

OPTIMIZED AND VALIDATED SPECTROPHOTOMETRIC DETERMINATION OF BUTAMIRATE CITRATE IN BULK AND DOSAGE FORMS USING ION-PAIR FORMATION WITH METHYL ORANGE AND BROMOTHYMOLO BLUE

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Abstract

Two easy, sensitive, precise and valid spectrophotometric techniques are described for the determination of butamirate citrate (BC) in pure form and pharmaceutical formulations. These methods are based on the formation of ion-pair between butamirate citrate with methyl orange (MO) and bromothymol blue (BTB). The techniques involve the formation of yellow coloured ion-pair in aqueous solution, extraction with dichloromethane and absorbance measurement at 424 nm for BC-MO and 410 nm for BC-BTB ion pairs against a blank solution. Molar ratio of the ion-pairs was determined using Job's Continuous Variation Method. The proposed methods were validated for linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. Beer's law was obeyed in the ranges of 4.0 - 20.0 µg/mL for BTB and 2.5 - 12.5 µg/mL for MO.

Rezumat

Sunt descrise două tehnici spectrofotometrice simple, sensibile, precise și valide pentru determinarea citratului de butamirat (BC) sub formă pură și din formulări farmaceutice. Aceste metode se bazează pe formarea de perechi de ioni între citratul de butamirat cu metiloranj (MO) și albastrul de bromotimol (ABT). Tehnicile implică formarea perechii de ioni colorați în galben în soluție apoasă, extracția cu diclorometan și măsurarea absorbantei la 424 nm pentru BC-MO și 410 nm pentru perechile de ioni BC-ABT față de o soluție martor. Raportul molar al perechilor de ioni a fost determinat utilizând metoda de variație continuă a lui Job. Metodele propuse au fost validate privind liniaritatea, limita de detecție (LOD), limita de cuantificare (LOQ), acuratețea și precizia. Legea Beer a fost respectată în intervalul de 4,0 - 20,0 µg/mL pentru ABT și 2,5 - 12,5 µg/mL pentru MO.

Keywords: butamirate citrate, spectrophotometric assay, ion-pair complexes, methyl orange

Introduction

Butamirate citrate, α -ethylbenzeneacetic acid 2-[2-(diethylamino)ethoxy] ethyl ester citrate, is a central-acting antitussive drug [1, 2]. It is a cough suppressant, mainly used in acute lower respiratory tract infections (tracheitis, laryngitis, bronchitis). The drug is also effective in the treatment of irritative cough due to seasonal respiratory disorders or chronic cough [3]. A survey of the literature revealed that a few analytical methods have been reported for the determination of butamirate citrate: HPLC methods from pharmaceutical dosage forms and derivative UV-spectrophotometry [1, 4-6] from human blood plasma [7]. There is no ion pair extraction method reported for butamirate citrate in the literature.

Extractive spectrophotometric procedures are the most widely used techniques because of their simplicity, cost-effectiveness, sensitivity in many pharmaceutical analysis including ofloxacin and lomefloxacin [8], felodipine [9], ranitidine [10], sertoconazole nitrate

and miconazole nitrate [11], enoxacin [12], levofloxacin [13]. Thus ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. In this study we performed the development of an extractive spectrophotometric method based on the absorbance measurement of coloured BC-MO and BC-BTB ion-pair complexes.

Materials and Methods

Apparatus: spectrophotometric measurements were made using a Shimadzu UVmini-1240 PC UV-visible spectrophotometer with 1 cm matched quartz cells. Bio vortex (V1) was used to vortex and pH measurements were carried out with Jenco (6179) pH meter, calibrated with buffer solution (pH 4.0 and 7.0). The HPLC system (Schimadzu Corporation Analytical System) consisting of Rheodyne syringe sample injector (20 µL), LC-20AT pump system, DGU-20A5R degassing unit, SPD-M20A PDA detector, GL Sciences

Inertsil ODS-3 column (46 x 260 mm, 5 μ m), CTO-10AS column oven.

Reagents: bulk butamirate citrate (BC) and its syrup (Notuss Fort[®], labelled content of BC 22.5 mg/5 mL) were kindly supplied by Tripharma. All the chemicals and reagents were obtained from Merck (Darmstadt, Germany). All reagents and chemicals used were of analytical or pharmaceutical grade.

Preparation of stock standard solutions and reagents: a stock standard solution of 100 μ g/mL BC was prepared in distilled water. Working standard solutions were obtained by appropriate dilutions with distilled water. Bromotymol blue (BTB) reagent solution was prepared by dissolving 0.025 g of BTB in 5 mL methanol and diluting to 100 mL with distilled water. Methyl orange (MO) reagent was prepared by dissolving 0.008 g of MO in distilled water and diluted to 100 mL with the same solvent. The phosphate buffer solutions were prepared according to European Pharmacopoeia 8th Edition.

General procedures

BC-BTB ion complex: 1 mL of standard BC solution (20 - 100 μ g/mL), 1 mL of 0.025% BTB solution and 1 mL of pH = 3 phosphate buffer solution were added in a 15 mL centrifuge tube and extracted with 5 mL dichloromethane vortexing for 1 minute and then centrifuging at 3000 rpm for 2 minutes. The absorbance of organic phase was measured at 410 nm against a blank reagent.

BC-MO ion complex: 1 mL of standard BC solution (12.5 - 62.5 μ g/mL), 1 mL of 0.008% MO solution and 1 mL of pH = 5.0 phosphate buffer solution were added in a 15 mL centrifuge tube and extracted with 5 mL dichloromethane vortexing for 1 minute and then centrifuging at 3000 rpm for 2 minutes. The absorbance of organic phase was measured at 424 nm against a reagent blank.

Assay of pharmaceutical preparation: 1 mL aliquot of syrup was diluted to 100 mL with distilled water, mixed well and 1 mL of this solution was used for the analysis using the above procedures. The concentrations were calculated using the regression equation of the corresponding calibration curve.

Results and Discussion

Optimization of experimental variables

Selecting the optimum wavelength: the absorption spectra of the ion-pair complexes formed between BC and MO with BTB were recorded at 424 and 410 nm against respective blank reagent. The measurements were made at these wavelengths.

Effect of pH on the ion pair formations: The effect of the pH on the formation of ion-pairs was investigated in the interval of 2.0 to 8.0. Maximum absorbance was observed when the aqueous solution was buffered at pH 3.0 for BC-BTB and at pH 5.0 for BC-MO (Figure 1).

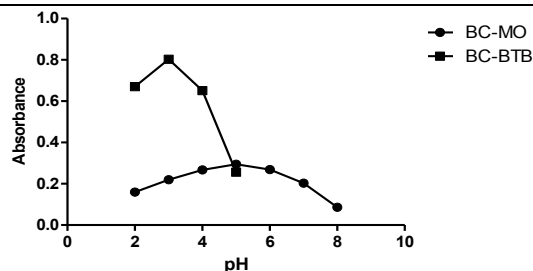


Figure 1.

Effect of the pH on the formation of BC-MO and BC-BTB complexes

Effect of the extracting solvent: the effect of extraction solvent on the ion-pair complexes was examined. Three organic solvents such as chloroform, dichloromethane and toluene were tested for the extraction of the ion-pair. Although, there were no significant differences in the absorbance values between the chloroform and dichloromethane, dichloromethane was chosen as solvent, due to its low toxicity (Figure 2).

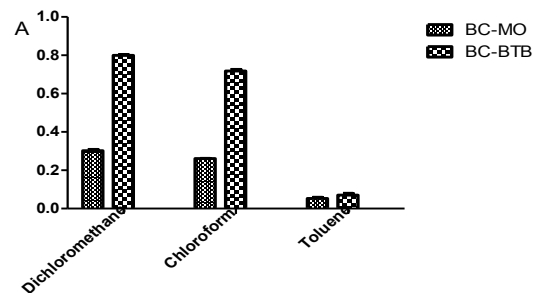


Figure 2.

Effect of different extracting solvents on the ion-pair formation

Effect of reagents concentration: the effect of reagent concentration was studied by repeating the determinations using increasing concentrations of BTB. 1 mL of 0.025% BTB solution is sufficient for 20 μ g/mL final BC concentration. The effect of reagent concentration was studied by repeating the determinations using increasing concentrations of MO. 1 mL of 0.008% MO solution is sufficient for 12.5 μ g/mL final BC concentration corresponding to the reagent/BC mole ratio of 2 for both reagents (Figure 3).

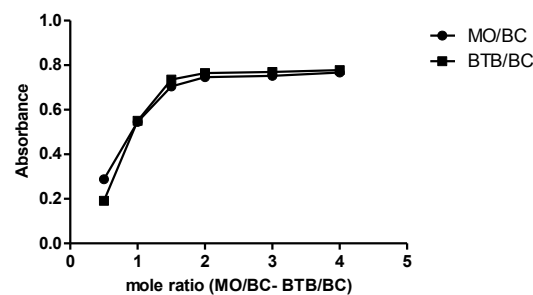


Figure 3.

Effect of the reagent concentration on the ion-pair complex formation

Stoichiometric relationship: the stoichiometric relationship was determined using the method of Job's Continuous Variations [11]. Job's curve was prepared using equimolar concentrations of BC, MO and BTB (2×10^{-4} M). As it is seen in Figure 4, 1:1 complex was formed with each reagent.

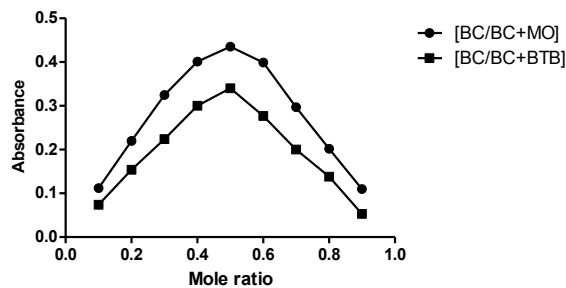


Figure 4.

Job's Plot of Continuous Variations for the reaction of the ion-pair complexes

Method validation

The validation of proposed methods was carried out in accordance with the ICH guidelines [14] in order to determine linearity, limit of detection, limit of quantification, precision and recovery.

Linearity and range: under optimum conditions, a linear relationship was obtained between absorbance (A) and BC concentration (c) for both reagents (Figures 5 and 6). Regression equations, linear concentration ranges and correlation coefficients are given in Table I.

Sensitivity: the limit of detection was calculated by $LOD = 3.3 \times \sigma/S$, where σ is the standard deviation of the intercept in the calibration curve and S is the slope of the calibration curve. The limit of quantification was

calculated $LOQ = 3 \times LOD$ (Table I). LOD and LOQ values were determined as 0.07 and 0.21 $\mu\text{g/mL}$ BC for MO; 0.29 and 0.87 $\mu\text{g/mL}$ BC for BTB respectively.

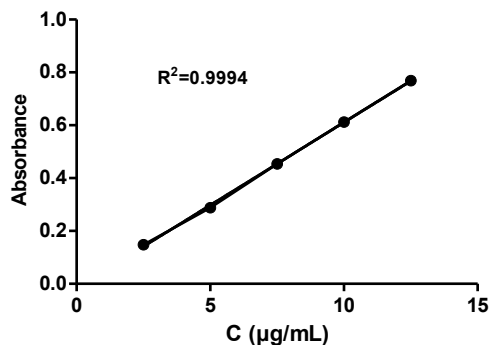


Figure 5.

Calibration curve of the BC-MO ion pair

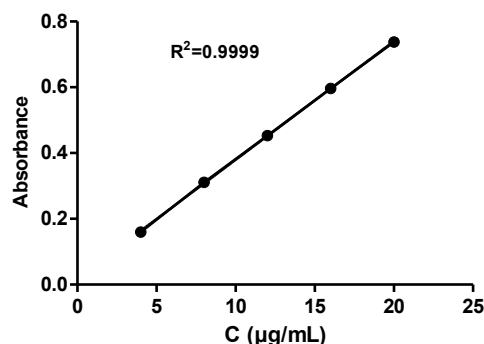


Figure 6.

Calibration curve of the BC-BTB ion pair

Table I

Statistical analysis of the calibration graphs and analytical data in the determination of BC using the proposed methods

Parameters	BC-MO	BC-BTB
Wavelengths λ_{max} (nm)	424	410
pH	5.0	3.0
Beer's law limits ($\mu\text{g/mL}$)	2.5 - 12.5	4.0-20.0
Regression equation	$A = 0.0640c - 0.0330$	$A = 0.0356c + 0.0193$
Intercept (a)	-0.0330	0.0193
Slope (b)	0.0640	0.0356
Correlation coefficient (R^2)	0.9994	0.9999
LOD ($\mu\text{g/mL}$) ^a	0.07	0.29
LOQ ($\mu\text{g/mL}$) ^b	0.21	0.87

^aLOD, limit of detection; ^bLOQ, limit of quantification

Precision and Accuracy: intra-day (three times a day operation under the same conditions) and inter-day (four different days) variations using three different concentrations 5.0, 7.5, 10.0 $\mu\text{g/mL}$ of BC-MO and 8.0, 12.0, 16.0 $\mu\text{g/mL}$ of BC-BTB respectively were

investigated. The accuracy of the proposed method was validated by standard addition method. Known amounts of standard BC was added to samples at a level of 50% (Table II).

Table II

Intra-day and inter-day precision and accuracy results for BC obtained using the proposed methods

Method	Concentration ($\mu\text{g/mL}$)					
	BTB			MO		
	Taken	Found	RSD (%) ^a	Taken	Found	RSD (%) ^a
Interday	8.0	8.08	0.91	5.0	5.07	0.7
	12	12.03	0.47	7.5	7.55	0.3
	16	16.01	0.53	10	9.97	0.2
Intraday	8.0	8.05	0.96	5.0	5.06	0.8
	12	11.89	0.84	7.5	7.53	0.1
	16	16	0.75	10	9.96	0.2
Recovery studies						
	Level (%)	Conc. taken ($\mu\text{g/mL}$)		Conc. Found ($\mu\text{g/mL}$)		Recovery (%)
	BC-MO	13.50		13.47		99.77 \pm 0.04
	BC-BTB	13.50		13.33		98.74 \pm 0.01

^aMean of three determination; RSD%, percentage relative standard deviation

Application to pharmaceutical formulation: the proposed method was applied to the determination of BC in commercially available syrup. The same samples were also analysed simultaneously by the method of Paul A.K *et al.* as a reference method [15],

since a pharmacopoeial method is not available for BC. The results obtained from the analyses were compared statistically. The Student's t-values and F-values at the 95% confidence level did not exceed the tabulated values. Table III summarizes the results.

Table III

Results of butamirate citrate syrup analysis

n	Proposed method BC-MO Conc.(mg/5 mL syrup)	Proposed method BC-BTB Conc.(mg/5 mL syrup)	Reference method HPLC Conc.(mg/5 mL syrup)
1	22.93	23.25	23.14
2	23.20	23.50	23.19
3	23.22	22.98	23.21
4	23.06	23.16	23.29
5	23.40	22.97	23.43
6	23.02	23.02	23.19
Mean	23.14	23.15	23.24
SD	0.17	0.20	0.10
RSD%	0.73	0.89	0.45
CI ^a	22.96 - 23.32	22.93 - 23.36	23.13 - 23.35
F test ^b	2.61	3.87	
t test ^c	1.16	1.00	

^a Confidence interval (95%); ^b (F = 5.05 for p = 0.05); ^c (t = 2.57 for p = 0.05)

Conclusions

The developed spectrophotometric methods for butamirate citrate are simple, sensitive, precise, rapid, reliable and requires no costly chemicals and equipment. The reagents used in the proposed methods are cheaper, readily available and the procedures do not caused any critical reaction conditions. The proposed methods were applied successfully for the determination of the butamirate citrate in pure and dosage form. The t test showed that there is no significant difference between the results of proposed and reference method. Also, good recovery results indicate that the excipients in syrup have no interference in the determination. The methods were validated by the statistical data and can be easily adapted for industrial analysis.

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