P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2009,(3), (3) :06-12

Spectrophotometric Determination of Nitrazepam in Pharmaceutical Tablets by Oxidative Coupling Reaction with Pyrocatechol

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ARTICLE INFO

Received: 19 / 5 /2022
Accepted: 28 / 5 /2022
Available online: 14/6/2012
DOI: 10.37652/juaps.2009.37781

Keywords:
Nitrazepam;
Spectrophotometric;
Pyrocatechol;
Pharmaceutical tablets.

ABSTRACT

A simple and sensitive spectrophotometric method was developed for the determination of nitrazepam (NZP) in pharmaceutical tablets. The method was based on oxidative coupling organic reaction of reduced NZP with pyrocatechol in the presence of ferric sulfate to form red water soluble product with maximum absorbance at 510 nm. The reaction conditions were studied and optimized. The linear range for the determination of NZP, and the detection limit were 1– 24 µg mL-1 and 0.698 µg mL-1, respectively. The proposed method has been applied successfully for the determination of NZP in pharmaceutical tablets. A statistical comparison of these results with those obtained by the British pharmacopoeia procedure using the Student t-test and variance ratio F-test shows a good agreement and indicates no significant difference in accuracy and precision at the 95% confidence

Introduction:

Nitrazepam (NZP) is 2H-1,4-benzodiazepin-2-one, 1,3-dihydro-7-nitro-5-phenyl-, C15H11N3O3 [1].

NZP is a powerful hypnotic drug, anticonvulsant and a sedative in the group of drugs, which is known as benzodiazepines[2].

Various methods have been reported for determining this drug in biological and pharmaceutical samples. These include: flow injection-voltammetric[3], micellar liquid chromatographic[4], micellar electrokinetic capillary chromatographic[5], thin layer chromatographic[6], thin layer chromatographic-densitometric[7], high performance liquid chromatographic[8], reverse phase-high performance liquid chromatographic[9,10], spectrophotometric[11, 12].

Many colorimetric methods for the determination of NZP in pharmaceutical preparations are described in Table (1). The BP recommends a spectrophotometric method for NZP tablets at 280 nm[23].

This search describes the development of simple and sensitive spectrophotometric method for the quantitative determination of NZP in pharmaceutical tablets. The proposed method was based on reduction of the nitro aromatic drug (NZP) to the corresponding primary aromatic amine followed by oxidative coupling reaction of the latter with pyrocatechol in the presence ferric sulfate and the measurement of the absorbance of the compounds thus, formed.

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Experimental:-

Apparatus

A Shimadzu UV-VIS 260 digital double-beam recording spectrophotometer (Kyoto, Japan) was used for all spectral and absorbance measurements with matched 1-cm quartz cells.

Chemicals and reagents

Chemicals and reagents of analytical grade used in present study. The standard material of NZP and excipients usually used in pharmaceutical tablets were provided from the State Company for Drug Industries and Medical Appliances (SDI), Samarra-Iraq.

Pharmaceutical tablets

Pharmaceutical tablets were obtained from commercial sources. Mogam Tablets: 5 mg Nitrazepam for each tablet (Domina Pharmaceuticals, Damascus-Syria).

Solutions

Nitrazepam (NZP) reduction solution (500 µg mL-1)[22]

This was prepared by dissolving 0.0500 g of NZP in ethanol. It was transferred into 50 mL volumetric flask, and diluted to the mark with the same solvent. The solution was transferred into beaker of 125 mL. A 20 mL of distilled water, 20 mL of hydrochloric acid (11.64 N), and 3 g of zinc powder were added. The beaker was allowed to stand for 15 min at room temperature, then the solution was filtered into 100 mL volumetric flask, washed the residues with distilled water, and diluted to the mark volume with distilled water to obtain 500 µg mL-1 of NZP reduction solution. More dilute solution was prepared daily by appropriate dilution using distilled water.

Pyrocatechol (PC) solution (5 mM)

This was freshly prepared by dissolving 0.1101 g of PC and diluting to 200 mL with distilled water in volumetric flask.

Ferric sulfate solutions (10 mM)

This was prepared by dissolving 0.5619 g of ferric sulfate and diluting to 100 mL with distilled water in volumetric flask.

Solutions of pharmaceutical tablets

Tablets samples: Twenty tablets were accurately weighted and finely powdered. An amount of the powder equivalent to 50 mg of NZP was dissolved in 30 mL of ethanol. The solution was filtered into a 50 mL volumetric flask, the residue was washed with ethanol and diluted to volume with the same solvent to obtain 1000 µg mL-1 of NZP. This solution was transferred into 125 mL beaker and was reduced as described above. Further appropriate solutions of pharmaceutical tablets were made by using distilled water.

Analytical procedure

Into a series of 25 mL volumetric flasks an increasing volume of the reduced solution of drug (100 µg mL-1) were transferred to cover the range of the calibration graph (1 – 24 µg mL-1). To each of these were added 1.5 of PC (5 mM) and 1 mL of ferric sulfate (10 mM) and diluted to the mark with distilled water, mixed well and left for 20 min at room temperature (25˚C). The absorbances were measured at 510 nm versus the reagent blanks, prepared in the same way but containing no drug.

RESULTS AND DISCUSSION

Absorption spectra of the colored product

When a solution of reduced NZP was mixed with PC reagent and oxidized with ferric sulfate, an intense red color forms immediately, which became stable after 20 min. The red solution has a maximum absorption at 510 nm. Fig. (1) shows the spectra of the red solution formed and of the reagent blank. The maximum absorption at 510 nm was used in all subsequent experiments.

Optimum conditions for product formation

The effect of various variables on the color development was tested to establish the optimum conditions for the determination of NZP by oxidative coupling with PC reagent in the presence of ferric sulfate.

In the subsequent experiments, 500 µg of the reduced NZP was taken in 25 mL final volumes and the absorbances of a series of solutions were measured by varying one and fixing the other parameters at 510 nm versus reagent blank after 20 min from the beginning of the reaction.

Effect of oxidant

Fe2(SO4)3.9H2O was found to be a useful oxidizing agent for oxidative coupling reaction, other oxidizing agents such as NBS, K2Cr2O7, K2S2O8, NaIO4, KIO4, and NaIO3 have also been tested, but none offered real advantages over ferric sulfate.

The effect of the different volumes (0.3 – 2.0 mL) of 10 mM ferric sulfate solution was examined on the maximum absorbance of the colored product in the presence 1.5 mL of PC (5 mM). Fig. (2) shows that 1.5 mL of the solution was enough to obtain a maximum absorbance, and it was used in the subsequent experiments.

Effect of the coupling reagent

Pyrocatechol (PC) was found to be a useful coupling reagent for oxidative coupling reaction, because it produced stable oxidative coupling organic products rapidly. Moreover, this reagent is easily to obtain and solve in water. Other coupling reagents such as resorcinol, phloroglucinol, phenol, 2-nitrophenol and 3-nitrophenol did not give product when tried in place of PC.

The effects of the different volumes (0.3 – 2.0 mL) of 5 mM PC solution were examined on the maximum formation of the colored product. Fig. (2) shows that 1 mL of the solution was optimum and was used in the subsequent experiments.

Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the

analytical procedure, otherwise a loss in color intensity and stability was observed. The order of addition of reagents cited under analytical procedure was used in all subsequent experiments.

Effect of temperature

The effect of temperature on the color intensity of the product was studied. In practice a maximum absorbance was obtained when the color was developed at room temperature (25˚C), but when the color was developed in an ice-bath (5˚C) or in a water-bath (45˚C) a loss in color intensity and stability were observed. It is therefore recommended that the color reaction should be carried out at room temperature (25˚C).

Effect of reaction time

The color intensity reached a maximum after reduced drug solution had been reacted immediately with PC and ferric sulfate in aqueous medium and became stable after 20 min and remained stable for at least 60 min. Therefore, 20 min development time was selected as optimum in the analytical procedure.

Structure of the product

Based on the mole ratio and continuous variation methods, it was found that reduced NZP reacted with PC in a ratio of 1:1 as shown in Fig. (3) and Fig. (4).

The reduced drug of NZP, by virtue of their strong electron donating ability, coupling with PC (oxidized to o-benzoquinone by ferric sulfate), leading to the formation of oxidative coupled products[24], as shown in Fig. (5).

Stability constant of the product

The product formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of NZP and PC (concentration of both NZP and PC are 2 mM) that of a solution containing a five-fold excess of PC reagent. The stability constant of the product in water under the described experimental of conditions was 1.004 × 105 L mol-1.

Optical characteristics

Employing the conditions described under the analytical procedure, a calibration graph for NZP was studied. The linearity of calibration graph, molar absorptivity, Sandell's sensitivity, limit of detection, and limit of quantitative are summarized in Table (2). The slope, the intercept, the standard deviations for residuals, slope and intercept and the correlation coefficient were evaluated by a least-squares regression analysis[25], and are also included in the same Table. The obtained correlation coefficient value is highly significant.

Accuracy and precision

The accuracy and precision of the determination of MPH was studied depending upon the value percentage of the relative error (E%), recovery (Rec.%), and relative standard deviation (RSD%), respectively. For five replicates of each concentration of MPH containing 8, 12, and 16 µg mL-1. The results in Table (3) show a good accuracy and precision.

Effect of interferences

To evaluate the selectivity of the proposed method for the analysis of pharmaceutical preparations containing NZP, the interfering effect of excipients were examined by determining NZP in the presence of the interference applying the analytical procedure.

The excipients studied were: lactose, talc, starch, magnesium stearate, and PVP. For this study, solution was containing NZP and each one of the excipients was taken separately in concentrations five-times greater than that of NZP were analyzed. Under the reaction conditions used all of them do no interfere as shown in Table (4).

Pharmaceutical applications

The proposed method was applied for the determination of NZP in tablets by the analysis of three different concentrations of sample using the analytical procedure. The results obtained are summarized in Table (5).

Evaluation of the proposed method

For evaluating the competence and the success of the proposed method, the results obtained were compared with those obtained by standard BP method[23].

The same pharmaceutical tablets for NZP were analyzed by standard BP method. The results obtained by the two different methods (Table (6)) were statistically compared, using the Student t-test and variance ratio F-test at 95% confidence level[25]. In all cases, the calculated t- and F-values (Table (6)) did not exceed the theoretical values, which indicate that there is no significant difference between either methods in accuracy and precision in the determination of NZP in pharmaceutical tablets.

From an analytical point of view, it is concluded that the described procedure allow for the determination of NZP in pharmaceutical tablets. Unlike the other procedures, the instrument is simple and inexpensive. Its importance lies in the chemical reaction upon which the procedure is based, rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of a major interest in analytical pharmacy, since it offers a distinct possibility in the assay of a particular component in complex pharmaceutical preparations. The reagents utilized in the proposed method are cheaper and readily available, and the procedure do not involve any critical reaction conditions, such as heating, extraction or removal of excipients, and hence could be used for routine quality control in drug industries. The method was found to be simple, low cost, and fairly selective than some of the reported colorimetric methods (Table (1)).

The proposed method advantage over the standard BP method (spectrophotometric method) are more selective, as they depend on the presence of the nitro group, and less prone to interfere, which are normally encountered in single wavelength UV measurements.

The proposed method was applied to analysis of NZP in tablets solutions, suggesting that it used as a reliable and advantageous alternative to the other previously exported methods for routine analysis of NZP in these samples.

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Table (1): Colorimetric methods for the determination of NZP in pharmaceutical preparations

Reagents	Comments	Colored species	λmax., nm	Linear range, µg mL^{-1}	Ref.
Dimethyl sulfate-methanol	Reaction of NZP in methanol with reagent by heating for 25 min in a boiling-water bath	Complex	353	8 – 20	13
HCl	Acidic hydrolysis of NZP by boiling with dilute HCl	Acidic hydrolysis product of NZP	360	2 – 20	14
1-Fluoro-2,4-dinitrobenzene and HCl in dimethyl sulfate	Reaction of NZP with reagent for 30 min on a boiling-water bath	Charge transfer complex	368	2 – 32	15
Picric acid in benzene medium	Direct reaction of NZP with reagent	Charge transfer complex	420	20 – 300	16
Picric acid in $CHCl_3$ medium	Direct reaction of NZP with reagent	Charge transfer complex	395	2 – 30	17
NaOH	Alkaline hydrolysis of NZP by boiling with 20% NaOH for 30 min to form 2-amino5-nitrobenzophenone	Alkaline hydrolysis product of NZP	400	Up to 30	18
Alizarin violet 3B or Alizarin Brilliant violet R	NZP was extracted from aqueous solution at pH 1.2 into $CHCl_3$ as a complex with reagent	Ion-pair complex	560	2 – 8	19
$Na_3[Fe(CN)_5NH_3]$	Reduction of NZP by Zn dust in $CaCl_2$ solution followed by reaction with reagent	Hydroxylamine derivative	560	1 – 20	20
Ethyl acetoacetate and NaOH	The product of hydrolyzed of NZP was diazotized and coupled with reagent	Diazotization and coupling	482	1 – 12	21

Ethyl acetoacetate and NaOH	Reduction of NZP with Zn / HCl followed by diazotization	Diazotization and coupling	410	2 – 16	22

Table (2): Analytical values of statistical treatments for the calibration graph

Parameter	Value
Correlation coefficient, r	9.995775×10^{-1}
Regression equation y = b x + a; y = absorbance, x = concentration (µg mL^{-1})	y = 0.017270 x + 0.031276
Slope, b (mL µg^{-1})	1.727021×10^{-2}
Intercept, a	3.127631×10^{-2}
Standard deviation of the residuals, $S_{y/x}$	4.020463×10^{-3}
Standard deviation of the slope, S_b	1.514278×10^{-4}
Standard deviation of the intercept, S_a	2.141916×10^{-3}
Linearity range (µg mL^{-1})	1 – 24
Molar absorptivity, ε (L mol^{-1} cm^{-1})	4.858109×10^{3}
Sandell's sensitivity, S (µg cm^{-2}) per 0.001 absorbance unit	5.790319×10^{-2}
Limit of detection, LOD (µg mL^{-1})	6.983929×10^{-1}
Limit of quantification, LOQ (µg mL^{-1})	2.327976

Table (3): Accuracy and precision of the proposed method

Concn., µg mL^{-1}		E%	Rec.%	RSD%
Present	Found			
8.000	8.024	+ 0.300	100.300	1.413
12.000	12.076	+ 0.633	100.633	0.809
16.000	16.068	+ 0.425	100.425	0.492

Table (4): Effect of excipients on the recovery of 12 µg mL^{-1} of NZP

Excipient, 60 µg mL^{-1}	Concn. of NZP, µg mL^{-1}	E%*	Rec.%*
	Found		
Lactose	11.949	– 0.425	99.575
Talc	12.030	+ 0.250	100.250
Starch	12.071	+ 0.592	100.592
Mg stearate	11.970	– 0.250	99.750
PVP	12.020	+ 0.167	100.167

* Average of five determinations.

Table (5): Application of the proposed method for determination of NZP in pharmaceutical tablets

Pharmaceutical tablets	Concn. of MPH, µg mL^{-1}		E%*	Rec.%*	RSD %*
	Present	Found			
Mogam Tablets	8.000	8.058	+ 0.725	100.725	1.127
	12.000	12.040	+ 0.333	100.333	0.818
	16.000	16.073	+ 0.456	100.456	0.647

* Average of five determinations.

Table (6): The comparison of the proposed method with standard BP methodusing t- and F-statistical tests

Pharmaceutical preparation	Proposed method	BP method	s	Value
	Rec.%* (x_i)$_1$	$(x_i-\bar{x})^2_1$	Rec.%* (x_i)$_2$	$(x_i-\bar{x})^2_2$		t (theor.)	F (theor.)
NZP pure	100.000	0.0640	100.000	0.0576	0.348	1.417 (4.303)	1.107 (161.4)
Mogam Tablets	100.505	0.0635	99.520	0.0576			
	$\bar{x}_1$ = 100.253	Σ = 0.1275	$\bar{x}_2$ = 99.760	Σ = 0.1152	$(n_1 + n_2 - 2) = 2$	$(n_1 - 1) = 1$ $(n_2 - 1) = 1$	

* Average of five determinations.

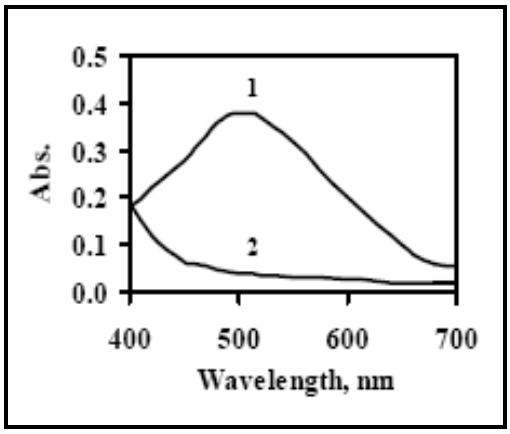

Fig. (1): Absorption spectra NZP a of the product obtained by the reaction of PC with 40 µg mL^{-1} of 1- reduced NZP in the presence of ferric sulfate versus reagent blank, 2- reagent blank versus distilled water

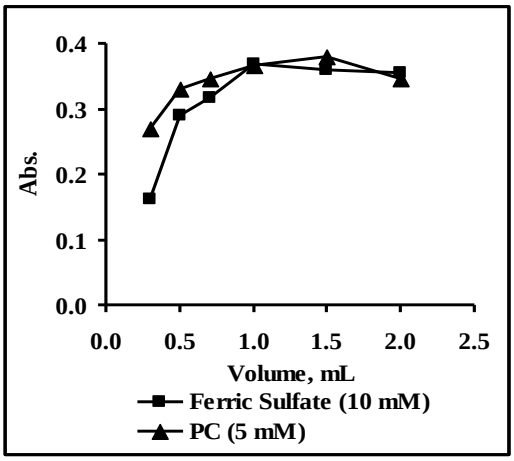

Fig. (2): Optimum conditions for determination of NZP

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2009,(3), (3) :06-12

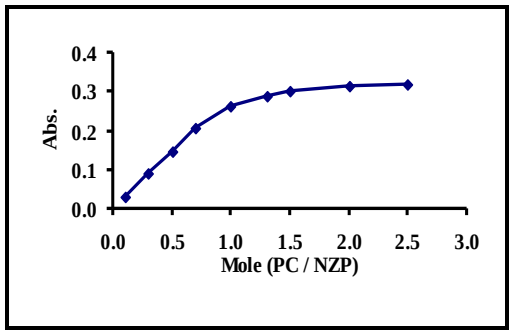

Fig. (3): Mole ratio plot

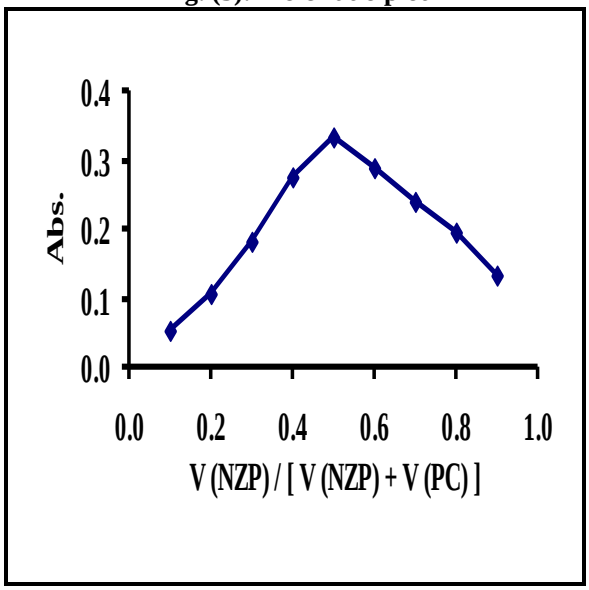

Fig. (4): Continuous variation plot

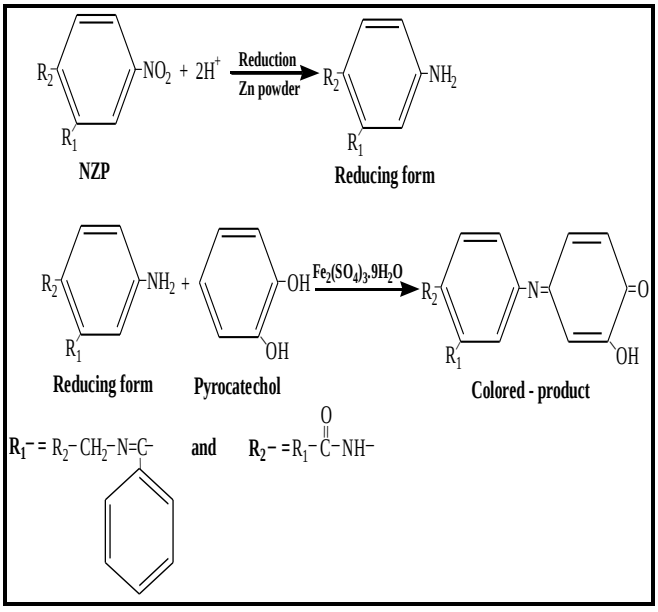

Fig. (5): Reaction scheme

التقدير الطيفي لنايترازيبام في الأقراص الصيدلانية بوساطة تفاعل الازدواج التأكسدي مع البايروكاتيكول

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الخلاصة

يتضمن البحث تطوير طريقة طيفية بسيطة وحساسة لتقدير النايترازيبام في الأقراص الصيدلانية. تعتمد الطريقة على تفاعل الازدواج التأكسدي العضوي لدواء النايترازيبام المختزل (بوساطة مسحوق الخارصين وحامض الهيدروكلوريك) مع البايروكاتيكول بوجود كبريتات الحديديك، إذ يتكون ناتج أحمر ذائب في الماء أظهر أقصى امتصاصية عند طول موجي 510 نانومتراً. تم دراسة وتثبيت ظروف التفاعل الفضلى. كان مدى الخطية لتقدير النايترازيبام بين 1 – 24 مايكروغرام مل-1 وبحد كشف 0.698 مايكروغرام مل-1. طبقت الطريقة بنجاح في تقدير النايترازيبام في الأقراص الصيدلانية، و تم مقارنة نتائجها إحصائياً باستعمال اختباري t و F مع نتائج طريقة دستور الأدوية البريطاني، و وجد أنه لا يوجد فرق معنوي في دقة و مصداقية الطريقة المقترحة مع طريقة دستور الأدوية البريطاني عند مستوى ثقة 95%.