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Original article

Use of charge transfer complexation reaction for the spectrophotometric determination of bupropion in pharmaceuticals and spiked human urine

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Abstract:

This is the first report on use of two visible spectrophotometric methods for the determination of bupropion hydrochloride (BUPH) in pharmaceuticals and spiked human urine. Bupropion is a second-generation antidepressant agent which is also used in the management of smoking cessation. The methods are based on charge transfer complexation reaction of bupropion base (BUP) as n-electron donor with either p-chloranilic acid (PCA) or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as π-acceptors to give highly colored radical anion species. The colored products were quantified spectrophotometrically at 520 and 585 nm in PCA and DDQ methods, respectively. Under the optimized experimental conditions, Beer's law is obeyed over the concentration ranges of 21.7-217 μg mL^{-1} and 5.65-67.8 μg mL^{-1} BUP for PCA and DDQ methods, respectively. The molar absorptivity, Sandell sensitivity, detection and quantification limits are also reported. The proposed methods were applied successfully to the determination of BUPH in pure form, commercial tablets and spiked human urine with good accuracy and precision. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and F-ratio at 95% confidence level. There is no significant difference between the reference and proposed methods with regard to accuracy and precision. Further, the validity of the methods was confirmed by recovery studies via standard addition technique.

Keywords: Bupropion hydrochloride; Charge transfer complexation; Pharmaceutical; Spectrophotometry; Spiked urine

Introduction

Bupropion hydrochloride (BUPH), chemically known as 1-(3-chlorophenyl)-2-[(1, 1-dimethylethyl) amino]-1-propanone hydrochloride [1], is a water soluble salt of an aminoketone [2], with a pKa of 7.9 [3] and it is also known with the generic name of amfebutamone hydrochloride. Bupropion is structurally related to phenylethylamines, cathinone (a CNS stimulant from leaves of *Catha edulis*) and to the anorectic drug diethylpropion [4, 5]. Bupropion is a second-generation antidepressant agent that is also used in the management of smoking cessation [6]. Since its introduction in 1989, the most extensively used technique for the quantitation of BUPH is HPLC but, most of the procedures using this technique are devoted to biological fluids like human plasma [7, 8], plasma and serum [9] and dog plasma [10]. Even such techniques as radioimmunoassay [11], GC [12], LC [13, 14], LC/ESI/MS/MS [15], LC-MS [16] and LC-MS/MS [17, 18] are mostly confined to biological fluids including dog plasma [11], human plasma [12-14], rat everted gut sacs [16], human plasma and urine [17] and plasma of human, mouse and rat [18]. A limited number of methods based on few techniques such as non aqueous titration [19], HPLC [19-21], GLC [22] and derivative UV spectrophotometry [23] are found in the literature for the determination of BUPH in pharmaceuticals.

To the best of our knowledge, no visible spectrophotometric method has ever been reported for the quantification of BUPH in pharmaceuticals. Visible spectrophotometry, because of its simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and easy access in most quality control laboratories, has remained competitive in an area of chromatographic techniques for pharmaceutical analysis.

This paper describes, for the first time, the application of p-chloranilic acid (PCA) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as π-acceptors to the spectrophotometric determination of BUPH based on the interaction between these π-acceptors and the secondary amine group of BUP as a good n-electron donor to form charge-transfer complexes. These π-acceptors have numerous applications as analytical reagents and they have been used for the spectrophotometric determination of many drugs in pharmaceutical formulations [24-32]. The purpose of this investigation was directed to develop simple, sensitive, precise and inexpensive procedures for the quantification of BUPH in pharmaceuticals and spiked human urine.

Materials and Methods

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) provided with 1-cm matched quartz cells was used for all absorbance measurements.

Materials

Pharmaceutical grade bupropion hydrochloride (BUPH) was received from GlaxoSmithKline Pharmaceuticals, Mumbai, India. The following pharmaceutical preparations were purchased from commercial sources in the local market and subjected to analysis: Bupron-SR-150® (150 mg BUPH per tablet) from Sun Pharmaceutical Industries, Jammu, India, and Ession-ER-150® (150 mg BUPH per tablet) from Psycoremedies, Ludhiana, Punjab, India. Urine was obtained from healthy volunteer (male, around 30 years old).

Reagents and chemicals

All the reagents and solvents used were of analytical-reagent grade and distilled water was used throughout the investigation.

1. PCA (Rolex Laboratory Reagent, Mumbai, India): 0.1% (w/v) solution in 1,4-dioxane (Merck, Mumbai, India) was prepared and kept in the dark when not in use.

2. DDQ (Avra Synthesis Pvt. Ltd., Hyderabad, India): 0.2% (w/v) solution in acetonitrile (Merck, Mumbai, India), was prepared afresh just before use.

3. Sodium hydroxide (Merck, Mumbai, India), 0.5 M aqueous solution.

Standard bupropion base solution

Fifty milligrams of pure bupropion hydrochloride was dissolved in about 20 mL of water and the solution was quantitatively transferred into a 125-mL separating

funnel containing 10 mL of 0.5 M sodium hydroxide. The base was extracted with 4 x 20 mL of chloroform. The two phases were allowed to separate and the chloroform layer was dried over anhydrous sodium sulphate and transferred into a 100-mL volumetric flask. The solvent was evaporated on a water bath and the resulting yellow oil (bupropion base) was dissolved in 2.0 mL of methanol and the volume was then completed to the mark with acetonitrile. The resulting solution (434 μg mL^{-1} in bupropion base) was used in method A and diluted appropriately with acetonitrile to get a working concentration of 113 μg mL^{-1} BUP for use in method B.

Recommended methods

Method A (using PCA)

Different aliquots (0.25, 0.5, 1.0,---2.5 mL) of a standard BUP (434 μg mL^{-1}) solution were accurately transferred into a series of 5-mL volumetric flasks and the total volume was adjusted to 2.5 mL by adding adequate quantity of acetonitrile. To each flask was then added 1.0 mL of 0.1% p-chloranilic acid, and the content was mixed well and kept aside for 10 min. The mixture was diluted to the volume with acetonitrile and the absorbance was measured at 520 nm against a reagent blank prepared simultaneously.

Method B (using DDQ)

Aliquots (0.25, 0.5, 1.0,---3.0 mL) of a standard BUP (113 μg mL^{-1}) solution were accurately transferred into a series of 5-mL volumetric flasks and the total volume was adjusted to 3.0 mL by adding adequate quantity of acetonitrile to each flask. One milliliter of 0.2% DDQ solution was added to each flask and the mixture was diluted to the volume with acetonitrile and the absorbance of each solution was measured at 585 nm against a reagent blank.

Procedure for commercial tablets

Ten tablets each containing 150 mg of BUPH were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50.0 mg of BUPH was dissolved in about 30 mL distilled water in a 50-mL volumetric flask. The mixture was shaken for 10 min and filtered using Whatman No. 42 filter paper in to a 125-mL separating funnel containing 10 mL of 0.5 M sodium hydroxide. The base was extracted as described under "Standard bupropion base solution". The base solution (i.e. 434 μg mL^{-1} BUP) was assayed by the method A, and diluted to get 113 μg mL^{-1} BUP before applying the method B for the assay.

Procedure for spiked human urine sample

Urine (5 mL) was spiked with 50 mg of pure BUPH and diluted to 25 mL with water and quantitatively transferred into a 125-mL separating funnel containing 10 mL of 0.5 M sodium hydroxide. The solution was mixed well and the base was then extracted as detailed under "Standard bupropion base solution". The final concentration of BUP in the resulting solution 434 μg mL^{-1} was used in method A and was diluted appropriately with acetonitrile to get a working concentration of 113 μg mL^{-1} BUP for use in method B.

Stoichiometric relationship

Job's method of continuous variations of equimolar solutions was employed to establish the stoichiometry of the colored products. The solutions equivalent to 1.81 x 10^{-3} and 2.17 x 10^{-3} M BUP were prepared. Further, 1.81 x 10^{-3} M PCA and 2.17 x 10^{-3} M DDQ solutions were prepared in 1, 4-dioxane and acetonitrile, respectively. A series of solutions was prepared in which the total volume of BUP and reagent was kept at 2 mL in the total volume of 5 mL. The solutions were mixed in various proportions; in method A, the volume was completed to the mark after 10 min with both acetonitrile and 1,4-dioxane keeping the ratio of the two solvents as 1:1 in each flask while for method B, the volume was completed to the mark with acetonitrile. The absorbance of the resulting solutions was measured at the respective wavelengths of maximum absorbance (λ_{max}) against the reagent blank under the same conditions.

Results and Discussion

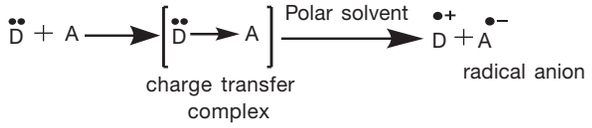
Absorption spectra

The reaction of PCA as a π-acceptor with bupropion base as n-electron donor results in the formation of an

intense orange-red product which exhibits absorption maxima at 520 nm (Figure 1) due to the formation of the corresponding PCA radical anion. DDQ also acts as a π-acceptor and the BUP-DDQ charge transfer complex resulted in the formation of an intense reddish violet color which exhibit three maxima at 585, 545 and 455 nm (Figure 2). These bands can be attributed to the formation of DDQ radical anions arising from the complete transfer of n-electrons from donor to acceptor moieties in acetonitrile. The absorption band at 585 nm was selected as analytical wavelength keeping in view the sensitivity of the reaction product and blank absorbance.

Reaction mechanism

The chemistry involved in the proposed methods is based on the reaction of the basic nitrogen of bupropion base as n-donor with the π-acceptors, namely, PCA and DDQ to form charge transfer complexes which subsequently dissociate into radical anions depending on the polarity of the solvent used. In polar solvents, such as acetonitrile, complete electron transfer from the donor to the acceptor moiety takes place with the formation of intensely colored radical anions [33], according to the following equation:



The dissociation of the ($D \longrightarrow A$) complex is promoted by the high ionizing power of the acetonitrile. The hydrochloride salts of amines do not react with π-acceptors due to non-availability of non-bonding electrons (n-electrons) on the nitrogen atom. To determine amine-HCl, it is necessary to first neutralize the hydrochloride and then extract the amine into a non-aqueous solvent [28]. The neutralization of the amine hydrochloride with sodium hydroxide and extraction of HCl-free-amine into chloroform followed by evaporating the chloroform was described by Mostafa *et al.* [34]. Since bupropion hydrochloride as such did not react with the π-acceptors, the salt was converted to base followed by extraction, evaporation and dissolving the residue in the solvents mentioned under "Standard bupropion base solution". The tentative reaction mechanisms for BUP-PCA and BUP-DDQ complexes were proposed and illustrated in schemes 1 and 2, respectively.

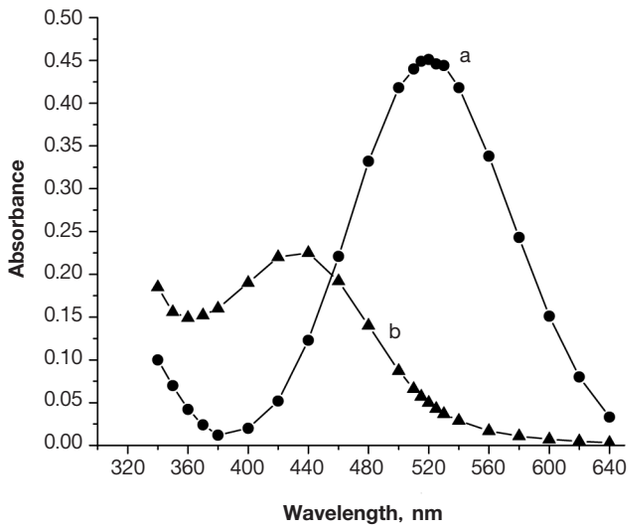
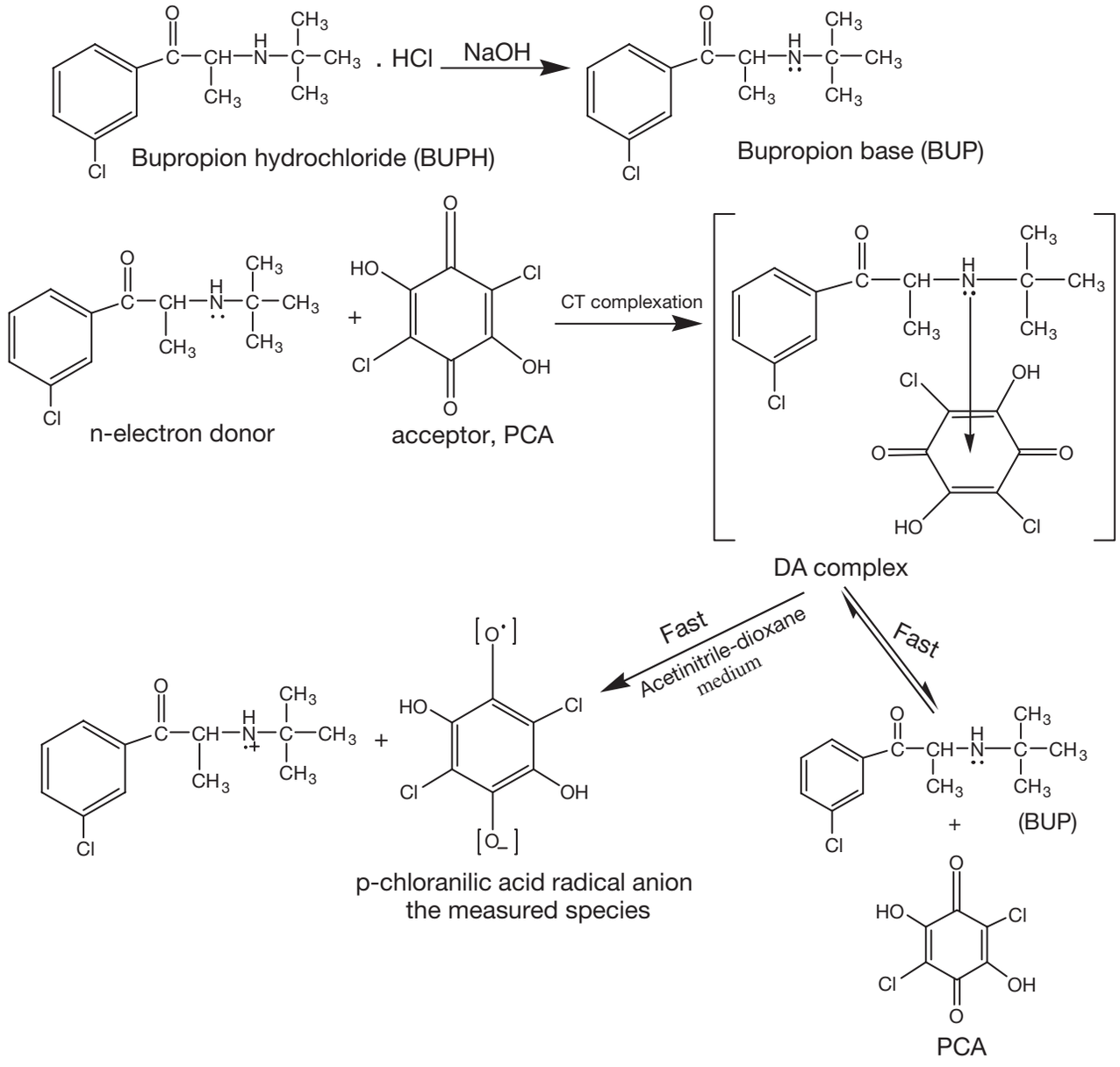


Figure 1 Absorption spectra of charge transfer complex of BUP-PCA (108.5 µg mL^{-1} BUP): (a) BUP-PCA complex, (b) PCA in 1,4-dioxane



Scheme 1 The tentative reaction mechanism for BUP-PCA complex

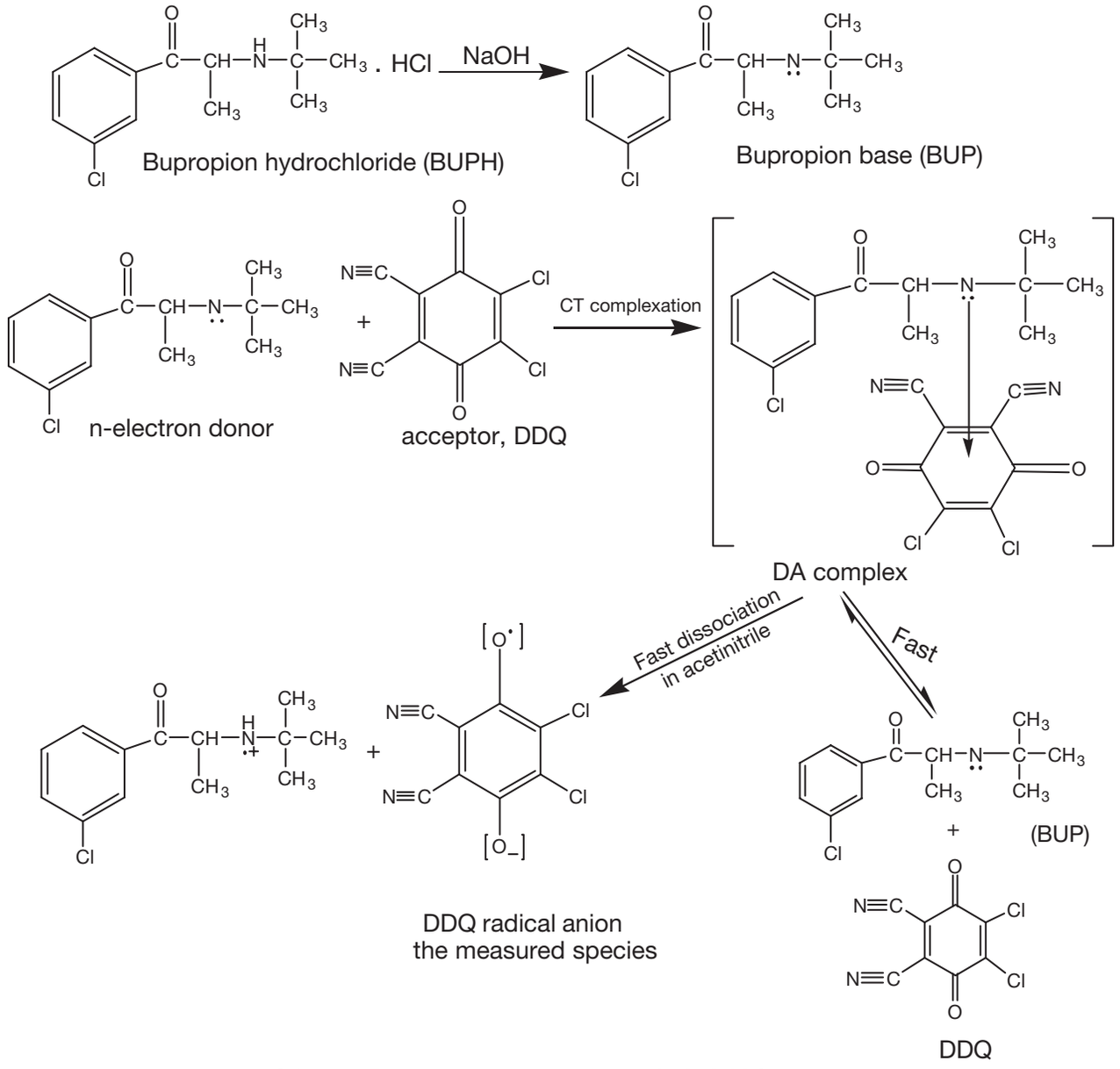
The effect of different experimental variables

Effect of reagent concentration

The effect of the reagent concentration on the intensity of the color developed at the selected wavelengths was ascertained by adding different amounts of the reagents PCA and DDQ to fixed concentrations of 108.5 and 56.5 μg mL^{-1} BUP in method A and method B, respectively. It was found that 1.0 mL of 0.1% PCA and 1.0 mL of 0.2% DDQ solutions were sufficient for the production of maximum and reproducible color intensity and the highest absorbance remained unaffected by further addition of these reagents (Figure 3).

Effect of solvent

In order to select the suitable solvent for charge transfer complex formation, the reaction of BUP with PCA and DDQ was carried out in different solvents. The acetonitrile-dioxane medium was found to be opt in the case of PCA because PCA in 1,4-dioxane exists in unionized form and acts as a π-acceptor like quinones [27] and the acetonitrile showed super priority over many solvents used such as chloroform, 2-propanol, dichloroethane, methanol and ethanol. Acetonitrile was found to be an ideal solvent in the case of DDQ, because it afforded the maximum sensitivity when compared with



Scheme 2 The tentative reaction mechanism for BUP-DDQ complex

all other solvents and it possesses the highest dielectric constant of all solvents examined [35], a property which is known to promote the dissociation of the original charge-transfer complexes to the radical anions.

Effect of reaction time

The optimum reaction time was determined by following the color development upon the addition of reagent solution to the BUP solution at room temperature. Complete color development was attained after 10 min with PCA while the reaction with DDQ was instantaneous. The absorbance of these radical anions remained stable for at least 60 and 30 min for method A and method B, respectively.

Molar ratio of the reaction

Job's continuous variations graph for the reaction between BUP and PCA or DDQ (Figure 4) shows that the interaction occurs on an equimolar basis via the formation of a charge-transfer complexes 1:1 (BUP: reagent). This finding was anticipated by the presence of one basic or electron donating centre (-NH) in the BUPH.

Method validation

Linearity

Under optimum experimental conditions for determination of the drug under study, the absorbance versus concentration plots were found to be linear over

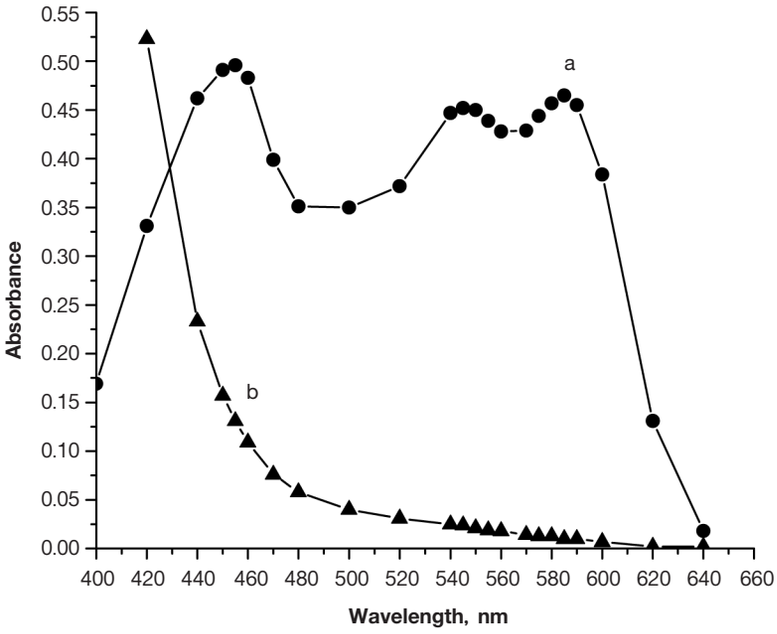


Figure 2 Absorption spectra of charge transfer complex of BUP-DDQ (56.5 μg mL^{-1} BUP): (a) BUP-DDQ complex, (b) DDQ in acetonitrile

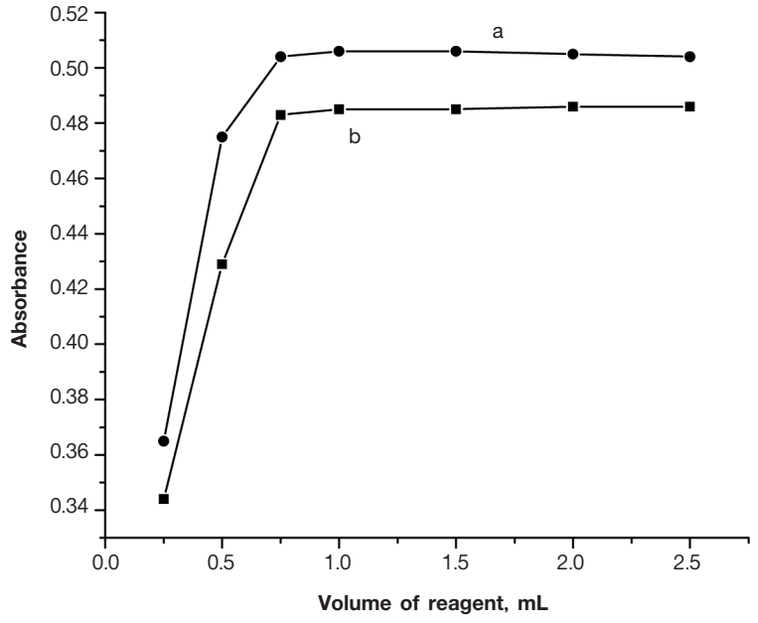


Figure 3 Effect of reagent concentrations on color development: (a) PCA (0.1%), (b) DDQ (0.2%)

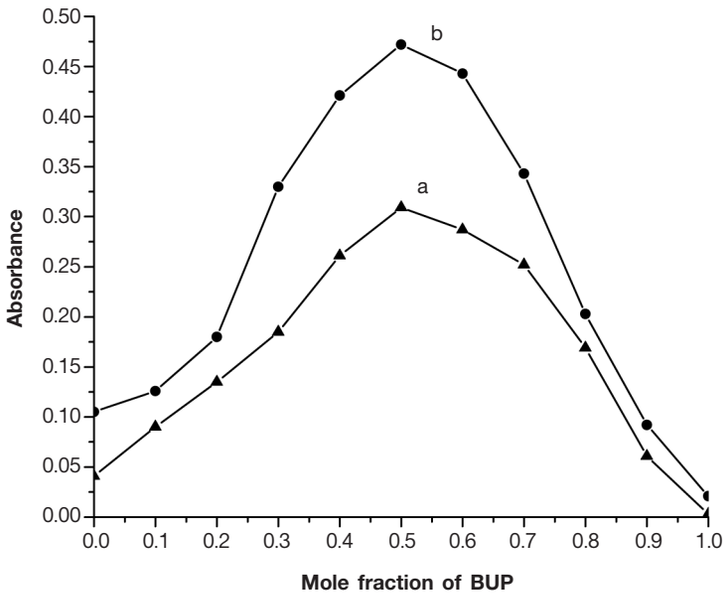


Figure 4 Job's continuous-variation plot for: (a) [BUP] and [PCA] = 1.81 x 10^{-3} M and (b) [BUP] and [DDQ] = 2.17 x 10^{-3} M

the concentration ranges stated in Table 1. The regression parameters calculated from the calibration graphs data, along with the standard deviations of the slope (S_b) and the intercept (S_a) are presented in Table 1. The linearity of the calibration graphs was demonstrated by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The molar absorptivity, Sandell sensitivity of the methods A and B are also shown in Table 1.

Table 1 Characteristic parameters for the charge transfer reaction of BUP with two π-acceptors

Parameter	Method A	Method B
λ_{max}, nm	520	585
Colour stability, min	60	30
Beer's law limits, μg mL^{-1}	21.7-217	5.65-67.8
Molar absorptivity, L mol^{-1} cm^{-1}	0.88 x 10^3	3.06 x 10^3
Sandell sensitivity*, μg cm^{-2}	0.2732	0.0783
Limit of detection, μg mL^{-1}	2.10	1.22
Limit of quantification, μg mL^{-1}	6.38	3.71
Regression equation, Y**		
Intercept, (a)	0.0209	0.0178
Slope, (b)	0.0034	0.0118
Correlation coefficient, (r)	0.9992	0.9997
Standard deviation of intercept (S_a)	0.00884	0.00554
Variance (S_a^2)	7.81 x 10^{-5}	3.07 x 10^{-5}
$\pm tS_a/\sqrt{n}$	9.28 x 10^{-3}	5.12 x 10^{-3}
Standard deviation of slope (S_b)	0.00007	0.00014
$\pm tS_b/\sqrt{n}$	7.34 x 10^{-5}	1.30 x 10^{-4}

*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 1.0 cm^2 and l = 1.0 cm. $Y^{**} = a + bX$, where Y is the absorbance and X concentration in μg/mL, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope

Sensitivity

Sensitivity of the methods can be determined, through the limit of detection (LOD) and limit of quantification (LOQ). The LOD for the proposed methods were calculated using the following equation [36]

$$LOD = \frac{3.3 \times \sigma}{S} \quad (1)$$

where σ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and S is the sensitivity, namely the slope of the calibration graph.

The LOQ, defined as [36]

$$LOQ = \frac{10 \times \sigma}{S} \quad (2)$$

Based on the above equations, the limits of detection and quantification were calculated and recorded in Table 1.

Assay precision and accuracy

In order to determine the precision of the proposed methods, solutions containing three different concentrations of BUP were prepared and analyzed in seven replicates and the analytical results are summarized in Table 2. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% R.E) also indicate the high precision and the good accuracy of the proposed methods. RSD (%) and RE (%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five days to evaluate intermediate precision (inter-day precision).

Selectivity

In order to evaluate the selectivity of the proposed methods for the analysis of pharmaceutical formulations, the effect of the presence of common excipients, such as talc, starch, lactose, glucose, sodium alginate, calcium gluconate and magnesium stearate was tested for possible interference in the assay by placebo blank and synthetic mixture analyses and no significant interference was observed from these excipients.

Applications to analysis of pharmaceutical formulations and spiked urine sample

The proposed methods were successfully applied to the determination of BUPH in two representative tablets Bupron-SR-150® and Ession-ER-150®. The results obtained are showed in Table 3 and were compared with those obtained by the reference method [19] by means of Student's t-and F-tests at 95% confidence level. The reference method involved the visual titration of the drug with acetous perchloric acid in non aqueous medium using crystal violet indicator. In all cases, the average results obtained by the proposed methods and reference method were statistically identical, as the difference between the average values had no significance

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

Method	BUP taken μg mL^{-1}	Intra-day (n = 7)			Inter-day (n = 5)		
		BUP found[a] μg mL^{-1}	% RE[b]	% RSD[c]	BUP found[a] μg mL^{-1}	% RE[b]	% RSD[c]
Method A	86.80	88.10	1.50	1.42	88.30	1.73	2.04
	130.20	131.61	1.08	1.60	131.80	1.23	1.83
	173.60	177.80	2.42	0.69	177.30	2.13	1.16
Method B	22.60	22.79	0.84	2.10	22.92	1.42	1.38
	33.90	34.16	0.77	1.32	34.22	0.94	2.00
	45.20	45.95	1.66	1.79	46.13	2.06	2.41

[a]= Mean value of n determinations, [b]= Relative error (%), [c]= Relative standard deviation (%)

at 95% confidence level with respect to accuracy and precision. The proposed methods were also applied to the determination of BUPH in spiked human urine sample and the results are presented in Table 4.

Recovery study

To ascertain the validity of the proposed methods, recovery experiment was performed via standard addition technique. To a fixed and known amount of BUP in tablet powder (pre-analyzed), pure BUP was added at three concentration levels (50, 100 and 150% of the level present in the tablet) and the total was measured by the proposed methods. The determination with each concentration was repeated three times and the results of this study presented in Table 5 indicated that the various excipients present in the formulations did not interfere in the assay.

Table 3 Results of assay of tablets and statistical evaluation

Commercial tablets	Found (% of nominal amount ± SD)*		
	Reference method	Proposed methods	
		Method A	Method B
Bupron SR 150®	98.14 ± 0.64	97.01 ± 1.24	97.43 ± 1.05
		t = 1.90	t = 1.33
		F = 3.75	F = 2.69
Ession ER 150®	100.10 ± 0.73	98.62 ± 1.32	99.17 ± 0.98
		t = 2.28	t = 1.72
		F = 3.27	F = 1.80

*Mean value of five determinations,

Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39

Table 4 Determination of bupropion in spiked human urine sample

Method	Spiked concentration (μg mL^{-1})	Found[a] ± S.D.	% Recovery ± RSD
A	130.20	125.50 ± 3.35	96.39 ± 2.67
B	33.90	33.32 ± 1.11	98.29 ± 3.33

[a]Mean value of five determinations; RSD is relative standard deviation

Table 5 Results of recovery study using standard addition method

Formulation studied	Method A				Method B			
	BUP taken, μg mL^{-1}	Pure BUP added, μg mL^{-1}	Total found, μg mL^{-1}	Pure BUP recovered*, Percent ± SD	BUP taken, μg mL^{-1}	Pure BUP added, μg mL^{-1}	Total found, μg mL^{-1}	Pure BUP recovered*, Percent ± SD
Bupron SR	84.19	43.40	127.00	98.64 ± 2.97	22.02	11.30	33.38	100.53 ± 1.94
150®	84.19	86.80	172.05	101.22 ± 2.21	22.02	22.60	44.33	98.72 ± 2.07
	84.19	130.20	219.14	103.65 ± 1.54	22.02	33.90	54.72	96.46 ± 2.43
Ession ER	85.84	43.40	129.58	100.78 ± 1.86	22.41	11.30	33.44	97.60 ± 2.75
150®	85.84	86.80	171.80	99.03 ± 2.05	22.41	22.60	45.51	102.21 ± 1.98
	85.84	130.20	219.61	102.74 ± 1.47	22.41	33.90	56.89	101.71 ± 2.31

*Mean value of three determinations

Conclusion

This is the first report on the application of visible spectrophotometry for the quantification of bupropion in pharmaceuticals and human urine. The methods are based on well-characterized charge-transfer complexation reaction, and have the advantages of simplicity, speed, accuracy and precision, and use of inexpensive equipment compared to the reported HPLC and GC methods. The DDQ method is more sensitive than the PCA method as seen from the higher molar absorptivity. UV spectrophotometry [23] method for bupropion quantitation in pharmaceuticals has been developed but this method has a narrow linear range (10-30 $\mu g\ mL^{-1}$) compared with the proposed methods. Moreover, the proposed methods can be performed at room temperature. The highlight of the proposed methods is their ability to quantify bupropion in human urine which in the past has been determined using very expensive and sophisticated technique like LC-MS/MS. Thus, the methods are useful for the quality control and routine analysis of BUPH in pharmaceuticals since there is no interference from the common excipients that might be found in commercial formulations.

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