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DEVELOPMENT OF COLORIMETRIC METHOD FOR DETERMINATION OF NITRAZEPAM IN TABLETS AND BULKS

RAKESH KUMAR TEKADE*[123], VIRENDRA GAJBHIYE[1], KAVITA RAI[1] AND MUKTIKA SHARMA[2]

Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, SAGAR 470 003, INDIA[2]Bhopal Institute of Technology and Sciences, Bangarasia, Bhopal (M.P), INDIA. *[3]School of Pharmacy and Pharmaceutical Sciences, University of Central Lancashire, Preston PR1 2HE, ENGLAND (United Kingdom). *Email:* rakeshtekade@yahoo.com; rtekade@uclan.ac.uk

ABSTRACT

A simple, economical, precise and reproducible colorimetric method with higher sensitivity as well as lower limit of detection (1.26 µg/ml) for the routine estimation of Nitrazepam has been developed. The method is based on the formation of a yellow coloured complex by Nitrazepam in presence of $FeCl_3$ and NaOH. The developed colored-complex showed λ_{max} at 490nm corresponding to Filter no.3 of colorimeter, and obeyed Lambert-Beer's law in the concentration range of 4-24 µg/ml. Results of analysis were authenticated statistically as well as by recovery studies, which gave mean recovery between 98.805±3.242 to 100.80±4.628, % ± standard deviation (SD). The method was successful in determining Nitrazepam in commercial formulations viz: Nitravet 5 mg (Anglo French); Nitrosun 10 mg (Sun Pharma) and Nipam 5 mg (La-Pharma), with an average recovery of 101.16±3.725, 98.12±1.012 and 98.55±3.145; %±SD, respectively. The proposed method could find application to product development scientists in on-going research; as well provide an additional tool for routine analysis of Nitrazepam in academia practical based on Nitrazepam formulations.

Keywords*:* Nitrazepam, Colorimetry, Accurate, Precise, Reproducible, Low SD

INTRODUCTION

Benzodiazepines are commonly prescribed as anticonvulsant, muscle relaxant, anxiolytic and sedative and have been considered largely free of neuro-endocrine effect [1-2]. Most of the pharmacological actions of Benzodiazepines are considered to be mediated via binding to BZ receptors, and the consequent increase in the effects of γ-aminobutyric acid (GABA) [3]. Moreover, Benzodiazepines are suggested to affect the monoamine transmitters, such as serotonin (5-HT) [4], noradrenaline [5], and dopamine (DA) [6], directly or by enhancing the GABA system. They represent a class of very useful medicines. Nitrazepam, which is 1,2 dihydro-7-nitro-2-Oxo-5-phenyl-3H-1,4-benzodiazepine is one of the efficient member of this class [7]. A number of methods available to quantify Nitrazepam in formulations unfortunately demand employment of costlier chemicals. In order to minimize the research and development costs, the techniques employed for preliminary analysis must be simple, precise, reproducible, and moreover should be economical.

Colorimetric determination refers to an analytical technique best suited for preliminary confirmations. This technique is concerned about the measurement of light in the visible region. We see objects because they transmit or reflect a certain portion of light in this region. When an ordinary light is passed through the object it absorbs certain wavelength, leaving certain un-absorbed wavelength to be transmitted. The transmitted light or we can say the transmitted wavelength

will be seen as color. The basic principle of Colorimetric analysis is the measurement of color of a solution, whose intensity corresponds to its concentration [8]. The colorimetric methods of analysis are at present undergoing considerable development and may be applied to a great number of estimations with adequate accuracy.

EXPERIMENTAL

Chemicals and reagents

Methanol (AR grade) was purchased from Qualigens Fine Chemicals (Mumbai, India), NaOH was purchased from E Merk (India) Ltd (Mumbai, India), HCl (Ranbaxy Fine Chemicals Ltd, New Delhi, India), Doubled distilled water was used throughout the study. Nitrazepam was received as a gift sample from M/s Bennett Pharmaceuticals, Vadodara, India. All other chemicals used were of analytical reagent (AR) grade.

Formation of colored complex and determination of absorption maxima (λ_{max})

Nitrazepam (40 mg) was accurately weighed and dissolved in methanol (100 ml) to make a standard stock solution of strength 400 μg/ml. From this, six standard sub-dilutions of concentrations 4, 8, 12 16, 20 and 24 μg/ml were prepared in 10 ml aliquot volumetric flasks. To each volumetric flask, NaOH solution (0.5 M, 0.2 ml) and $FeCl_3$ (0.05 M, 0.02 ml) were added, shaken uniformly and kept for 30 minutes to allow formation of colored complex. After 30 minutes, each aliquot was scanned over the entire colorimetric range (420-720 nm) employing Systronics Colorimeter and the wavelength of maximum absorbance (λ_{max}) was determined (Figure1).

Determination of stability of complex

The stability testing of developed complex is based on the principle of chemical kinetics, and was assessed under different conditions of temperature and light. For stability study, aliquots of concentration 2, 4, 8, 12, 16 and 24 μg/ml were prepared and complex was done, as stated in previous section. The samples were kept in transparent and amber colored glass vials under low (8±0.5°C), intermediate (room temperature; 25±0.5ºC) and elevated (50±0.5ºC) temperature conditions, in controlled oven for varying period of time. The samples were analyzed initially and then periodically (0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 hr) for absorbance determination. The degradation rate constant at various temperatures were determined. The data was analyzed to infer the effect of time as well as storage conditions on the stability of the formed complex (Figure 2-4).

Preparation of standard calibration curve and method validation

Standard aliquots of concentration 4, 8, 12, 16, 20 and 24 μg/ml were scanned at wavelength of maximum absorbance (λ_{max}), absorbance of solution was determined and standard calibration curve was plotted (Y=0.039X–0.039; R^2=1), the regression analysis as well as optical properties of which are depicted in Table 1. Validation, accuracy and presion of the developed analytical method was done by performing systematically designed studies, as described in following sections.

Recovery studies

For recovery studies four standard laboratory samples of Nitrazepam NR_1,

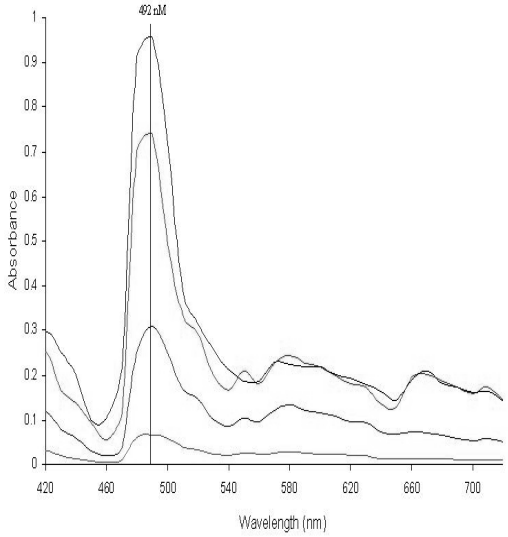

Fig. 1: Scan of various Nitrazepam solutions between between 420-700 nM.

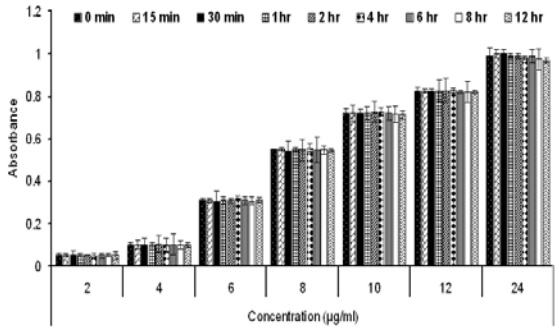

Fig. 2: Stability profile of Nitrazepam complex at low temperature (8±1°C)

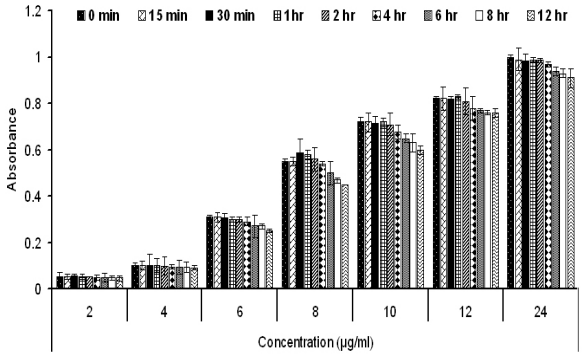

Fig. 3: Stability profile of Nitrazepam complex at Room temperature (25±2°C)

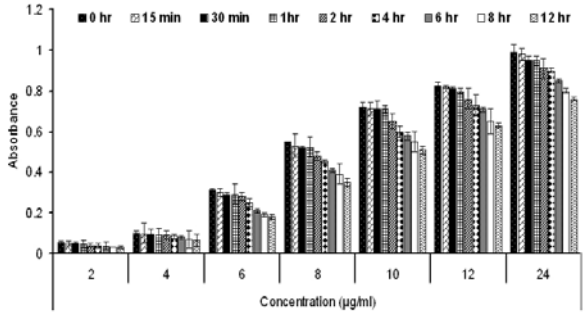

Fig. 4: Stability profile of Nitrazepam complex at high temperature (50±2°C)

NR_2, NR_3 and NR_4 having strength of 6, 10, 12 and 24 µg/ml, respectively, were randomly prepared in triplicate and the concentrations corresponding to these solutions were determined with the help of developed method. Statistical analysis was performed with Graph Pad Instat Software (version 3.0, Graph Pad Software San Diego, California, USA) using one-way ANOVA followed by Tukey-Kramer multiple comparison test. Difference with $P>0.05$ was considered statistically insignificant, whereas $P<0.001$ was considered a very significant difference. The results obtained in each instance were compared with theoretical value of 100.0% and represented as mean±SD; SEM (Table 2).

Accuracy and precision

For establishment of accuracy and precision of the developed method, standard laboratory samples of Nitrazepam having concentration of 2 µg/ml prepared (N_1). The strength of this solution was gradually raised (applying alligation medial method; Jain and Sharma, 2000) to yield solutions of strength 6, 10, 16 and 24 µg/ml, respectively, which were labeled as N_2,

N_3, N_4 and N_5. Finally, the cumulative strength of these solutions was determined by developed method and percent Nitrazepam detected was calculated (Table 3). Statistical analysis was done as stated in previous section.

Estimation of Nitrazepam in commercial formulations

Twenty tablets were weighed and powdered in a pestle-mortar. The quantity of the powder equivalent to amount labeled for each tablet was weighed, mixed with 5 ml of water in conical flask and allowed to stand for 15 min. Then, HCl (0.5 ml, 0.5 M) and methanol (quantity sufficient; q.s) were added to produce 100 ml solution. This solution was finally filtered to collect the filtrate containing extracted Nitrazepam (I.P-1996, with slight modification). Nitrazepam from different marketed brands viz: Nitravet 5 mg (Anglo French); Nitrosun 10 mg (Sun Pharma) and Nipam 5 mg (La-Pharma), were extracted and appropriately diluted to determine the absorbance at λ_{max} and the corresponding amount of Nitrazepam in formulations (Table 4). Statistical analysis was done as stated in previous section.

RESULTS AND DISCUSSION

The motif behind the project was to develop an accurate, precise, sensitive and reproducible method for determination of Nitrazepam. For this, colored complex of Nitrazepam was formed with the help of $FeCl_3$ in presence of NaOH, and its application in analytical detection was explored. This formed complex was intense yellow in color and showed wavelength of maximum absorbance (λ_{max}) at 492 nm (Figure 1). The stability of color as well as the developed complex is a prerequisite towards such motif. Hence, the stability of this complex was assessed under different conditions, wherein it was found

Table 1: Regression and Optical Characteristics of Nitrazepam

Parameters	Report
Absorption maxima (λ_{max}, nm)	492
Lambert-Beer's law range (µg/ml)	4-24
Regression values:	
Slope (m)	0.04
Intercept (c)	0.039
Coefficient of determination (R^2)	0.999
Normality test KS value	0.1269
Fischers extract test 'P' value.	0.6728

Table 2: Recovery studies of standard laboratory samples of Nitrazepam

Code	Actual concentration of drug in solution (µg/ml)	Drug concentration recovered after analysis (µg/ml)	% Recovery
NR_1	6	6.0548±0.2865	100.80±4.628
NR_2	10	9.8837±0.3295	98.805±3.242
NR_3	12	11.894±0.4946	99.121 ±4.114
NR_4	24	23.366±1.1887	97.355±2.984

values are represented as Mean±SD, *(n=3)*; NR_1, NR_2, NR_3, NR_4 and NR_5 signify authentic laboratory samples.

Table 3: Establishment of accuracy and precision of the method

Nitrazepam Composition (μg/ml)	Code	Cumulative strength of mixture (μg/ml)	Observed cumulative Strength (μg/ml)	% Detection
2	N_1	2	1.83±0.69	95.75
N_1+2	N_2	4	3.69±0.85	96.12
N_2+4	N_3	8	7.65±0.58	97.75
N_3+6	N_4	14	15.01±1.1	105.05
N_4+8	N_5	22	21.7±0.99	98.5

values are represented as Mean±SD, *(n=3)*; N_1, N_2, N_3, N_4 and N_5 signify authentic laboratory samples with predefined overages.

Table 4: Estimation of Nitrazepam in Various Marketed Tablets (*n=3)*

Brand Name	Company	Labelled Claim/Tablet (mg)	Amount of Nitrazepam Detected (mg)	Nitrazepam Recovery (%)
Nitravet	Anglo French	5	5.058±0.1862	101.16±3.725
Nitrosun	Sun Pharma	10	9.812±0.1012	98.12±1.012
Nipam	La-Pharma	5	4.9275±0.1597	98.55±3.145

that the complex was stable in low temperature, and showed no change in absorbance value throughout the study. The solution was also found to be retain its stability for resonable period of time, when stored at 25±2ºC (Figure 2). But, it should be noted that the color of complex starts fading when exposed to higher temperature. The order of stability of developed complex was found to be 8±1ºC>25±2ºC>50±2ºC (Figure 3-4). This suggests that the exposure of these colored solutions to high temperature should be avoided during the analysis.

The colorimetric method so developed was found to obey Lambert-Beer's law in the concentration range of 4-24 μg/ml, with coefficient of determination closer to

1 ($Y=0.039X–0.039$, $R^2=0.999$; Table 1). To validate this method for its accuracy and precision, systematically designed studies were performed, wherein it was found that the method may successfully determine the concentration of unknown samples closer to their theoretical strength, as is evinced from the outcomes of recovery studies (Table 2).

To establish the accuracy of method, standard laboratory sample of known strength (2 μg/ml) was prepared, and the strength of this solution was raised gradually from 2 to 22 μg/ml. During each addition, the corresponding increase in concentration of solution was confirmed by estimating the sample for drug content. The method was found satisfactory in detecting the cumulative rise in strength of solutions between 95.75 to 105.05% (Table 3). Similarly, the recovery studies were also performed on commercial formulations, wherein, Nitravet 5 mg (Anglo French); Nitrosun 10 mg (Sun Pharma) and Nipam 5 mg (La-Pharma) showed an average recovery of 101.16±3.725, 98.12±1.012 and 98.55±3.145; %±SD, respectively (Table 4). In both the studies, the recovery was found closer to 100%, with smaller SD.

The outcome revealed that the developed method is accurate, precise, rapid, reproducible and economic for routine detection of Nitrazepam in sample solutions. During product development research, as well believed to provide a supplementary alternative for day-to-day

analysis of Nitrazepam in academia practical based on Nitrazepam formulations.

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