Journal of Pre-Clinical and Clinical Research, 2010, Vol 4, No 1, 024-031
www.jpccr.eu

ORIGINAL ARTICLE

Sensitive and selective extraction-free spectrophotometric determination of quetiapine fumarate in pharmaceuticals using two sulphonthalein dyes

Nagaraju Rajendraprasad, Kanakapura Basavaiah, Kanakapura B. Vinay

Department of Chemistry, Manasagangothri, University of Mysore, Mysore, India

Abstract: Two direct, simple, sensitive and rapid extraction-free spectrophotometric methods have been developed for the determination of quetiapine fumarate (QTF) in pure form and in its dosage forms. The methods are based on the formation of ion-pair complex between the drug and two sulphonthalein acidic dyes, namely, bromophenol blue (method A) and thymol blue (method B), followed by the measurement of absorbance at 410 and 380 nm, respectively. Conformity to Beer's law enabled the assay of the drug in the range 1-20 and 1.5-30 µg mL^{-1} in method A and method B with apparent molar absorptivities of 2.97×10^4 and 1.97×10^4 L $mol^{-1}cm^{-1}$, respectively. The Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) values have also been reported for both the methods. The stoichiometry of the reaction in both cases was accomplished adopting the limiting logarithmic method and was found to be 1:2 (drug:dye). The accuracy and precision of the methods were evaluated on intra-day and inter-day basis; the relative error (%RE) was ≤ 3.5% and the relative standard deviation (RSD) was < 3%. The methods were successfully applied to the determination of drug in tablets without interference by the common co-formulated substances. Statistical comparison of the results with the reference method showed good concurrence and indicated no significant difference in accuracy and precision.

Key words: quetiapine fumarate, spectrophotometry, sulphonthalein dye, ion-pair, pharmaceuticals

INTRODUCTION

Quetiapine fumarate (QTF) – chemically known as {2-(2-(4-dibenzo[b,f] [1,4]thiazepine-11-yl-1-piperazinyl)eth oxy)ethanol, fumaric acid (1 : 2 salt; formula $C_{29}H_{33}N_3O_{10}S$; molecular weight: 615.66)} – a dibenzothiazepine derivative is one of the most recent 'atypical' antipsychotic drugs [1]. It is a selective monoaminergic antagonist with high affinity for the serotonin Type 2 ($5HT_2$) and dopamine type 2 (D_2) receptors. QTF is prescribed for the treatment of schizophrenia and other psychotic or schizoactive disorders [2-4]. QTF was approved by the FDA for the treatment of Bipolar I (Bipolar II) disorder as a monotherapeutic agent [5].

QTF is not officially recognized in any pharmacopoeia. Several analytical methods, such as HPLC [6-13], chemiluminescence spectrometry [14], electrospray ionization MS [15-18], tandem MS/MS detection [19-22], UPLC with tandem MS detection [23, 24], GC [25, 26] and voltammetry [27], are found in the literature for the determination of QTF in biological materials. Different techniques, such as polarography [28], capillary zone electrophoresis [29, 30], HPTLC [31-33], HPLC [34-37], UV-spectrophotometry [29, 38] and visible Spectrophotometry [39], have been used earlier for the determination of QTF in pharmaceuticals.

Ion-pair extractive spectrophotometry is commonly used for the assay of pharmaceuticals [40-42] due to its sensitivity and selectivity and has received considerable attention for the quantitative determination of many organic compounds [43-48]. In these cases, an ion-pair is formed between a basic compound and an anionic dye (e.g., bromophenol blue, bromocresol purple, methyl orange, etc.). At a specific pH, the ion-pair, which is immiscible with water, is extracted into an organic solvent and the concentration of the drug is determined spectrophotometrically. Though, ion-pair extractive spectrophotometry has several advantages, it has some difficulties and inaccuracies arising from incomplete extraction or the formation of emulsions between the organic solvent and the basic compound containing solution. In response to the problem resulting from extraction of the ion-pair, a few articles have been published for the analysis of pharmaceutical compounds through ion-pair formation without involving extraction [49-52].

In this paper, we describe the application of acidic dyes to the spectrophotometric determination of quetiapine fumarate without using buffers. The ion-pair formed between the drug and two sulphonthalein dyes, namely, bromophenol blue (method A) and thymol blue (method B), requires no extraction and is measured directly in dioxane and acetone in method A and method B, respectively. The proposed methods were applied successfully for the determination of the drug, either in pure or in dosage forms, with good accuracy and precision. Also, the methods were demonstrated to be both robust and rugged, and found to be free from interference by co-formulated substances when applied to dosage forms.

Experimental. *Apparatus:* All absorption measurements were made using a Systronics model 106 digital

Corresponding author: Prof. Kanakapura Basavaiah, Department of Chemistry, Manasagangothri, University of Mysore, Mysore-570 006, India.
E-mail: basavaiahk@yahoo.co.in

Received: 24 June 2010; accepted: 30 June 2010

spectrophotometer (Systronics Ltd, Ahmedabad, India) with 1 cm path length quartz cells.

Materials and reagents. Chemicals used were of analytical reagent grade. 1,4-Dioxane and acetone (spectroscopic grade) were purchased from Merk (Mumbai, India).

Materials. Phramaceutical grade QTF was procured from Cipla Ltd, Bangalore, India, and certified to be 99.5% pure. It was used without further purification. Qutipin-200 and Qutipin-100 tablets (both from Sun Pharmaceuticals Ltd, India) were purchased from a local market.

Reagents. A 0.1% bromophenol blue (Loba Chemie Ltd, Mumbai, India, 98% pure) and 0.05% thymol blue [Thomas Bakers (Chemicals) Ltd, Mumbai, India, 99% pure] stock solutions were prepared freshly in acetone.

For method A, a stock solution of QTF (50 μg mL^{-1}) was prepared by dissolving 5 mg of pure drug in 1,4-dioxane followed by gentle warming for 5 min, and the final volume was made up to mark in a 100 mL volumetric flask using the same solvent, whereas for method B, a 50 μg mL^{-1} QTF stock solution was prepared in acetone.

General procedures for the construction of calibration curves.

Method A. Varying aliquots of standard QTF solution equivalent to 1-25 μg mL^{-1} (0.1- 2.0 mL of 50 μg mL^{-1}) were accurately measured by means of a microburette and transferred into a series of 5 ml calibrated flasks, and the total volume in each flask was brought to 2 mL by adding 1,4-dioxane. After the addition of 1mL of 0.1 % BPB solution diluted to mark with 1,4-dioxane, the content was mixed well and the absorbance was measured at 410 nm against a reagent blank similarly prepared without adding QTF solution.

Method B. Into a series of 5 ml calibration flasks, aliquots (0.15-3.0 mL) of standard QTF solution (50 μg mL^{-1}) equivalent to 1.5-30.0 μg mL^{-1} QTF were accurately transferred, and to each flask 1 ml of 0.05% TB solution was added and the mixture was diluted to 5 ml with acetone. After 2 min, the absorbance of the yellow-coloured ion-pair complex was measured at 380 nm against the reference blank similarly prepared.

Standard graph was prepared in each case by plotting the absorbance *vs.* QTF concentration, and the concentration of the unknown read from the calibration graphs or computed from the respective regression equation derived using the absorbance-concentration data.

Procedure for tablets. Twenty tablets were weighed and pulverized. Weighed amount of tablet powder equivalent to 5 mg of QTF was transferred into a 100 mL volumetric flask containing ~70 mL of 1,4-dioxane followed by heating for 5 min (method A) or acetone (method B). After shaking the content for 20 min, the solution was diluted to the mark with the same solvent. This was filtered using Whatman No 42 filter paper. The first 10 mL portion of the filtrate was discarded and a suitable aliquot was used for the assay by applying procedures described earlier.

Procedure for the analysis of placebo blank and synthetic mixture. A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution prepared as described for tablets and then subjected to analysis.

A synthetic mixture was prepared by adding pure QTF (100 mg) to the above- mentioned placebo blank and the mixture was homogenized. The synthetic mixture containing 5 mg of QTF was weighed and its solution in a 100 mL volumetric flask was prepared as described under the procedure for tablets. Three different aliquots were subjected to analysis by the general recommended procedure. The concentration of QTF was found from the calibration graph or from the regression equation.

RESULTS AND DISCUSSION

Chemically, the structure of QTF features its basic nature. This structure suggests the possibility of utilizing an anionic dye as chromogenic reagent. In 1,4-dioxane/acetone, QTF does not absorb in the visible region; the dye employed has insignificant absorbance. In contrast, when a solution of BPB/TB in 1,4-dioxane/acetone is added to the drug solution, an intense yellow-coloured product – measurable at 410 nm or 380 nm, in method A or method B – is immediately produced (Fig 1 and Fig 2). This is due to an opening of the lactoid ring

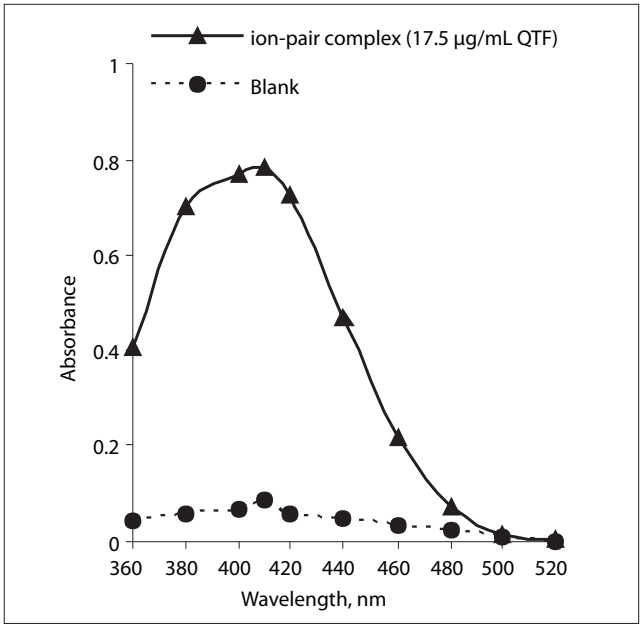


Figure 1 Absorption spectra of QTF-BPB ion-pair complex and blank.

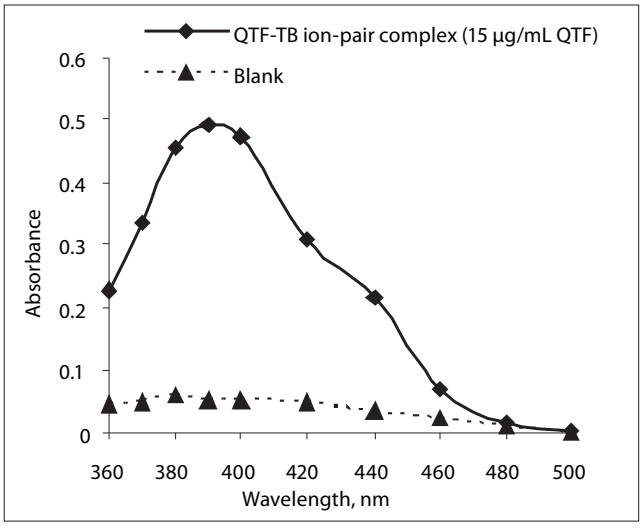
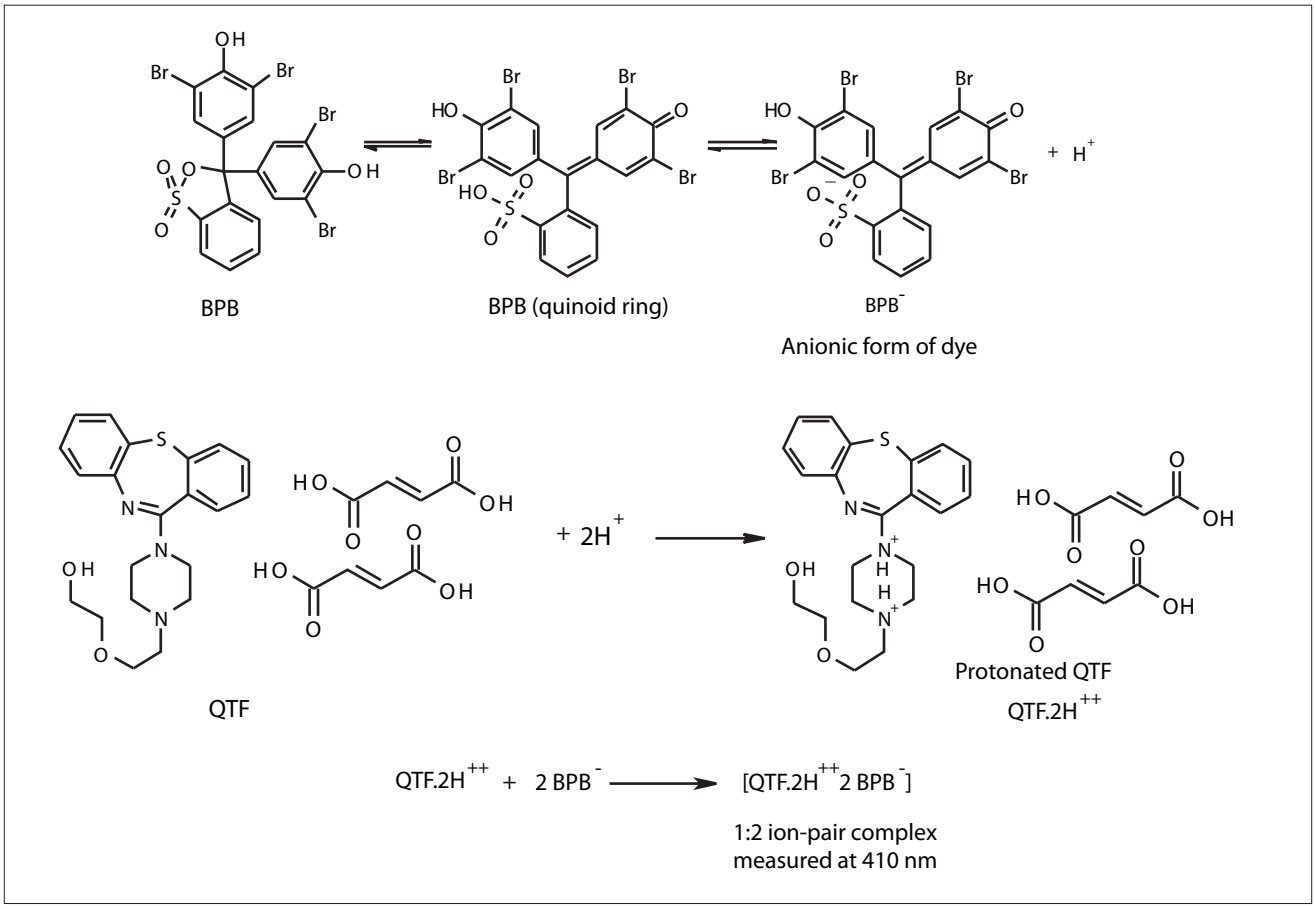


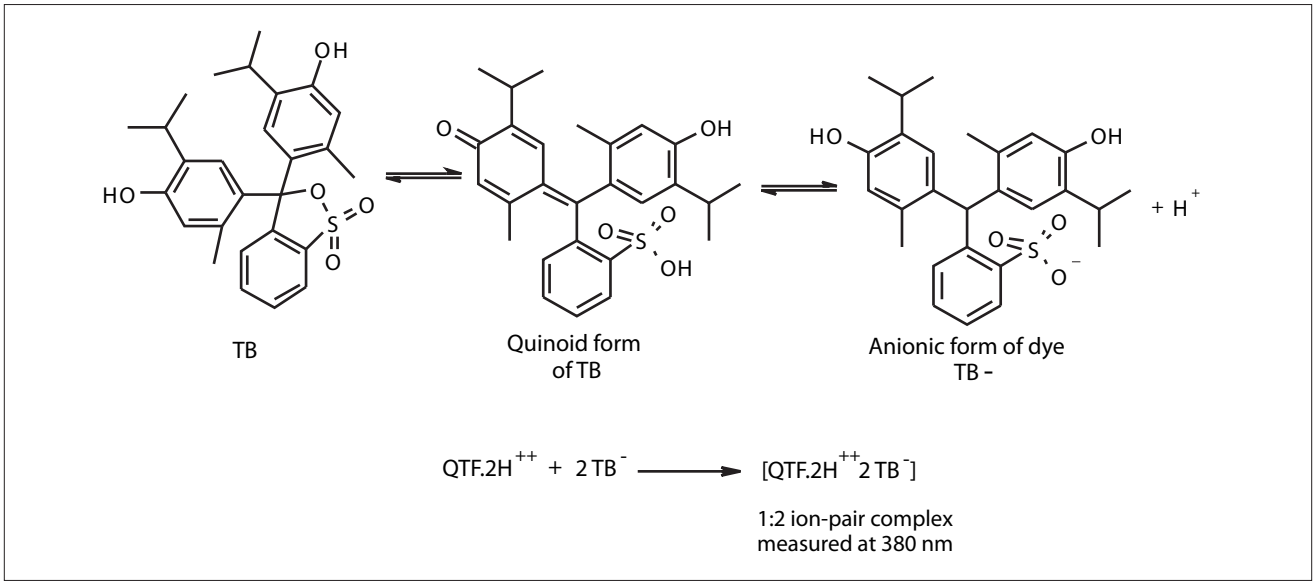
Figure 2 Absorption spectra of QTF-TB ion-pair complex and blank.

and subsequent formation of quinoid group [53]. It is supposed that the two tautomers are present in equilibrium, but due to the strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally, protonated QTF forms ion-pair with the dye. The possible reaction mechanisms are shown in Schemes 1 and 2. Anionic dye, such as BPB or TB, forms an ion-pair complex with the positively charged drug. Each drug-dye ion-pair complex molecule, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction.

Optimisation of Variables and Method Development. The experimental conditions were studied separately by measuring the absorbance of the final solution resulting from the reaction mixtures containing a fixed concentration of QTF and various amounts of the dyes.



Scheme 1 Possible reaction pathway for the formation of QTF-BPB ion-pair complex.



Scheme 2 Possible reaction schemes for the formation of QTF-TB ion-pair complex.

Effect of solvent. In order to select a suitable solvent for the formation of the ion-pair complex, the reaction of QTF with BPB or TB was studied in different solvents. Better results were obtained when QTF was dissolved in 1,4-dioxane and acetone in method A and method B, respectively, than other solvents like chloroform, 1,2-dichloroethane, acetonitrile or carbon tetrachloride. In the case of dyes, acetone was preferred to chloroform, dichloromethane, acetonitrile, 2-propanol, dichloroethane, 1,4-dioxane, methanol and ethanol, because the complex formed in these solvents had very low sample absorbance values or higher blank absorbance values. Therefore, 1,4-dioxane and acetone were chosen as solvents.

Effect of dye concentration, reaction time and stability of the ion-pair complex. It was found that 1 mL of 0.1% BPB or 1 mL of 0.05% TB solution was sufficient to produce maximum and reproducible absorbance (Fig 3a and Fig 3b) at 410 or 380 nm. The reaction time or standing time after the addition of dye was also examined. It was found that 5 min standing time was sufficient for the complete formation of the ion-pair complex in both the methods. The absorbance of the formed ion-pair complex was observed to be stable for 2.5 and 1.5 h, in method A and method B, respectively, at room temperature.

Investigation of composition of ion-pair complex. The composition of the ion-pair complex was established by adopting the limiting logarithmic method [54]. Two sets of experiments were carried out employing the general recommended procedures described above for method A and method B. The first set of experiments was carried out using increasing QTF concentrations ($4.06 \times 10^{-6} - 3.25 \times 10^{-5}$ M and $8.12 \times 10^{-6} - 4.17 \times 10^{-5}$ M, in method A and method B, respectively) at fixed reagent concentration (1.5×10^{-3} M BPB or 1.1×10^{-3} M TB in a total volume of 5 mL). The second set of experiments were carried out using increasing reagent concentrations (BPB: $3.7 \times 10^{-5} - 1.9 \times 10^{-4}$ and TB: $2.2 \times 10^{-5} - 1.3 \times 10^{-5}$ M) at fixed QTF concentration (1.04×10^{-4} and 1.56×10^{-4} M for method A and method B, respectively). The *log* absorbance values were plotted as a function of the *log* of the QTF concentration and reagent concentration in the first and second sets of experiments, respectively, in each method (fig 4 and 5). The ratios of the slopes of two straight lines were 1.95 and 2.01, for method A and method B, respectively. This means that the reaction proceeds in 1:2 (QTF:dye) stoichiometric ratio.

Method Validation. *Linearity, sensitivity, limits of detection and quantification.* A linear correlation was found between absorbance at λ_{max} and concentration of QTP in the ranges given in Table 1. The graphs are described by the regression equation:

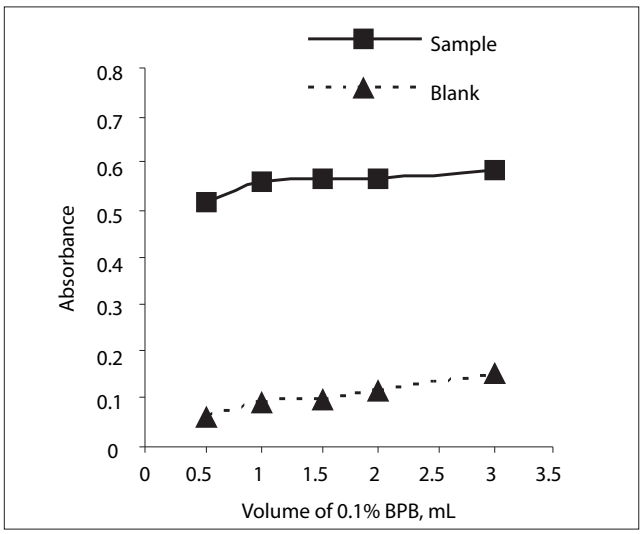
$$Y = a + bX$$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in μg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. A plot of *log* absorbance and *log* concentration, yielded straight lines with slopes equal to 1.07 and 0.96 for method A and method B, respectively, further establishing the linear relation between the two variables. The optical characteristics, such

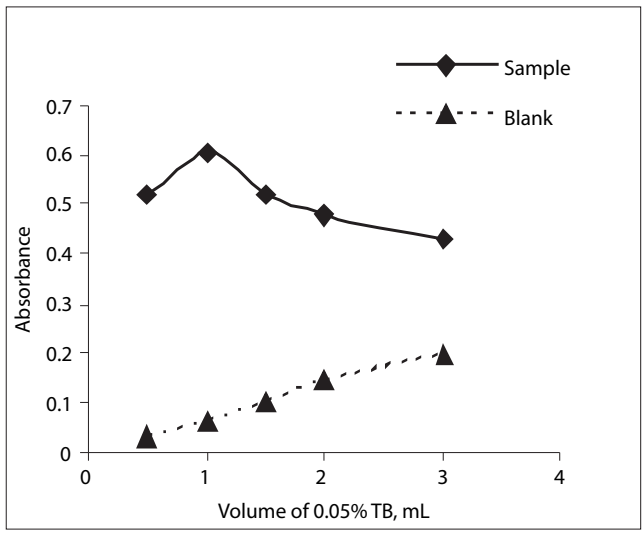
Table 1 Sensitivity and regression parameters.

Parameter	Method A	Method B
λ_{max}, nm	410	380
Colour stability, h	2.5	1.5
Linear range, μg mL^{-1}	1.0–20.0	1.5–30.0
Molar absorptivity(ε), L mol^{-1}cm^{-1}	2.97×10^4	1.97×10^4
Sandell sensitivity*, μg cm^{-2}	0.0207	0.0314
Limit of detection (LOD), μg mL^{-1}	0.21	0.54
Limit of quantification (LOQ), μg mL^{-1}	0.62	1.24
Regression equation, Y**		
Intercept (a)	0.0182	-0.0053
Slope (b)	0.0461	0.0321
Standard deviation of a (S_a)	0.0239	0.0194
$\pm tS_a/\sqrt{n}$	0.0296	0.0194
Standard deviation of b (S_b)	0.0017	0.0009
$\pm tS_b/\sqrt{n}$	0.0021	0.0009
Variance (S_a^2)	0.0006	0.0004
Regression coefficient (r)	0.9980	0.9987

* Limit of determination as the weight in μg per ml of solution, corresponding to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.
** Y = a + bX, where Y is the absorbance, X is concentration in μg/ml, a is intercept, b is slope, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope.



(a)



(b)

Figure 3 Effect of volumes of (a) BPB and (b) TB solution on the absorbance of QTF-dye ion-pair complex and blank.

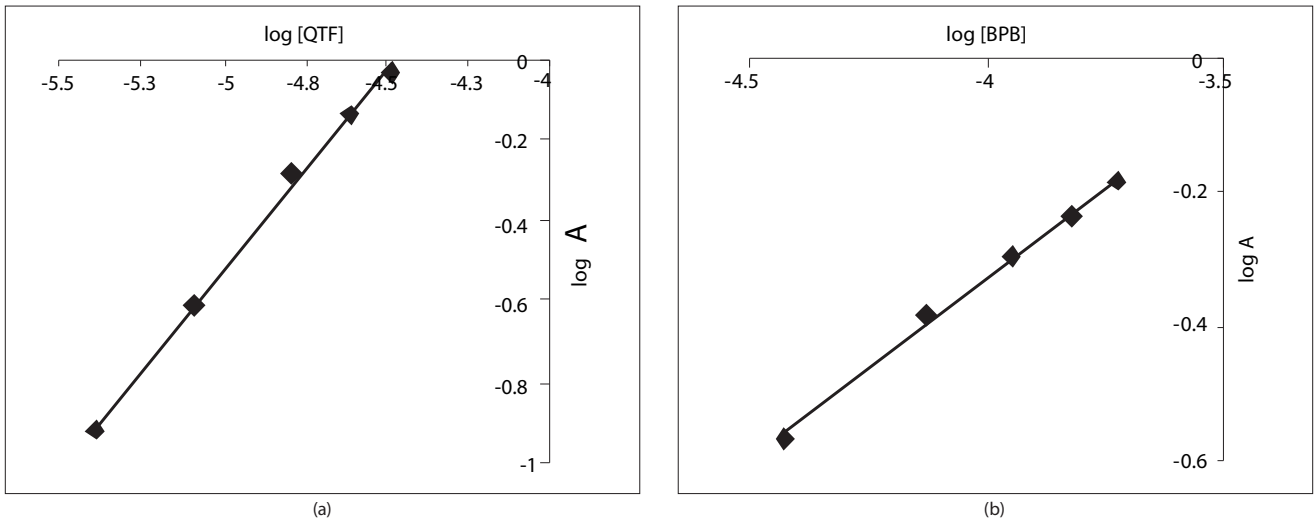


Figure 4 Limiting logarithmic plots to evaluate the stoichiometry of QTF-BPB ion-pair complex in method A: (a) variation of [QTF] and (b) variation [BPB].

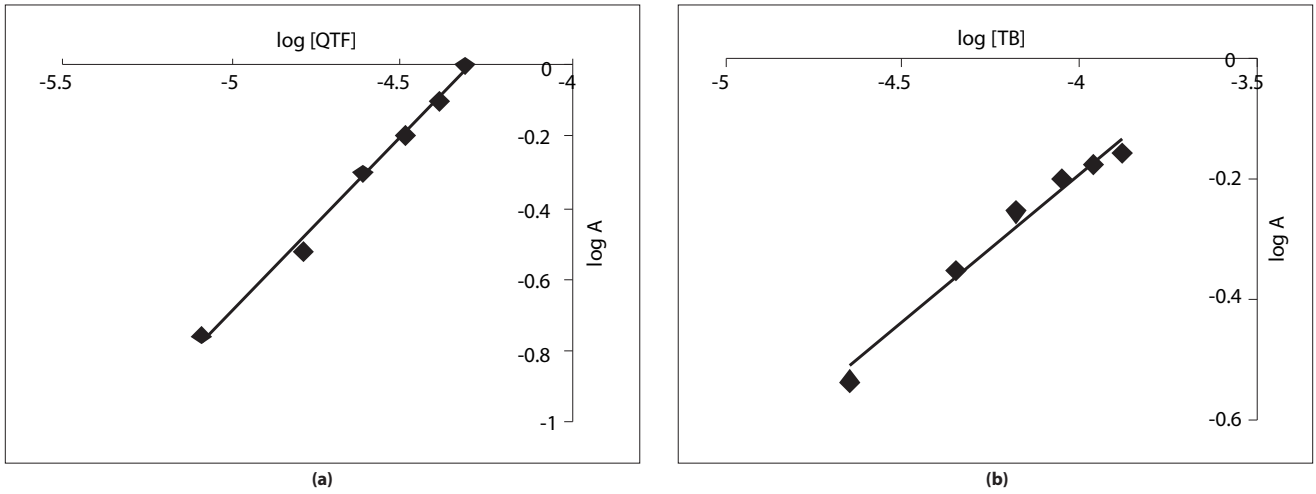


Figure 5 Limiting logarithmic plots to evaluate the stoichiometry of QTF-TB ion-pair complex in method B: (a) variation of [QTF] and (b) variation [TB].

as Beer's law limits, molar absorptivity and Sandell sensitivity values [55] of both the methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [56] using the formulae:

LOD = 3.3 S/b and LOQ = 10 S/b, (where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table 1. The high values of ε and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

Precision and accuracy. The assays described under "general procedures" were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ⩽ 2.66% (intra-day) and ⩽ 2.86% (inter-day) indicating high precision of the

Table 2 Evaluation of intra-day and inter-day accuracy and precision.

Method	QTF taken, μg mL^{-1}	Intra-day accuracy and precision, (n=7)			Inter-day accuracy and precision, (n=5)		
		QTF found±CL, μg mL^{-1}	%RE	%RSD	QTF found±CL, μg mL^{-1}	%RE	%RSD
A	5.0	4.87±0.12	2.63	2.40	5.08±0.14	1.60	2.15
	10.0	10.32±0.27	3.20	2.66	10.28±0.36	2.80	2.86
	15.0	15.15±0.27	1.00	1.80	15.25±0.37	1.67	1.98
B	10.0	10.22±0.26	2.20	2.53	10.35±0.27	3.50	2.11
	20.0	20.30±0.38	1.50	1.85	20.35±0.47	1.75	1.88
	30.0	30.98±0.39	3.27	1.25	30.58±0.81	1.93	2.14

% RE. Percent relative error, %RSD. relative standard deviation and CL. Confidence limits were calculated from: CL = ± tS/√n.
The value of t is 2.45 and 2.77 for 6 and 4 degrees of freedom respectively, at the 95% confidence level;
S = standard deviation;
n = number of measurements.

methods. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for QTP. Bias {bias % = [(Concentration found – known concentration) × 100 / known concentration]} was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ⩽ 3.5% demonstrates the high accuracy of the proposed methods.

Selectivity. The results obtained from placebo blank and synthetic mixture analyses revealed that the inactive ingredients used in the preparation did not interfere in the assay of active ingredient. The absorbance values obtained from the placebo blank solution were almost equal to the absorbance of the blank which revealed no interference from the adjuvants. To study the role of additives added to the synthetic sample, 2 mL of the resulting solution prepared by using synthetic mixture (50 μg mL^{-1} in QTF from method A and method B) was assayed (n = 4). The percentage recoveries of 96.34 –104.15 with %RSD values in the range 1.92–2.83 demonstrated the accuracy as well as the precision of the proposed method and complement the findings of the placebo blank analysis with respect to selectivity.

Robustness and ruggedness. The robustness of the methods was evaluated by making small incremental changes in the volume of dye and contact time, and the effect of the changes was studied on the absorbance of the ion-pair complex systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (⩽ 2.14%). Method ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis on four different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were in the range 2.02–3.05% indicating acceptable ruggedness. The results are presented in Table 3.

Application. The proposed methods were applied for the quantification of QTF in commercial tablets. The results obtained were compared with those obtained using a conventional UV spectrophotometric method [29], where the absorbance of the methanolic solution of QTF was measured at 246 nm. Statistical analysis of the results did not detect

Table 3 Method robustness and ruggedness expressed as intermediate precision (% RSD).

Method	QTF taken, μg mL^{-1}	Robustness		Ruggedness	
		Parameters altered		Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
		Volume of dye*	Reaction time$^{\Psi}$		
A	5.0	1.85	1.02	2.22	3.00
	10.0	1.26	0.89	2.16	2.55
	15.0	0.98	0.91	2.02	2.86
B	10.0	1.45	2.11	2.15	2.91
	20.0	2.14	1.87	2.18	2.56
	30.0	1.11	1.54	2.95	3.05

* Volumes of BPB or TB added were 1±0.2.
$^{\Psi}$ Reaction times were 5±1 min.

Table 4 Results of analysis of tablets by the proposed methods and statistical comparison of results with the reference method.

Tablet brand name$^{\Psi}$	Nominal amount (mg/tablet)	Found* (Percent of label claim ± SD)		
		Reference method	Method A	Method B
Qutipin-200	200	100.8±0.64	101.2±0.87 t= 0.84 F= 1.85	101.4±0.54 t= 1.61 F= 1.40
Qutipin-100	100	99.38±0.72	100.8±1.09 t= 2.48 F= 2.29	98.68±1.26 t= 1.11 F= 3.06

*Mean value of 5 determinations.
Tabulated t-value at the 95% confidence level and for 4 degrees of freedom is 2.77.
Tabulated F-value at the 95% confidence level and for 4 degrees of freedom is 6.39.
$^{\Psi}$ Marketed by Sun Pharmaceuticals.

Table 5 Results of recovery study via standard-addition method.

Tablets studied	Method A				Method B			
	QTF in tablet, μg mL^{-1}	Pure QTF added, μg mL^{-1}	Total found, μg mL^{-1}	Pure QTF recovered (Percent±SD*)	QTF in tablet, μg mL^{-1}	Pure QTF added, μg mL^{-1}	Total found, μg mL^{-1}	Pure QTF recovered (Percent±SD*)
Qutipin-200	5.0	2.50	7.47	98.80±1.58	10.0	5.00	14.89	97.8±1.01
	5.0	5.00	10.23	104.6±1.25	10.0	10.00	20.19	101.9±1.31
	5.0	7.50	12.63	101.7±0.88	10.0	15.00	25.84	105.6±2.11
Qutipin-100	5.0	2.50	7.56	102.4±1.11	10.0	5.00	15.12	102.4±0.89
	5.0	5.00	9.89	97.80±1.45	10.0	10.00	20.13	101.3±1.22
	5.0	7.50	12.48	99.73±1.22	10.0	15.00	25.88	105.9±0.89

* Mean value of 3 determinations.

any significant difference in the performance of the proposed method to the reference method with respect to accuracy and precision, as revealed by the Student's t-value and variance ratio F-value [57]. The results of this study are given in Table 4.

Recovery study. To further assess the accuracy of the proposed methods, the recovery experiment was performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was performed by spiking the pre-analysed tablet powder with pure QTF at three different levels: 50, 100 and 150 % of the content present in the tablet powder (taken), and the total was found by the proposed method. Each test was repeated three times. From this test the percentage recovery values were found to be in the range of 97.8–105.6, with standard deviation values from 0.88–1.58%. Closeness of the results to 100% showed the fairly good accuracy of the method. These results are shown in Table 5.

CONCLUSIONS

Two spectrophotometric methods for the determination of quetiapine fumarate in bulk drug and in pharmaceuticl dosage forms were developed and validated for accuracy, precision, linearity, robustness and ruggedness. The methods employ normal conditions rather than those previously reported, and rely on well-characterised ion-pair formation reactions. Besides, the methods have the advantages of simplicity without involving heating or extraction steps and high sensitivity. The proposed methods are rapid, simple, and in addition, offer advantages in determining QTF, (in pharmaceutical preparations), when difficulties arise with the reported visible spectrophotometric method [39] and the separation techniques such as HPLC, and include reduced cost, and speed with high accuracy. Hence, the proposed methods could be adopted for quality control in the pharmaceutical industries.

ACKNOWLEDGEMENT

Authors wish to thank Cipla Ltd, Bangalore, India, for providing pure QTF sample, and the University of Mysore, Mysore, India, for providing facilities to carry out this work. One of the authors (NRP) is grateful to the University Grants Commission, New Delhi, India, for providing a Meritorious Research Fellowship.

REFERENCES

1. Arnt J, Skarsfeldt T: **Do Novel Antipsychotics Have Similar Pharmacological Characteristics? A Review of the Evidence.** *Neuropsychopharmacology* 1998, **18**, 63-101.
2. Balestrieri M, Vampini C, Bellantuno C: Efficacy and safety of novel antipsychotics: a critical review. *Hum Psychopharmacol* 2000, **15**, 499-512.
3. Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitmann B: Effectiveness of Second-Generation Antipsychotics in Patients With Treatment-Resistant Schizophrenia: A Review and Meta-Analysis of Randomized Trials. *American J Psychiatry* 2001, **158**, 518-526.
4. Keck Jr PE, McElroy SL, Strakowski SM: Schizoaffective disorder: role of atypical antipsychotics. *Schizophrenia Res Suppl* 1999, **35** S5-S12.
5. AstraZeneca (13.1.2004): *AstraZeneca Receives FDA Approval for SEROQUEL in Bipolar Mania*, at: http://en.wikipedia.org/wiki/Quetiapine.
6. Belal F, Elbrashy A, Eid M, Nasr JJ: Stability-indicating HPLC method for the determination of quetiapine: application to tablets and human plasma. *J Liquid Chromatogr Rel Technol* 2008, **31,** 1283-1298.
7. Davis PC, Wonga AJ, Gefvertb O: Analysis and pharmacokinetics of quetiapine and two metabolites in human plasma using reversed-phase HPLC with ultraviolet and electrochemical detection. *J Pharma Biomed Anal* 1999, **20,** 271-282.
8. Sachse J, Köller J, Härtter S, Hiemke C: Automated analysis of quetiapine and other antipsychotic drugs in human blood by high performance-liquid chromatography with column-switching and spectrophotometric detection. *J Chromatogr B* 2006, **830**, 342-348.
9. Saracino MA, Mercolini L, Flotta G, Albers LJ, Merli R, Raggi MA: Simultaneous determination of fluvoxamine isomers and quetiapine in human plasma by means of high-performance liquid chromatography. *J Chromatogr B Anal Tech Biomed Life Sci* 2006, **843**, 227-233.
10. Mandrioli R, Fanali S, Ferranti A, Raggi MA: HPLC analysis of the novel antipsychotic drug quetiapine in human plasma. *J Pharma Biomed Anal* 2002, **30,** 969-977.
11. Frahnert C, Rao ML, Grasmader K: Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. *J Chromatogr B* 2003, **794,** 35-47.
12. Hasselstroem J, Linnet K: Fully automated on-line quantification of quetiapine in human serum by solid phase extraction and liquid chromatography. *J Chromatogr B Anal Technol Biomed Life Sci* 2003, **798**, 9-16.
13. Li WB, Xue YZ, Zhai YM, Zhang J, Guo GX, Wang CY, Cai ZJ: Determination of quetiapine fumarate in serum by HPLC with ultraviolet detection. *Yaowu Fenxi Zazhi* 2003, **23,** 247-251.
14. Bellomarino SA, Brown AJ, Conlan XA, Barnett NW: Preliminary evaluation of monolithic column high-performance liquid chromatography with tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence detection for the determination of quetiapine in human body fluids. *Talanta* 2009, **77**, 1873-1876.
15. Li KY, Cheng ZN, Li X, Bai XL, Zhang BK, Wang F, Li HD: Simultaneous determination of quetiapine and three metabolites in human plasma by high-performance liquid chromatography-electrospray ionization mass spectrometry. *Acta Pharmacol Sin* 2004, **25,** 110-114.
16. Zhou Z L, Li X, Li KY, Xie ZH, Cheng ZN, Peng WX, Wang F, Zhu RH, Li HD: Simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in plasma by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 2004, **802,** 257-262.
17. Li Z, Tan ZR, Ouyang DS, Wang G, Wang LS, Zhou G, Guo D, Chen Y, Zhou HH: HPLC - MS/MS determination of quetiapine in human plasma. *Yaowu Fenxi Zazhi* 2008, **28,** 706-708.
18. Lin SN, Chang Y, Moody DE, Foltz RL: A liquid chromatographic-electrospray-tandem mass spectrometric method for quantitation of quetiapine in human plasma and liver microsomes: application to a study of *in vitro* metabolism. *J Anal Tox* 2004, **28**, 443-448.
19. Barrett B, Holcapek M, Huclova J, Borek-Dohalsky V, Fejt P, Nemec B, Jelinek I: Validated HPLC-MS/MS method for determination of quetiapine in human plasma. *J Pharm Biomed Anal* 2007, **44,** 498-495.
20. Nirogi R, Bhyrapuneni G, Kandikere V, Mudigonda K, Ajjala D, Mukkanti K: Sensitive liquid chromatography tandem mass spectrometry method for the quantification of Quetiapine in plasma. *Biomed Chromatogr* 2008, **22,** 1043–1055.
21. Tan A, Pellerin B, Couture J, Vallée F: An automated SPE extraction method for the rapid analysis of quetiapine in human EDTA K2 plasma by LC/MS/MS. *SFBC Anafarm*, at: http://www.aapsj.org/abstracts/AM_2006/staged/AAPS2006-000989.PDF
22. Kundlik ML, Kambli S, Shah V, Patel Y, Gupta S, Sharma R, Zaware B, Kuchekar SR: Quantification of Quetiapine in Human Plasma by LC–MS–MS. *Chromatographia* 2009, **70**, 1587-1592.
23. Li KY, Zhou YG, Ren HY, Wang F, Zhang BK, Li HD: Ultra-performance liquid chromatography–tandem mass spectrometry for the determination of atypical antipsychotics and some metabolites in *in vitro* samples. *J Chromatogr B* 2007, **850**, 581-585.
24. Tu JY, Xu P, Xu DH, Li HD: UPLC-MS-MS analysis of quetiapine and its two active metabolites, 7-hydroxyquetiapine and 7-hydroxy-N-dealkylquetiapine, in rat plasma and cerebrospinal fluid. *Chromatographia* 2008**,** **68**, 525-532.
25. McMullin MM: Development of a gas chromatographic method for the determination of quetiapine in human serum, and a report of patient values. *Ther Drug Monit* 1999, **21**, 459-459

26. Atanasov VN, Kanev KP, Mitewa MI: Detection and identification of atypical quetiapine metabolite in urine. *Central Europ J Med* 2008, **3,** 327-331.
27. Ozkan SA, Dogan B, Uslu B: Voltammetric analysis of the novel atypical antipsychotic drug quetiapine in human serum and urine. *Microchim Acta* 2006, **153**, 27-35.
28. El-Enany N, El-Brashy A, Belal F, El-Bahay N: Polarographic analysis of quetiapine in pharmaceuticals. *Portugaliae Electrochimica Acta* 2009, **27**, 113-125.
29. Pucci V, Mandrioli R, Ferranti A, Furlanetto S, Raggi MA: Quality control of commercial tablets containing the novel antipsychotic quetiapine. *J Pharm Biomed Anal* 2003, **32**, 1037-1044.
30. Hillaert S, Snoeck L, van den Bossche W: Optimization and validation of a capillary zone electrophoretic method for the simultaneous analysis of four atypical antipsychotics. *J Chromatogr* 2004, **1033,** 357-362.
31. Dhandapani B, Somasundaram A, Raseed SH, Raja M, Dhanabal K: Development and validation of HPTLC method for estimation of quetiapine in bulk drug and in tablet dosage form. *Int J PharmTech Res* 2009, **1**, 139-141.
32. Skibiński R, Komsta Ł, Kosztyła I: Comparative validation of quetiapine Determination in tablets by NP-HPTLC and RP-HPTLC with densitometric and videodensitometric detection. *J Planar Chromatogr Modern TLC* 2008, **21,** 289-294.
33. Dhaneshwar SR, Patre NG, Mahadik MV: Stability-indicating HPTLC method for quantitation of quetiapine fumarate in the pharmaceutical dosage form. *Acta Chromatographia* 2009, **21**, 83-93.
34. Radha Krishna S, Rao BM, Someswara Rao N: A validated stability indicating hplc method for the determination of related substances in quetiapine fumarate. *Rasayan J Chem* 2008, **1**, 466-474.
35. Bharathi CH, Prabahar KJ, Prasad CHS, Srinivasa Rao M, Trinadhachary GN, Handa VK, Dandala R, Naidu A: Identification, isolation, synthesis and characterization of impurities of quetiapine fumarate. *Pharmazie* 2008, **63**, 14-19.
36. Fu CM, Wang RZ: Reversed phase-HPLC determination of quetiapine fumarate in tablets. *Zhongguo Xinyao Zazhi* 2002, **11**, 144-146.
37. Raju IVS, Raghuram P, Sriramulu J: Development and Validation of a New Analytical Method for the Determination of Related Components in Quetiapine Hemifumarate. *Chromatographia* 2009, **70**, 545.
38. Fursule RA, Rupala DK, Mujeeb Gulzar Khan Md, Shirkhedkar AA, Surana SJ: Determination of quetiapine fumarate and cilostazol in bulk and tablet by uv-spectrophotometry. *Biosci Biotechnol Res Asia* 2008 (May), at: http://www.biotech-asia.org/display.asp?id=429.
39. Arulappa RX, Sundarapandian M, Venkataraman S, Boopathi M, Kaurav M: Spectrophotometric Determination of Quetiapine Fumarate in Bulk and Dosage Form. *Res J Pharm Tech* 2009, **2,** 884.
40. Onal A, Kepekci SE, Oztunc A: Spectrophotometric methods for the determination of the antidepressant drug paroxetine hydrochloride in tablets. *J AOAC Intl* 2005, **88,** 490-495.
41. Rahman N, Ahmad Khan N, Hejaz ASN: Extractive spectrophotometric methods for the determination of nifedipine in pharmaceutical formulations using bromocresol green, bromophenol blue, bromothymol blue and eriochrome black T. *IL Farmaco* 2004, **59,** 47-54.
42. Ramesh KC, Gowda BG, Melwanki MB, Seetharamappa J, Keshavayya J: Extractive spectrophotometric determination of antiallergic drugs in pharmaceutical formulations using bromopyrogallol red and bromothymol blue. *Anal Sci* 2001, **17,** 1101-1103.
43. Rahman N, Hejaz-Azmi SN: Extractive spectrophotometric methods for determination of diltiazem HCl in pharmaceutical formulations using bromothymol blue, bromophenol blue and bromocresol green. *Pharm J Biomed Anal* 2000, **24,** 33-41.
44. Silva N, Schapoval EES: Spectrophotometric determination of etidocaine in pharmaceutical (dental) formulation. *J Pharm Biomed Anal* 2002, **29,** 749-754.
45. Perez-Ruiz T, Martinez-Lozano C, Tomás V, Sanz A, Sahuguillo E: Flow-injection extraction-spectrophotometric method for the determination of ranitidine in pharmaceutical preparations. *J Pharm Biomed Anal* 2001, **26,** 609-615.
46. Basavaiah K, Charan VS: Titrimetric and spectrophotometric assay of some antihistamines through the determination of the chloride of their hydrochlorides. *IL Farmaco* 2002, **57,** 9-17.
47. Maghsoudi H, Fawzi A: Direct spectrophotometric determination of thebaine in Arya II population capsules of Papaver bracteatum Lindi. *J Pharm Sci* 1978, **67,** 32-35.
48. Farsam H, Yahya-saeb HH, Fawzy A: Spectrophotometric determination of codeine in pharmaceutical preprations. *Int J Pharm* 1981, **7,** 343-348.
49. El-Kerdawy MM, Moustafa MA, El-Ashry SM, El-Waseef DR: Spectrophotometric determination of certain phenothiazines and their dosage forms using Bromophenol blue. *Anal Lett* 1993, **26,** 1669-1680.
50. Abdine HH: Spectrophotometric determination of cisapride using some sulfonphthalein dyes. *Alex J Pharm Sci* 2000, **14**, 75-78.
51. Abdine H, Belal F, Zoman N: Simple spectrophotometric determination of cinnarizine in its dosage forms. *Il Farmaco* 2002, **57,** 267-271.
52. Manjunatha DH, Shaikh SMT, Harikrishna K, Sudhirkumar R, Kandagal PB Seetharamappa: Simple and sensitive spectrophotometric methods for the determination of acebutolol hydrochloride in bulk sample and pharmaceutical preparations. *Ecl Quim Sao Paulo* 2008, **33,** 37-40.
53. Safwan Ashour, Fawaz Chehna M, Roula Bayram: Spectrophotometric Determination of Alfuzosin HCl in Pharmaceutical Formulations with some Sulphonephthalein Dyes. *Intl J Biomed Sci* 2006, **2,** 273-278.
54. Rose J: *Advanced Physico-chemical Experiments*, Pitman and Sons, London 1964, 67.
55. Zavis H, Ludvik D, Milan K, Ladislaw S, Frantisck V: *Handbook of Organic Reagents in Inorganic Analysis*. (Transl. & Ed.: Chalmers, SK), Ellis Horwood Ltd., Chichester (UK), a Division of John Wiley & Sons Inc., New York, London, Sydney, Toronto 1976, p.364.
56. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology, 6 November 1996, incorporated in November 2005, London.
57. Inczedy J, Lengyel T, Ure AM, *IUPAC Compendium of Analytical Nomenclature : Definitive Rules,* Blackwell Science Inc., Boston 1998.