P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2011,(5), (3) :13-17

Spectrophotometric Assay of Noradrenaline in Pharmaceutical Formulation with Quinalizarin in Aqueous Solution

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ARTICLE INFO

Received: 5 / 5 /2011
Accepted: 19 / 10 /2011
Available online: 14/6/2012
DOI: 10.37652/juaps.2011.44301

Keywords:
Noradrenaline;
Spectrophotometric method,
Quinalizarin;
Pharmaceutical formulation.

ABSTRACT

A simple, rapid and sensitive spectrophotometric method for the determination of noradrenaline was developed. The method is based on the proton transfer reaction with quinalizarin in aqueous neutral solution to form a violet product showing maximum absorbance at 560 nm with molar absorptivity of 6680 l.mol-1.cm-1. The method follows Beer's law over the concentration range (5.91×10-6 -5.91×10-5) mol.L-1 The accuracy (average recovery) of the method is 99.72% and the precision (RSD) of the method is less than 1.5%.The method was successfully applied for the determination of noradrenaline in pharmaceutical formulation as injection and the results were in a good agreement with the standard addition procedure.

Introduction

Noradrenaline (NA) is a catecholamine which has been largely recognized to play a very important role in biological system(1). The cells that produce and release NA, namely adrenal chromaffin cells (ACCS)(2), sympathetic postganglionic neurons and central neurons. It plays important roles in learning and memory, and in regulation of cardiovascular activity and nervous system(2,3).

A variety of spectrophotometric methods have been described for the determination of NA. A flow injection analysis (FIA) technique was used for the determination of NA(4). Spectro-photometric(5-7)

NA was determined via oxidative coupling reaction using 2,6-dichloroquinone-chlorimide(8). Ammonium metavanadate, sodium bismuthate and sodium periodate were used to determine NA by oxidation producing aminochrome derivative which can be measured spectrophotometrically at 485, 486 and 490 nm respectively(9-11).

Recently NA was determinated spectrophotometrically using alizarin red sulphonate reagent(12).

In this paper quinalizarin reagent was used for the spectrophotometric determination of NA via proton transfer reaction in aqueous solution.

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Experimental:-

Apparatus:

A Shimadzu UV-210 A digital double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral and absorbance measurements.

Reagents

Noradrenaline, quinalizarin (BDH) and all other chemicals used were of analytical-reagent grade. Distilled water was used to prepare all solutions except quinalizarin was prepared in absolute ethanol, NA was dissolved in 5 ml of ethanol then completed with distilled water.

Solutions

NA: 100 g.ml-1 of the pure drug was freshly prepared in aqueous solution (protected from sun light) and used as the standard solution for the analytical purposes.

Quinalizarin: 2 × 10-4 mol.L-1 was prepared by dissolving 0.0136 gm in 250 ml of absolute ethanol. This solution was kept in brown bottle and was stable for, at least, one month(13).

Pharmaceutical injections:

Pharmaceutical injections were obtained from commercial sources.

Noradrenaline injections: 1mg/ 1ml (Lab. Renaudin- France).

Assay procedure for injection:

Five ampoules of NA (1 mg/ml) marked by Lab Renaudin-France were mixed well, diluted with distilled water and completed to the mark in a 50 ml volumetric flask. Different volumes were used from this solution containing the concentration range (5.91×10-6 -5.91×10-5) mol.L-1. After that, the calibration procedure described above was carried out.

Analytical procedure

To a series of 10 ml calibration flasks, transfer increasing volumes of NA working standard solution to cover the range (5.91×10-6 -5.91×10-5) mol.L-1 in final dilution. Add 5 ml of 2 × 10-4 mol.L-1 quinalizarin. Dilute the solution to the mark with D.W. Stopper the flasks and shake well, then allow to stand for 20 min. at 40 °C, and the absorbance is measured at 560 nm against the reagent blank, using 1 cm cells.

RESULTS AND DISCUSSION

Absorption spectra

NA was reacted with quinalizarin to produce a violet coloured proton transfer product with maximum absorption at 560 nm while the reagent blank shows low absorption at this wavelength (Fig. 1).

Optimization of Conditions

Effect of buffer

The pH of the pure drug was measured, the reading was 7.8, after addition of 3 ml of quinalizarin the pH was 7.0, therefore different buffers of pH 7.0 were prepared such as borate, carbonate, acetate and phosphate. It was found that these buffers decrease the absorbance (Table 1).

Effect of quinalizarin solubility

A 1 × 10-4, 2 × 10-4, 5 × 10-4, 1 × 10-3 and 2 × 10-3 mol.L-1 solutions of quinalizarin are prepared in absolute ethanol. Only 2 × 10-4 mol.L-1 gave a clear solution when reacts with NA. Therefore, this concentration was used in all the subsequent experiments.

Effect of solvent

Ethanol, methanol, propanol, dimethyl sulfoxide (DMSO) and acetone were examined as solvents for quinalizarin in the determination of NA. The use of ethanol provided maximum absorbance in comparison with other solvents, Table 2.

Effect of temperature and reaction time

The reaction time was determined by following the colour development at room temperature and in thermostatically controlled water-bath adjusted at 0, 40 and 50 °C. The absorbance was measured in 5 mins. intervals against reagent blank treated similarly. It was observed that the absorbance reached maximum after 20 mins. at 40 °C and remains constant more than 50 mins., and the absorbance decreased slowly thereafter. Hence 40 °C and reaction time 20 mins., were chosen for colour development (Table 3).

Effect of quinalizarin concentration

The effect of different quinalizarin concentrations on the absorbance of solution containing 4 g.ml-1 NA was studied, it is evident from the results that the absorbance increases with increasing quinalizarin concentration and reached maximum on using 5 ml of 2 × 10-4 mol.L-1 quinalizarin. Therefore, this concentration was used in all subsequent work (Table 4).

Effect of surfactant

The effect of different types of surfactants were used for the improvement of the absorption but the results shown in Table (5) confirm that there is no improvement in the absorption, therefore they were excluded.

Nature of product and reaction mechanism

The stiochiometry of the reaction between the drug and quinalizarin was investigated using Job's method(14), the results obtained show that 1:1 drug to reagent was formed (Fig. 3).

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 NA and quinalizarin (concentration of both NA and quinalizarin are 2 × 10-4 mol.L-1)with a solution containing five fold of quinalizarine. The stability constant of the product in water under the described experimental of conditions was 2.16 x 105 l.ml-1 (15).

Optical characteristics:

Under the proposed experimental conditions, a linear relation between the absorbance and the concentration of NA was observed cover the

concentration range (5.91×10-6 -5.91×10-5) mol.L-1 (Fig. 2) with correlation coefficient of 0.9965 and intercept of -0.0237. A negative deviation from Beer's law was observed at higher concentrations of NA. The molar absorptivity was 6680 l.mol-1.cm-1.

Accuracy and precision

To determine the accuracy and precision of the method, NA was determined at three different concentrations. The results shown in Table(6) referring a satisfactory precision and accuracy.

Application

The proposed method was applied to the determination of NA in pharmaceutical injection. Good recovery was obtained and the results compared with the standard addition method (Fig. 4 and Table 7).

Comparison of Methods

Table (8) gives the results obtained by application of this method and literature method to the determination of noradrenaline in pharmaceutical preparations.

From the results shown in Table(8) there is similarity between the two methods as molar absorptivity, linearity and recovery in addition to the simplicity, low cost and does not require solvent extraction.

CONCLUSION:

The reported method is rapid, simple and sensitive. The product formed is stable for at least 50 mins., thus permitting quantitative analysis to be carried out with good reproducibility.

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Table (1): Effect of buffer on the absorption of product

Buffer solutions (pH 7.0)	Borate	Carbonate	Acetate	Phosphate	Without
Absorbance	0.011	0.014	0.028	0.023	0.169

Table (2): Effect of solvent

Quinalizarin 2×10^{-4} mol.L^{-1} in	Ethanol	Methanol	Propanol	DMSO	Acetone
Absorbance	0.169	0.143	0.159	0.162	0.120

Table (3): Effect of temperature and reaction time

Temp. (°C)	Absorbance							
	Time (min)							
	5	10	15	20	30	40	50	60

0	0.150	0.150	0.148	0.150	0.150	0.149	0.147	0.145
RT	0.152	0.155	0.155	0.155	0.160	0.161	0.161	0.160
40	0.151	0.160	0.162	0.169	0.169	0.169	0.169	0.165
50	0.160	0.162	0.169	0.169	0.167	0.167	0.166	0.164

Table (4): Effect of quinalizarin concentration

Quinalizarin solution 2×10^{-4} mol.L^{-1} (ml)	2	3	4	5	6	7
Absorbance	0.148	0.169	0.171	0.176	0.158	0.152

Table (5): Effect of surfactant

Surfactant 0.1%	Absorbance / ml					
	Order	Without	0.5	1	2	3
Tween 80*	I	0.176	0.164	0.165	0.161	0.159
	II		0.162	0.162	0.160	0.158
CPC	I		0.164	0.164	0.162	0.160
	II		0.163	0.162	0.159	0.157
SDS	I		0.164	0.164	0.161	0.160
	II		0.160	0.161	0.161	0.158

* 1%
I NA + quinalizarin + surfactant
II NA + surfactant + quinalizarin

Table (6): Accuracy and precision of the proposed method

Amount of NA taken µg.ml^{-1}	Recovery* %	Relative standard deviation* (RSD%)
4	99.28	1.46
6	99.54	1.05
8	100.34	0.90

* Average for six determinations

Table (7): Assay of Noradrenaline in injection

Method	Amount (mg.ml^{-1})		Recovery* %	Average recovery* %
	Certified	Found		
Present	1.0000	0.9928	99.28	99.80
		1.004	100.46	
		0.9966	99.66	
Standard addition	1.0000	0.985	98.50	99.24
		1.022	102.25	
		0.985	98.50	

* Average of three determinations

Table (8): Comparison of the present method with other method

Analytical parameter	Present method	Alizarin Red Sulphonate method[12]
λ_{max} (nm)	560	530
Temp. (°C)	40	40
pH	Without	Without
Development time (min)	20	5
Stability period (min)	50	60
Molar absorptivity l.mol^{-1}.cm^{-1}	6680	6720
Linearity (µg.ml^{-1})	1-10	0.5-10
Recovery (%)	99.72	100.29
E%	0.28	-0.29
RSD (%)	< 1.5	< 1
Analytical application	Injection	Injection

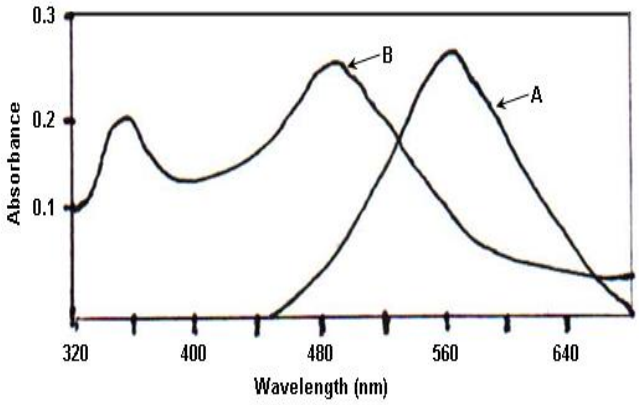


Fig. (1): Absorption spectra:
A: Noradrenaline (7 µg.ml^{-1})-quinalizarin (2×10^{-4} mol.L^{-1}) product versus reagent blank
B: Reagent blank versus distilled water

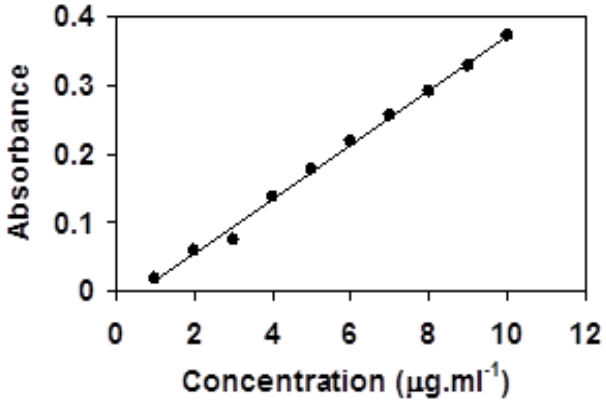


Figure (2): Calibration graph of Noradrenaline

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2011,(5), (3) :13-17

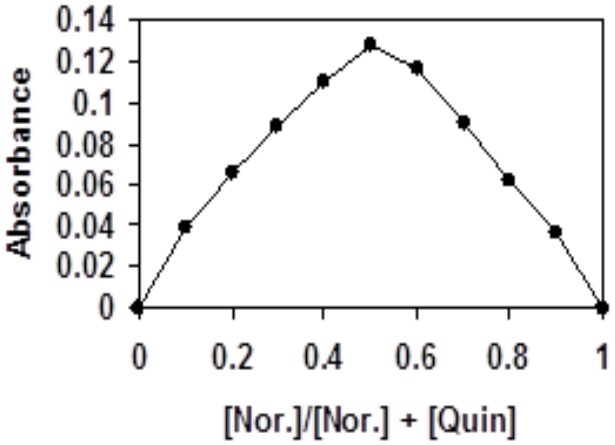


Figure (3): Job's method for noradrenaline-quinalizarin product

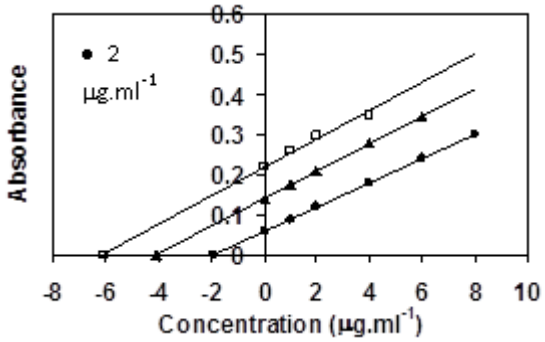
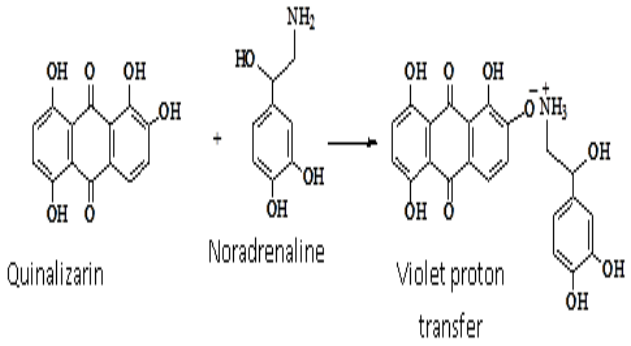


Figure (4): Standard addition graphs of Noradrenaline in armaceutical formulation



Fig(5): Reaction scheme

التقدير الطيفي للنورادرينالين في المستحضرات الصيدلانية باستخدام كاشف كوين اليزارين في المحلول المائي

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الخلاصة

تم تطوير طريقة طيفية بسيطة وسريعة وحساسة لتقدير النورادرينالين. تعتمد الطريقة على تفاعل انتقال البروتون مع كوين اليزارين في وسط متعادل لتكوين ناتج ذي لون بنفسجي ذائب في الماء يقاس اقصى امتصاص له عند طول موجي 560 نانوميتر وبامتصاصية مولارية 6680 لتر.مول−1.سم−1 وكان قانون بير ينطبق ضمن مدى التراكيز (5.91 × 10−6 – 5.91 × 10−5) مول.لتر−1 . لقد بلغت دقة الطريقة (معدل نسبة الاسترجاع) 99.72% وتوافق الطريقة (الانحراف القياسي النسبي) اقل من 1.5%. وطبقت الطريقة بنجاح في تقدير النورادرينالين في المستحضر الصيدلاني كحقن وتم مقارنة النتائج مع طريقة الاضافة القياسية.