Research article

Drug Testing and Analysis

Received: 18 November 2010 Revised: 10 January 2011 Accepted: 11 January 2011 Published online in Wiley Online Library: 21 February 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.266

Application of ion-pair complexation reaction for the spectrophotometric determination of bupropion hydrochloride in pharmaceuticals

Kanakapura Basavaiah* and Sameer A. M. Abdulrahman

This is the first report on the use of visible spectrophotometry for the determination of bupropion hydrochloride (BUPH), a second-generation antidepressant, in pharmaceuticals. Two sensitive, selective, and cost-effective spectrophotometric methods are described. The first method (method A) is based on the formation of yellow-coloured ion-pair complex between the BUPH and methyl orange (MO) at pH 3.80 ± 0.10 which was extracted into dichloromethane and the absorbance measured at 425 nm. The second method (method B) is based on the breaking of the yellow BUPH-MO ion-pair complex in acid medium followed by the measurement of the red-pink colour at 520 nm. Beer's Law is obeyed over the concentration ranges of 1.00–12.0 and 0.48–7.20 μg ml^{-1} BUPH for method A and method B, respectively. The molar absorptivities are calculated to be 2.18 × 10^4 and 3.79 × 10^4 l mol^{-1} cm^{-1} for method A and method B, respectively, and the corresponding Sandell sensitivity values are 0.0127 and 0.0073 μg cm^{-2}. The limits of detection and quantification have also been reported. The proposed methods were applied successfully to the determination of BUPH in pure drug and commercial tablets. The accuracy and reliability of the proposed methods were further ascertained by recovery studies *via* standard addition technique.

Keywords: bupropion hydrochloride; methyl orange; ion-pair complex; pharmaceuticals

Introduction

Bupropion hydrochloride (BUPH), chemically known as (±)-1-(3-chlorophenyl)-2-(tert-butylamino)propan-1-one hydrochloride,[1] 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.[2,3] Bupropion is a second-generation antidepressant agent that is also used in the management of smoking cessation.[4] Since its introduction in 1984, several methods have been reported for its determination in biological fluids including high performance liquid chromatography (HPLC),[5–8] gas chromatography (GC),[9] liquid chromatography (LC),[10–12] liquid chromatography-mass spectrometry (LC-MS),[13] liquid chromatography-tandem mass spectrometry (LC-MS/MS),[14,15] and radioimmunoassay.[16] Several methods have been reported for the determination of BUPH in pharmaceuticals such as non-aqueous titration,[17] HPLC,[17–20] gas liquid chromatography (GLC),[21] reversed-phase LC,[22] thin-layer chromatography,[23] potentiometry,[24] conductometry,[24] and UV-spectrophotometry.[24,25] In spite of the suitability of the proposed methods, most of these methods employ expensive instruments or materials, and need extra pure solvents which are hazardous material (i.e. HPLC methods). In addition, chromatographic methods need a suitable compound as internal standard, which makes the analytical procedure more complex.

To the best of our knowledge, no visible spectrophotometric method has ever been reported for the determination 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.

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of some drugs;[26–30] therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. This technique depends on the reaction of a drug that has a basic cationic nitrogen and an anionic dye at a suitable pH, where a highly coloured ion-pair complex is formed. Also, methyl orange (MO) has been widely used as ion-pairing reagent for quantitative analysis of many drugs in pharmaceutical formulations.[29,31–35] However, no report dealing with the extractive spectrophotometric determination of BUPH in drug forms has appeared so far. Therefore, the purpose of this investigation was directed to develop accurate, sensitive, selective, precise, and inexpensive procedures for the determination of BUPH in pharmaceuticals based on ion-pair complex formation using MO as a reagent.

* *Correspondence to: Prof. Kanakapura Basavaiah, Department of Chemistry, University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India. E-mail: basavaiahk@yahoo.co.in*

Department of Chemistry, University of Mysore, Manasagangotri, Mysore-570 006, Karnataka, India

Experimental

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped 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) and certified to be 99.78% pure. The following pharmaceutical preparations were purchased from commercial sources in the local market and subjected to analysis: Bupron-SR-150 from Sun Pharmaceutical Industries (Jammu, India) and Ession-ER-150 from Psycoremedies (Ludhiana, Punjab, India).

Reagents and chemicals

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

1. MO (S.d. Fine Chem., Mumbai, India): 0.1% (*w/v*) solution in water.
2. Sulfuric acid (Merck, Mumbai, India, sp. gr. 1.84): 5.0% (*v/v*) in ethanol.
3. Buffer solution of pH 3.80 was prepared by mixing 50 ml of 0.2 N sodium acetate (Merck, Mumbai, India) and 42.5 ml of 0.2 N HCl (Merck, Mumbai, India, sp. gr. 1.18), the solution was diluted to 250 ml with water, and the pH was adjusted with a pH meter.

Standard stock solution

A stock standard solution of 200 μg ml^{-1} of BUPH was prepared by dissolving accurately weighed 20 mg of pure drug in water and diluting to the mark with water in a 100-ml calibrated flask. This solution was diluted appropriately with water to get a working concentration of 20 μg ml^{-1} BUPH for use in both the methods.

Recommended methods

Method A (based on the measurement of ion-pair)

Different aliquots (0.75, 1.5, 3.0, −9.0 ml) of a standard BUPH (20.0 μg ml^{-1}) solution were accurately transferred into a series of 125-ml separating funnels and the total volume was adjusted to 11.0 ml by adding adequate quantity of water. To each funnel 3 ml of buffer solution of pH 3.80 was added, followed by 1 ml of 0.1% MO solution. The content was mixed well and after 10 min, the ion-pair was extracted with 15 ml of dichloromethane after shaking for 1 min. The two phases were allowed to separate and the dichloromethane layer was dried over anhydrous sodium sulfate and the absorbance of the yellow BUPH-MO ion-pair complex was measured at 425 nm against a corresponding reagent blank.

Method B (based on the measurement of acidic form of dye from the broken ion-pair)

Aliquots (0.2, 0.5, 1.0, −3.0 ml) of BUPH-MO ion-pair complex (12 μg ml^{-1} in BUPH; prepared in method A) were transferred into a series of 5-ml standard flasks and the total volume was adjusted to 3.0 ml by adding dichloromethane. To each flask, 1 ml of 5.0% alcoholic H_2SO_4 was added, the content was mixed well and kept aside for 5 min. Finally, the volume was made up to the mark with ethanol and the absorbance of the red-pink-coloured species was measured at 520 nm against the reagent blank.

Procedure for commercial tablets

Ten tablets, each containing 150 mg of BUPH, were weighed and finely powdered. An amount of the powder equivalent to 10.0 mg of BUPH was accurately weighed and transferred to a 100-ml volumetric flask, 60 ml of water was added, and the content was shaken thoroughly for about 10 min. The volume was diluted to the mark with water, mixed well, and filtered using Whatman No.42 filter paper. The first 10-ml portion of the filtrate was rejected and a suitable aliquot of the filtrate (containing 100 μg ml^{-1} BUPH) was diluted with water to get a working concentration of 20.0 μg ml^{-1} and used for the assay by method A. The ion-pair complex BUPH-MO (12.0 μg ml^{-1}; in BUPH) of the tablets was used for assay by applying the procedure described in method B.

Results and discussion

Absorption spectra

Since BUPH forms an ion-pair complex with MO, the extraction of the yellow ion-pair complex from the aqueous reaction medium into dichloromethane was investigated. The ion-pair formed was found to be quantitatively extracted into dichloromethane and its absorption spectrum (Figure 1) displays an absorption peak at 425 nm (method A). Neither BUPH nor MO alone exhibits any significant absorption at 425 nm under the same conditions. In method B, this BUPH-MO ion-pair complex was treated with alcoholic sulfuric acid to yield a chromogen, the acidic form of the MO, which exhibits bathochromic shift to maximum absorbance 520 nm (Figure 1).

Reaction mechanism

The nitrogenous drug is present in positively charged protonated form and anionic dye is present mainly in anionic form in acidic medium. So when the BUPH treated with an acid dye such as MO in acidic medium (pH 3.80 ± 0.10), a yellow ion-pair complex extractable into dichloromethane is formed. The

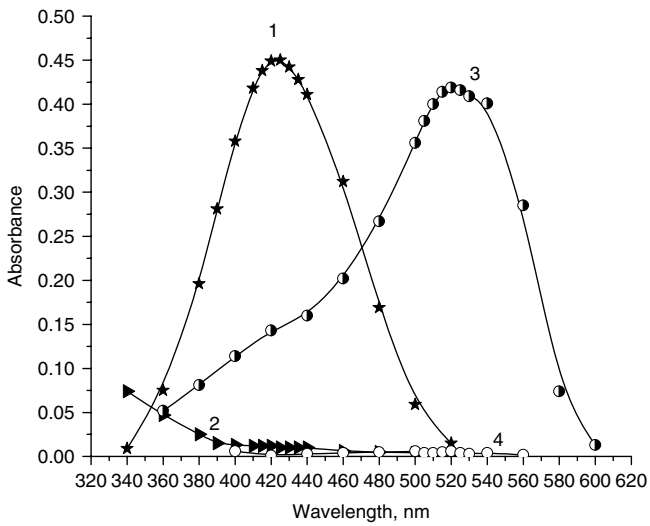
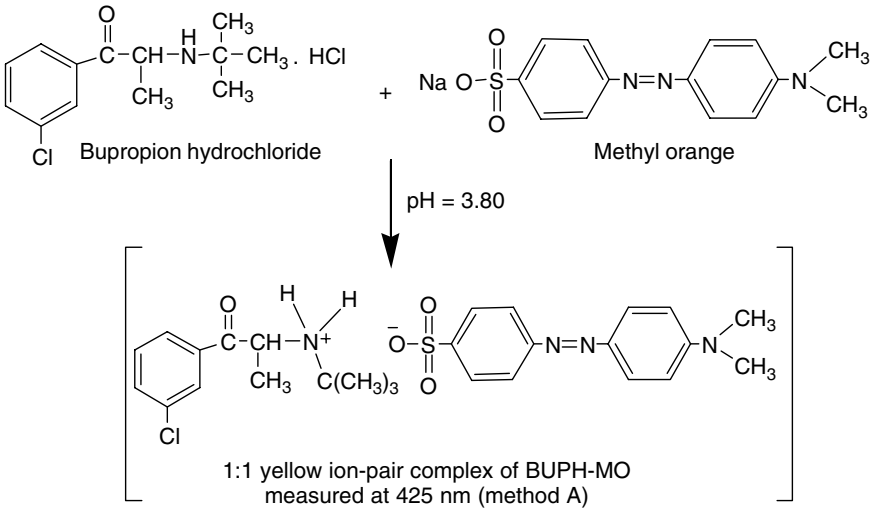
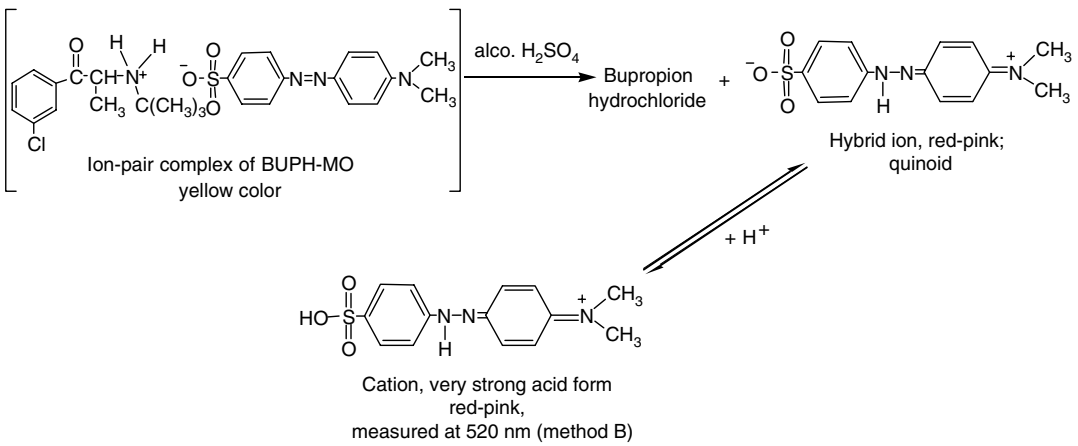


Figure 1. Absorption spectra of: 1. ion-pair complex of BUPH-MO (7.5 μg ml^{-1} BUPH); 2. reagent blank in method A; 3. acidic form of the dye (4.8 μg ml^{-1}, in BUPH) in method B; 4. reagent blank in method B.



Scheme 1. The possible mechanism for ion-pair complex formation.



Scheme 2. The possible mechanism for the formation of acidic form of MO.

possible mechanism for method A is given in Scheme 1. When the BUPH-MO ion-pair complex, formed in method A, treated with alcoholic sulfuric acid, the ion-pair complex will break and the yellow colour will change to red-pink due to the formation of acidic form of MO and exhibits absorbance maximum at 520 nm. The possible mechanism[36] for method B is illustrated in Scheme 2.

Optimization of reaction variables

The optimization of the methods was carefully studied to achieve complete reaction formation, quantitative extraction of the ion-pair complex, and highest sensitivity. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH, type of organic solvent, volumes of the dye, and extent of shaking time for the extraction of ion-pair complex. In method B, alcoholic sulfuric acid concentration required for complete breaking of the ion-pair complex was optimized.

Effect of reagents concentration

In method A, the effect of the dye concentration on the intensity of the colour developed at the selected wavelength was ascertained by adding different amounts of MO to fixed concentration of 10 μg ml^{-1} BUPH. It was found that 1.0 ml of 0.1% MO solution was sufficient for the production of maximum and reproducible colour intensity and the highest absorbance remained unaffected by further addition of the reagent (Figure 2). For method B, the effect of alcoholic sulfuric acid concentration required to break the ion-pair complex and formation of the acidic form of the dye was studied by measuring the absorbance of the solutions containing a fixed concentration of ion-pair complex (6.0 μg ml^{-1}; in BUPH) and different volumes of alcoholic H_2SO_4. It was found that 1 ml of 5.0% alcoholic H_2SO_4 was sufficient to yield a maximum absorbance at 520 nm, although larger volumes of acid had no pronounced effect on the absorbance of the measured species (Figure 2).

Effect of pH on the ion-pair formation

The effect of pH was studied by extracting the coloured complex in the presence of various buffers viz., KCl-HCl, NaOAc-HCl, and NaOAc-AcOH. It was noticed that the maximum colour intensity and constant absorbance was observed in NaOAc-HCl buffer of pH 3.80. Further, 3 ml of this buffer of pH 3.80 gave maximum absorbance and reproducible results. Low absorbance values were observed at pH values higher than 3.90 or lower than 3.70.

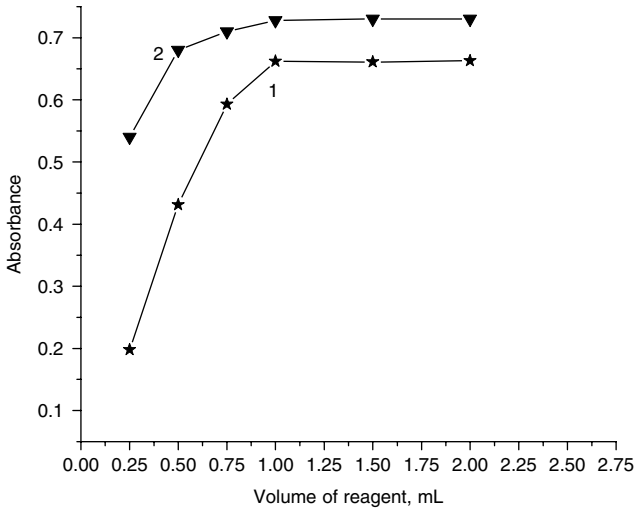


Figure 2. Effect of reagent concentration on colour development: 1. MO (0.1%), 2. alcoholic H_2SO_4 (5.0% *v/v*).

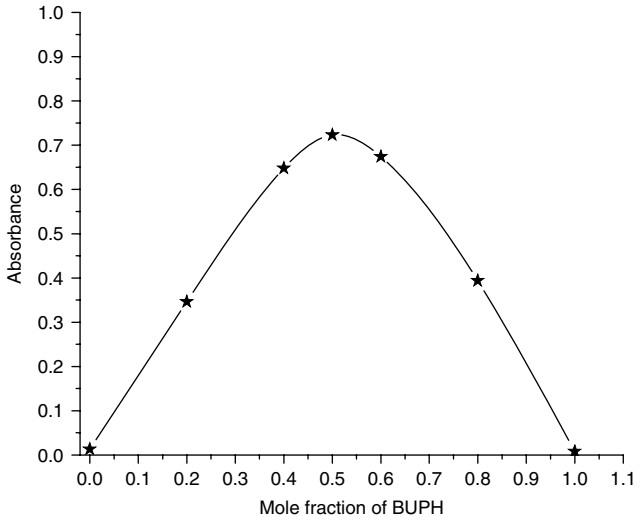


Figure 3. Job's continuous-variations plot for [BUPH] + [MO] = 1.45×10^{-4} M.

Table 1. Effect of the extracting solvent on absorbance of the BUPH-MO ion-pair.[a]

Solvent	A_{blank}	$A_{ion-pair}$
Chloroform	0.010	0.745
Dichloromethane	0.014	1.066
1, 2- dichloroethane	0.016	0.623
Ethyl acetate	0.068	0.253
Benzene	0.007	0.004

[a] BUPH concentration: 15 μg ml^{-1}.

Selecting of the extracting solvent

In order to select the suitable solvent for ion-pair extraction, a number of organic solvents such as chloroform, dichloromethane, 1,2-dichloroethane, ethyl acetate and benzene were examined in order to provide an applicable extraction procedure. The results in Table 1 showed that dichloromethane is the best solvent, although it is not an environmental friendly solvent; it was preferred for its efficient and quantitative extraction of ion-pair complex and the greater stability of the extracted ion-pair (>24 h), its high sensitivity, maximum absorbance of the measured species, and shortest time to reach the equilibrium between both phases.

Effect of time and the stability

The effect of contact time between BUPH and MO in the presence of buffer of pH 3.80 (method A) was studied in the time range of 0–30 min before extraction and it was found that 10 min is sufficient to achieve maximum absorbance at 425 nm. Shaking times of 0.5–3 min produced a constant absorbance, and hence a shaking time of 1 min was used throughout. In method B, the effect of the time required to break the complex was studied after the addition of alcoholic H_2SO_4 to the ion-pair complex and it was found that 5 min was sufficient for complete breaking. The absorbance of the yellow ion-pair complex remained stable for more than 24 h without any change in the absorbance reading at room temperature (method A); also the absorbance of the red-pink colour of acidic form of MO (method B) was found to remain stable for at least 90 min.

Composition of the ion-pair complex

The composition of the ion-pair complex formed in method A between BUPH and MO was established by applying Job's method of continuous variations. In this method, 1.45×10^{-4} M solutions of BUPH and MO were used and mixed in varying volume ratios in such a way that the total volume of the drug and MO was kept at 12 ml in the total volume of 15 ml of the aqueous layer. The absorbance of extracted ion-pair in each instance was measured and plotted against the mole fraction of the drug (Figure 3). The plot reached a maximum value at a mole fraction of 0.5 which indicates that a 1:1 (BUPH: MO) ion-pair complex is formed through the electrostatic attraction between positive protonated BUPH and MO anion. This finding was anticipated by the presence of one basic or electron donating centre (-NH) in the drug under study.

Method validation

Linearity

At described experimental conditions for BUPH determination, the absorbance–concentration plots were found to be linear over the concentration ranges stated in Table 2. The statistical parameters were given in the regression equation calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a). The linearity of calibration graphs was proven by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The apparent molar absorptivity, Sandell sensitivity, and limits of detection and quantification of the proposed methods were also calculated and are recorded in Table 2.

Accuracy and precision

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different amounts of drug were analyzed in seven replicates during the

Table 2. Optical characteristic and statistical data of the regression Equation

Parameter	Method A	Method B
λ_{max}, nm	425	520
Colour stability	>24 hr	90 min
Beer's Law limits, µg ml^{-1}	1.00–12.0	0.48-7.20
Molar absorptivity, l mol^{-1} cm^{-1}	2.18×10^4	3.79×10^4
Sandell sensitivity,* µg cm^{-2}	0.0127	0.0073
Limit of detection, µg ml^{-1}	0.07	0.05
Limit of quantification, µg ml^{-1}	0.22	0.15
Regression equation, Y**		
Intercept, (a)	0.0044	0.0307
Slope, (b)	0.0773	0.1148
Correlation coefficient, (r)	0.9999	0.9989
Standard deviation of intercept (S_a)	0.00210	0.01016
Variance (S_a^2)	4.41×10^{-6}	1.03×10^{-4}
$\pm tS_a/\sqrt{n}$	1.94×10^{-3}	9.40×10^{-3}
Standard deviation of slope (S_b)	0.00029	0.00235
$\pm tS_b/\sqrt{n}$	2.68×10^{-4}	2.17×10^{-3}

* 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^{-1}, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope.

same day (intra-day precision) and five consecutive days (inter-day precision). The percentage relative standard deviation (RSD %) values were ≤2.74 % (intra-day) and ≤2.82% (inter-day) indicating high precision of the methods. Also, the accuracy of the methods was evaluated as percentage relative error (RE %) and from the results shown in Table 3, it is clear that the accuracy is satisfactory (RE ≤2.22 %).

Selectivity

In order to evaluate the selectivity of the proposed methods for the analysis of BUPH in pharmaceutical formulations, the effect of the presence of the excipients, such as talc, starch, lactose, glucose, sodium alginate, calcium gluconate, carnauba wax, hypromellose, microcrystalline cellulose, polyethylene glycol, polysorbate 80, titanium dioxide 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.

Table 4. Results of assay of tablets and statistical evaluation

Tablet brand name	Found (% of nominal amount ± SD)*		
	Reference method	Proposed methods	
		Method A	Method B
Bupron SR 150	98.14 ± 0.64	96.75 ± 0.97 **t** =2.67 **F** = 2.30	99.58 ± 1.15 **t** =2.45 **F** = 3.23
Ession ER 150	100.1 ± 0.73	98.50 ± 1.67 **t** =1.96 **F** = 5.23	98.75 ± 1.04 **t** =2.38 **F** = 2.03

* 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.

Applications to analysis of pharmaceutical formulations

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 shown in Table 4 and were compared with those obtained by the reference method[17] 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 a non-aqueous medium using a 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 at 95% confidence level with respect to accuracy and precision. Accuracy of the proposed methods was further confirmed using the standard addition procedure. Pre-analyzed tablet powder (Bupron SR 150 and Ession ER 150) was spiked with pure BUPH at three different concentration levels (50, 100, and 150% of the quantity present in the tablet powder) and the total was measured by the proposed methods. The determination with each level was repeated three times and the results of this study presented in Table 5 indicate that the various excipients present in the formulations did not interfere in the assay.

Table 3. Evaluation of intra-day and inter-day precision and accuracy

Method	BUPH[a] taken µg ml^{-1}	Intra-day (n=7)			Inter-day (n=5)		
		BUPH found[a] µg ml^{-1}	% RE[b]	% RSD[c]	BUPH Found[a] µg ml^{-1}	% RE[b]	% RSD[c]
Method A	4.00	3.93	1.75	2.58	3.92	2.00	2.03
	7.00	6.90	1.43	1.38	6.87	1.86	1.21
	10.0	9.88	1.20	2.74	9.85	1.50	2.82
Method B	2.40	2.36	1.67	2.00	2.35	2.08	1.63
	3.60	3.53	1.94	0.51	3.52	2.22	0.83
	4.80	4.79	0.21	2.25	4.77	0.62	2.05

[a] Mean value of n determinations.
[b] Relative Error (%).
[c] Relative standard deviation (%).

Table 5. Results of recovery study using standard addition method

Formulation studied	Method A				Method B			
	BUPH taken μg ml^{-1}	Pure BUPH added, μg ml^{-1}	Total found, μg ml^{-1}	Pure BUPH recovered*, Percent ± SD	BUPH taken μg ml^{-1}	Pure BUPH added, μg ml^{-1}	Total found, μg ml^{-1}	Pure BUPH recovered*, Percent ± SD
Bupron SR 150	3.87	2.00	5.82	97.50 ± 2.13	2.39	1.20	3.58	99.17 ± 1.92
	3.87	4.00	7.71	96.00 ± 1.91	2.39	2.40	4.72	97.08 ± 0.68
	3.87	6.00	9.82	99.17 ± 2.07	2.39	3.60	6.04	101.4 ± 1.34
Ession ER 150	3.94	2.00	5.90	98.00 ± 1.47	2.37	1.20	3.58	100.8 ± 0.93
	3.94	4.00	7.85	97.75 ± 2.36	2.37	2.40	4.68	96.25 ± 1.75
	3.94	6.00	10.02	101.3 ± 2.00	2.37	3.60	5.90	98.06 ± 2.03

* Mean value of three determinations.

Conclusions

This is the first report on the use of visible spectrophotometry for the determination of bupropion hydrochloride in pharmaceuticals. A significant advantage of the extractive spectrophotometric methods is that it can be applied for the determination of individual compounds in a multicomponent mixture. The proposed methods are based on well-characterized ion-pair complexation reaction, and have the advantages of sensitivity, selectivity, speed, accuracy, precision, and use of inexpensive equipment compared to the reported HPLC and GC methods. Comparison of the proposed methods with those of the reported methods revealed that the present methods offer a higher sensitivity than HPLC (10–200 μg ml^{-1}),[18] HPLC (10–120 μg ml^{-1}),[19] HPLC (2.12–21.2 μg ml^{-1}),[20] GC (100–3000 μg ml^{-1}),[21] and derivative UV spectrophotometry (10–30 μg ml^{-1}).[25] Moreover, the proposed methods can be performed at room temperature and are unaffected by slight variations in experimental conditions, such as pH and reagent concentration. 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.

Acknowledgments

One of the authors (SAMA) wishes to express his thanks to Al-Bayda' University, Republic of Yemen for awarding a research fellowship and to the authorities of the University of Mysore for permission and facilities to carry out the research work.

References

[1] H. Buschmann, J. L. Díaz, J. Holenz, A. Párraga, A. Torrens, J. M. Vela. *Antidepressants, Antipsychotics, Anxiolytics: from Chemistry and Pharmacology to Clinical Application*. Wiley-VCH: Weinheim, **2007**, pp. 242.
[2] H. G. Kinnell, Bupropion for smokers: drug is almost identical in structure to diethylpropion, a controlled drug. *Brit. Med. J.* **2001**, *322*, 431.
[3] R. M. Lane, G. B. Baker. Chirality and drugs used in psychiatry: nice to know or need to know? *Cell. Mol. Neurobiol.* **1999**, *19*, 355.
[4] A. J. Johnston, J. Ascher, R. Leadbetter, V. D. Schmith, D. K. Patel, M. Durcan, *et al*, Pharmacokinetic optimisation of sustained-release bupropion for smoking cessation. *Drugs* **2002**, *62*, 11.
[5] K. I. Al-khamis. Rapid determination of bupropion in human plasma by high performance liquid chromatography. *J. Liq. Chromatogr. R. T.* **1989**, *12*, 645.
[6] T. A. Jennison, P. Brown, J. Crossett, F. M. Urry. A high-performance liquid chromatographic method for quantitating bupropion in human plasma or serum. *J. Anal. Toxicol.* **1995**, *19*, 69.
[7] K. K. Loboz, A. S. Gross, J. Ray, A. J. McLachlan. HPLC assay for bupropion and its major metabolites in human plasma. *J. Chromatogr. B* **2005**, *823*, 115.
[8] D. Zhang, B. Yuan, M. Qiao, F. Li. HPLC determination and pharmacokinetics of sustained-release bupropion tablets in dogs. *J. Pharmaceut. Biomed.* **2003**, *33*, 287.
[9] T. P. Rohrig, N. G. Ray. Tissue distribution of bupropion in a fatal overdose. *J. Anal. Toxicol.* **1992**, *16*, 343.
[10] T. B. Cooper, R. F. Suckow, A. Glassman. Determination of bupropion and its major basic metabolites in plasma by liquid chromatography with dual-wavelength ultraviolet detection. *J. Pharm. Sci.* **1984**, *73*, 1104.
[11] R. F. Suckow, M. F. Zhang, T. B. Cooper. Enantiomeric determination of the phenylmorpholinol metabolite of bupropion in human plasma using coupled achiral-chiral liquid chromatography. *Biomed. Chromatogr.* **1997**, *11*, 174.
[12] D. Yeniceli, D. Doğrukol-Ak. An LC method for the determination of bupropion and its main metabolite, hydroxybupropion in human plasma. *Chromatographia* **2009**, *70*, 1703.
[13] C. Arellano, C. Philibert, C. Vachoux, J. Woodley, G. Houin. Validation of a liquid chromatography-mass spectrometry method to assess the metabolism of bupropion in rat everted gut sacs. *J. Chromatogr. B* **2005**, *829*, 50.
[14] V. Borges, E. Yang, J. Dunn, J. Henio. High-throughput liquid chromatography-tandem mass spectrometry determination of bupropion and its metabolites in human, mouse and rat plasma using a monolithic column. *J. Chromatogr. B* **2004**, *804*, 277.
[15] R. Coles, E. D. Kharasch. Stereoselective analysis of bupropion and hydroxybupropion in human plasma and urine by LC/MS/MS. *J. Chromatogr. B* **2007**, *857*, 67.
[16] R. F. Butz, D. H. Schroeder, R.M. Welch, N. B. Mehta, A. P. Phillips, J. W. A. Findlay. Radioimmunoassay and pharmacokinetic profile of bupropion in the dog. *J. Pharmacol. Exp. Ther.* **1981**, *217*, 602.
[17] L. Delazzeri. Development of methods for the quality control of bupropion hydrochloride and paroxetine hydrochloride in compounding pharmacies. *Caderno de Farmácia* **2005**, *21*, 37.
[18] I. Bhattacharyya, S. P. Bhattacharyya, S. Sen. Reverse phase high performance liquid chromatographic method for the analysis of bupropion hydrochloride in pharmaceutical dosage form. *Int. J. Pharm. Technol.* **2010**, *2*, 224.
[19] L. Delazzeri, S. B. Borba, A. M. Bergold. Development and validation of a chromatographic method for the determination of bupropion hydrochloride. *Rev. Ciênc. Farm. Básica Apl.* **2005**, *26*, 211.
[20] Q. Meiling, W. Peng, G. Yingshu, G. Junling, F. Ruonong. Development and validation of an HPLC method for the determination of bupropion hydrochloride in tablets. *J. Chin. Pharmaceut. Sci.* **2002**, *11*, 16.
[21] R. T. Sane, M. Francis, S. Khedkar, A. Menezrs, A. Moghe, P. Patil. Gas chromatographic determination of bupropion hydrochloride from its pharmaceutical formulations. *Indian Drugs* **2003**, *40*, 231.

[22] D. Yeniceli, D. Doğrukol-Ak. The retention behaviour of bupropion hydrochloride in reversed phase ion pair LC and validated analysis of the drug in pharmaceuticals. *Chromatographia* **2010**, *71*, 79.
[23] D. Yeniceli, D. Doğrukol-Ak. Validated thin-layer chromatographic method for the determination of bupropion hydrochloride in pharmaceutical dosage form. *J. Planar Chromatogr.* **2010**, *23*, 212.
[24] D. Yeniceli, D. Doğrukol-Ak. The determination of bupropion hydrochloride in pharmaceutical dosage forms by original UV- and second derivative UV spectrophotometry, potentiometric and conductometric methods. *Turk. J. Pharm. Sci.* **2010**, *7*, 99.
[25] K. N. Patel, J. K. Patel, I. S. Rathod. Derivative spectrophotometric method for simultaneous estimation of nicotine and bupropion hydrochloride in synthetic mixture by derivative spectrophotometric method. *J. Pharm. Res.* **2009**, *2*, 1525.
[26] M. Amanlou, P. Khosravian, E. Souri, O. G. Dadrass, R. Dinarvand, M. M. Alimorad, *et al*, Determination of buprenorphine in raw material and pharmaceutical products using ion-pair formation. *Bull. Korean Chem. Soc.* **2007**, *28*, 183.
[27] S. Ashour, M. F. Chehna, R. Bayram. Spectrophotometric determination of alfuzosin HCl in pharmaceutical formulations with some sulphonephthalein dyes. *Int. J. Biomed. Sci.* **2006**, *2*, 273.
[28] K. Harikrishna, B. S. Nagaralli, J. Seetharamappa. Extractive spectrophotometric determination of sildenafil citrate (viagra) in pure and pharmaceutical formulations. *J. Food Drug Anal.* **2008**, *16*, 11.
[29] J. Milano, S. G. Cardoso. Spectrophotometric determination of oxiconazole in topical lotion using methyl orange. *J. Pharm. Biomed. Anal.* **2005**, *37*, 639.
[30] N. Rahman, S. N. Hejaz-Azmi. Extractive spectrophotometric methods for determination of diltiazem HCl in pharmaceutical formulations using bromothymol blue, bromophenol blue and bromocresol green. *J. Pharm. Biomed. Anal.* **2000**, *24*, 33.
[31] A. M. El-Didamony. Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange. *Spectrochim. Acta A* **2008**, *69*, 770.
[32] D. H. Manjunatha, J. Seetharamappa, P. B. Kandagal, S. S. Kalanur. New extractive spectrophotometric methods for the determination of nortriptyline hydrochloride in pure form and pharmaceutical dosages. *J. Anal. Chem.* **2009**, *64*, 462.
[33] A. H. Prabhakar, V. B. Patel, R. Giridhar. Spectrophotometric determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. *J. Pharmaceut. Biomed.* **1999**, *20*, 427.
[34] S. Mostafa, M. El-Sadek, E. A. Alla. Spectrophotometric determination of enrofloxacin and pefloxacin through ion-pair complex formation. *J. Pharmaceut. Biomed.* **2002**, *28*, 173.
[35] W. E. Hassan. Extractive colorimetric method for the determination of dothiepin hydrochloride and risperidone in pure and in dosage forms. *Chem. Pharm. Bull.* **2008**, *56*, 1092.
[36] I. M. Kolthoff. *Acid-Base Indicators*. Macmillan: New York, **1937**, pp. 228.