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Comparative Analytical Study of Bromazepam in Pharmaceutical Dosage Forms

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Abstract

UV spectrophotometric comparative analytical study of was presented Bromazepam in pharmaceutical dosage forms. The proposed method was validated for application in routine analysis. Satisfactory results were obtained by conducting the spectrophotometry at fixed conditions. There was found analytical parameters precision, accuracy, linearity and specificity which is in great significance for optimization in the pharmaceutical and toxicological analysis of Bromazepam.

Key words: Bromazepam, UV-spectrometry, Validation

Introduction

Drugs of the benzodiazepine class have already significantly altered the field of psychopharmacology. At last few years Bromazepam (7-bromo-2,3-dihydro-5-pyridin-2-yl-1H-1,4-benzodiazepin-2-one) is one of widely used in the medicinal therapy. It is important that Bromazepam is an object in the toxicological practice too.

Different analytical methods have been reported for the identification and quantitative determination of benzodiazepine derivatives – non-aqueous titration (BP 1998), IR- and mass-spectrometry (Kintz et al., 1996; 1993; Zhang et al., 1996), HPLC and GC (Bogusz, 2001; El-Haj et al., 2001; Robertson et al., 1995). The non-aqueous titration is very simplicity but nonspecific method. The related substances and impurities (BP 1998) with similar physic-chemical properties like 2-amino-5-bromophenylpyridin-2-yl ketone; N - [4 – bromo – 2 - (pyridin – 2 - ylcarbonyl)phenyl – 2 -chloracetamide; 7-bromo - 2,3 – dihydro - 5(6-methylpyridin-2-yl)-1H- 1,4 - benzodiazepine -2- one; 3-amino-6-bromo-1,2-dihydro-4-pyridin-2-ylquinolin-2-one are determined in summary by this method. From the other side large number of analytical methods is not suitable in the performing on the routine analysis.

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The aim of this study is to validate UV spectrophotometric method for quantitative determination of Bromazepam in pharmaceutical drug dosage forms.

Materials and methods

Instruments:

All analysis were performed using a Hewllet-Packard 8452A Diode-Array single-beam UV/VIS spectrophotometer with 1 cm path length quartz cells.

Reagents:

All reagents were of analytical grade quality. For preparing of standard solutions Bromazepam CRS were used. For preparing the sample solutions 3 different dosage forms Bromazepam tablets 3 mg were used. For TLC identification Silicagel 60 F254 Merck plates with layer thickness 0.25 mm were used.

Standard solutions:

1. For TLC identification: Accurately weighed Bromazepam CRS BP (20 mg ± 0.0001) was transferred into a 20.0-ml volumetric flask, dissolved in acetone and diluted with the same solvent to volume.
2. For UV determination accurately weighed Bromazepam CRS BP (20 mg ± 0.0001) was transferred into a 200.0-ml volumetric flask, dissolved in 0.04 M HCL and diluted with the same solvent to volume. 10.0 ml from this solution was diluted to 100.0-ml with 0.04 M HCL. The UV spectra were performed by measuring the sample solutions at 270 nm in a 1-cm cell against 0.04 M HCL as the blank.

Sample solutions:

3. For TLC identification: A quantity weighed tablet powder equivalent to 10 mg Bromazepam was transferred into an Erlenmeyer flask with a ground glass-stopper, 10.0 ml of acetone was added and shacked for 10 minutes. After that the sample was filtered through filter paper black ribbon and 25 µL of the clear filtrate was applied in a point on the TLC plate.
4. For UV determination: 20 tablets, containing Bromazepam with determined average weight were pulverized and from tablet powder a quality equivalent to 10 mg Bromazepam was weighed and transferred into a 200.0-ml volumetric flask. 100 ml of 0.04 M HCL was added, the sample was shacked for 20 minutes and diluted to volume. After that the obtained solution was filtered through filter paper blue ribbon. 20.0 ml from the solution was diluted to 100.0-ml with 0.04 M HCL. The UV spectra were performed by measuring the sample solutions at 270 nm in a 1-cm cell against 0.04 M HCL as the blank.

TLC identification:

Reagents for TLC identification were acetone, chloroform, methanol and NH_3 (min 25 %); the TLC plates was activated at 105 °C for 30 minutes; the mobile phase was chloroform : methanol : NH_3 (min 25 %) in ratios 80 : 20 : 2.5 v/v/v. The developed plates were visualized by observing under UV light at 254 nm (run = 15 cm).

Precision

The homogenous samples from Bromazepam tablets were analyzed six times by the same UV spectrophotometry method and the standard deviation and related standard deviations were found. The replicate analysis were performed for the 3 Bromazepam dosage forms and compared.

Accuracy

The model mixtures of placebo (drug-free tablets) with adding of active substance Bromazepam were prepared for determinate of the accuracy. The samples, containing a quality of Bromazepam equivalent to 50 %, 100 % and 150 % were analyzed 3 times and the concentrations were found by using the method of external standard. The replicate analysis were performed for the Bromazepam dosage forms and compared.

Linearity

Six standard solutions with decreasing concentration of Bromazepam were prepared and analyzed for determinate the linearity. Beer's low is valid in concentration ratio 0.5–10 μg/ml. The standard deviation and the correlation coefficient were calculated.

Specificity

The sample from Bromazepam dosage forms, the reference substance of Bromazepam (CRS BP standard) and the placebo (drug-free tablets) were analyzed for determinate of the analytical parameter specificity. The obtained UV spectra of the placebo solutions did not show the absorption at 270 nm. There is no dependence of tablet's ingredients on the behavior of Bromazepam in the UV analytical zone.

Results and discussion

The results obtained in determining of analytical parameters accuracy, precision and linearity are shown on Fig.1 and Tables 1, 2.

Figure 1. Calibration curve of Bromazepam CRS BP in 0.04 M HCL.

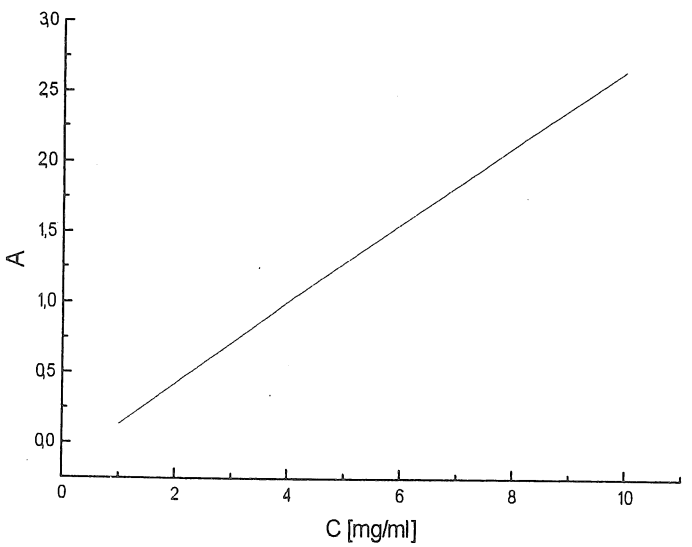

Table 1. Precision of UV spectrophotometric method for quantitative determination of Bromazepam tablets 3 mg.

Number of analysis (n)	Containing of Bromazepam in tablet dosage form (g)	$\bar{X}$	Standard deviation (SD)	Relative standard deviation (RSD)
1	0.306	3.05×10^{-1}	1.64×10^{-3}	5.37×10^{-1}
2	0.306			
3	0.305			
4	0.308			
5	0.305			
6	0.303			

Table 2. Accuracy of UV spectrophotometryc method for quantitative determination of Bromazepam tablets 3 mg.

Added quantity of Bromzepam (g)	Number of analysis (n)	Found quantity of Bromazepam (g)	Recovery (%) ± RSD
0.00150	1.	0.00150	100.00
	2.	0.00153	102.00 ± 1.68
	3.	0.00148	98.66
0.00300	1.	0.00306	102.00
	2.	0.00302	100.66 ± 1.29
	3.	0.00300	100.00
0.00450	1.	0.00447	99.33
	2.	0.00451	100.22 ± 1.12
	3.	0.00455	101.11

The correlation coefficient obtained in the studying of linearity was 0.99923 and standard deviation was 0.003 (six samples). The equation of the calibration curve seemed as follow:

$Y = A + B.x$

$A = 0.1107$ (SD = 0.0023);

$B = 0.0187$ (SD = 0.00037).

The analytical recovery found in studying of accuracy was 0 – 2 % ± 1.7 ; ± 1.3 and ± 0.96 percents of variation coefficient for each of the analyzed concentrations of Bromazepam – 50, 100 and 150 %.

All analytical parameters verified for different pharmaceutical dosage forms allow the validation of UV spectrophotometric method of analysis in the pharmaceutical practice.

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