.23	97.57	100.74	101.29	102.38
Lactose	101.49	100.75	102.73	103.73	99.43	99.25	102.99	101.12
Arabic Gum	99.00	100.50	101.49	103.48	98.48	99.44	101.48	101.29
Sodium Chloride	98.26	101.74	102.96	104.23	97.00	96.44	99.44	98.31
Sucrose	98.76	100.25	103.73	97.90	97.90	100.56	100.19	101.66
Starch	100.99	102.23	103.23	98.86	98.86	99.81	101.12	101.49

Stoichiometry and Stability constant

The molar ratio of the complexes formed between the DN and RNZ with vanillin reagent were investigated by applying the mole ratio and continuous variation (Job's) methods [31], using equimolar solutions of each (1×10^{-2} M). The results indicated that the products were formed in the ratio of 1:1 for DN and RNZ to the reagent (Figures 8,9). This finding supports that The NH_2 group in DN or which produced from the reduction of the NZ drug was sharing in the formation of Schiff's base products.
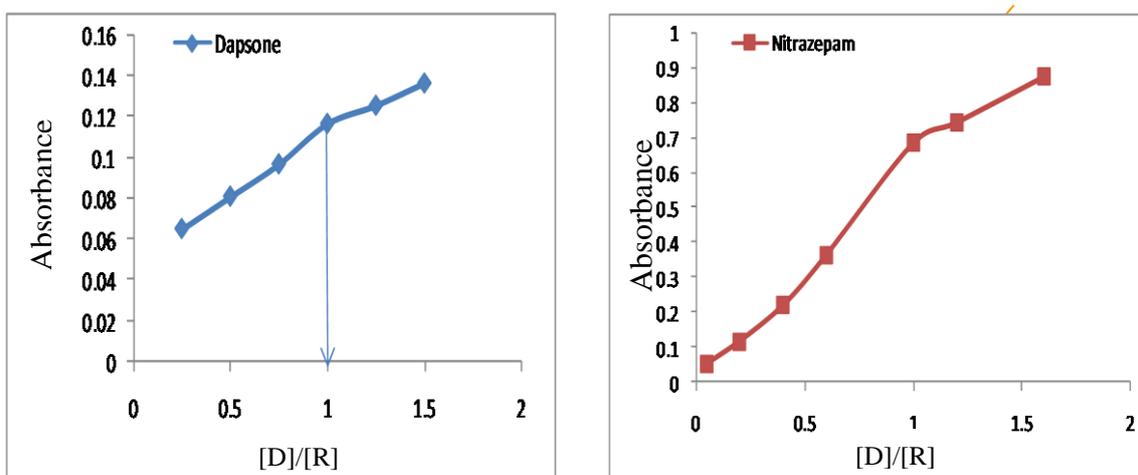
Figure 8: Mole ratio plots for products of DN and RNZ with vanillin reagent under the optimum reaction conditions.
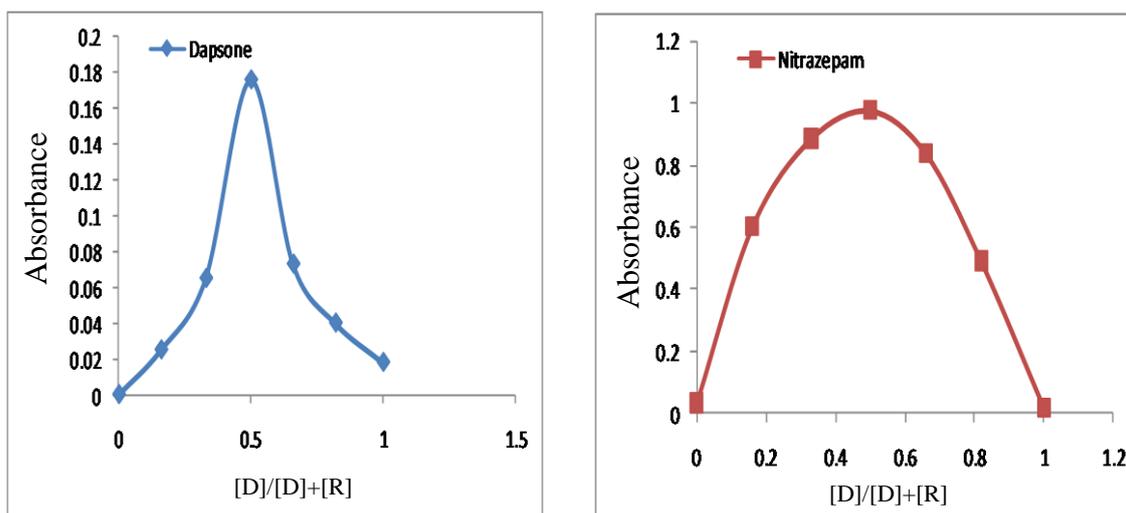
Figure 9: Continuous variation plots for products of DN and RNZ with vanillin reagent under the optimum reaction conditions.

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the drug and vanillin (As) to one containing an excessive amount of vanillin reagent (Am). The average conditional stability constant of the complex was calculated, according to the 1:1 ratio, by the following equation:

$$Kc = 1-\alpha/\ \alpha^2\ C$$
$$\alpha = Am-As/Am$$

where Kc is the stability constant (l.mol^{-1}), α the dissociation degree and C the concentration of the product which is equal to the concentration of drug. However; the average of stability constant for three different concentration was found 1.607×10^4 and 4.631×10^4 l.mol^{-1} for DN and RNZ respectively indicating the good stabilities.

Analytical applications

The proposed method was successfully applied to determine DN and NZ in its pharmaceutical preparation as tablet. The obtained results were compared statistically by student′s t-test for accuracy and a variance ratio F-test for precision with the official methods [1], which are depending on spectrophotometric method for NZ tablet and potentiometric titration for DN tablet, at 95% confidence level with six degrees of freedom, as cited in Table 4. The result showed that the t- test and F-test were less than the theoretical value (t= 2.57, F= 5.05), indicating that there was no significant difference between the proposed method and official methods.

Table 4: Assay of DN and RNZ in tablet using the proposed method and comparison with the official methods

Procedure applied	Pharmaceutical preparation	Drug amount present ($\mu g.ml^{-1}$)	Recovery[a] (%)	Drug content found(mg)	Average recovery (mg)	Certified value (mg)	RSD[a]
Proposed method	Dapsone[c] Tablet	6.0	101.67	101.67	100.54 (0.312,2.6)[b]	100 mg	1.27
		14.0	100.41	100.41			
		20.0	99.55	99.55			
	Mogadon[d] Tablet	10.0	100.19	5.01	5.01 (0.562,2.9)[b]	5 mg	0.291
		14.0	99.21	4.96			
		18.0	100.84	5.06			
British Pharmacopoeia	Dapsone Tablet	250(mg)	99.36	99.36	100.06	100 mg	1.24
			101.84	101.84			
			100.85	100.85			
	Mogadon Tablet	5 (mg)	99	4.95	4.97	5 mg	1.37
			101	5.05			
			98.4	4.92			

a= Average of four determinations
b= Figures in parenthesis are the calculated values for t and F tests respectively
c = Domina pharmaceuticals , Damascus
d= Marketed by Roche Co. Switzerland

Conclusions

A simple, sensitive and rapid method is described for the determination of DN and NZ in its dosage forms as tablets. The method is based on the formation of Schiff's base products by reaction of vanillin as a chromogenic agent with DN and RNZ. The method is rapid and applied at ambient temperature, color development is instantaneous and does not require strict pH control or extraction step. The color formed is highly stable leading to very precise results.

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