Direct, Fast Spectrophotometric Determination of Bromazepam in Its Pharmaceutical Dosage Forms Based On Ion–Pair Association Reaction

Niyazi A.S. Al–Areqi[1,*], Ajmal Koya Pulikkal[2], Hakim Q. N. M. Alarique[3], Sami Al-Naqeeb[4,*]

Abstract— A rapid and validated spectrophotometric method has been developed for determination of Bromazepam in pure and pharmaceutical dosage forms. The method was based on ion–pair association reaction of Bromazepam with iron (III) thiocyanate complex in acidic medium. The color development was monitored spectrophometrically at the maximum absorption, λ_{max} = 498 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 Bromazepam in five marketed brands with three labeled dosages (1.5, 3, 6 mg per tablet/ capsule). 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 Bromazepam in bulks as well as in the commercial formulations.

Index Terms— Bromazepam; Spectrophotometry; Ion–pair associate; HPLC

1 Introduction

BROMAZEPAM is one of the most important benzodiazepine derivatives, widely administrated to generate a variety of pharmacological effects, including anxiolytic, sedative, tranquilizer, muscle–relaxant, anti–convulsion or hypnotic [1,2]. In addition to being used to treat anxiety or panic states, Bromazepam may be used as a pre– medicant prior to minor surgery [3]. However, Bromazepam causes similar side effects to Diazepam (Valium).The most common side effects reported are drowsiness, sedation, ataxia, memory impairment, and dizziness [4,5]. Impairments to memory functions are common with bromazepam and include a reduced working memory and reduced ability to process environmental information [6]. Bromazepam typically comes in doses of 1.5 mg, 3 mg and 6 mg tablets or capsules, marketed under several brand names, including Lectopam, Lexotan, Lexilium, Lexaurin, Brazepam, Rekotnil,Bromaze and Lexotanil.

Because of the therapeutic importance of Bromazepam, many methods have been developed for its determination in pharmaceutical dosage forms and / or biological fluids. These methods include potentiometric titrations [7,8], high performance liquid chromatography (HPLC) [9–12], liquid chromatography– mass spectroscopy (LC– MS) [13], gas chromatography– mass spectroscopy (GC– MS) [14]. However, most of these methods are tedious and involve expensive and sophisticated experimental set up which many ordinary quality control laboratories cannot afford. To the best of our knowledge, none of the reported procedures describe spectrophotometric method for the determination of Bromazepam. Thus, the present work was made to develop a new, rapid, sensitive and validated spectrophotometric procedure for the determination of Bromazepam in its commercial , pharmaceutical dosage form. The method described here is based on the formation of ion–association complex of Bromazepam with iron (III) thiocyanate complex in acidic medium [15]. The color development was monitored spectrophometrically at the maximum absorption, λmax = 498 nm. The analytical results obtained from the proposed spectrophotometric method were statistically compared with those obtained from a reference HPLC method [12], the most commonly used analytical method for determination and quantification of Bromazepam. The application of proposed method to the assay of Bromazepam in commercial samples indicated no significant difference in precision and accuracy, and was in a satisfactory agreement with a reference HPLC method.

- [1]*Department of Chemistry, Faculty of Applied Science, Taiz University, Taiz, Yemen. Corresponding E-mail: niyazi75.alareqi@gmail.com* (N.A.S Al-Areqi).
- [2]*Department of Chemistry, National Institute of Technology Mizoram, Aizawl 796012, India.*
- [3]*Department of Chemistry, Faculty of Education, Taiz University, Taiz, Yemen.*
- [4]*Department of Pharmacy, Al-Saeed University, Taiz, Yemen. Corresponding E-mail: Samialnaqeeb1985@gmail.com (S. Al-Naqeeb).*

2 Materials and Methods

2.1 Instrumentations

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

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

2.2 Reagents and drugs

Bromazepam reference standard (99.60 %) and its com-

monly used excipients (the mixture consisting of microcrystalline cellulose, lactose monohydrate, magnesium stearate, and Talc) were kindly provided from some approved drug control authorities. Bromazepam– containing samples of different pharmaceutical dosage forms (tablets and capsules) and of several company brands (five companies and three labeled dosages ; 1.5, 3, 6 mg per tablet/ capsule) were purchased from several local markets. 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 Bromazepam hydrochloride (A), 10^{-3} M (for the proposed spectrophotometric method) was prepared by dissolving an appropriate amount of the reference standard Bromazepam in 100– ml aqueous hydrochloric acid (0.1 M). For the reference HPLC method, a stock solution of Bromazepam (B) was prepared dissolving the same amount of Bromazepam in 100– ml mobile phase consisted of acetonitrile, methanol, and 0.05M 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 µg/mL) of Bromazepam 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 Bromazepam concentrations were measured spectrophotometrically at λ_{max} = 498 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 Bromazepam 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 277 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 and capsules

Twenty tablets/ capsules of drug were weighed, powdered, and thoroughly mixed. An accurately weighed portion of the powdered tablets equivalent to 25 mg Bromazepam 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

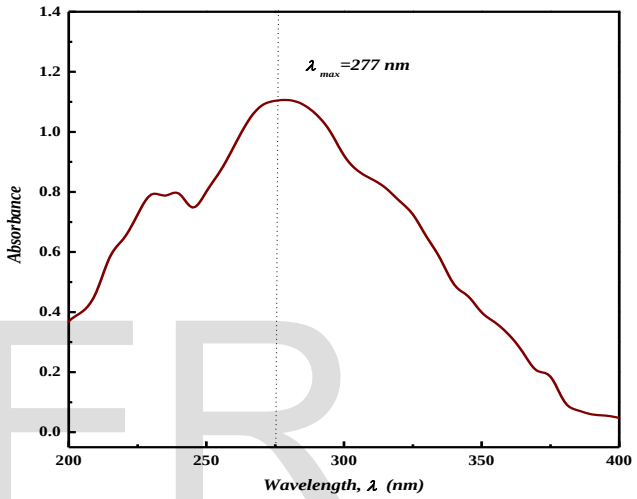

Fig. 1. UV– Visible absorption spectrum of 1× 10^{-6} M Bromazepam. HCl

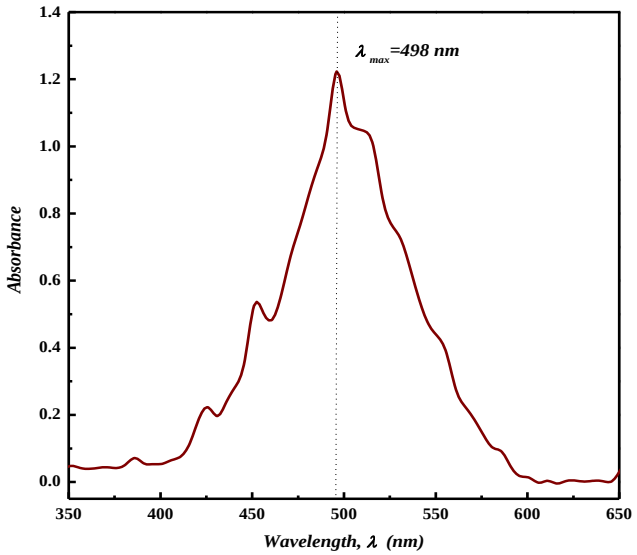

Fig. 2. UV– Visible absorption spectrum of 1× 10^{-6} M Bromazepam– Fe(III) thiocyanate ion– association complex.

a tablet–extract filtrate containing Bromazepam. 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 Bromazepam 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 Bromazepam.HCl and Bromazepam– Fe(III) thiocyanate complex solution are presented in **Fig.s 1** and **2**, respectively. The remarkable bathochromic shift, narrower absorption peak (from λ_{max}= 277 nm to λ_{max}= 498 nm), and increasing molar absorptivity seen upon the addition of excess NH_4SCN solution, indicate that the complex between Bromazepam and Fe(III) thiocyanate 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. **Fig. 3** depicts the variation of absorbance at λ_{max}= 498 nm with the addition of varying volumes of Bromazepam.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 Bromazepam: 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 (**Fig. 4**) shows that the molar ratio of SCN^-: Fe(III) in the ion– association complex remains unchanged as in the case of unbound Fe(III) thiocyanate ion (i.e., 6:1). This indicates that the formation of Bromazepam– 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 Bromazepam. HCl solution (1× 10^{-6}M) and of 60–120 mL of HSCN (1× 10^{-6} M)

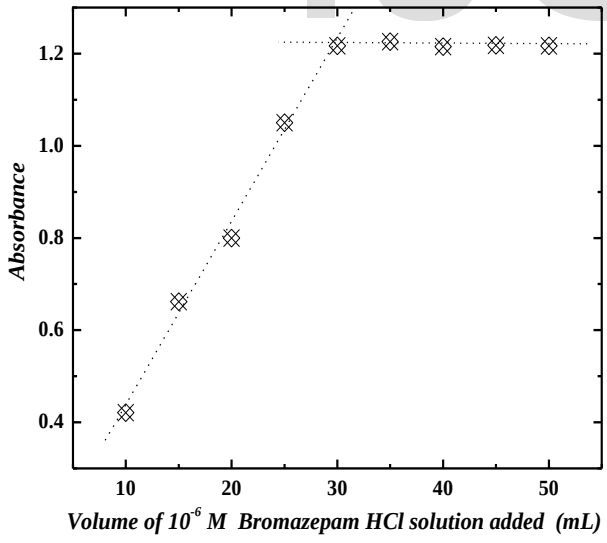

Fig. 3. The variation in absorbance of Bromazepam– Fe(III) thiocyanate complex as a function of Bromazepam. HCl addition (10 mL Fe(III), 1× 10^{-6} M + 10 mL NH_4SCN, 1× 10^{-6} M).

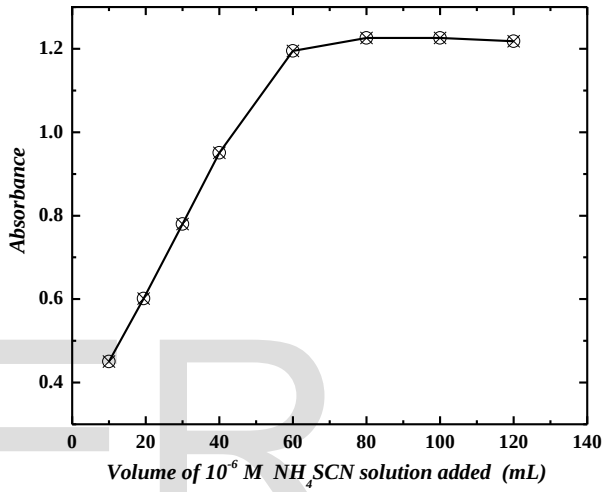

Fig. 4. The variation in absorbance of Bromazepam– Fe(III) thiocyanate complex as a function of NH_4SCN addition (10 mL Fe(III), 1× 10^{-6} M + 30 mL Bromazepam. HCl, 1× 10^{-6} M).

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 Bromazepam molecule, resulting in the formation of $(Bromazepam.H)^+$ cation. The Bromazepam– Fe(III) thiocyanate complex is thereafter formed by electrostatic interaction between these cations and anions of Fe(III) thiocyanate, $[Fe(SCN)_6]^{3-}$. The reaction scheme is also illustrated in **Fig. 5**.

3.2 Chromatographic conditions for the reference HPLC analysis

To demonstrate the potential of the proposed spectrophotometric determination of Bromazepam based on the formation of ion– association complex, the results obtained from the quantitative analysis of Bromazepam 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 Bromazepam and some other benzodiazepines [9,12]. In this study, idealized HPLC analysis was performed on Phenomenex C18 analytical column (50 mm ×4.6 mm, 5 μm),

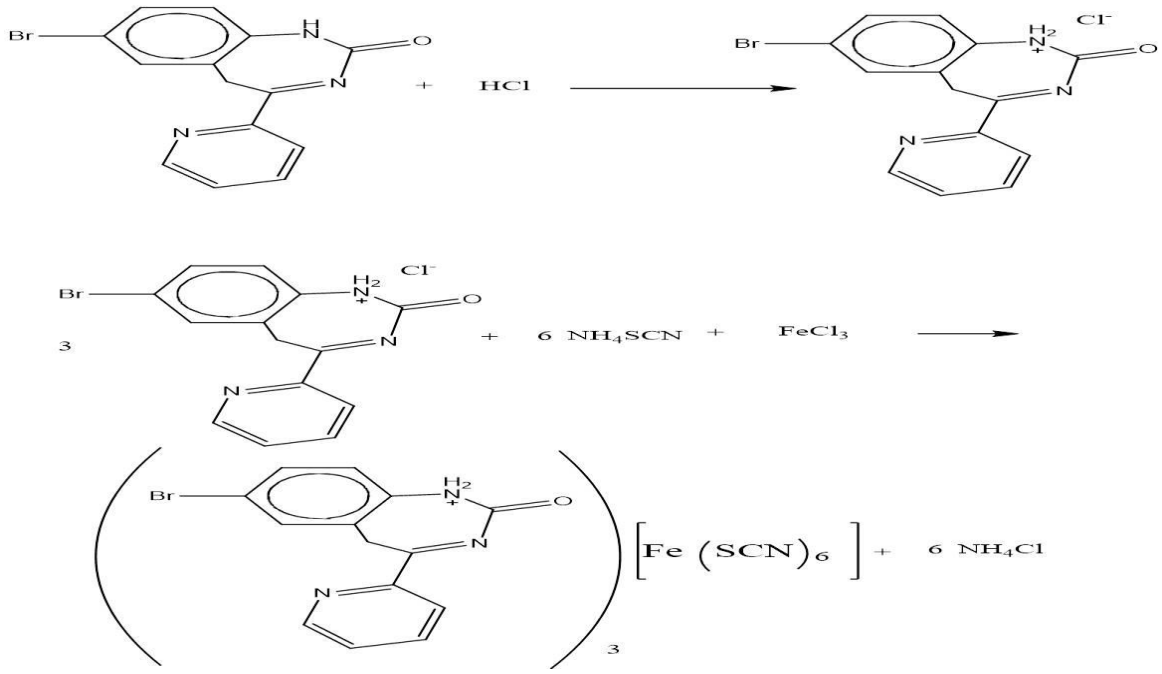

Fig. 5. Reaction scheme for Bromazepam– Fe(III) thiocyanate complex formation.

setting an SPD–M 20AUV/VIS detector at 295 nm. All the experiments were conducted under idealized, optimal conditions; the mobile phase consisted of acetonitrile, methanol, and 0.05M 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 Bromazepam was 3.82 min. Data acquisition and processing and calculation of peak area were conducted using the Analyst1.5.1 software on a Dell computer (Digital equipment Co).

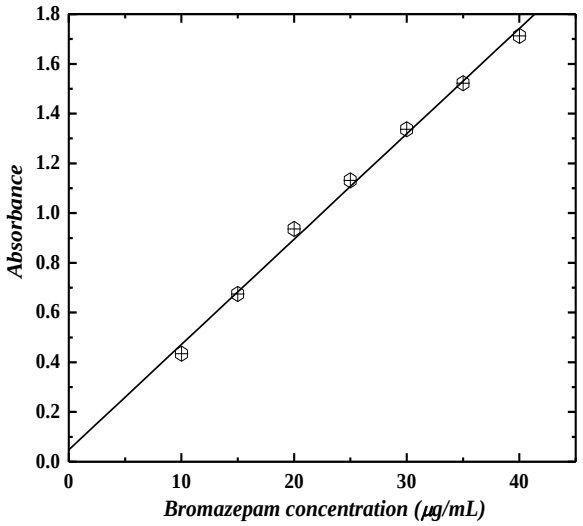

Fig. 7. Standard calibration curve of the proposed spectrophotometric method.

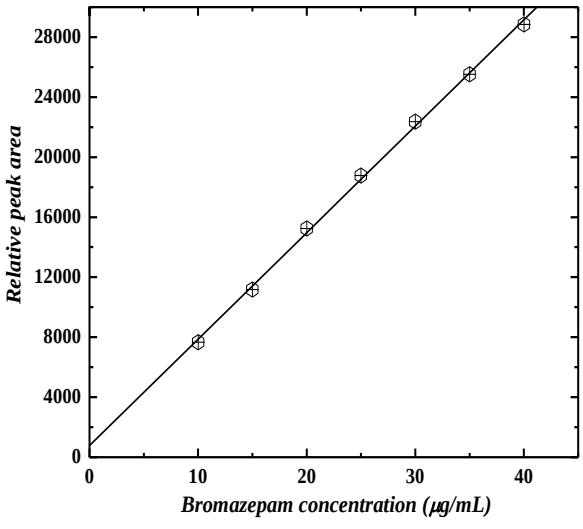

Fig. 8. Standard calibration curve of the reference HPLC method.

3.3 Method validation

3.3.1 Linearity and range

Fig.s 6 and 7 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 Bromzepam using the proposed and reference method are summarized in **Tables 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 Bromazepam concentrations with no significant difference from the reference HPLC method . However, the estimated limits were verified by analyzing a suitable number of Bromazepam– containing samples at the corresponding concentrations.

TABLE 1
REGRESSION AND ANALYTICAL PARAMETERS FOR THE DETERMINATION OF BROMZEPAM USING THE PROPOSED SPECTROPHOTOMETRIC METHOD.

Wavelength, λ_{max} (nm)	**498**
Concentration range (μg/mL)	10–40
Molar absorptivity (L mol^{-1} cm^{-1})	8.9×10^4
Intercept (*a*)	+ 0.04814
Slope (*b*)	0.04235
Standard deviation (*SD*)	0.03208
Correlation coefficient (*R*)	0.99796
Detection limit (μg/mL)	4.5
Quantification limit (μg/mL)	8
Relative standard deviation ([a] RSD%)	0.58

[a] *RSD was estimated from six replicate determinations.*

for Bromazepam– Fe(III) thiocyanate 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 Bromazepam– Fe(III) thiocyanate complex is shown in **Fig. 5**. It is apparent that the maximum absorbance of λ_{max} observed, when the concentration of HCl used for the preparation of Bromazepam. 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 Bromazepam–Fe(III) thiocyanate complex into its corresponding contact ion– pairs and hydrothiocyanic acid [18].

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 Bromazepam molecule, resulting in the formation of (Bromazepam.H)$^+$ cation. The Bromazepam– Fe(III) thiocyanate complex is thereafter formed by electrostatic interaction between these cations and anions of Fe(III) thiocyanate , $[Fe(SCN)_6]^{3-}$. The reaction scheme is also illustrated in **Fig. 5**.

TABLE 2
REGRESSION AND ANALYTICAL PARAMETERS FOR THE DETERMINATION OF BROMZEPAM USING THE REFERENCE HPLC METHOD.

Concentration range (μg/mL)	**10–40**
Mean retention time, t_R (min)	3.82
Mean tailing factor, T_f (min)	1.13
Capacity factor, k'	22.61
[b] Range of theoretical plates	12,432 – 11,258
Intercept (*a*)	+ 776.14306
Slope (*b*)	709.48853
Standard deviation (*SD*)	300.86319
Correlation coefficient (*R*)	0.99936
Detection limit (μg/mL)	0.15
Quantification limit (μg/mL)	0.53
Relative standard deviation ([a] RSD%)	0.34

[a] *RSD was estimated from six replicate determinations*

[b] *Range of theoretical plates corresponds to the concentration range of Bromazepam standard solutions.*

.

3.2 Chromatographic conditions for the reference HPLC analysis

To demonstrate the potential of the proposed spectrophotometric determination of Bromazepam based on the formation of ion– association complex, the results obtained from the quantitative analysis of Bromazepam 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 Bromazepam and some other benzodiazepines [9,12]. In this study, idealized HPLC analysis was performed on Phenomenex C18 analytical column (50 mm ×4.6 mm, 5 μm), setting an SPD–M 20AUV/VIS detector at 295 nm. All the experiments were conducted under idealized, optimal conditions; the mobile phase consisted of acetonitrile, methanol, and 0.05M 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 Bromazepam was 3.82 min. Data acquisition and processing and calculation of peak area were conducted using the Analyst1.5.1 software on a Dell computer (Digital equipment Co).

3.3.2. Precision

The repeatability of the proposed spectrophotometric method was assessed through analysis of Bromazepam within the whole investigated range of concentration three times intra– daily, according to the guidelines of ICH [20].

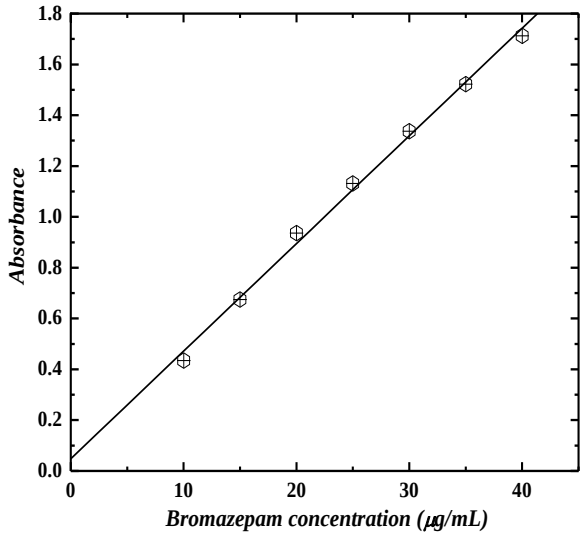

Fig. 6. Standard calibration curve of the proposed spectrophotometric method.

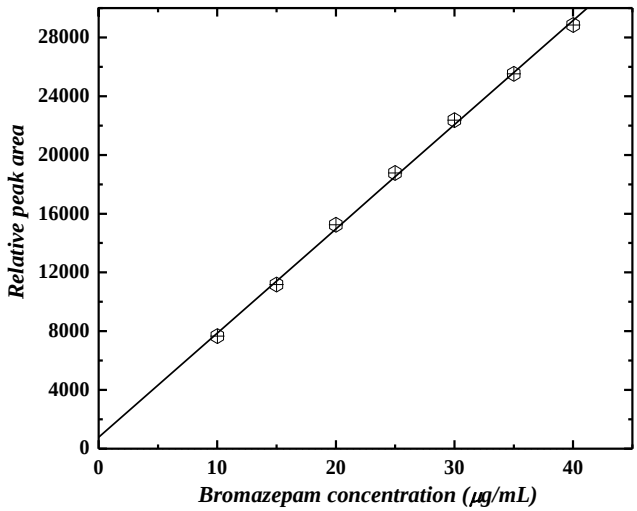

Fig. 7. Standard calibration curve of the reference HPLC method

TABLE 3
ASSAY VALIDATION SHEETS OF THE PROPOSED SPECTROPHTOMETRIC METHOD IN COMPARISON WITH THE REFERENCE HPLC METHOD FOR BROMAZEPAM DETERMINATION

[a] *Mean ± standard deviation estimated from six replicate determinations.*

[b] *Percentage relative standard deviation.*

[c] *Percentage relative error.*

	Proposed spectrophtometric method			Reference HPLC method		
	[a]Found±SD (μg/mL)	[b]RSD (%)	[c]E_r (%)	[a]Found±SD (μg/mL)	[b]RSD (%)	[c]E_r (%)
15 μg/mL Bromazepam	14.98± 0.06	0.40	–0.13	14.94± 0.13	0.87	–0.40
15 μg/mL Bromazepam + 0.25 mg/mL Excipients	14.95±0.10	0.67	–0.33	14.97±0.15	1.00	–0.20
15 μg/mL Bromazepam + 0.50 mg/mL Excipients	14.93±0.11	0.74	–0.46	14.96±0.08	0.53	–0.27

TABLE 4
ANALYTICAL RECOVERY AND INTERFERING EXCIPIENT LIABILITIES.

Taken (μg/mL)	Proposed spectrophtometric method						Reference HPLC method					
	Intra– day			Inter– day			Intra– day			Inter– day		
	[a]Found±SD (μg/mL)	[b]RSD (%)	[c]Er (%)	[a]Found±SD (μg/mL)	[b]RSD (%)	[c]Er (%)	[a]Found±SD (μg/mL)	[b]RSD (%)	[c]Er (%)	[a]Found±SD (μg/mL)	[b]RSD (%)	[c]Er (%)
10	9.95 ±0.10	1.01	–0.50	9.96 ±0.05	0.50	–0.40	10.07 ±0.13	1.29	0.70	9.94 ±0.05	0.50	–0.60
15	14.98± 0.06	0.40	–0.13	14.94± 0.13	0.87	–0.40	14.96± 0.11	0.74	–0.27	15.05± 0.10	0.66	0.33
20	19.98±0.04	0.20	–0.10	20.14±0.15	0.74	0.70	19.94±0.08	0.40	–0.30	19.98±0.05	0.25	–0.10
25	25.06±0.09	0.36	0.24	24.96±0.08	0.32	–0.16	25.12±0.19	0.76	0.48	25.07±0.06	0.24	0.28
30	29.94±0.17	0.57	–0.20	30.15±0.07	0.23	0.50	29.96±0.15	0.50	–0.13	29.95±0.05	0.17	–0.17
35	35.09±0.22	0.63	0.26	34.92±0.06	0.17	–0.23	35.32±0.32	0.90	0.91	34.96±0.24	0.69	–0.11
40	39.97± 0.05	0.12	–0.08	40.21± 0.10	0.25	0.53	39.98±0.43	1.08	–0.05	39.97± 0.08	0.20	– 0.07

[a] *Mean ± standard deviation estimated from six replicate determinations.*
[b] *Percentage relative standard deviation.*
[c] *Percentage relative error estimated.*

TABLE 5
QUANTITATIVE DETERMINATION OF BROMAZEPAM IN ITS PHARMACEUTICAL FORMULATIONS BY THE PROPOSED SPECTROPHTOMETRIC METHOD IN COMPARISON WITH REFERENCE METHOD.

Drug product (company)	Labeled dosage per tablet or capsule (mg)	Proposed spectrophtometric method	Reference HPLC method	*t– test* [b](2.57)	*F–test* [c](5.05)
		[a]Found±SD (mg)	[a]Found±SD (mg)		
Tablet (A)	6	6.06± 0.08	5.98± 0.05	2.45	0.39
Tablet (B)	1.5	1.48± 0.03	1.51± 0.06	2.45	0.25
Tablet (C)	3	3.04± 0.10	3.02 ± 0.08	0.49	0.64
Capsule (E)	3	2.97± 0.07	2.98± 0.05	0.35	0.51
Capsule (F)	1.5	1.55± 0.13	1.52± 0.11	0.56	0.72

[a] *Mean ± standard deviation estimated from six replicate determinations.*
[b] *The tabulated t– value at 95% confidence limit.*
[c] *The tabulated F– value at p=0.05.*

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 Brozepam spectrophotometric determination, 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 (E_r%), which are significantly comparable to those estimated for the refrence method (**Table 3**), also evidence an acceptable level of the accuracy of the proposed spectrophotometric determination of Bromazepam.

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 Bromazepam (15 μg/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 a satisfactory levels of accuracy and precision with no significant influence of the co–existence of the assigned excipients on the stability of the Bromazepam– Fe(III) thiocyanate complex.

3.5 Application of the proposed spectrophotometirc method to the analysis of pharmaceutical products

The proposed spectrophotometric method based on the ion– pair associate formation was successfully applied to the analysis of Bromazepam 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 Bromazepam was calculated for the both methods from their corresponding regression equations, and was then expressed as mg per tablet/ capsule for the sake of comparison with the claimed amounts of Bromazepam 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 bromazepam in its pharmaceutical dosage forms based on the ion–pair associate formed between Bromazepam and iron(III) thiocyanate. 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 Bromazepam in bulks or its commercial products.

References

[1] British Pharmacopoeia (2009) the British Pharmacopoeia Secretariat of the Medicines and Healthcare products Regulatory Agency, UK.

[2] Rang H.P., Dale M.M., Ritter J.M., et al. (2007) Rang and Dale's Pharmacology, Elsevier health, 6th edition, 535–542.

[3] Fontaine R., Annable L., Beaudry P., Mercier P., Chouinard G. (1985) Efficacy and withdrawal of two potent benzodiazepines: bromazepam and lorazepam, Psychopharmacology bulletin, **21**, 91–2.

[4] Münte T.F., Gehde E., Johannes S., Seewald M., Heinze H.J. (1996) Effects of alprazolam and bromazepam on visual search and verbal recognition memory in humans: a study with event–related brain potentials, Neuropsychobiology, **34**, 49–56. http://dx.doi.org/10.1159/000119291

[5] Hirata K., Murata M., Kurakawa A., et al. (1998) The survey of acute benzodiazepines poisoning in Japan, Jpn. J. Toxicol, **11**, 425–426.

[6] M. Cunha, C. Portela, V.H. Bastos, et al., Responsiveness of sensorimotor cortex during pharmacological intervention with bromazepam, Neurosci. Lett., 2008, 448 (1): pp. 336. doi: 10.1016/j.neulet.2008.10.024. PMID 18938214. http://dx.doi.org/10.1016/j.neulet.2008.10.024

[7] European Pharmacopoeia (2007) European Directorate for the Quality of Medicines and HealthCare, 6th edition.

[8] Salem A.A., Barsoum B.N., Izake E.L. (2003) Potentiometric determination of diazepam, bromazepam and clonazepam using solid

contact ion–selective electrodes, Anal. Chim. Acta, **498**, 79–91. http://dx.doi.org/10.1016/j.aca.2003.08.070

[9] Samanidou V.F., Pechlivanidou A.P., Papadoyannis I.N. (2007) Development of a validated HPLC method for the determination of four 1,4–Benzodiazepines in human biological fluids, J. Sep. Sci., **30**, 87–679. http://dx.doi.org/10.1002/jssc.200600365

[10] El– Mahjoub A., Staub C. (2000) High– performance liquid chromato graphic method for the determination of Benzodiazepines in plasma or serum using the column–switching technique, J. Chromatogr. B: Biomed. Sci. Appl., **742,** 381–390. http://dx.doi.org/10.1016/S0378-4347(00)00185-7

[11] Bugey A., Staub C. (2004) Rapid analysis of Benzodiazepines in whole blood by high–performance liquid chromatography: use of a monolithic column, J. Pharm. Biomed. Anal., **35**, 555–562. http://dx.doi.org/10.1016/j.jpba.2004.01.023

[12] Al–Hawasli H., Ammaral–Khayat M., Ameral–Mardini M. (2012) Development of a validated HPLC method for the separation and analysis of a Bromazepam, Medazepam and Midazolam mixture, J. Pharm Analysis, **2**, 484–491.

[13] Moore, C., Coulter C., Crompton K. (2007) Determination of Benzo–diazepines in Urine and Blood Using Rapid Resolution Liquid Chromatography/Triple Quadrupole Mass Spectrometry. Agilent Technologies, Immunalysis Corporation, Pomona, CA, 5989–7201.

[14] Aebi, B., Sturny–jungo R., Bernhard W., et al.(2002) Quantitation using GC–TOF–MS: example of Bromazepam, Forensic Sci. Int., **128**, 84–89. http://dx.doi.org/10.1016/S0379-0738(02)00165-2

[15] Misiuk W.(2005) Extractive spectrophotometric methods for the determination of doxepin hydrochloride in pharmaceutical preparations using titanium (IV) and iron (III) thiocyanate complexes, IL FARMACO., **60**, 61–69.http://dx.doi.org/10.1016/j.farmac.2004.09.004

[16] Basavaiah K., Krishnamurthy G. (1998) Extractive spectrophotometric determination of some phenothiazine derivatives in pharmaceutical preparations, Talanta, **46**, 665–670.http://dx.doi.org/10.1016/S0039-9140(97)00323-8

[17] Gans, P. Gill J.B., Holden K.M.L. (1994) Spectrochemistry of solutions. Part 27–Formation of $[Mg(NCS)]^+$ in solutions of $Mg(NCS)_2$ in methanol, J. Chem. Soc. Faraday Trans., **90** , 2351–2352. http://dx.doi.org/10.1039/ft9949002351

[18] Winstein, S. Clippinger, E. Fainberg, A.H., Heck R., Robinson G.C. (1956) Salt effects and ion pairs in solvolysis and related reactions. III.1 Common ion rate depression and exchange of anions during acetolysis, J. Amer. Chem. Soc., **78**, 328–335. http://dx.doi.org/10.1021/ja01583a022

[19] Validation of analytical procedures: text and methodology, in: International Conference on Harmonization (ICH), Q2(R1), IFPMA (2005) Geneva, Switzerland.

[20] Validation of analytical procedures: text and methodology, in: International Conference on Harmonisation, (ICH), Q2 (R1) (2012) Geneva, Switzerland.