

Methodology Article

Spectrophotometric Determination of Diazepam in Pharmaceutical Forms by Ion-Pairing with Ferrithiocyanide Complex

Ali Abduh Mutair^{1,*}, Pulikkal Ajmal Koya², Niyazi Abdulmawla Sallam Al-Areqi¹

¹Department of Chemistry, Faculty of Applied Science, Taiz University, Taiz, Republic of Yemen

²Department of Chemistry, National Institute of Technology, Mizoram, India

Email address:

alimutair01@yahoo.com (A. A. Mutair)

*Corresponding author

To cite this article:

Ali Abduh Mutair, Pulikkal Ajmal Koya, Niyazi Abdulmawla Sallam Al-Areqi. Spectrophotometric Determination of Diazepam in Pharmaceutical Forms by Ion-Pairing with Ferrithiocyanide Complex. *Science Journal of Analytical Chemistry*. Vol. 4, No. 4, 2016, pp. 52-58. doi: 10.11648/j.sjac.20160404.12

Received: June 25, 2016; **Accepted:** July 9, 2016; **Published:** July 23, 2016

Abstract: A rapid and validated spectrophotometric method has been developed for determination of Diazepam (DZP) in pure and pharmaceutical dosage forms. The method was based on ion-pair association reaction of DZP with ferrithiocyanide complex in acidic medium. The color development was monitored spectrophotometrically at the maximum absorption, $\lambda_{max} = 506$ nm. The stoichiometry of the ion-pair associate was determined, and the reaction path way was postulated. The proposed method was successfully applied to the determination of DZP in five marketed brands with three labeled dosages; 2, 5, 10 mg per tablet. The analytical results of the proposed spectrophotometric method were statistically compared with those obtained from standard HPLC procedures used as a reference method. Application of the proposed procedure shows acceptable linearity, precision, repeatability, and reproducibility. The analytical results were in good agreement with the label claims. *F*-and Student's *t*-tests proved that no significant difference regarding both accuracy and precision between the proposed and reference method, and that this spectrophotometric method can be employed for the routine analysis of DZP in bulks as well as in the commercial formulations.

Keywords: DZP, Spectrophotometry, Ion-Pair Associate, HPLC

1. Introduction

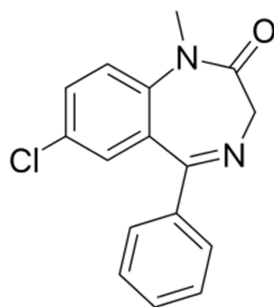


Figure 1. Chemical structure of Diazepam.

Diazepam (DZP), most commonly known by its trade name Valium, is a benzodiazepine and chemically it is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2-one. The structural formula of Diazepam with molecular formula $C_{16}H_{13}ClN_2O$, is shown in Figure 1. It is commonly used to treat a wide range of conditions including anxiety, panic attacks, insomnia, seizures (including status epilepticus), muscle spasms (such as in tetanus cases), restless legs syndrome, alcohol withdrawal syndrome, benzodiazepine withdrawal syndrome, opioid withdrawal syndrome, and Ménière's disease. However, it is a potent sedative-hypnotic, and is one of the most prescribed drugs in the world. It is also one of the top five most abused benzodiazepines, and misuse can lead to both psychological dependence and/or physical addiction [1].

Because of the therapeutic importance of DZP, many methods have been developed for its determination in pharmaceutical dosage forms and/or biological fluids. Literature survey reveals that various methods like Spectrophotometry [2-4], gas liquid chromatography [5], fluorimetry [6], first derivative spectroscopy [7], capillary electrophoresis [8] and HPLC [9, 10] are reported for the estimation of Diazepam in single dosage form. However, most of these methods are tedious and involve expensive and sophisticated experimental set up which many ordinary quality control laboratories cannot afford. Spectrophotometry occurs in the forefront of the most sensitive and widely used analytical techniques. In recent years, it has found wide applications for the determination of many important drugs [11-14]. Various spectrophotometric methods have been used for determination of DZP. The method proposed by El-Hawary [2] is based on the reaction of DZP with picric acid or dinitrobenzoic acid to form ion-pair complex. Another spectrophotometric technique [4] is based on extraction of diazepam from hydrochloric acid medium into dichloromethane (CH_2Cl_2) as a colored ion pair complex with orange II. To the best of our knowledge, none of the reported procedures described spectrophotometric method for the determination of DZP based on ion-association of DZP with ferrithiocyanide. Thus, the present work was made to develop a new, rapid, sensitive and validated spectrophotometric procedure for the determination of DZP in its commercial, pharmaceutical dosage form. The method described here is based on the formation of ion-association complex of DZP with ferrithiocyanide complex in acidic medium [15].

The color development was monitored spectrophotometrically at the maximum absorption, $\lambda_{\text{max}} = 506$ nm. The analytical results obtained from the proposed spectrophotometric method were statistically compared with those obtained from a reference HPLC method [10], the most commonly used analytical method for determination and quantification of DZP. The application of proposed method to the assay of DZP in commercial samples indicated no significant difference in precision and accuracy, and was in a satisfactory agreement with a reference HPLC method. Comparing the proposed method with some of the published ones reveals that this method is simple, need no prior separation steps and can be applied for determination of low concentrations. Also, the reagents used are common and available. In conclusion, the proposed method is comparable in accuracy and precision with the published ones.

2. Materials and Methods

2.1. Instrumentations

SHIMADZU UV-1601 double beam UV-visible spectrophotometer, equipped with 150W Xenon arc lamp. The slit widths for absorption monochromator were set at 10nm.

SHIMADZU HPLC system consisting of two LC-20AD pumps, an SPD-M 20AUV/VIS detector, a rheodyne injector, a SPD-M20A diode array detector (PDA), and a DGU-20A3 degasser.

2.2. Reagents and Drugs

DZP reference standard (99.25%) and its commonly used excipients (the mixture consisting of microcrystalline cellulose, lactose monohydrate, magnesium stearate, and Tale) were kindly provided from some approved drug control authorities. DZP-containing samples of different pharmaceutical dosage forms and several company brands (three companies and three labeled dosages) were purchased from several local markets; Placidox[®], from (Nupin Co.), labeled to contain 2 mg Diazepam per tablet, Anxol[®], from (Svizera Healthcare Co.), labeled to contain 5 mg Diazepam per tablet and Valium[®], from (Nicholas Co.), labeled to contain 10 mg Diazepam per tablet. All reagents used were of analytical grade or HPLC grade and all were purchased from Sigma Aldrich. Double-distilled deionized water was used in preparations of all the solutions.

2.3. Preparation of Solutions

2.3.1. Preparation of Stock Solutions

A stock solution of DZP hydrochloride (A), 10^{-3} M (for the proposed spectrophotometric method) was prepared by dissolving an appropriate amount of the reference standard DZP in 100 ml aqueous hydrochloric acid (0.1 M). For the reference HPLC method, a stock solution of DZP (B) was prepared dissolving the same amount of DZP in 100-ml mobile phase consisted of acetonitrile, methanol, and 0.05 M ammonium acetate (3:4:3, v/v/v). The working solutions of lower concentration were freshly prepared by dilution of the stock solution.

Fe (III) stock solution, 10^{-3} M was prepared by dissolving iron (III) chloride in double-distilled deionized water. The working solutions were prepared by the dilution of the standard solution. Stock solution of ammonium thiocyanate, 0.01 M was prepared using double-distilled deionized water.

2.3.2. Preparation of a Series of Standard Solutions for Proposed Spectrophotometric Method

A series of standard solutions were prepared in the following concentrations (10-15-20-25-30-35-40 $\mu\text{g/mL}$) of DZP hydrochloride by transferring an appropriate volume of the stock solution (A) into a 250-mL volumetric flask, containing 10 mL ammonium thiocyanate solution (0.01 M) and 10 mL Fe (III) stock solution (10^{-3} M). The resulting mixture was shaken for 5 minutes and then diluted to the mark with double-distilled deionized water. The absorbance of the prepared ion-association complex solutions of assigned DZP concentrations were measured spectrophotometrically at $\lambda_{\text{max}} = 506$ nm in a 1.0 cm cell using a reagent blank as reference. The calibration curve relating the measured absorbance to corresponding concentration was constructed. The linearity of calibration curve was examined by studying the correlation coefficient between the concentrations and the response absorbance of each concentration.

2.3.3. Preparation of a Series of Standard Solutions for Reference HPLC Method

The same series of standard solutions of DZP were prepared by diluting the stock standard solution (B) with the mobile phase, and mixing. Triplicate 20 μL injections were made for each concentration. Chromatograms were recorded under the following instrumental parameters: the flow rate was 1.5 mL min^{-1} at ambient temperature and the effluent was monitored at 251 nm. The calibration curve relating the obtained peak area ratio to corresponding concentration was constructed. The linearity of calibration curve was examined by studying the correlation coefficient between the concentrations and the response area of each concentration.

2.3.4. Analysis of Tablets

Fifteen tablets of drug (5×2 mg tablets + 5×5 mg tablets + 5×10 mg tablets) were weighed, powdered, and thoroughly mixed. An accurately weighed portion of the powdered tablets equivalent to 25 mg DZP was transferred into a 50 mL volumetric flask and extracted in 30 mL chloroform with the aid of a vortex mixer. The mixture was completed to volume with chloroform and filtered to produce a tablet-extract filtrate containing DZP. The filtrate was evaporated to dryness under vacuum and the resulting residue was dissolved in an appropriate volume of either hydrochloric acid solution (0.1 M) or mobile phase, according to the method used for the determination. Resulting sample solutions were treated under general procedure as previously described in section 2.3.2 or 2.3.3. The analytical data and results for the application of spectrophotometric procedure for the determination of DZP were compared with those obtained from HPLC method.

3. Results and Discussion

3.1. Investigation of Ion-Association Complex Stoichiometry and Optimization of Conditions for Proposed Spectrophotometric Method

UV-visible absorption spectra of free DZP, HCl and DZP-ferrithiocyanide complex solution are presented in Figure 2 and Figure 3, respectively. The remarkable bathochromic shift, narrower absorption peak (from $\lambda_{\text{max}} = 251$ nm to $\lambda_{\text{max}} = 506$ nm), and increasing molar absorptivity seen upon the addition of excess NH_4SCN solution, indicate that the complex between DZP and Ferrithiocyanide is effectively formed in acidic medium via the ion-pair association mechanism [15, 16]. The stoichiometry of ion-pair associates was investigated by Job's method of continuous variation and spectrophotometric titration. Figure 4 depicts the variation of absorbance at $\lambda_{\text{max}} = 506$ nm with the addition of varying volumes of DZP, HCl standard solution (1×10^{-6} M) to a fixed volume of equimolar Fe(III) solution. It is clearly evident that the molar ratio of DZP: Fe(III) in ion-pair associates is equal to 3:1. However, the variation of absorbance with the addition of varying volumes of NH_4SCN standard solution (Figure 5) shows that the molar ratio of SCN^- : Fe(III) in the ion-association complex remains unchanged as in the case of unbound Ferrithiocyanide ion (i.e., 6:1). This

indicates that the formation of DZP-Fe(III) ion-pair associates is more favorable than that of the inner-sphere coordination compound [17]. Regarding method optimization, it was found that the absorbance of λ_{max} remains constant and maximal in the range of 30-50 mL of DZP, HCl solution (1×10^{-6} M) and of 60-120 mL of HSCN (1×10^{-6} M) for DZP-ferrithiocyanide complex. The proposed method was also optimized with respect to the concentration of HCl solution used. The influence of the concentration of HCl on absorbance of DZP-Ferrithiocyanide complex is shown in Figure 6. It is apparent that the maximum absorbance of λ_{max} observed, when the concentration of HCl used for the preparation of DZP, HCl standard solutions was 0.1 M, is a clear evidence for the highest stability of ion-pair associates at moderate acidic media. It is worthwhile to appoint that the lowering in the stability of ion-association complex with the increase of HCl concentration beyond 0.1 M may be attributed to the increased dissociation of DZP-ferrithiocyanide complex into its corresponding contact ion-pairs and hydrothiocyanic acid [18].

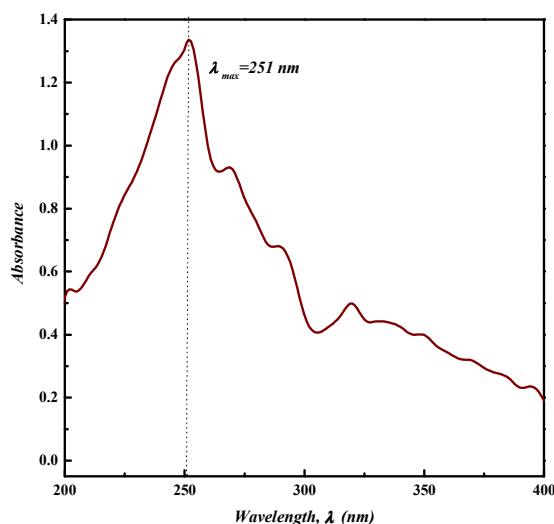


Figure 2. UV-Visible absorption spectrum of 1×10^{-6} M DZP, HCl solution.

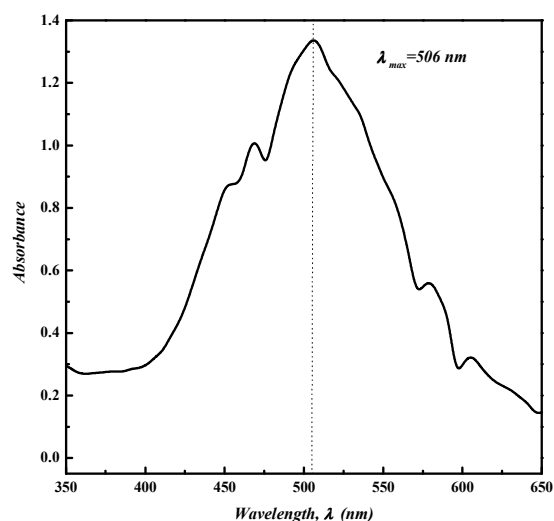


Figure 3. UV-Visible absorption spectrum of 1×10^{-6} M DZP-ferrithiocyanide ion-association complex.

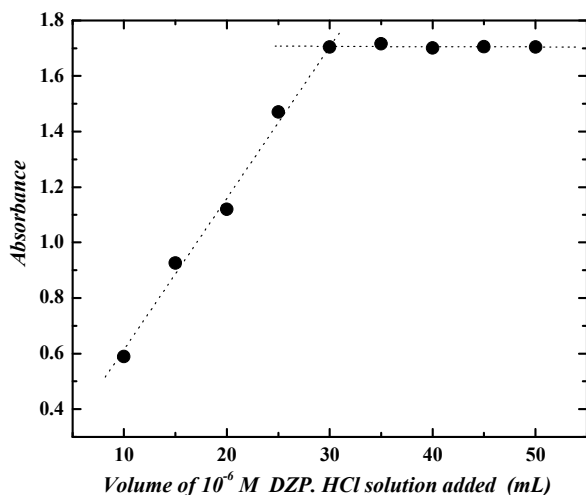


Figure 4. The variation in absorbance of DZP-ferrithiocyanide complex as a function of DZP: HCl addition (10 mL Fe(III), 1×10^{-6} M + 10 mL NH_4SCN , 1×10^{-6} M).

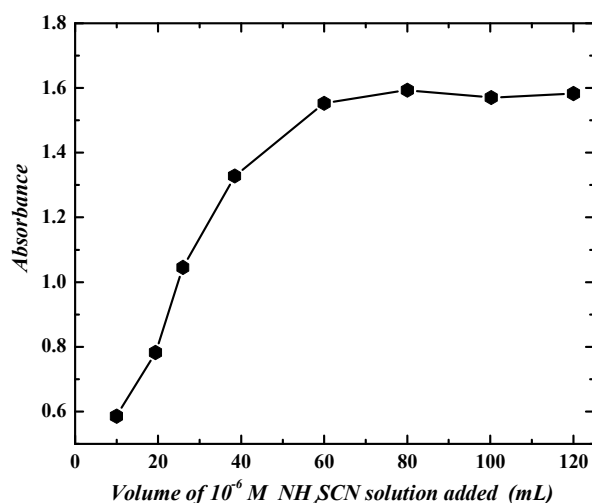


Figure 5. The variation in absorbance of DZP-ferrithiocyanide complex as a function of NH_4SCN addition (10 mL Fe(III), 1×10^{-6} M + 30 mL DZP: HCl, 1×10^{-6} M).

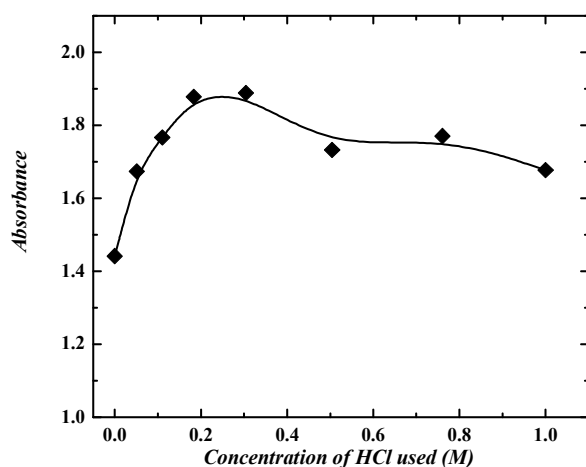


Figure 6. The variation in absorbance of DZP-ferrithiocyanide complex as a function of HCl concentration (10 mL Fe(III), 1×10^{-6} M + 30 mL DZP: HCl, 1×10^{-6} M + 10 mL NH_4SCN , 1×10^{-6} M).

The aforementioned results suggest that the reaction is first initiated by the protonation of nitrogen atom of secondary amine group in the aliphatic ring of the DZP molecule, resulting in the formation of $(\text{DZP} \cdot \text{H})^+$ cation. The DZP-Ferrithiocyanide complex is thereafter formed by electrostatic interaction between these cations and anions of ferrithiocyanide, $[\text{Fe}(\text{SCN})_6]^{3-}$. The reaction scheme is also illustrated in Figure 7.

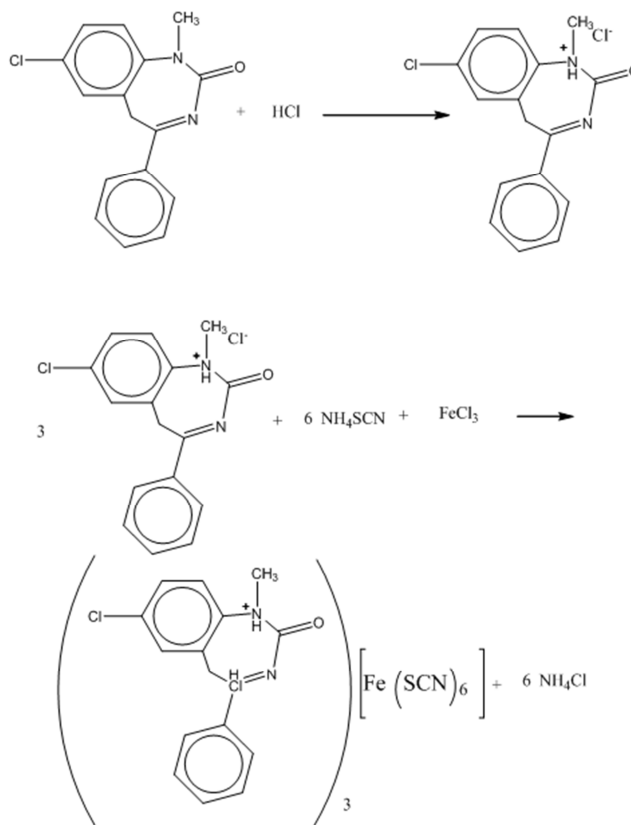


Figure 7. Reaction scheme for DZP-ferrithiocyanide complex formation.

3.2. Chromatographic Conditions for the Reference HPLC Analysis

To demonstrate the potential of the proposed spectrophotometric determination of DZP based on the formation of ion-association complex, the results obtained from the quantitative analysis of DZP in its standard solutions and pharmaceutical products by the proposed method were compared with a reference HPLC method that exhibited appreciably sensitive and accurate quantitative determination of DZP and some other benzodiazepines [10]. In this study, idealized HPLC analysis was performed on Phenomenex C18 analytical column (50 mm \times 4.6 mm, 5 μm), setting an SPD-M 20AUV/VIS detector at 255 nm. All the experiments were conducted under idealized, optimal conditions; the mobile phase consisted of acetonitrile, methanol, and 0.05 M ammonium acetate (3:4:3, v/v/v) was operated in isocratic mode at 27°C using HPLC solvent program. Six replicate 20 μL injections were made for each concentration at a fixed flow rate of 1.5 mL/min. Under these conditions, the mean

retention time of DZP was 3.82 min. Data acquisition and processing and calculation of peak area were conducted using the Analyst 1.5.1 software on a computer.

3.3. Method Validation

3.3.1. Linearity and Range

Figures 8 and 9 show the calibration curves for the proposed spectrophotometric and HPLC reference method, both constructed in the same concentration range. Regression and analytical parameters for the determination of Diamzepam using the proposed and reference method are summarized in Table 1 and 2, respectively. Satisfactory linearity, detection limit, DL , and quantification limit, QL [19], are obtained for the two methods. The regression equation parameters demonstrated for the spectrophotometric determination revealed that this proposed method is accurate, precise and specific over the specified range of DZP concentrations with no significant difference from the reference HPLC method. However, the estimated limits were verified by analyzing a suitable number of DZP-containing samples at the corresponding concentrations.

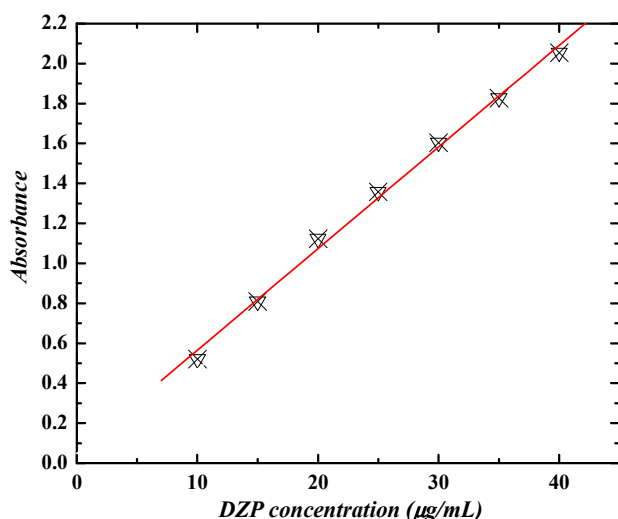


Figure 8. Standard calibration curve of the proposed spectrophotometric method.

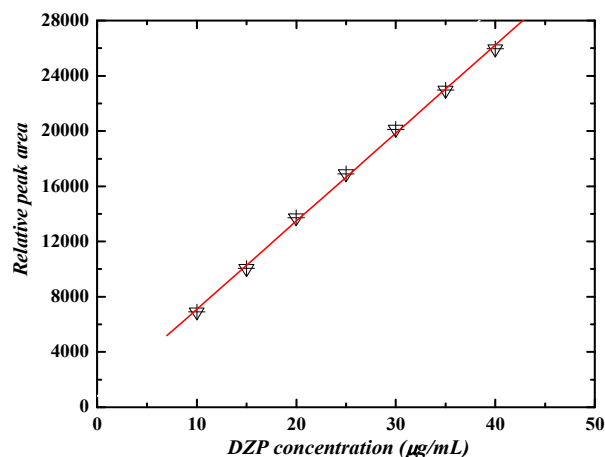


Figure 9. Standard calibration curve of the reference HPLC method.

Table 1. Regression and analytical parameters for the determination of DZP using the proposed spectrophotometric method.

Wavelength, λ_{max} (nm)	506
Concentration range ($\mu\text{g/mL}$)	10-40
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	97751.6
Intercept (a)	+ 0.11662
Slope (b)	0.05034
Standard deviation (SD)	0.02681
Correlation coefficient (R)	0.99689
Detection limit ($\mu\text{g/mL}$)	3.0
Quantification limit ($\mu\text{g/mL}$)	5.5
Relative standard deviation ($^a\text{RSD}\%$)	0.47

^aRSD was estimated from six replicate determinations.

Table 2. Regression and analytical parameters for the determination of DZP using the reference HPLC method.

Concentration range ($\mu\text{g/mL}$)	10-40
Mean retention time, t_R (min)	4.61
Mean tailing factor, T_f (min)	2.34
Capacity factor, k'	35.12
^b Range of theoretical plates	11,123-11,982
Intercept (a)	+ 776.14306
Slope (b)	911.23419
Standard deviation (SD)	407.33192
Correlation coefficient (R)	0.99897
Detection limit ($\mu\text{g/mL}$)	0.20
Quantification limit ($\mu\text{g/mL}$)	0.65
Relative standard deviation ($^a\text{RSD}\%$)	0.31

^aRSD was estimated from six replicate determinations.

^bRange of theoretical plates corresponds to the concentration range of DZP standard solutions.

3.3.2. Precision

The repeatability of the proposed spectrophotometric method was assessed through analysis of DZP within the whole investigated range of concentration three times intra-daily, according to the guidelines of ICH [20]. The results obtained from the proposed method were also statistically compared with the intra-day precision of the reference HPLC analysis performed simultaneously. The values of percentage relative standard deviation (RSD%) of the proposed method were found to be not exceeding 1.7%, and not significantly different as compared to the HPLC analysis (Table 3). This suggests a good, validated repeatability of the spectrophotometric determination of DZP, based on the ion-pair associate formation.

In other hand, inter-day precision was also investigated for all standard solutions prepared freshly over three consecutive days at the same maintained conditions. It is interesting to note that the application of the proposed method for the analysis of all the studied concentrations almost exhibits relatively small RSD% values, indicating an acceptable level of the intermediate precision of the proposed method.

3.3.3. Accuracy

The accuracy of the proposed method was examined and compared with that of the HPLC analysis, in terms of percentage recoveries. The important point to be emphasized here is that the adequate recovered concentrations along with the low values of percentage relative error ($Er\%$), which are

significantly comparable to those estimated for the reference method (Table 3), also evidence an acceptable level of the accuracy of the proposed spectrophotometric determination of DZP.

Table 3. Assay validation sheets of the proposed spectrophotometric method in comparison with the reference HPLC method for DZP determination.

Taken ($\mu\text{g/mL}$)	Proposed spectrophotometric method						Reference HPLC method					
	Intra-day			Inter-day			Intra-day			Inter-day		
	^a Found \pm SD ($\mu\text{g/mL}$)	^b RSD (%)	^c E _r (%)	^a Found \pm SD ($\mu\text{g/mL}$)	^b RSD (%)	^c E _r (%)	^a Found \pm SD ($\mu\text{g/mL}$)	^b RSD (%)	^c E _r (%)	^a Found \pm SD ($\mu\text{g/mL}$)	^b RSD (%)	^c E _r (%)
10	10.06 \pm 0.08	1.01	0.40	9.94 \pm 0.06	0.50	-0.60	10.09 \pm 0.09	0.93	0.81	9.94 \pm 0.08	0.30	-0.56
15	14.96 \pm 0.06	0.33	-0.16	15.04 \pm 0.08	0.46	0.30	15.11 \pm 0.06	0.48	0.32	15.07 \pm 0.12	0.45	0.42
20	19.93 \pm 0.08	0.26	-0.22	19.94 \pm 0.10	0.38	-0.56	19.94 \pm 0.08	0.35	-0.21	20.06 \pm 0.05	0.18	0.22
25	24.97 \pm 0.07	0.29	-0.18	24.95 \pm 0.10	0.36	-0.31	24.92 \pm 0.10	0.51	-0.28	25.17 \pm 0.08	0.34	0.38
30	30.09 \pm 0.08	0.48	0.23	30.05 \pm 0.10	0.18	0.50	29.96 \pm 0.12	0.43	-0.30	30.10 \pm 0.12	0.24	0.19
35	34.94 \pm 0.06	0.54	-0.25	35.09 \pm 0.22	0.23	0.21	34.96 \pm 0.24	0.66	-0.21	35.32 \pm 0.25	0.81	0.76
40	39.92 \pm 0.12	0.32	-0.15	40.08 \pm 0.06	0.17	0.44	39.92 \pm 0.13	0.28	-0.25	39.98 \pm 0.05	0.16	-0.08

^aMean \pm standard deviation estimated from six replicate determinations.

^bPercentage relative standard deviation.

^cPercentage relative error.

3.4. Influence of Excipients on the Selectivity of the Proposed Method

The selectivity of the proposed spectrophotometric procedures towards excipients was investigated by the analysis of prepared standard solution of intact DZP (25 $\mu\text{g/mL}$) in the absence and the presence of varying amounts (0.25 and 0.50 mg/mL) of the commonly used excipients (the mixture consisting of microcrystalline cellulose, lactose monohydrate, magnesium stearate, and Talc), that are existent in the most drug products. Recoveries and RSD (%) values

obtained by application of the proposed method are shown in Table 4. The good recoveries obtained using both ion-pair associate formation in comparison with the reference HPLC procedures suggest that there was no interference from the co-formulated inactive excipients. It is also evident from these results that the proposed spectrophotometric method is applicable to the assay of drug at satisfactory levels of accuracy and precision with no significant influence of the co-existence of the assigned excipients on the stability of the DZP-ferrithiocyanide complex.

Table 4. Analytical recovery and interfering excipient liabilities.

	Proposed spectrophotometric method			Reference HPLC method		
	^a Found \pm SD ($\mu\text{g/mL}$)	^b RSD (%)	^c E _r (%)	^a Found \pm SD ($\mu\text{g/mL}$)	^b RSD (%)	^c E _r (%)
25 $\mu\text{g/mL}$ DZP	24.97 \pm 0.10	0.43	-0.26	24.94 \pm 0.08	0.47	-0.32
25 $\mu\text{g/mL}$ DZP+0.25 mg/mL Excipients	25.05 \pm 0.15	0.54	0.42	25.07 \pm 0.12	0.70	-0.37
25 $\mu\text{g/mL}$ DZP+0.50 mg/mL Excipients	24.92 \pm 0.15	0.71	-0.36	24.98 \pm 0.08	0.35	-0.33

^aMean \pm standard deviation estimated from six replicate determinations.

^bPercentage relative standard deviation.

^cPercentage relative error estimated.

3.5. Application of the Proposed Spectrophotometric Method to the Analysis of Pharmaceutical Products

Table 5. Quantitative determination of DZP in its pharmaceutical formulations by the proposed spectrophotometric method in comparison with reference method.

Drug product (company)	Labeled dosage per tablet (mg)	Proposed spectrophotometric method	Reference HPLC method	<i>t</i> -test ^b (2.57)	<i>F</i> -test ^c (5.05)
		^a Found \pm SD (mg)	^a Found \pm SD (mg)		
Valium 10 mg/tab.	10	10.05 \pm 0.12	9.98 \pm 0.08	1.75	0.46
Anxo 5 mg/tab.	5	4.98 \pm 0.07	4.93 \pm 0.11	1.23	0.35
Placidox 2 mg/tab.	2	2.04 \pm 0.08	1.95 \pm 0.12	0.84	0.41

^aMean \pm standard deviation estimated from six replicate determinations.

^bThe tabulated *t*-value at 95% confidence limit.

^cThe tabulated *F*-value at *p*=0.05.

The proposed spectrophotometric method based on the ion-pair associate formation was successfully applied to the analysis of DZP in its commercial pharmaceutical tablets and capsules. The results of the proposed method were statistically compared with those of the reference HPLC method, in respect to the accuracy and precision. The concentration of DZP was calculated for the both methods from their

corresponding regression equations, and was then expressed as mg per tablet for the sake of comparison with the claimed amounts of DZP in the studied samples of pharmaceutical formulations. Statistical comparison of the results of the proposed spectrophotometric analysis with those obtained by the reference HPLC method was performed through Student's *t*-test for accuracy and variance ratio *F*-test for precision. It is

interesting to note that the values of Student's t-test at 95% confidence level, as well as the variance ratio F-values calculated for $p=0.05$ did not exceed the corresponding theoretical values (Table 5), indicating no significant difference in accuracy and precision, respectively, between the proposed method and the reference method.

4. Conclusion

This paper described a simple, rapid, validated spectrophotometric method developed for the determination of DZP in its pharmaceutical dosage forms based on the ion-pair associate formed between DZP and ferrithiocyanide. The factors affecting the ion-pair associate formation were studied and the conditions were optimized. The stoichiometry of the ion-pair associate was determined, and the reaction path way was postulated. The application of the proposed method in a statistical comparison with the reference HPLC procedure showed satisfactory data for all the validation parameters tested. Recovery studies indicated that there is no remarkable interference of the most commonly used excipients, so this method can be satisfactorily adopted for routine quality control analysis of DZP in bulks or its commercial products.

References

- [1] British Pharmacopoeia, the British Pharmacopoeia Secretariat of the Medicines and Healthcare products Regulatory Agency, UK, 2009.
- [2] El-Hawary WF, Issa YM, Talat A, Spectrophotometric Determination of Diazepam in Pure Form, Tablets and Ampoules. *International Journal of Biomedical Science* 2007, 3, pp. 50-55.
- [3] Dastidar D G and Biswanath S, A Comparative Study of UV-Spectrophotometry and First-Order Derivative UV-Spectrophotometry Methods for the Estimation of Diazepam in Presence of Tween-20 and Propylene Glycol, 2009, 10, pp. 1396
- [4] Manes J, Civera J, Font G, Bosch F, Spectrophotometric determination of benzodiazepines in pharmaceuticals by ion pairing, *Cienc. Ind. Farm* 1987, 6, pp 333-338.
- [5] Arthur J, De silva F, Morton A, Stefanovic V, Kaplan J. The Liquid Chromatography of Some Benzodiazepines. Hoffmann-la Roche Inc., 1964, 36, pp. 2099.
- [6] Salem AA, Barsoum BN, Izake EL. Spectrophotometric and fluorimetric determination of diazepam, bromazepam and clonazepam in pharmaceutical and urine samples. *Spectrochim Acta A Mol Biomol Spectrosc.*, 2004, 60 (4), pp. 771-780.
- [7] Mannucci C, Bertini J, Cocchini A, Perico A, Salvagnini F, Triolo A. Simultaneous determination of otilonium bromide and diazepam by first derivative spectroscopy. *J Pharm Sci.*, 1992, 81 (12), pp. 1175-1177.
- [8] Furlanetto S, Orlandini S, Massolini G, Faucci MT, La Porta E, Pinzaui S. Optimisation and validation of a capillary electrophoresis method for the simultaneous determination of diazepam and otilonium bromide. *Analyst*, 2001, 126 (10), pp 1700-1706.
- [9] Samia M, El-Gizawy M. Simultaneous Determination of Diazepam, Oxazepam and Temazepam in Spiked Urine By HPLC. *Analytical Letters*, 2000; 33 (4), pp. 629-638.
- [10] Moore C, Coulter C, Crompton K, Determination of Benzo-diazepines in Urine and Blood Using Rapid Resolution Liquid Chromatography/Triple Quadrupole Mass Spectrometry. Agilent Technologies, Immunalysis Corporation, Pomona, CA, 2007, pp. 5989-7201.
- [11] Gehad G M, Nour El-Dien F A, Khalil S M, Nehad A M, Spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochlorides through ion-pair formation with molybdenum and thiocyanate *Spectrochimica Acta Part A*, 2006, 65, pp. 1221-1226
- [12] Kaura A, Gupta V, Roy G S, Kaura M, Spectrophotometric determination of chlorpheniramine maleate and phenylpropanolamine hydrochloride in dosage forms, *International Current Pharmaceutical Journal*, 2013, 2 (5), pp. 97-100
- [13] Naveed, S, Simple UV Spectrophotometric Assay of Atorvastatin API Formulation and Their Comparative Study. *Global Journal of Medical Research*, 2014, 14, pp. 35-38
- [14] Akhoundi-Khalafi A M, Masoud R S, New Technique for Quantitative Determination of Dexamethasone in Pharmaceutical and Biological Samples Using Kinetic Spectrophotometric Method, *International Journal of Analytical Chemistry*, 2015, 2015, pp. 1-6
- [15] Misiuk W, Extractive spectrophotometric methods for the determination of doxepin hydrochloride in pharmaceutical preparations using titanium (IV) and ferricyanide complexes, *IL FARMACO.*, 2005, 60, pp. 61-69.
- [16] Basavaiah K, Krishnamurthy G, Extractive spectrophotometric determination of some phenothiazine derivatives in pharmaceutical preparations, *Talanta*, 1998, 46, pp. 665-670.
- [17] Gans P, Gill JB, Holden KML, Spectrochemistry of solutions. Part 27-Formation of $[Mg(NCS)]^+$ in solutions of $Mg(NCS)_2$ in methanol, *J. Chem. Soc. Faraday Trans.*, 1994, 90 (16), pp. 2351-2352.
- [18] Winstein S, Clippinger E, Fainberg AH, Heck R, Robinson GC, Salt effects and ion pairs in solvolysis and related reactions. III. 1 Common ion rate depression and exchange of anions during acetolysis, *Journal of the American Chemical Society*, 1956, 78 (2), pp. 328-335.
- [19] Validation of analytical procedures: text and methodology, in: International Conference on Harmonization (ICH), Q2 (R1), IFPMA, Geneva, Switzerland, 2005.
- [20] ICH, Q2 (R1), Validation of analytical procedures: text and methodology, in: International Conference on Harmonisation, Geneva, November 2005, [/http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdfS](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdfS), (accessed 1 May, 2012).