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Determination of Nitrazepam in its pure form, formulations and in biological samples

Kanchan Upadhyay*

*Department of chemistry, Govt. V.Y.T.PG Autonomous College, Durg, India, 491001

Abstract
A simple and sensitive spectrophotometric determination method for the determination of nitrazepam in bulk, in pharmaceutical dosage forms and in biological fluids was developed. The method is based upon reduction of nitro group of nitrazepam by zinc and hydrochloric acid followed by diazotization and coupling with orcinol in basic medium to form a stable chromophore which absorbs at 470 nm. The method showed a good linearity in the range 0.3 – 3.5µgmL^{-1}. The method is free from the interference of common excipients used in pharmaceutical dosage. The method was also used for the determination of nitrazepam in pharmaceutical as well as in biological samples.

Keywords: Nitrazepam, spectrophotometric, diazotization, orcinol

INTRODUCTION

Nitrazepam (NTZ), chemically known as 1,3-dihydro-1-nitro-2-oxo-5-phenyl-2H-1,4- benzodiazepine-2-one (Figure 1) is a hypnotic agent belongs to the benzodiazepine class and it has been used in the treatment of stress related disorders [1]. The official method for its determination is non-aqueous titrimetry reported in British Pharmacopoeia [2]. Various other methods based on HPLC [3-5], complexometry with cadmium-2-methyl-5- nitrobenzenesuphonate [6], polarography [7-8], difference uv- spectrophotometry [9], densitometric TLC [10], gas-chromatography [11], micellar electrokinetic capillary chromatography [12], and derivative UV-spectrophotometry [13] have been reported for the assay of NTZ in pharmaceutical formulations. Different manipulation steps are involved in some of these methods, which are not simple for the routine analysis of pharmaceutical formulations.

Spectrophotometric methods are still considered to be a very convenient and cost-effective technique, and hence widely used for the determination of therapeutics in pure as well as in dosage forms. Very few spectrophotometric methods have been reported for estimation of NTZ [14-17]. These methods were suffering from one or other disadvantages such as lack of selectivity [14-16], the use of costly reagent [16] and lower sensitivity [15-16]. Thus, there is a need to develop a simple, selective, cost-effective and sensitive procedure for the determination of NTZ in pure and in pharmaceutical formulations.

The present paper describes the development and optimization of spectrophotometric methods based on diazo-coupling reaction using orcinol as the coupling agents. The developed methods are simple, sensitive and accurate for the determination of NTZ in pure and in pharmaceutical formulations.

EXPERIMENTAL SECTION

Apparatus

A Systronic spectrophotometer was used for absorbance measurement. The pH measurements were made with a Systronic digital pH- meter (model-335).

Reagents and Standards

Analytical reagent grade chemicals were used, and double distilled water was used throughout the experiment to prepare all solutions.

Standard NTZ solution:The pharmaceutical grade NTZ, certified to be 99.99% pure were received from Anglo French Drug & Industries Ltd., Bangalore India, as a gift sample and were used as received. Accurately 10 mg of NTZ was weighed into a 100 mL beaker and dissolved NTZ in 5 mL acetone. To this, 5 mL 4 N hydrochloric acid and 1 g 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 calibrated flask (100 µg/mL), and filter through Whatman No.41 filter paper. Working solutions were prepared as required by dilution.

Orcinol solution: 0.1% orcinol solution was prepared in double distilled water. Aqueous solutions of sodium nitrite (Merck) [0.1%], sulphamic acid (Qualigens) [3.0% w/v], sodium hydroxide (Merck) [4 M] and hydrochloric acid (Merck) [1 M and 6 M] were used.

General procedure

Preparation of calibration curve

Aliquots of a reduced standard nitrazepam solution (0.4 - 4.0µgml^{-1}) were placed in a series of 25ml calibrated tube. Then 1 ml of 5M HCl and 0.5ml of sodium nitrite solution were added successively and allowed to stand for 5 min with occasional shaking in an ice bath. Excess nitrite was removed by addition of 1 ml of sulphamic acid then 1 ml of orcinol solution was added. An orange yellow solution was obtained after addition of 1 ml of 2M sodium hydroxide solution. The solution was made up to the mark with distilled water and the absorbance was measured at 465 nm against a reagent blank, which gave negligible absorbance at this wavelength.

Procedure for pharmaceutical preparation

Twenty tablets were weighed accurately and ground into a fine powder. Tablets powder equivalent to 10 mg of the NTZ was accurately weighed and transferred into a 100 mL beaker. To this 5 mL 4 N hydrochloric acid and 1 g of zinc dust were added and stirred thoroughly for about 30 min. The contents were diluted with 50 mL distilled water and filtered using Whatman No. 41 filter paper. The filtrate was received into a 100 mL calibrated flask and it was made up to the mark with water. Appropriate aliquots of the drug solution were taken and the proposed standard procedures were followed for analysis of the drug content.

RESULTS AND DISCUSSION

A new colorimetric method developed for the determination of nitrazepam. The method depends upon diazotization of reduced Nitrazepam followed by coupling with orcinol in basic medium due to which an orange yellow dye formed. The dye has an absorption maximum at 465 nm.

Effect of varying reaction conditions

Effect of reagent concentration: - For reduction of nitrazepam molecule use of 10ml of 1M hydrochloric acid and 0.25 gm of zinc dust were found optimal. It was found that 0.5ml of sodium nitrite and 1ml of orcinol were required for maximum colour intensity. Excess amount of nitrite caused no effect on absorbance value as the addition of sulphamic acid solution removed excess nitrite. There was no significant change in the absorbance even if a large excess of orcinol was taken. 1 ml of 2M NaOH was required for full colour development, excess NaOH decreases the intensity of the colour. 0.5-5.0 ml of sulphamic acid do not affect the intensity of dye so 1ml of sulphamic acid was added for removal of excess nitrite.

Effect of pH time and temperature

The reduction time 15 min is sufficient to yield maximum absorbance. Since for diazotization 2 min or more gave the same results after addition of orcinol, it required 5 min for complete colour development.

Effect of temperature on reduction diazotization and coupling was studied. Effect of temperature on reduction rate was studied at various temperature ranges. Reduction rate was slow below 35^{o}C while it was instantaneous at temperature above 35^{o}C hence 45^{o}C was selected for reduction. Diazotization at 0-5^{o}C gives maximum colour intensity whereas coupling rate below 10^{o}C was slow hence room temperature as selected for coupling.

The effect of pH on the reaction was studied maximum intensity was found at pH range 10-11 pH hence 1ml of 2M NaOH was used in study.

INTERFERENCE

The effects of common excipients such as talc, glucose, dextrose etc. commonly used in pharmaceutical preparations were investigated under the optimal conditions. An amount in 1000 fold excess of that used in pharmaceutical preparation was added in 0.1 µgml^{-1} nitrazepam solution and no effect due to these excipients was found under the proposed experimental conditions.

Analytical data

Proposed method follows Beer's law over the concentration range of 0.4-4.0µgml^{-1}. The molar absorptivity, sandell's sensitivity, limits of detection, limit of quantification, regression equation, correlation coefficients values given in table 1. The precession of the method was tested by 6 replicate analysis of solution containing 2.0µgml^{-1}. The standard deviation and RSD values are given in table 1.

Application

Analysis of pharmaceutical preparations:

The applicability of the proposed method for the assay of different pharmaceutical formulations containing nitrazepam examined for tablets. For this, 20 tablets crushed and drug equivalent to 100 mg was weighed accurately and dissolved in methanol and then diluting up to the mark with distilled water. Concentration is determined by applying the proposed method.

The results were statistically compared with those obtained by the official method [4] and by calculating student's t-test and F-test found not to differ significantly. The results summarized in table 2.

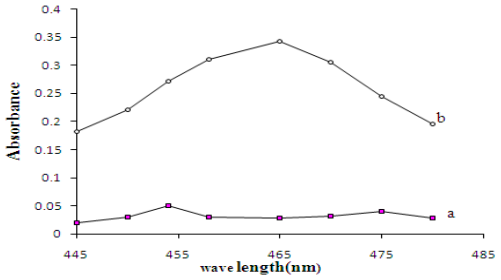

Fig 1. Absorption spectra of (a) Blank solution: 0.5mL of 0.1%(w/v) sodium nitrite, 1 mL of 0.1%(w/v) orcinol, 1ml of 3% (w/v) sulphamic acid and 1mL of 2M NaOH solution, (b) Sample solution: Nitrazepam 1.6 µgmL^{-1} with blank solution in 25 mL volumetric flask and diluted to volume with distilled water.

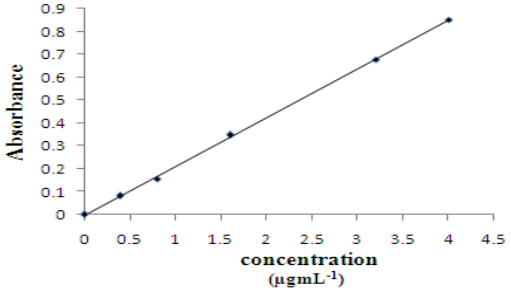

Fig 2. Optical absorption spectra with different concentration

Table 1. Analytical parameters for spectrophotometric determination

Parameters	Results
$\lambda_{max,}$ (nm)	465
Beer's law limits (µg mL^{-1})	0.4-4.0
Molar absorptivity (L mol^{-1} m^{-1})	0.832 x 10^{5}
Sandell's sensitivity (µg cm^{-2})	0.00338
Standard deviation(±)	0.008
Relative standard deviation (%)	2.43
Regression equation (Y= bX*+a)	
Slop(b)	1.213212
Intercept(a)	-0.00249
Correlation coefficient	0.9998

X = concentration in µg mL^{-1}

Table 2. Determination of nitrazepam in pharmaceutical preparations

Tablet brand Name	Nominal amount mg per tablet	Reference method [17]	Proposed method
Nitravet*	5 mg	101.2±1.3	99.96±0.561 t=1.23, F=5.37

CONCLUSIONS

The developed visible spectrophotometric method is simple, sensitive, accurate, precise, reproducible and economical and can be successfully applied to the routine estimation of nitrazepam in bulk and in pharmaceutical dosage forms without the need of extraction or heating the reaction mixture. Diazotization was carried out at room temperature and cooling to 0-5˚C was not necessary. The methods are unaffected by slight variations in the experimental conditions such as basicity, reagent concentrations and temperature. The value of standard deviation was satisfactorily low and recovery was close to 100% which indicates the reproducibility and accuracy of the three methods.

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