INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DIAZEPAM AND PROPRANOLOL HYDROCHLORIDE IN COMBINED DOSAGE FORM

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Article Received on: 09/06/11 Revised on: 12/07/11 Approved for publication: 08/08/11

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ABSTRACT
The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of diazepam and propranolol hydrochloride in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in 0.05M methanolic sulphuric acid and the determinations were made at 248 nm (ZCP of propranolol hydrochloride) for diazepam and 242 nm (ZCP of diazepam) for propranolol hydrochloride. The linearity was obtained in the concentration range of 2.5-30 µg/ml for both diazepam and propranolol hydrochloride. The mean recovery was 99.77 ± 1.39 and 100.6 ± 1.18 % for diazepam and propranolol hydrochloride, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of diazepam and propranolol hydrochloride in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.
KEYWORDS: Diazepam, Propranolol hydrochloride, Derivative spectrophotometry, Zero crossing point, Methanolic sulphuric acid, Tablet

INTRODUCTION

Diazepam (DZP) is chemically 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-1, 4- benzodiazepin-2-one; $C_{16}H_{13}ClN_2O$[1], used as an anxiolytic agent[2]. It is official in IP, USP and BP. IP[3] and BP[4] describes non-aqueous titration method and USP[5] describe liquid chromatography method for its estimation. Literature survey reveals spectrophotometric[6-9], spectrofluorimetric[10], GC[11], HPLC[12], HPTLC[13-14], LC/MS[15] and radioimmunoassay[16] methods for the estimation of DZP in single dosage form. Propranolol hydrochloride (PRO) is chemically (*RS*)-1-isopropylamino-3- (1-naphthyloxy) propan-2-ol hydrochloride; $C_{16}H_{21}NO_2$, HCl[17], is beta-adrenoceptor antagonist[18]. The combination of DZP and PRO has been shown to be effective in the management of chronic anxiety. The combination was generally more effective than diazepam[18]. Propranolol hydrochloride is official in IP, USP and BP. IP[19] and BP[20] describes potentiometric titration method and USP[21] describe liquid chromatography method for its estimation. Literature survey reveals spectrophotometric[22-24], fluorimetric[25], HPLC[26-27] and chemiluminescence[28] methods for estimation of propranolol hydrochloride in single dosage form. This combination is not official in any pharmacopoeia, so no official method is available for the estimation of these two drugs in combined dosage forms. Literature survey reveals spectrophotometric[29] method for the simultaneous estimation of DZP and PRO in combined dosage form. The present manuscript describes simple, sensitive, accurate, precise, rapid and economic first order derivative spectrophotometric method for simultaneous determination of diazepam and propranolol hydrochloride in pharmaceutical tablet dosage form.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

DZP and PRO bulk powder was kindly gifted by Santham Pharmaceutical Ltd, Gandhinagar, Gujarat,

India. The commercial fixed dose combination product containing 2 mg DZP and 10 mg PRO was procured from the local pharmacy. Methanol and sulphuric acid, AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Preparation of standard stock solutions

An accurately weighed quantity of DZP (10 mg) and PRO (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with 0.05M methanolic sulphuric acid to obtain standard solution having concentration of DZP (100 µg/ml) and PRO (100 µg/ml).

Methodology

The standard solutions of DZP (10 µg/ml) and PRO (10 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 2 nm. The two spectra were overlain and it appeared that DZP showed zero crossing at 242 nm, while PRO showed zero crossing at 248 nm. At the zero crossing point (ZCP) of DZP (242 nm), PRO showed a first-derivative absorbance, whereas at the ZCP of PRO (248 nm), DZP showed a first-derivative absorbance. Hence 248 and 242 nm was selected as analytical wavelengths for determination of DZP and PRO, respectively. These two wavelengths can be employed for the determination of DZP and PRO without any interference from the other drug in their combined dosage formulations.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[30].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2.5-30 µg/ml for each DZP and PRO. Accurately measured standard solutions of DZP and PRO (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) were transferred to a series of 10 ml of volumetric flasks, separately and diluted to the mark with 0.05M methanolic sulphuric acid, and first-derivative absorbances (D1) were measured at 248 nm for DZP and 242 nm for PRO. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution (*n* = 6) for DZP and PRO (10 µg/ml) without changing the parameter of the first-derivative spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of DZP and PRO (5, 10 and 15 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of DZP and PRO by the standard addition method. Known amounts of standard solutions of DZP and PRO were added at 50, 75 and 100 % level to prequantified sample solutions of DZP and PRO (3 µg/ml for DZP and 15 µg/ml for PRO). The amounts of DZP and PRO were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[30].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of DZP and PRO in combined tablet dosage form

Twenty Tablets were weighed and powdered. The powder equivalent to 2 mg of DZP and 10 mg of PRO was transferred to a 100 ml volumetric flask. Methanolic sulphuric acid (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanolic sulphuric acid. This solution is expected to contain 100 µg/ml of DZP and 100 µg/ml of PRO. This solution (1.5 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanolic sulphuric acid to get a final concentration of DZP (3 µg/ml) and PRO (15 µg/ml). The responses of the sample solution were measured at 248 nm and 242 nm for quantitation of DZP and PRO, respectively. The amounts of the DZP and PRO present in the sample solution were calculated by fitting the responses into the regression equation for DZP and PRO in the proposed method.

RESULTS AND DISCUSSION

The standard solutions of DZP and PRO were scanned separately in the UV range, and zero-order spectra (Figure 1) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 2 nm. The two derivative spectra showed maximum absorbance at 248 nm (ZCP of PRO) for DZP and 242 nm (ZCP of DZP) for PRO. First-derivative absorbances (D1) were recorded 248 nm for DZP and 242 nm for PRO (Figure 2). First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulations. Second and third-ordered derivative spectra of the drugs were not tested because the first-order spectra give satisfactory ZCPs and good quantitative determination of both the drugs without any interference.

Linear correlation was obtained between absorbances and concentrations of DZP and PRO in the concentration ranges of 2.5–30 µg/ml for both drug. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values for DZP and PRO were found to be 1.59 and 1.44%, respectively (Table 1). The low values of relative standard deviation (less than 2 %) indicate that the proposed method is repeatable. The low RSD values of interday (0.84 – 1.96 and 1.20 – 1.98 %)) and intraday (0.54–1.72 and 0.57–1.65 %) for DZP and PRO, respectively, reveal that the proposed method is precise (Table 1). LOD values for DZP and PRO were found to be 0.45 and 0.63 µg/ml, respectively and LOQ values for DZP and PRO were found to be 1.49 and 2.08 µg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of DZP and PRO.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.77 ± 1.39 and 100.6 ± 1.18 % for DZP and PRO, respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine DZP and PRO in their combined dosage form. The results obtained for DZP and PRO were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of DZP and PRO in pharmaceutical dosage forms.

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 2.5-30 µg/ml for both DZP and PRO with co-efficient of correlation, (r^2) = 0.9978 and (r^2) = 0.9980 for DZP and PRO, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of DZP and PRO. The method can be used for the routine analysis of the DZP and PRO in combined dosage form without any interference of excipients.

ACKNOWLEDGEMENT

The authors are thankful to Santham Pharmaceutical Ltd, Gandhinagar, Gujarat, India for providing gift sample of DZP and PRO for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

PARAMETERS		First-derivative UV Spectrophotometry	
		DZP at 248 nm	PRO at 242 nm
Concentration range (µg/ml)		2.5 - 30	2.5 - 30
Regression equation (y = a + bc) Slope (b) Intercept (a)		y = 0.0045x + 0.0051 0.0045 0.0051	y = 0.0047x + 0.0031 0.0047 0.0031
Correlation Coefficient (r^2)		0.9978	0.9980
Accuracy (% recovery) (n = 5)	Level I	98.65 ± 1.68	100.3 ± 1.35
	Level II	101.2 ± 1.84	99.65 ± 1.22
	Level III	99.45 ± 0.64	101.8 ± 0.98
Repeatability (%RSD[a], n = 6),		1.59	1.44
Interday (n = 3) (%RSD)		0.84 – 1.96	1.20 – 1.98
Intraday(n = 3) (%RSD)		0.54 – 1.72	0.57 – 1.65
LOD[b] (µg/ml)		0.45	0.63
LOQ[c] (µg/ml)		1.49	2.08

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

TABLE 2: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Mean recovery ± S.D. (n = 5)
DZP	I	3	1.5	4.44	98.65 ± 1.68
	II	3	2.25	5.31	101.2 ± 1.84
	III	3	3	5.97	99.45 ± 0.64
PRO	I	15	7.5	22.57	100.3 ± 1.35
	II	15	11.25	26.16	99.65 ± 1.22
	III	15	15	30.54	101.8 ± 0.98

S. D. is Standard deviation and n is number of determinations

TABLE 3: ANALYSIS OF PRO AND DZP BY PROPOSED METHOD

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 3)	
	DZP	PRO	DZP	PRO)	DZP	PRO
I	2	10	2.00	10.06	100.3 ± 0.93	100.6 ± 1.11
II	2	10	1.97	9.93	98.25 ± 1.32	99.32 ± 1.65

S. D. is Standard deviation and n is number of determinations

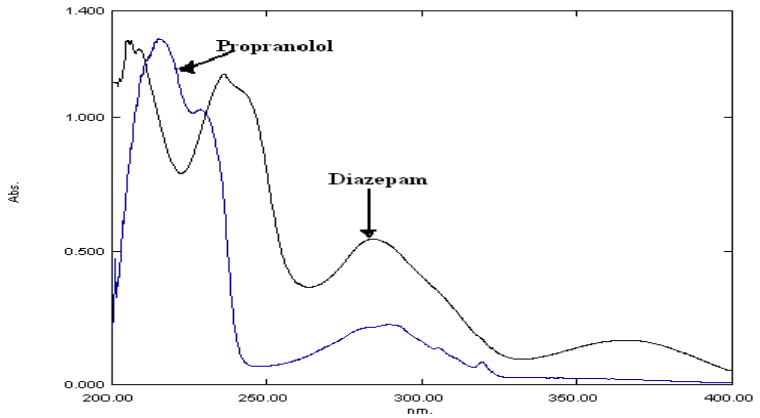

FIGURE 1: Overlain zero-order absorption spectra of DZP and PRO

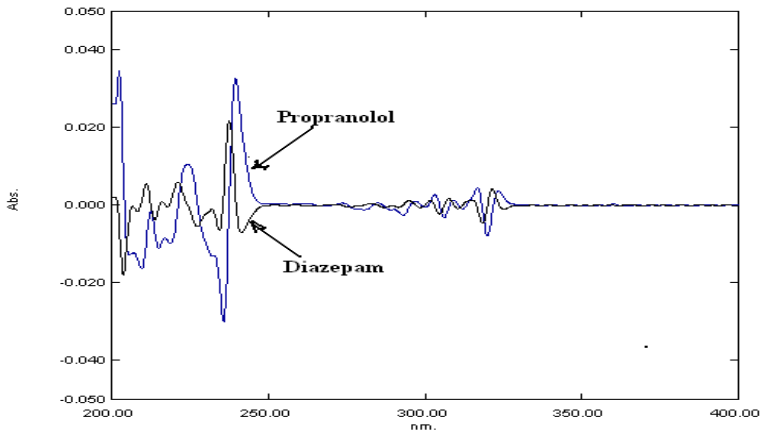

FIGURE 2: Overlain first-order derivative spectra of DZP and PRO

Source of support: Nil, Conflict of interest: None Declared