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SPECTROPHOTOMETRIC DETERMINATION OF QUETIAPINE FUMARATE IN PHARMACEUTICALS AND HUMAN URINE BY TWO CHARGE-TRANSFER COMPLEXATION REACTIONS

Two simple, rapid and accurate spectrophotometric procedures are proposed for the determination of quetiapine fumarate (QTF) in pharmaceuticals and in spiked human urine. The methods are based on charge transfer complexation reactions of free base form of the drug (quetiapine, QTP), as n-electron donor (D), with either p-chloranilic acid (p-CAA) (method A) or 2,3-dichloro-5,6-dicyanoquinone (DDQ) (method B) as π-acceptors (A). The coloured charge transfer complexes produced exhibit absorption maxima at 520 and 540 nm, in methods A and B, respectively. The experimental conditions such as reagent concentration, reaction solvent and time have been carefully optimized to achieve the maximum sensitivity. Beer's law is obeyed over the concentration ranges of 8.0-160 and 4.0-80.0 μg mL^{-1}, for methods A and B, respectively. The calculated molar absorptivity values are 1.77×10^3 and 4.59×10^3 $l\ mol^{-1}cm^{-1}$, for methods A and B, respectively. The Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) have also been reported. The stoichiometry of the reaction in both cases was accomplished adopting the limiting logarithmic method and was found to be 1:2 (D:A). The accuracy and precision of the methods were evaluated on intra-day and inter-day basis. The proposed methods were successfully applied for the determination of QTF in pharmaceutical formulations and spiked human urine.

Keywords: quetiapine fumarate, determination, spectrophotometry, C-T complex, pharmaceuticals, spiked human urine.

Quetiapine fumarate (QTF), 2-(2-(4-dibenzo[b,f] [1,4]thiazepine-11-yl-1-piperazinyl)ethoxy)-ethanol, fumaric acid (1:2 salt; formula $C_{29}H_{33}N_3O_{10}S$; molecular weight: 615.66) (Figure 1), is a dibenzothiazepine derivative and is one of the most recent "atypical" antipsychotic drugs [1]. QTF was introduced in the clinic as a new antipsychotic drug for the treatment of schizophrenia and other psychotic [2,3] or schizoaffective disorders [4]. Recently, it was approved by the FDA for the treatment of depressive episodes associated with Bipolar I (Bipolar II) disorder as a monotherapeutic agent [5]. QTF is not official in any pharmacopoeia.

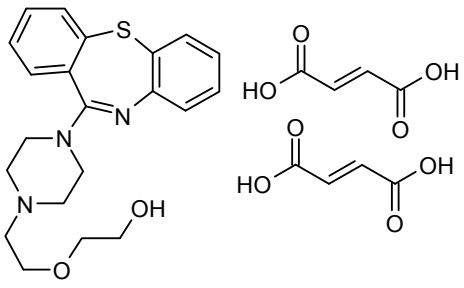

Figure 1. Chemical structure of QTF.

Several analytical methods have been found in the literature for the determination of QTF in biological fluids and include HPLC [6-13], chemiluminescence spectrometry [14], electrospray ionization MS [15-18],

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tandem MS/MS detection [19-22], UPLC with tandem MS detection [23,24], GC [25,26] and voltammetry [27]. Various techniques like polarography [28], capillary zone electrophoresis [29,30], HPTLC [31-33], HPLC [34-37], UV spectrophotometry [29,38-41], titrimetry [41] and visible spectrophotometry [42-44] have been used to the determination of QTF in pharmaceuticals.

To the best of our knowledge, there are three reports on the use of visible spectrophotometry for the determination of QTF in pharmaceuticals. All three reports were based on ion-pair complexation reaction with different regents such as bromocresol green [42], wool fast blue [43] and calmagite [44] in acid medium. The ion-pair complexes formed using bromocresol green [42] and wool fast blue [43] as reagents were extracted into chloroform and the absorbance was measured at 415 and 585 nm, respectively, over the concentration ranges of 5-25 and 50-250 μg ml^{-1} QTF. The ion-pair complexes formed using calmagite [44] as reagent were extracted into dichloromethane and the absorbance was measured at 480 nm over a concentration range of 3-30 μg ml^{-1} QTF. The results of these methods are mainly dependent on the efficiency of extraction of ion-pair extracted into the organic solvents and the process is cumbersome.

Molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely coloured charge-transfer complexes, which absorb radiation in the visible region [45,46]. These C-T reactions were of particular interest in the analysis of many pharmaceutical compounds [47-51]. Therefore, the aim of the present study was directed to investigate two simple, rapid, sensitive and cost-effective spectrophotometric methods based on the charge-transfer complexation reactions using *p*-chloranilic acid (*p*-CAA) and 2,3-dichloro-5,6-dicyanoquinone as π-acceptors. In the first method (method A), the free base form of the drug (QTP) in dichloromethane was reacted with *p*-CAA in acetonitrile to form a charge transfer complex with an absorption maximum peaking at 520 nm. The second method (method B) employed DDQ (in acetonitrile) as π-acceptor to form a red chromogen with strong absorption maximum at 540 nm. The methods were successfully applied to quantify QTF in pharmaceutical formulations and in spiked human urine. The results obtained were satisfactorily precise and accurate.

EXPERIMENTAL

Apparatus

All absorption measurements were made using a Systronics model 106 digital spectrophotometer (Systronica Ltd., Ahmedabad, India) with 1 cm path length quartz cells.

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 (both from Sun Pharmaceuticals Ltd., India) tablets were purchased from local market. Dichloromethane and acetonitrile (spectroscopic grade) were purchased from Merck, Mumbai, India. Distilled water was used wherever required. All other chemicals used were of analytical reagent grade. The urine was collected from a healthy volunteer (28-year-old male) and kept frozen until use after gentle thawing.

Reagents

p-Chloranilic acid and 2,3-dichloro-5,6-dicyanoquinone solutions

A 0.2% *p*-chloranilic acid (*p*-CAA) and 0.1% 2,3--dichloro-5,6-dicyanoquinone (DDQ) (both from S.D. Fine Chem Ltd., Mumbai), respectively, were prepared freshly in acetonitrile.

Sulphuric acid (0.1 M)

Concentrated acid (S.D. Fine Chem, Mumbai, India, Sp. gr. 1.84) was appropriately diluted with water to get 0.1 M.

Sodium hydroxide (1 M)

Accurately weighed 4 g of the pure NaOH (Merck, India) was dissolved in water, the solution was made up to 100 ml with water.

Standard free base form solution (QTP)

Into a 125 ml separating funnel, an accurately weighed amount of 16.1 mg of pure QTF was transferred and its solution was made by adding 2 ml of 0.1 M H_2SO_4 and 10 ml of water. A 5 ml of 1 N NaOH was added and the content was shaken for 5 min. Then the free base (QTP) was extracted with three 15 ml portions of dichloromethane, the extract was passed over anhydrous sodium sulphate and collected in a 50 ml volumetric flask, the volume was made up to mark with dichloromethane and the resulting solution (200 μg ml^{-1} QTP) was used for the assay in method A. This solution was diluted with dichloromethane to get a working concentration of 100 μg ml^{-1} QTP for method B.

General procedures for the construction of calibration curves

Method A

Varying aliquots of standard QTP solution equivalent to 8.0–160 μg ml^{-1} (0.2–4.0 ml of 200 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 4 ml by adding dichloromethane. After the addition of 1ml of 0.2% *p*-CAA solution, the content was mixed well and the absorbance was measured at 520 nm against a reagent blank similarly prepared without adding QTP solution.

Method B

Into a series of 5 ml calibration flasks, aliquots (0.2–4.0 ml) of standard QTP solution (100 μg ml^{-1}) equivalent to 4.0–80.0 μg ml^{-1} QTP were accurately transferred, and to each flask 1 ml of 0.1% DDQ solution was added and the mixture was diluted to 5 ml with dichloromethane. After 2 min, the absorbance of the red coloured C-T complex was measured at 540 nm against the reference blank similarly prepared.

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

Procedure for commercial dosage forms

Twenty tablets were weighed and pulverized. The amount of tablet powder equivalent to 16.1 mg of QTP was transferred into a 25 ml volumetric flask containing 2 ml of 0.1 M H_2SO_4 and 10 ml of water. The content was shaken well for 20 min. The resulting solution was filtered through Whatman No. 42 filter paper and the filtrate was collected in to a 125 ml separating funnel. QTP solutions of concentrations 200 and 100 μg ml^{-1}, for methods A and B, respectively, were prepared as described under the general procedure for pure drug and a suitable aliquot was used for assay by applying procedures described earlier.

Procedure for spiked human urine

To prepare spiked urine sample, 16.1 mg of the pure QTF, 2 ml of 0.1 M H_2SO_4 and 5 ml of urine sample were taken in a separating funnel. 10 ml of water was added followed by 5 ml of 1 M NaOH. The content was shaken for 5 min and QTP base formed was extracted with three 15 ml portions of dichloromethane. The organic layer was passed over anhydrous sodium sulphate and collected in a 50 ml volumetric flask. The solution was made up to the mark with dichloromethane, mixed well and 200 μg ml^{-1} QTP solution so obtained was used for the assay in method A. The above solution was diluted appropriately to get a working concentration of 100 μg ml^{-1} and a suitable aliquot (say 2 or 3 ml) was then subjected to analysis by following the procedure described in method B.

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 was prepared as described under 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 homogenised. Synthetic mixture containing 16.1 mg of QTP was weighed and its solution was prepared as described for tablets. Three different aliquots were subjected to analysis by the general procedure. The concentration of QTP was found from the calibration graph or from the regression equation.

RESULTS AND DISCUSSION

Spectral characteristics and reaction mechanism

The charge transfer complex forming reactions occur when π-acceptors react with the basic nitrogenous compounds which act as n-donors. Charge--transfer complex formation is characterized by electronic transition(s) to an excited state in which there is a partial transfer of electronic charge from the donor to the acceptor moiety. As a result, the excitation energy of this resonance occurs very frequently in the visible region of the electro-magnetic spectrum. This produces the usually intense colours characteristic for these complexes. Therefore, QTP, a nitrogenous base acting as n-donor was made to react with *p*-CAA and DDQ (π-acceptors) to produce a coloured charge transfer complexes in dichloromethane-acetonitrile solvent system.

QTF is a fumarate salt. Salts of amines do not react faster with π-acceptors [50]. Therefore it was necessary to first convert the salt form into free base (QTP) and then extract the free base into a non-aqueous solvent. QTP, being an n-electron donor, reacts with π-acceptors giving CT complexes of n-π type which dissociate to give the coloured free radical anions of the acceptors according to the following equation:

$$\underset{}{QTP + A} \rightleftharpoons \underset{\text{C-T complex}}{QTP\text{-}A} \rightleftharpoons \underset{\text{Radical anion}}{QTF^{\bullet +}} + A^{\bullet -}$$

Interaction of QTP with *p*-CAA gives a red chromogen which exhibits strong absorption maxima at 520 nm (Figure 2) with reproducible results. The band

may be attributed to the formation of the radical anion (p-CAA$^{\bullet-}$), which was probably formed by the dissociation of the original (QTP-p-CAA) complex promoted by the high ionizing power of the acetonitrile solvent [52]. The interaction of QTP with DDQ in dichloromethane-acetonitrile at room temperature gave a red colored chromogen with strong absorption maxima at 460, 540 and 590 nm due to the formation of the free radical anion [53] and the wavelength 540 was selected for the further studies because of higher sample absorbance and lower blank absorbance readings (Figure 3).

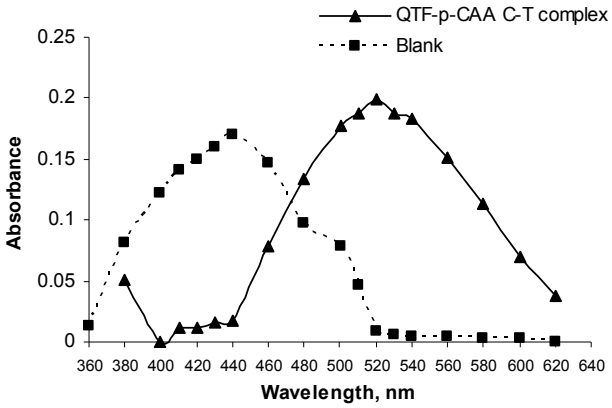


Figure 2. Absorption spectra of the QTP-p-CAA C-T complex (40 μg ml^{-1} QTP).

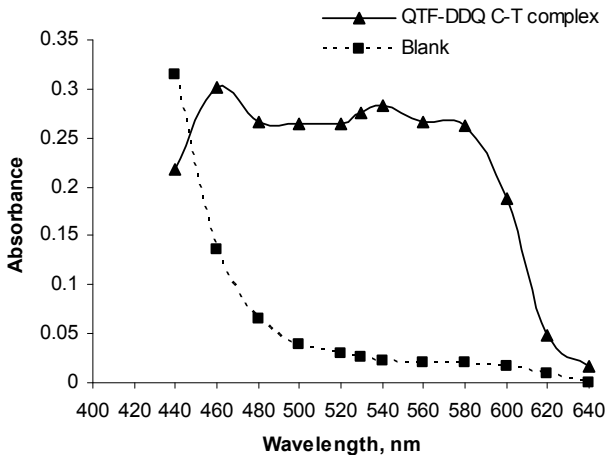


Figure 3. Absorption spectra of the QTP-DDQ C-T complex (25 μg ml^{-1} QTP).

Optimization of reaction conditions

Optimum conditions were established by measuring the absorbance of C-T complexes at 520 and 540 nm, for methods A and B, respectively, by varying one and fixing other parameters.

Effect of reagent concentration

To establish optimum concentrations of the reagents for the sensitive and rapid formation of the QTP charge transfer complexes, the free base of the drug (QTP) was allowed to react with different volumes of the reagents (0.5-3 ml of 0.2% p-CAA and 0.5-3 ml of 0.1% DDQ in methods A and B, respectively). In both the cases, maximum and minimum absorbance values were obtained for sample and blank, respectively, only when 1 ml of the reagent was used (Figures 4a and 4b). Therefore, 1 ml of 0.2% p-CAA in method A and 1 ml of 0.1% DDQ in method B in a total volume of 5 ml were used throughout the investigation.

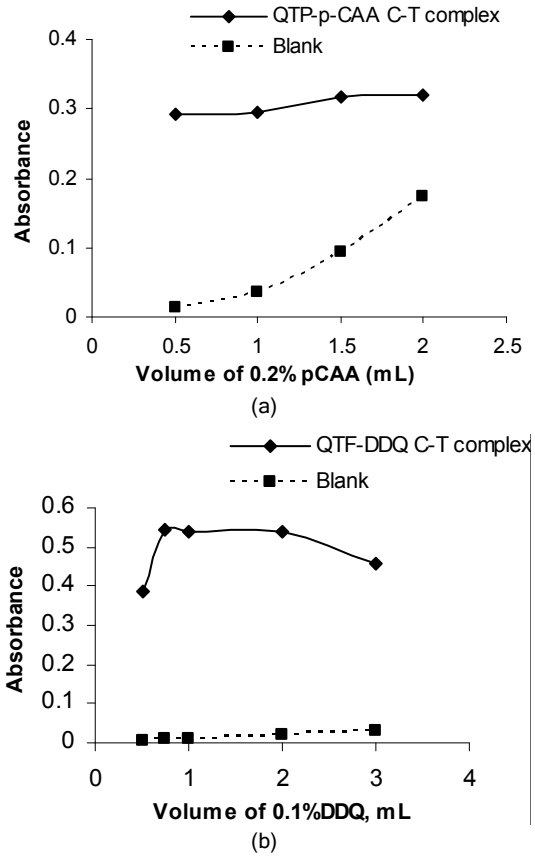


Figure 4. Effect of reagent concentration on the absorbance of C-T complex.

Effect of solvent

In order to select the suitable solvent for CT complex formation, the reaction of QTP with p-CAA or DDQ was studied in different solvents. Better results were obtained when QTP was extracted into dichloromethane than other solvents like chloroform, 1,2-dichloroethane or carbon tetrachloride. In case of reagents, acetonitrile was preferred to chloroform, dichloromethane, acetone, 2-propanol, dichloroethane, 1,4-dioxane, methanol and ethanol because as the complex formed in these solvents either had very low absorbance values or precipitated upon dilution. Therefore, dichloromethane and acetonitrile were chosen

as solvents to extract QTP base and to dissolve the reagents, respectively.

Effect of reaction time and stability of the C-T complexes

The optimum reaction time was determined by measuring the absorbance of the formed complex upon the addition of reagent solution to QTP solution at room temperature. In both methods the formation of C-T complex was complete within 5 min and the absorbance values of QTP-*p*-CAA and QTP-DDQ complexes were stable for 2.5 h and 20 min, respectively.

Composition of the C-T complex

The composition of the C-T complex was established by adopting limiting logarithmic method [54]. Two sets of experiments were carried out employing the general recommended procedures described above for methods A and B. The first set of experiments was carried out using increasing QTP concentrations (5.2×10^{-5}-4.2×10^{-4} M and 2.02×10^{-5}-8.1×10^{-5} M, in methods A and B, respectively) at fixed reagent concentration (9.6×10^{-3} M *p*-CAA or 4.4×10^{-3} M DDQ in a total volume of 5 ml). The second set of experiments were carried out using increasing reagent concentrations (*p*-CAA: 4.8×10^{-4}-1.9×10^{-4} and DDQ: 4.1×10^{-4}-3.1×10^{-3} M) at fixed QTP concentration (1.04×10^{-4} and 1.56×10^{-4} M for methods A and B, respectively). The log absorbance values were plotted as a function of the log of the QTP and reagent concentration in the first and second sets of experiments, respectively, in each method. The ratios of the slopes of two straight lines were 2.14 and 1.96, for methods A and B, respectively. This means that the reaction proceeds in 1:2 (QTP-reagent) stoichiometric ratio. Based on this fact the following reaction pathway for the formation of C-T complex is proposed and shown in Scheme 1.

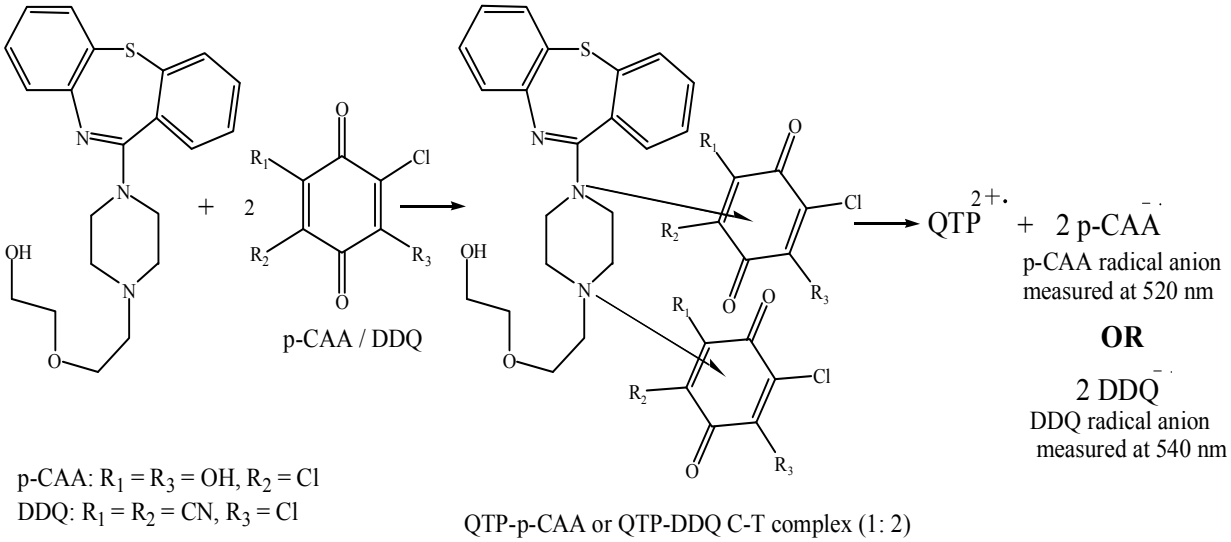
Method validation

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 0.991 for methods A and B, respectively, further establishing the linear relation between the two variables. The optical characteristics such 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.



Scheme 1. Proposed reaction pathway for the formation of C-T complex between QTP and p-CAA/DDQ.

Table 1. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max} / nm	520	540
Color stability	2.5 h	20 min
Linear range, μg ml^{-1}	8.0 - 160	4.0 - 80.0
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.77×10^3	4.59×10^3
Sandell sensitivity[a], μg cm^{-2}	0.2172	0.0836
Limit of detection (*LOD*), μg ml^{-1}	1.86	0.36
Limit of quantification (*LOQ*), μg ml^{-1}	5.64	1.08
Regression equation, *Y*[b]		
Intercept (*a*)	-0.0066	0.0057
Slope (*b*)	0.0048	0.0118
Standard deviation of *a* (S_a)	0.0108	0.0241
$\pm tS_a/\sqrt{n}$	0.0133	0.030
Standard deviation of *b* (S_b)	1×10^{-4}	4.2×10^{-4}
$\pm tS_b/\sqrt{n}$	1.24×10^{-4}	5.2×10^{-4}
Variance (S_a^2)	1.17×10^{-4}	5.81×10^{-4}
Regression coefficient (*r*)	0.9994	0.9981

[a]Limit of determination as the weight in μg per ml of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and $l = 1$ cm; [b]$Y = a + bX$, 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

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 ≤ 1.36% (intra-day) and ≤ 3.22% (inter--day) indicating high precision of the methods. Accuracy was evaluated as percentage relative error (*RE*) between the measured mean concentrations and taken concentrations for QTP. Bias (%) was calculated at each concentration and these results are also presented in Table 2. Percent relative error (*RE*, %) values of ≤ 2.75% 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, 3 ml of the resulting solution prepared by using synthetic mixture (200 and 100 μg ml^{-1} in QTP from methods A and B, respectively) was assayed (n = 4). The percentage recoveries of 98.44–103.65 with *RSD* values in the range 2.02–3.83% 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.

Table 2. Evaluation of intra-day and inter-day accuracy and precision (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 six and four degrees of freedom respectively, at the 95% confidence level; S = standard deviation and n = number of measurements)

Method	QTP taken, μg ml^{-1}	Intra-day accuracy and precision (n = 7)			Inter-day accuracy and precision (n = 5)		
		QTP found ±*CL*, μg ml^{-1}	*RE*, %	*RSD*, %	QTP found ±*CL*, μg ml^{-1}	*RE*, %	*RSD*, %
A	40.0	39.49±0.49	1.28	1.36	40.89±1.09	2.22	2.15
	80.0	79.35±0.89	0.81	1.22	81.38±3.25	1.73	3.22
	120.0	118.85±1.18	0.96	1.07	121.58±4.35	1.32	2.89
B	20.0	19.71±0.52	1.50	2.86	20.15±0.55	0.75	2.21
	40.0	40.99±0.60	2.48	1.59	40.89±1.80	2.22	3.56
	60.0	61.65±1.27	2.75	2.22	61.20±0.97	2.00	1.28

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent and contact time, and the effect of the changes was studied on the absorbance of the complex systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as *RSD* (≤ 3%). 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 1.99-3.26% indicating acceptable ruggedness. The results are presented in Table 3.

Application

The proposed methods were applied for the quantification of QTP 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 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. The results of this study are given in Table 4.

Application to spiked human urine

The proposed methods were applied to the determination of QTP in spiked human urine by following the general procedures described above. The recovery of the drug from spiked urine analysis was calculated by triplicate analysis of urine sample (containing 40, 120 and 160 μg ml^{-1} QTP and 40, 60 and 80 μg ml^{-1} QTP in methods A and B, respectively) separately. The percentage recovery values of 95.0--111.3 with standard deviation 0.5-2.11% showed the non-interference of other materials present in urine to the assay of QTP with considerable accuracy. The analytical results obtained for QTP in human urine sample are presented in Table 5.

Recovery study

To further assess the accuracy of the proposed methods, 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 done by spiking the pre-analysed tablet powder with pure QTP 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 in the range of 97.88-106.2 with standard deviation values from 0.89

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

Method	QTP taken μg ml^{-1}	Robustness		Ruggedness	
		Parameters altered		Inter-analysts (*RSD*, %) (n = 4)	Inter-instruments (*RSD*, %) (n = 4)
		Volume of *p*-CAA/DDQ[a]	Reaction time[b]		
A	40.0	1.85	1.56	2.89	3.26
	80.0	1.44	1.88	3.21	3.10
	120.0	0.98	2.31	2.56	2.56
B	20.0	1.12	2.22	2.88	1.99
	40.0	1.59	2.11	2.45	2.51
	60.0	1.78	1.89	2.22	2.31

[a]The volumes of *p*-CAA or DDQ added were 1±0.2 mL; [b]the reaction times were 5±1 min

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

Tablet brand name[a]	Nominal amount (mg/tablet)	Found[b] (label claim ± *SD*, %)		
		Reference method	Method A	Method B
Qutipin-200	200	102.3±0.68	101.7±1.17	100.46±1.46
			t = 1.02	t = 2.72
			F = 2.96	F = 4.61
Qutipin-100	100	96.72±0.72	97.48±0.89	95.68±0.89
			t = 1.49	t = 2.04
			F = 1.53	F = 1.53

[a]Marketed by Sun pharmaceuticals; [b]Mean value of 5 determinations. Tabulated *t*-value at the 95% confidence level and for four degrees of freedom is 2.77. Tabulated *F*-value at the 95% confidence level and for four degrees of freedom is 6.39

Table 5. Application of the proposed methods to the QTP concentration measurements in spiked urine

Method	QTP added, μg ml^{-1}	QTP found[a], μg ml^{-1}	Recovery of QTP ± *SD*, %
A	40.0	38.0	95.0±0.89
	120.0	124.5	103.8±1.22
	160.0	164.0	102.5±0.50
B	40.0	44.51	111.3±2.11
	60.0	64.00	106.7±1.45
	80.0	84.62	105.78±1.88

[a]Mean value of three determinations

Table 6. Results of recovery study via standard-addition method

Tablets studied	Method A				Method B			
	QTP in tablet μg ml^{-1}	Pure QTP added μg ml^{-1}	Total found μg ml^{-1}	Pure QTP recovered[a] ± *SD*, %	QTP in tablet μg ml^{-1}	Pure QTP added μg ml^{-1}	Total found μg ml^{-1}	Pure QTP recovered[a] ± *SD*, %
Qutipin-200	40.68	20.0	61.92	106.2±2.62	20.09	10.0	30.12	100.35±0.89
	40.68	40.0	79.83	97.88±2.10	20.09	20.0	39.88	98.99±2.13
	40.68	60.0	102.4	102.87±1.02	20.09	30.0	50.48	101.30±1.19
Qutipin-100	38.99	20.0	59.03	100.19±1.50	19.14	10.0	29.00	98.56±1.43
	38.99	40.0	79.92	102.33±1.28	19.14	20.0	39.21	100.35±1.29
	38.99	60.0	98.14	98.59±2.11	19.14	30.0	50.21	103.56±2.19

[*]Mean value of three determinations

to 2.62%. Closeness of the results to 100% showed the fairly good accuracy of the method. These results are shown in Table 6.

CONCLUSIONS

Two simple, sensitive, extraction-free, rapid and cost-effective spectrophotometric methods based on charge transfer complex formation reactions were developed and validated for the determination of QTF. The reagents utilized in the proposed methods are cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The methods are more selective than many of the reported spectrophotometric methods and employs higher wavelength to measure absorbance readings where the errors due to inactive ingredients are minimized to a large extent. The methods are free from interferences from the common excipients and other basic substances present in urine. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. These methods can be used as general methods for the determination of QTP in bulk powder, dosage forms and spiked human urine. The methods have many advantages over the separation techniques such as HPLC and include reduced cost, and speed with high accuracy. Hence, the methods can be used in routine analysis of drug in quality control laboratories and physiotherapeutical administration of the present drug in patients.

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REFERENCES

[1] J. Arnt, T. Skarsfeldt, Neuropsychopharmacology **18** (1998) 63-101

[2] M. Balestrieri, C. Vampini, C. Bellantuno, Hum. Psychopharmacol. **15** (2000) 499-512

[3] M. Chakos, J. Lieberman, E. Hoffman, D. Bradford, B. Sheitmann, Am. J. Psychiatry **158** (2001) 518-526

[4] P.E. Keck Jr., S.L. McElroy, S.M. Strakowski, Schizophrenia Res. Suppl. **35** (1999) S5-S12

[5] AstraZeneca (2004-01-13), "AstraZeneca Receives FDA Approval for SEROQUEL in Bipolar Mania", http://en.wikipedia.org/wiki/Quetiapine

[6] F. Belal, A. Elbrashy, M. Eid, J.J. Nasr, J. Liquid Chromatogr. Rel. Technol. **31** (2008) 1283-1298

[7] P.C. Davis, A.J. Wonga, O. Gefvertb, J. Pharm. Biomed. Anal. **20** (1999) 271-282

[8] J. Sachse, J. Köller, S. Härtter, C. Hiemke, J. Chromatogr., B **830** (2006) 342-348

[9] M.A. Saracino, L. Mercolini, G. Flotta , L.J. Albers, R. Merli, M.A. Raggi, J. Chromatogr., B **843** (2006) 227-233

[10] R. Mandrioli, S. Fanali, A. Ferranti, M. A. Raggi, J. Pharm. Biomed. Anal. **30** (2002) 969-977

[11] C. Frahnert, M.L. Rao, K. Grasmader, J. Chromatogr., B **794** (2003) 35-47

[12] J. Hasselstroem, K. Linnet, J. Chromatogr., B **798** (2003) 9-16

[13] W. B. Li, Y. Z. Xue, Y. M. Zhai, J. Zhang, G.X.Guo, C.Y. Wang, Z.J. Cai, Yaowu Fenxi Zazhi **23** (2003) 247-251

[14] S.A. Bellomarino, A.J. Brown, X.A. Conlan, N.W. Barnett, Talanta **77** (2009) 1873-1876

[15] K.Y. Li, Z.N. Cheng , X. Li, X.L. Bai , B.K. Zhang, F. Wang, H.D. Li, Acta Pharmacol. Sin. **25** (2004) 110-114

[16] Z.L. Zhou, X. Li, K.Y. Li, Z.H. Xie, Z.N. Cheng, W.X. Peng, F. Wang, R.H. Zhu, H.D. Li, J. Chromatogr., B **802** (2004) 257-262

[17] Z. Li, Z. R. Tan, D.S. Ouyang, G. Wang, L.S. Wang, G. Zhou, D. Guo, Y. Chen, H.H. Zhou, Yaowu Fenxi Zazhi **28** (2008) 706-708

[18] S.N. Lin, Y. Chang, D.E. Moody, R.L. Foltz, J. Anal. Tox. **28** (2004) 443-448

[19] B. Barrett, M. Holcapek, J. Huclova, V. Borek-Dohalsky, P. Fejt, B. Nemec, I. Jelinek, J. Pharm. Biomed. Anal. **44** (2007) 498-495

[20] R. Nirogi , G. Bhyrapuneni , V. Kandikere, K. Mudigonda, D. Ajjala, K. Mukkanti, Biomed. Chromatogr. **22** (2008) 1043-1055

[21] A. Tan, B. Pellerin, J. Couture, F. Vallée, SFBC Anafarm, available at: http://www.aapsj.org/abstracts/AM_2006/ /staged/AAPS2006-000989.PDF.

[22] M.L. Kundlik, S. Kambli, V. Shah, Y. Patel, S. Gupta, R. Sharma, B. Zaware, S.R. Kuchekar, Chromatographia **70** (2009) 1587-1592

[23] K.Y. Li, Y.G. Zhou, H.Y. Ren, F. Wang, B.K. Zhang, H.D. Li, J. Chromatogr., B **850** (2007) 581-585

[24] J.Y. Tu, P. Xu, D.H. Xu, H.D. Li, Chromatographia **68** (2008) 525-532

[25] M.M. McMullin, Ther. Drug Monit. **21** (1999) 459

[26] V. N. Atanasov, K. P. Kanev, M.I. Mitewa, Central Europ. J. Med. **3** (2008) 327-331

[27] S.A. Ozkan, B. Dogan, B. Uslu, Microchim. Acta **153** (2006) 27-35

[28] N. El-Enany, A. El-Brashy, F. Belal, N. El-Bahay, Portugaliae Electrochimica Acta **27** (2009) 113-125

[29] V. Pucci, R. Mandrioli, A. Ferranti, S. Furlanetto, M.A. Raggi, J. Pharm. Biomed. Anal. **32** (2003) 1037-1044

[30] S. Hillaert, L. Snoeck, W. van den Bossche, J. Chromatogr. **1033** (2004) 357-362

[31] B. Dhandapani, A. Somasundaram, S. H. Raseed, M. Raja, K. Dhanabal, Int. J. PharmTech Res. **1** (2009) 139-141

[32] R. Skibiński, Ł. Komsta, I. Kosztyła, J. Planar Chromatogr. Modern TLC **21** (2008) 289-294

[33] S.R. Dhaneshwar, N.G. Patre, M.V. Mahadik, Acta Chromatographia **21** (2009) 83-93

[34] S.R. Krishna, B.M. Rao, N.S. Rao, Rasayan J. Chem. **1** (2008) 466-474

[35] C.H. Bharathi, K.J. Prabahar, C.H.S. Prasad, M. Srinivasa Rao, G.N. Trinadhachary, V.K. Handa, R. Dandala, A. Naidu, Pharmazie **63** (2008) 14-19

[36] C.M. Fu, R.Z. Wang, Zhongguo Xinyao Zazhi **11** (2002) 144-146

[37] I.V.S. Raju, P. Raghuram, J. Sriramulu, Chromatographia **70** (2009) 545

[38] R.A. Fursule, D.K. Rupala, Md.M.G. Khan, A.A. Shirkh-edkar, S. J. Surana, Biosci. Biotechnol. Res. Asia 05 (2008), http://www.biotech-asia.org/display.asp?id=429

[39] K. Basavaiah, N. Rajendraprasad, P. J. Ramesh, K. B. Vinay, Thai J. Pharm. Sci. **34** (2010) 146-154

[40] S.B. Bagade, S.P. Narkhede, D.S. Nikam, C.K. Sachde, Int. J. ChemTech Res. **1** (2009) 898-904

[41] K.B. Vinay, H.D. Revanasiddappa, P.J. Ramesh, N. Rajen-draprasad, Chem. Ind. Chem. Eng. Q. **17** (2011) 99-106

[42] R.X. Arulappa, M. Sundarapandian, S. Venkataraman, M. Boopathi, M. Kaurav, Res. J. Pharm. Tech. **2** (2009) 884

[43] G. Srihari, I.E. Chakravarthia, Int. J. Chem. Sci. **9** (2011) 949-952

[44] N. Rajendraprasad, K. Basavaiah, K. B. Vinay, Chem. Ind. Chem. Eng. Q. **17** (2011) 259-267

[45] R.S. Mulliken, J. Am. Chem. Soc. **72** (1950) 600-608

[46] R. Foster, Organic Charge-Transfer Complexes, Academic Press, New York, 1969

[47] M. Krishnamurthy, U. Muralikrishna, Indian Drugs **22** (1985) 171-180

[48] M.S. Luo, Yaowu Fenxi Zazhi **15** (1995) 52

[49] E. Khaled, Talanta **75** (2008) 1167-1174

[50] M. Walash, M. Sharaf-El Din, M.E.S. Metwalli, M. Reda-shabana, Arch Pharm Res. **27** (2004) 720-726

[51] K. Basavaiah, Farmaco **59** (2004) 315-321

[52] M.E. Abdel-Hamid, M.A. Abuirjeie, Talanta **35** (1988) 242--244

[53] N. Rahman, Md.N. Hoda, J. Pharm. Biomed. Anal. **31** (2003) 381-392

[54] J. Rose, Advanced Physico-Chemical Experiments, Pitman and Sons, London, 1964

[55] H. Zavis, D. Ludvik, K. Milan, S. Ladislaw, V. Frantisck, Handbook of Organic Reagents in Inorganic Analysis. Translated by Stanislav, K, Dr. Chalmers (The Series and Translation Editor: University of Aberdem, Ellis Horwood Limited, Chichester, A Division of John Wiley & Sons IC, 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 dated 06 November 1996, incorporated in November 2005, London.

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NAUČNI RAD

SPEKTROFOTOMETRIJSKO ODREĐIVANJE KUETIAPIN-FUMARATA U FARMACEUTSKIM PREPARATIMA I HUMANOM URINU KORIŠĆENJEM DVE KOMPLEKSOMETRIJSKE REAKCIJE UZ TRANSFER NAELEKTRISANJA

Predložene su dve ekonomične i osetljive spektrofotometrijske procedure za određivanje kuetiapin-fumarata (QTF) u farmaceutskim preparatima i humanom urinu. Metode se zasnivaju na kompleksometrijskim reakcijama uz transfer naelektrisanja između leka (kuetiapina, QTP), kao n-electron donora (D), sa p-hloranilnom kiselinom (p-CAA) (metoda A) ili sa 2,3-dihloro-5,6-dicianohinona (DDQ) (metoda B) kao π-acceptora (A). Obojeni proizvodi imaju apsorpcione maksimume na 520 nm u metodi A i na 540 nm u metodi B. U radu su opimizovani eksperimenalni uslovi, kao što su koncentracije reagenasa i upotreba odgovarajućih rastvarača, kako bi se postigla maksimalna osteljivost metoda. Saglasnost sa Beer-ovim zakonom je postignuta u opsegu koncentracija 8,0--160 za metodu A i 4,0-80,0 μg ml^{1} za metodu B. Izračunate vrednosti molarne apsorptivnosti su 1,77×10^{3} l mol^{1}cm^{-1} za metodu A i 4,59×10^{3} l mol^{1}cm^{-1} za metodu B. Takođe, u radu su izračunate vrednosti Sandel-ovog indeksa, granica detekcije i granica kvantifikacije. Predložene metode su uspešno primenjene za određivanje QTF u farmaceutskim formulacijama i humanom urinu.

Ključne reči: kuetiapin-fumarat; određivanje; spektrofotometrija C-T kompleksa; farmaceutski preparati; humani urin.