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Original article

SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF QUETIAPINE HEMIFUMARATE IN PHARMACEUTICAL PREPARATIONS USING BROMCRESOL PURPLE AND BROMCRESOL GREEN

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Abstract

Simple and selective extractive spectrophotometric methods for the determination of quetiapine hemifumarate (QF) were developed and validated. The methods were based on the formation of yellow ion-pair complexes between QF and acidic dyes namely bromcresol purple (BCP) and bromcresol green (BCG) at room temperature in phosphate buffer (pH 3.0). The formed complexes were extracted with chloroform and the absorbances were measured at 406.5 nm for BCP and at 416 nm for BCG complexes. The compositions of the ion-pairs were found as 1:1 by mole-ratio method. The reaction conditions such as concentration, pH, color formation time, temperature and chromogen stability were optimized. Good linear relationship was obtained between the absorbance and the concentration of QF in the range of 0.5 – 20 µg/mL for both BCP and BCG ($r \geq 0.9974$). LOD values were found as 0.12 and 0.16 µg/mL *for BCP and BCG complexes, respectively. Intra-day precisions were found less than 1 % in the methods. The developed methods were applied successfully to the determination of QF in tablets marketed in Turkey.*

Key words: *Quetiapine, Bromcresol purple, Bromcresol green, Spectrophotometry, Pharmaceutical preparations.*

Ketiapin Hemifumarat'ın Bromkrezol Moru ve Bromkrezol Yeşili Kullanılarak Farmasotik Preparatlarda Tayini İçin Spektrofotometrik Yöntemler

Ketiapin hemifumaratın (QF) tayini için basit ve seçici ekstraktif spektrofotometrik metotlar geliştirilmiş ve valide edilmiştir. Metotlar, QF ile bromkresol moru (BCP) ve bromkresol yeşili (BCG) asidik boyalarının oda sıcaklığında ve fosfat tampon (pH 3.0) içerisinde sarı renkli iyon çifti kompleksi oluşturmasına dayanır. Oluşan kompleksler kloroform ile ekstrakte edildikten sonra absorbansları BCP için 406.5 nm'de ve BCG için 416.0 nm'de ölçülmüştür. İyon çiftinin bileşimi mol-oranları yöntemiyle 1:1 olarak bulunmuştur. Konsantrasyon, pH, renk oluşma zamanı, reaksiyon koşulları, sıcaklık ve kromojen kararlılığı gibi reaksiyon koşulları optimize edilmiştir. 0.5-20 µg/mL aralığındaki QF konsantrasyonu ile absorbans arasında, hem BCP hem de BCG için doğrusal ilişki gözlenmiştir($r \geq 0.9974$). LOD değerleri BCP ve BCG için sırasıyla 0.12 ve 0.16 µg/mL olarak bulunmuştur. Metotlarda günler arası kesinlik %1'in altındadır. Geliştirilen metotlar QF'ın Türkiye'de bulunan tabletlerinin tayininde başarılı bir şekilde uygulanmıştır.

Anahtar kelimeler: *Ketiapin, Bromkrezol moru, Bromkrezol yeşili, Spektrofotometri, Farmasötik preparat*

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INTRODUCTION

Quetiapine (Q), (2-(2-(4-dibenzo[*b*,*f*][1,4]thiazepine-11-yl-1-piperazinyl)ethoxy) ethanol) (Fig 1), is an antipsychotic drug which is used for the treatment of schizophrenia and depressive episodes associated with bipolar disorder.

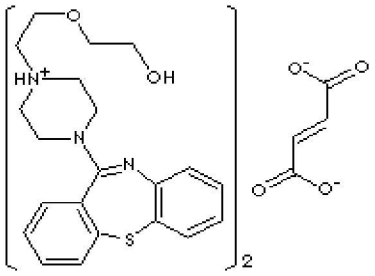

Figure 1. Quetiapine hemifumarate

Extractive spectrophotometric procedures are generally used for their sensitivity and selectivity in the assay of some drugs; therefore, ion-pair extractive spectrophotometry has been received considerable attention for the quantitative determination of many pharmaceutical compounds.

Methods for determination of QF by spectrophotometry (1-6), liquid chromatography (7-16), voltammetry (17), polarography (18) and capillary zone electrophoresis (19) has been reported. To our knowledge, there is no study in the literatures for the determination of quetiapine by spectrophotometric methods using BCP and BCG in pharmaceutical preparations.

The aim of this study was to develop an accurate, reproducible and selective extractive spectrophotometric methods based on the formation of ion-pair complexes between QF and bromcresol purple (BCP) and bromcresol green (BCG) for the determination of QF in pharmaceutical formulations.

EXPERIMENTAL

Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.WTW 538 pH meter was used for pH measurements.

Materials and reagents

Quetiapine hemifumarate reference standard was kindly supplied by EGIS Pharmaceuticals, (Budapest, Hungary) and used without further purification.

Sodium dihydrogen phosphate dihydrate, orthophosphoric acid, bromcresol purple and bromcresol green (Merck) were used without further purification.

Pharmaceutical preparations (Ketilept® 25 mg(Batch No. 9302D0108), 100mg (Batch No. 1848A0208), 200 mg (Batch No. 1849A0208), 300 mg (Batch No.1514A0108), film tablets; Seroquel® 25 mg (Batch No. FV405) film tablet) were purchased from local pharmacies.

All chemicals and reagents were analytical reagent grade.

Reagent solutions

Stock solutions of 200 µg/mL BCP and BCG were prepared by dissolving the dyes in water in a 100 mL volumetric flask separately. For pH:3 buffer solution, 15,601 g sodium dihydrogen phosphate dihydrate was dissolved in 900 mL water and pH adjusted to 3 with orthophosphoric acid then the volume was completed to 1L.

Standard solution

A standard stock solution of QF (100 µg/mL) was prepared in pH: 3.0 phosphate buffer. These solutions were kept at +4 °C. Various aliquots of standard solution were transferred into volumetric flasks and total volumes were brought to 100 mL with pH: 3.0 phosphate buffer to give a desired concentration of analyte.

RESULTS AND DISCUSSION

Selection of the extraction solvent

The effect of several organic solvents such as chloroform, carbon tetrachloride, ethyl acetate, diethylether, toluene, and dichloromethane were tried for effective extraction of the colored species from aqueous phase. Chloroform was found to be the most suitable solvent. Double extraction with total volume 25 mL yields maximum absorbance intensity.

Effect of time and temperature

The optimal reaction time was investigated in 0 – 4.0 min with 0.5 min interval by following the color development at ambient temperature (25°C). Permanent color intensity was attained just after mixing and no change was observed afterward. Raising the temperature up to 30°C has no effect on the absorbance of the formed complexes, whereas above 30 °C, the absorbance starts to decay. The absorbance remains stable for at least 72 h.

Effects of pH

The effect of pH was studied by extracting the colored complexes in the presence of various buffers and the maximum color intensity and highest absorbance value was observed in phosphate buffer (pH 3.0). In addition we obtained reproducible results in this pH and the buffer. Effect of the pH due to the absorbance measured at 406.5 nm and 416.0 nm (λ_{max}) were shown in Fig. 2.

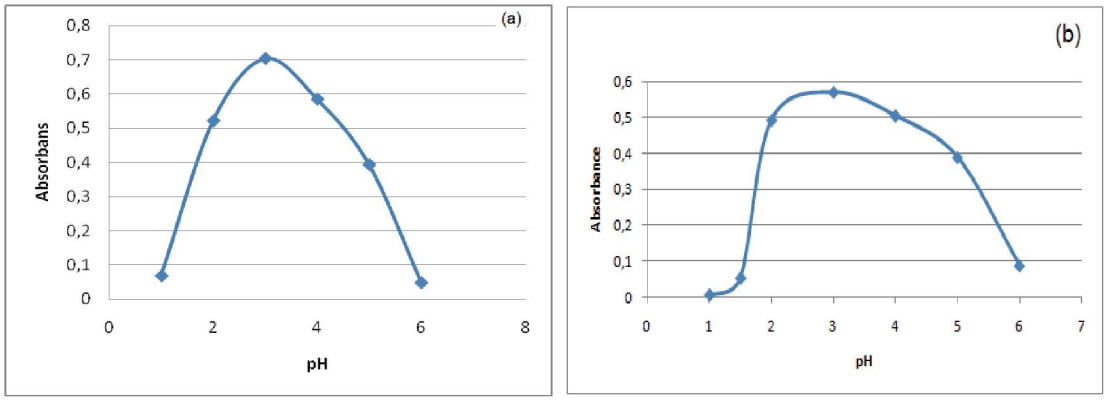

Figure 2. Effect of pH in (a) BCG and (b) BCP methods

Effects of reagent concentration

The effects of the reagent concentrations were studied by measuring the absorbances of solutions containing a fixed concentration of QF (10 µg/mL) and varied amounts of the reagent BCP and BCG. Maximum color intensity of the complex was achieved with 6 mL of BCP and BCG standard reagent solutions. A larger volume of the reagent had no significant effect on the absorbances of the formed ion-pair complex (Fig. 3).

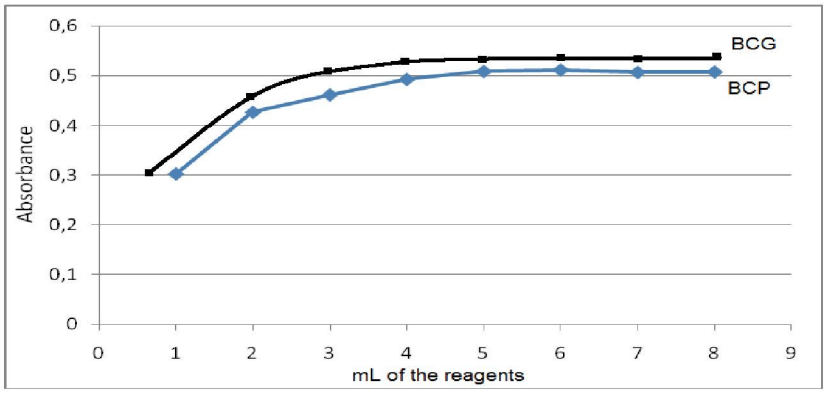

Figure 3. Effect of the reagent concentration on the absorbance (mL of the standard solutions of BCG and BCP) .

Stoichiometric relationship

In mole ratio method; A series of solutions was prepared with changing concentrations of reagent (BCP and BCG) with fixed volume of QF (2.0×10^{-4} M) . The absorbance was measured at 406.5 nm for BCP and at 416.0 nm for BCG. The molar ratio of the reagents (drug/dye) in the ion-pair complexes was determined by the mole - ratio method (Fig.4). The results (intersection of two lines) indicate that 1:1 (drug/dye) ion-pair are formed through the electrostatic attraction between positive protonated Q and negative BCP and BCG. The extraction equilibrium can be represented as follows:

$$Q_{(aq)}^{+} + BCP^{-}_{(aq)-} \leftrightarrow Q^{+}D_{(aq)}^{-} \leftrightarrow Q^{+}D_{(org)}^{-}$$

where Q^{+} and D^{-} represent the protonated quetiapine and the anion of the dye, respectively, and the subscripts (aq) and (org) refer to the aqueous and organic phases, respectively.

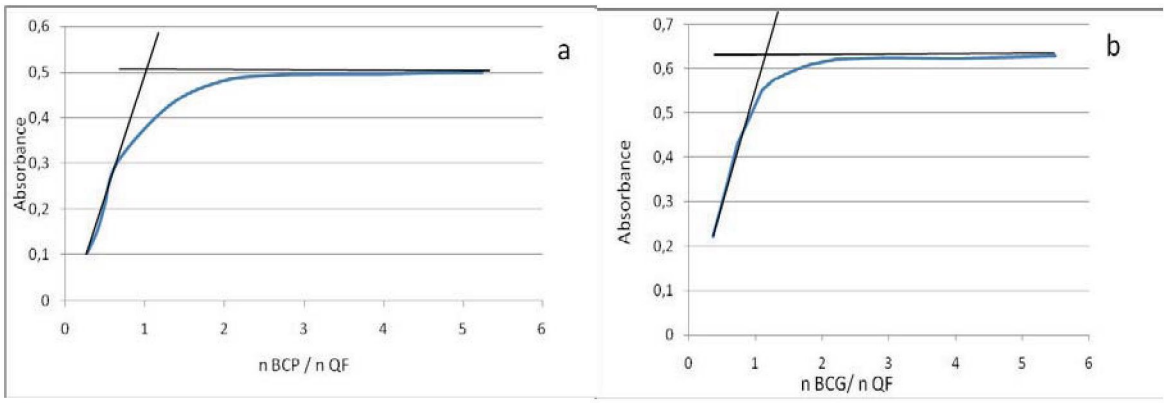

Figure 4. Stochiometric relationship (a) between Q and BCP, (b) between Q and BCG by mole-ratio method

Conditional stability constants (K_f) of the ion-pair complexes

The stability constants of the ion-pair complexes were calculated from the mole-ratio data using the following equation:

$$K_{drug\text{-}dye} = \frac{\frac{E}{E_{ex}}C_x}{\left[C_{drug} - \frac{E}{E_{ex}}C_x\right]\left[C_{dye} - \frac{E}{E_{ex}}C_x\right]}$$

where E_{ex} stands for the extrapolated, and E for the actual absorbance at the same abscissa value (20). Before intersection $C_x=C_{dye}$, after that $C_x=C_{drug}$. The log K values for drug-dye were 5.77 and 6.46 for Q+BCP and Q+ BCG respectively.

The standard free energy changes of complexation (G^o) were calculated from the association constants by the following equation:

$$\Delta G^o = -2.303RT \log K$$

where R is the gas constant (1.987 cal/mol/degree), T is the temperature in Kelvin and K is the association constant of drug-reagent ion-pair complexes (21). ΔG^o values for drug-dye were found as 7.868 and 8.809 $kJmol^{-1}$ for Q+BCP and Q+ BCG respectively.

General procedure

Aliquots of the standart QF solutions (100 µg/mL) were transferred to 25 mL volumetric flasks and completed with phosphate buffer (pH 3.0), then 6 mL of both BCP or BCG standard solutions (200 µg/mL) were added separately. The mixtures were extracted twice, first with 15 mL then 10 mL of chloroform by shaking for 1 min. and then allowed to stand for clear separation of two phases. Yellow colored chloroform layers (bottom) were transferred to another flask and, then they were passed through anhydrous sodium sulphate. The absorbances of the yellow complexes were measured at 416.0 and 406.5 nm for BCP and BCG complexes respectively against a blank prepared in the same manner except for the addition of drug. All measurements were made at room temperature (25◦C).

Construction of calibration curves

Aliquots of (0,125 - 5 mL) the standart QF solutions (100 µg/mL) were transferred to 25 mL volumetric flasks and completed with phosphate buffer (pH 3.0), then 6 mL of both BCP and BCG standard solutions (200 µg/mL) were added separately. Then the procedure was applied as explained in section *general procedure*. The calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

Calibration curves for the ion-pair complexes were constructed by plotting the absorbances as a function of the corresponding concentrations. The regression equations for the results were: (±Standard error)

$A = 0.045 (\pm 1.56 \times 10^{-3}) x + 0.028 (\pm 1.37 \times 10^{-3})$ (r=0.9974) for BCG and
$A = 0.055 (\pm 3.15 \times 10^{-4}) x + 0.018 (\pm 5.28 \times 10^{-4})$ (r=0.9991) for BCP

where A is the absorbance measured at 416.0 and 406.5 nm, x is the concentration of QF in µg/mL in the range of 0.5 – 20 µg/mL and r is the correlation coefficient. Log ε values were found to be 4.41 for BCP and 4.33 for BCG. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formulas: LOD = 3 SD/m, LOQ = 10 SD/m, SD is

the standard deviation of the intercept and m is the slope. The LOD and LOQ were found as 0.12 and 0.41 µg/mL for BCP and 0.16 and 0.49 µg/mL for BCG, respectively.

Accuracy and precision

The accuracy and precision of the proposed methods were determined by analyzing six replicate samples of standard QF solution at three concentration levels (4.0, 12.0 and 16.0 µg/mL) on three successive days for BCG and BCP for synthetic mixtures. The assay gave satisfactory results; the relative standard deviations (RSD) were less than 1.5 % (Table 1).

Table 1. Intra-day and inter-day precisions

Added µg/mL	Found (µg/mL) ± Standart error	Intra-Day Precision (RSD %)	Accuracy (recovery %)	Found (µg/mL) ± Standart error	Inter-Day Precision (RSD %)	Accuracy (recovery %)
BCP						
4.00	4.04 ± 0.01	0.88	101.00	4.08 ± 0.01	1.02	102.00
12.00	12.12 ± 0.02	0.67	101.00	12.26 ± 0.02	1.07	102.17
16.00	16.14 ± 0.04	0.93	100.88	16.23 ± 0.04	1.41	101.44
BCG						
4.00	4.03 ± 0.01	0.75	100.75	4.09 ± 0.01	0.99	102.25
12.00	12.17 ± 0.04	0.83	101.42	12.29 ± 0.03	1.03	102.41
16.00	16.11 ± 0.04	0.65	100.69	16.25 ± 0.03	0.84	101.56

Procedures for pharmaceutical formulations.

Ten tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred to 100 mL volumetric flasks and diluted to the mark with phosphate buffer (pH:3). The flasks were stirred for 30 min in magnetic stirrer and sonicated for 1 min. in ultrasonic bath and then the solutions were filtered through Whatman filter paper No.42. An appropriate aliquot of the filtrate was further diluted with buffer to obtain varied concentrations of calibration graphs and assayed as described above (*general procedure*). Assay results were shown in Table 2.

Table 2. Assay results of commercial preparations selected

	BCP			BCG			HPLC*			BCP-LC		BCG-LC	
Preparations	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	t val.	F val.	t val.	F val.
Ketilept© 25	25.25	1.39	-1.00	25.07	1.12	-0.28	25.15	1.33	-0.60	0.31	1.10	0.39	1.42
Ketilept© 100	100.47	1.29	-0.47	99.85	1.06	0.15	100.52	0.73	-0.72	0.26	3.18	1.21	2.14
Ketilept© 200	199.05	1.03	0.48	196.03	1.19	1.99	203.25	0.58	-1.63	1.38	3.14	1.78	4.04
Ketilept© 300	301.00	1.07	-0.33	298.08	0.89	0.64	299.79	0.56	0.07	1.25	3.67	0.99	2.50
Seroquel© 25	25.84	1.89	-3.36	25.14	1.62	-0.56	25.16	0.96	-0.64	1.45	3.66	0.56	2.84

*literature method (7)
theoretical value for *t* at p : 0.05 level = 2.26 for F=6,26

Applications

Accuracy of the proposed methods was also tested by recovery experiments. According to official validation guidelines (22), in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product for determining recovery. For this reason the recovery test was done by standard addition method in order to know whether the excipients in the pharmaceutical preparation show any interference with the analysis. Recoveries obtained from six replicates were summarized in Table 3. Recovery values were close to 100 % (RSD values < 1.4 %) indicating that there is no interference from the excipients in the formulations selected.

CONCLUSION

In this paper, two new spectrophotometric methods for determination of QF in bulk and in pharmaceutical preparations were developed. Developed methods were applied successfully to five tablets marketed in Turkey. Proposed methods were compared with a literature method (HPLC) (7) and statistically no significant difference was observed for the amount of drugs found by t test at p = 0.05 level for commercial formulations. The reagents used in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The methods have been proved sensitive, accurate and precise to be applied in routine analysis of QF in tablets.

Table 3. Recovery results obtained from standard addition method for BCG and BCP in pharmaceutical formulations.

BCG										
	Ketilept®								Seroquel®	
	25		100		200		300		25	
Added (µg/mL)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)
4.00	3.97	99.29	4.03	100.64	4.08	101.97	4.01	100.17	4.02	100.57
4.00	4.02	100.44	4.01	100.19	4.04	101.12	4.06	101.50	4.05	101.14
6.00	6.02	100.26	6.00	99.94	6.10	101.61	6.09	101.45	6.00	100.03
6.00	5.98	99.63	5.91	98.51	6.03	100.44	6.07	101.19	6.05	100.79
8.00	7.85	98.07	7.97	99.57	7.98	99.81	7.98	99.72	7.94	99.31
8.00	8.06	100.80	7.97	99.57	7.96	99.55	8.00	100.01	7.93	99.08
	Average	99.75		99.74		100.75		100.67		100.15
	RSD	0.99%		0.73%		0.97%		0.79%		0.83%

BCP										
	Ketilept®								Seroquel®	
	25		100		200		300		25	
Added (µg/mL)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)
4.00	3.95	98.67	4.04	101.03	4.07	101.79	4.05	101.15	4.02	100.50
4.00	4.02	100.50	4.00	99.91	4.06	101.50	4.06	101.52	3.93	99.23
6.00	5.98	99.65	6.08	101.35	6.17	102.87	5.96	99.34	5.89	98.17
6.00	5.95	99.10	6.03	100.47	6.08	101.38	5.92	98.59	5.88	98.04
8.00	8.08	101.05	8.15	101.82	8.04	100.50	7.89	98.58	8.14	100.69
8.00	8.11	101.43	8.08	100.98	8.05	100.63	8.10	101.28	8.13	101.56
	Average	100.07		100.92		101.44		100.08		99.87
	RSD	1.10 %		0.66 %		0.85 %		1.39 %		1.26 %

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