Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

SELECTIVE DETERMINATION OF BAMBUTEROL HYDROCHLORIDE IN THE PRESENCE OF ITS ACTIVE METABOLITE TERBUTALINE

Amr M Badawy, Nadia M Mostafa, Abd El-Aziz B Abd El-Aleem and Nesrine T Lamie*

Cairo University, Faculty of Pharmacy, Department of Analytical Chemistry, Kasr El-Aini Street, ET 11562, Cairo, Egypt.

Received: 21 April 2011; Revised: 26 May 2011; Accepted: 30 May 2011; Available online: 5 June 2011

ABSTRACT

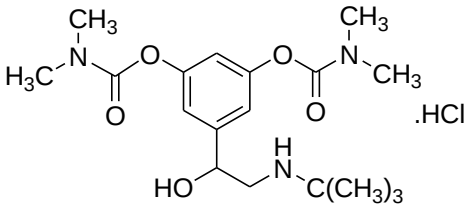
Stability-indicative determination of bambuterol hydrochloride (BH) in the presence of its degradation product (terbutaline) , which is also the metabolite, is investigated. The degradation product has been isolated, via acid-degradation, characterized and elucidated. Selective quantification of BH, singly in bulk form, pharmaceutical formulations and/or in the presence of its major degradation product is demonstrated. The indication of stability has been undertaken under conditions likely to be expected at normal storage conditions. Among the spectrophotometric methods adopted for quantification are second derivative (^{2}D), first derivative of ratio spectra (^{1}DD), ratio subtraction and bivariate analysis.

Keywords: Bambuterol hydrochloride, terbutaline, second derivative spectrophotometry, derivative-ratio, ratio subtraction, bivariate.

INTRODUCTION

Bambuterol hydrochloride (BH), (RS)-5-(2-tert-butylamino-1-hydroxyethyl)-m-phenylene bis(dimethyl-carbamate) hydrochloride[1], Figure **1**.

Figure 1. Structural formula of bambuterol hydrochloride



BH is a direct acting sympathomimetic with predominantly -adrenergic activity (β_2-agonist).[1] It is an ester prodrug of β_2 adrenergic agonist terbutaline.[2] Bambuterol hydrochloride is official in British Pharmacopeia and determined by non aqueous titration method.[3] Different HPLC methods have been reported for the estimation of BH in pharmaceutical dosage form.[4–6] The drug has been also estimated by solid-state NMR spectroscopy.[7] It is used for the prophylaxis and treatment of chronic asthma and chronic bronchitis in pediatrics. Literature survey reveals that there is no stability indicating spectrophotmetric methods reported for the determination of BH in presence of its degradation product terbutaline.

In modern analytical laboratory, there is always a need for significant stability-indicating methods of analysis. The present work aimed to develop simple spectrophotometric methods for the quantification of BH in pure form or even in the presence of its degradation product. The described methods include second derivative (^{2}D), first derivative of the ratio spectra (^{1}DD) , ratio subtraction and bivariate analysis.

***Corresponding Author:**
Dr Nesrine Talaat Lamie
Department of Analytical Chemistry, Faculty of Pharmacy,
Cairo University, Kasr El-Aini Street, ET 11562, Cairo, Egypt.
Contact no: +20-122711259, Email: nesrinelamie@hotmail.com

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were of analytical grade and the solvents were of spectroscopic grade. Pure sample was kindly supplied by the by Alborg Pharmaceutical industry, Alexandria, Egypt; it was assayed for its purity according to a pharmacopoeial method[3] and found to contain 100.15 ±1.258 %. Methanol (E. Merck, Darmstadt, Germany), 1M HCl, ethyl acetate, concentrated ammonia (specific gravity 0.91) were obtained from El- Nasr pharm co, Egypt.

- Bambedil 10 tablets: Manufactured by Western Pharmaceutical industry, Batch No. 09018, labeled to contain 10 mg BH/tablet.
- Bambec 10 tablets: Produced by Astrazeneca, Sodertalje, Sweden, imported by health family. Batch number: 740, labeled to contain 10 mg BH per tablet.
- Lela Free 20 tablets: Manufactured by Multiapex Pharma, (Badr city, Egypt), Batch No. MT1070409, labeled to contain 20 mg/tablet.

Preparation of standard solutions

BH standard solution (1 mgmL^{-1}) and drug degradation product (BHD) standard solution (1mgmL^{-1}) were prepared in methanol.

Instrumentation

Spectrophotometer: Shimadzu UV-1601 PC, dual-beam UV–visible spectrophotometer (Japan), with matched cm^{-1} quartz cells, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software Version 3.7 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2nm with wavelength-scanning speed of 2800 nm min^{-1}. *IR Spectrophotometer:* Mattson Genesis II FTIRTM (USA), sampling was undertaken as potassium

bromide discs. *UPLC MS-MS:* Acquity TQ-Waters (USA).

Degradation of bambuterol hydrochloride

Accelerated acid-degradation was performed by refluxing 500 mg of pure BH with 50 mL of 1 M HCl solution for 3 hours. Where complete degradation was achieved, as investigated by thin layer chromatography using ethyl acetate + methanol + ammonium hydroxide (7:3: 0.01, v/v/v) as a developer solvent. The solution was concentrated to a small volume and extracted with methanol. The methanolic extract was evaporated under vacuum. The structure of the isolated degradation product was elucidated using IR, and MS spectrometry.

Second derivative (^{2}D) method

Spectral characteristics of BH and its degradation product: Two aliquots equivalent to 3 mg of BH and 1 mg of its degradation product standard stock solutions (each, 1mgmL^{-1}) were transferred separately into two 10-mL volumetric flasks. Then the volumes were completed with methanol. The zero order (^{0}D) and the second derivative (^{2}D) spectra of the prepared solutions were recorded.

Linearity: Portions equivalent to (1–10mg) of BH standard solution (1mgmL^{-1}) were separately transferred to a series of 10-mL volumetric flasks. Each flask was completed to the volume with methanol to reach the concentration range of 100–1000µgmL^{-1}. The amplitudes of the second derivative peaks were measured at 272 nm with Δλ = 4 nm and a scaling factor = 100. Calibration graph was constructed by plotting peak amplitude *versus* concentration. The regression equation was then computed at the specified wavelength and used for determination of unknown samples containing BH.

First derivative of ratio spectra (^{1}DD) method

Linearity: Standard serial concentrations in the range of 100–1000µgmL^{-1} solutions of BH were prepared as above. Accurately 3mL of the degradation product standard solution (1mgmL^{-1}) was transferred into a 10-mL volumetric flask and the volume was completed with methanol to get a final concentration of 300 µgmL^{-1} to be used as a divisor. The spectra of the prepared standard solutions were scanned (200–400 nm) and stored into the computer.

The stored spectra of BH were divided (amplitude at each wavelength) by the spectrum of 300µgmL^{-1} of the degradation product. The first derivative of the ratio spectra (^{1}DD) with Δλ= 4 nm and a scaling factor = 10 was obtained. The amplitudes of the first derivative peaks of BH were measured at 250 nm. Calibration graphs were constructed relating the peak amplitudes of (^{1}DD) to the corresponding concentrations. The regression equations were then computed at the specified wavelength and used for determination of unknown samples containing BH.

Ratio subtraction spectrophotometric method

Linearity: Aliquots equivalent to 100–1000µgmL^{-1} from BH standard solution (1mgmL^{-1}) were transferred into a series of 10-ml volumetric flasks then completed to volume with methanol; the spectra of the prepared standard solutions were scanned. A calibration curve was constructed relating the absorbance of zero order spectra of BH at λ_{max} 265 nm to the corresponding concentrations and the regression equation was computed. Aliquot equivalent to 600µg from BH degradation product standard solution (1mgmL^{-1}) was transferred into 10-ml volumetric flask and completed to volume with methanol to be used as a divisor.

Bivariate method

Two series of standard solutions containing aliquots (100–1000µgmL^{-1}) of BH and (100–700µgmL^{-1}) of its degradation product were prepared from the stock solution (1mgmL^{-1}, each) for the bivariate calibration. Spectra of the obtained solutions were recorded and stored into the computer. The regression equations were computed at λ= 265 and 280 nm. The concentrations of BH and its degradation product were calculated using the parameters of the linear regression functions evaluated individually for each component at the same wavelength and substituting in the following equations:

$$C_{degradate} = \frac{mA_2(A_{AB1} - e_{AB1}) + mA_1(e_{AB2} - A_{AB2})}{m_{A2}m_{B1} - m_{A1}m_{B2}}$$

$$C_{BH} = \frac{A_{AB1} - e_{AB1} - m_{B1}C_{degradate}}{m_{A1}}$$

where A_{AB1} and A_{AB2} are the absorbance's of A and B at λ_1 and λ_2, respectively, e_{AB1} and e_{AB2} are the sum of the intercepts of the linear calibration at two, wavelengths λ_1 and λ_2 ($e_{AB1} = e_{A1} + e_{B1}$), m_A and m_B are the slopes of linear regression and C is the concentrations (µgmL^{-1}) of BH and its degradate.

The accuracy of the results was checked by applying the proposed bivariate calibration method for determination of different blind samples of pure BH and its degradate. The concentrations were obtained from the corresponding regression equations from which percentage recoveries were calculated.

Analysis of laboratory prepared mixtures containing different ratios of BH and its degradation product using the suggested methods

Aliquots of intact drug and its degradate were mixed to prepare different mixtures containing 10–70% (w/w) of the degradation product, and proceed as mentioned under each method. The concentrations were calculated from the corresponding regression equations.

Assay of pharmaceutical formulations

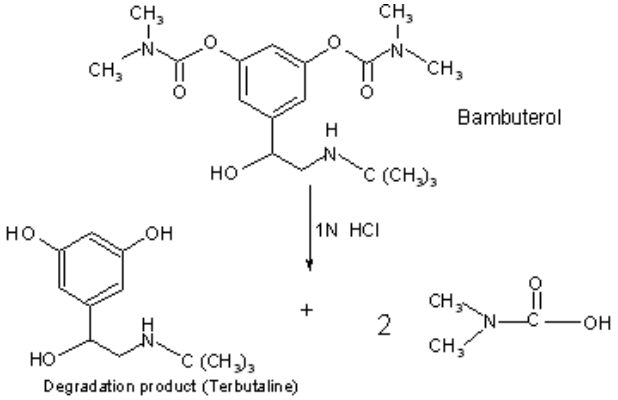
Twenty tablets were accurately weighed and powdered. A portion of the powder equivalent to 100 mg BH was accurately weighed into a 100-mL beaker, dissolved in methanol and filtered into a 100-mL measuring flask. The volume was completed by the same solvent to reach a final drug concentration of 1mgmL^{-1} for the proposed methods and proceed as mentioned under each method.

RESULTS AND DISCUSSION

Degradation of BH

The main degradation product of BH is terbutaline which is formed by hydrolysis of the two ester linkages. Degradation was examined under acidic and elevated temperature.(Scheme **1**)

Scheme 1. Suggested scheme for the degradation of bambuterol hydrochloride.



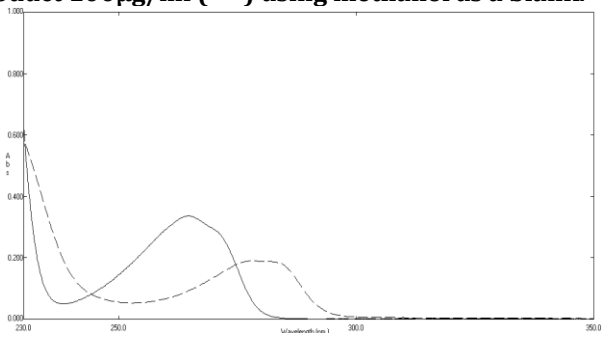
It has been confirmed that the main degradate is terbutaline which is also the major metabolite of the drug inside the human body. Once complete degradation was reached, the carbonyl stretching band at 1689cm^{-1} disappeared, also a broad band of alcoholic OH stretching vibration at 3394cm^{-1} confirmed the acidic hydrolysis at the two ester linkages. In the GC/MS-chart, the parent peak was identified at *m/z 225* (mol. w. of degradate). TLC monitoring of the drug degradation was done on thin layer plates of silica gel F254 using ethyl acetate+ methanol + ammonium hydroxide (7:3: 0.01, v/v/v) as a developing solvent. The developed plates were visualized under short UV-lamp. The degradate (R_f value = 0.4) could be separated elegantly from the intact drug (R_f value = 0.6).

Second derivative (^{2}D) method

Derivative spectrophotometry is a useful tool in quantification of mixture of drugs. It could be even used as a stability-indicating technique for the analysis of drugs in presence of their degradation products, by solving the problem of the overlapping absorption bands.

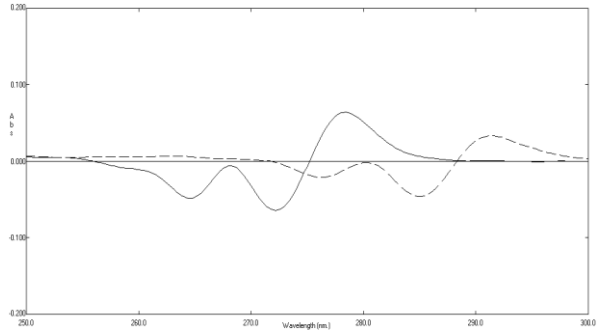
A simple, rapid and selective spectrophotometric procedure was proposed and applied for the determination of BH in the presence of its degradation product, either as raw material or in pharmaceutical formulations. This was done by applying the second derivative (^{2}D) UV spectrophotometry. The method can solve the problem of spectral bands overlapping between BH and its degradate without sample pretreatment or extra separation steps. The absorption spectra of BH and its degradation product (Figure **2**) show overlapping, little interference and error probability that make the use of direct measurement of BH in the presence of its degradate inaccurate, especially at higher level of degradation.

Figure 2. Absorption spectra of bambuterol hydrochloride 300µg/ml (__) and its degradation product 100µg/ml (----) using methanol as a blank.



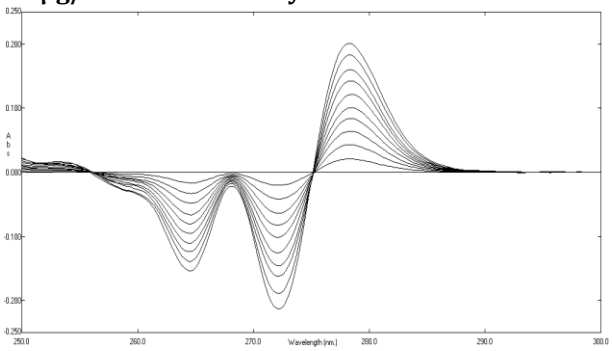
When the second-derivative spectra (Figure **3**) were examined, it was found that BH could be determined at 272 nm, where its degradate has no contribution (zero crossing) allowing accurate determination of BH in presence of its degradate.

Figure 3. Second-derivative absorption spectra of bambuterol hydrochloride 300µg/ml (_) and degradation product 100µg/ml (----) using methanol as a solvent.



A linear relationship was obtained in the range of 100–1000µgmL^{-1} BH (Figure **4**).

Figure 4. Second-derivative absorption spectra of 100-1000 µg/ml bambuterol hydrochloride



The regression equation was computed and found to be

^{2}D = -0.0002 C - 0.0005 (r = 0.9999), at 272 nm

Where ^{2}D is the peak amplitude of the second derivative curve at the corresponding wavelength, *C* the concentration of BH (µgmL^{-1}) and *r* is the correlation coefficient. The precision of the proposed method was confirmed by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recovery was found to be 99.97±0.818 at 272 nm.

Derivative-ratio spectrophotometric method

The derivative-ratio spectroscopy is a useful tool in quantification of drugs. It could be applied as a stability-indicating method for the determination of BH in presence of its degradate. The zero-order of the ratio spectra of BH and the first order of the ratio spectra are presented in Figure **5** and **6**, respectively. It was found that upon dividing by 300 µgmL^{-1} of the degradation product, best results were obtained in terms of sensitivity, repeatability and signal to noise ratio.

Figure 5. Ratio spectra of bambuterol hydrochloride (100-1000µg/ml) using the spectrum of 300µg/ml of degradation product as a divisor.

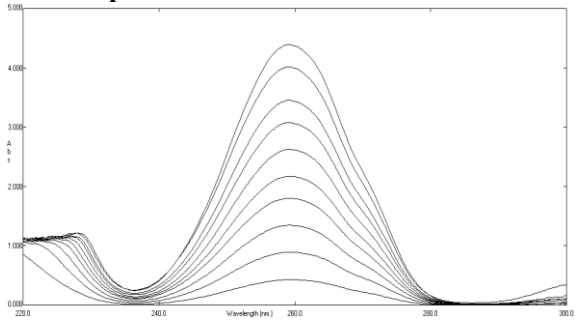
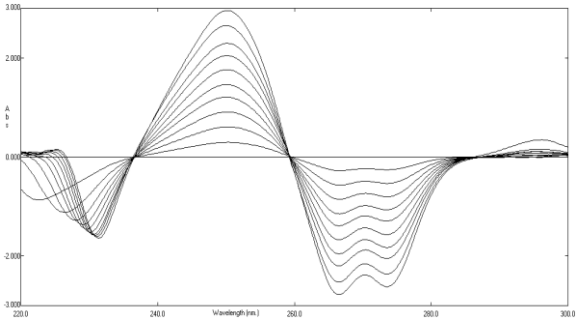


Figure 6. First derivative of ratio spectra of bambuterol hydrochloride (100-1000 µg/ml) using the spectrum of 300µg/ml of degradation product as a divisor.



Linear calibration graphs were obtained for BH in concentration range of 100–1000µgmL^{-1} by recording the peak amplitudes at 250 nm using 300µgmL^{-1} of the degradate as a divisor. The regression equations were computed and found to be

$^1DD = 0.0029\ C + 0.0153$ (r = 0.9996), at 250 nm

Where ^1DD is the peak amplitude of the first derivative curve for (BH/its degradate), C the concentration of BH (μgmL^{-1}) and r is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates.

Ratio subtraction spectrophotometric method

The method was applied for determination of mixture of BH(X) and its degradate (Y) when the spectrum of the degradate extended than the other, as shown in (Figure **2**). The determination of BH could be achieved by scanning the zero order absorption spectra of the laboratory-prepared mixtures in methanol, then dividing them by a carefully chosen concentration (600µg mL^{-1}) of standard BH degradation product to produce a new ratio spectra that represents BH/BHD + constant, as shown in (Figure **7**); then, subtraction of the absorbance values of these constants (BH/BHD) in plateau as shown in (Figure **8**) followed by multiplication of the obtained spectra by the divisor as shown in (Figure **9**); finally, the original spectra of BH, which are used for direct determination of BH at 265 nm, could be obtained and the concentration from the corresponding regression equation could be calculated. This can be summarized as follows:

$$\frac{X+Y}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'} + constant \quad \text{.....(1)}$$

$$\frac{X}{Y'} + constant - constant = \frac{X}{Y'} \quad \text{.....(2)}$$

$$\frac{X}{Y'} \times Y' = X \quad \text{.....(3)}$$

Figure 7. Division spectra of laboratory prepared mixtures of bambuterol HCl (Y) and degradation product (X) using 600µg/ml of its degradation product (Y') as a divisor and methanol as a blank

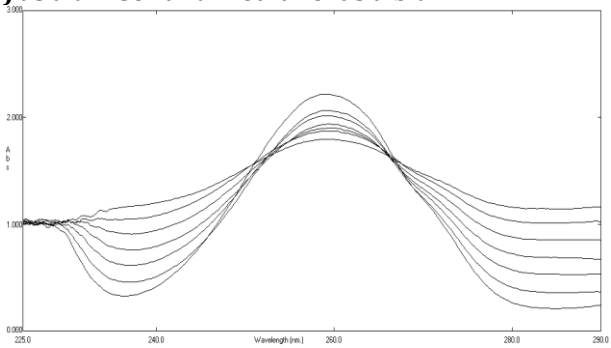
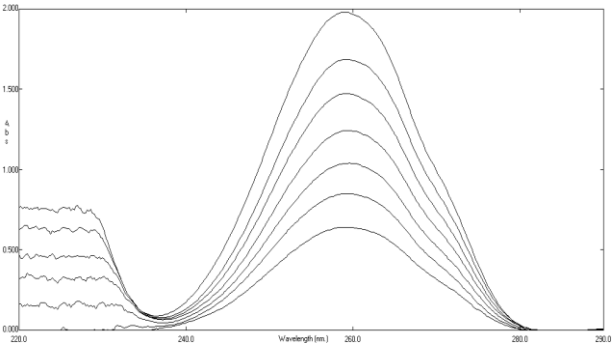


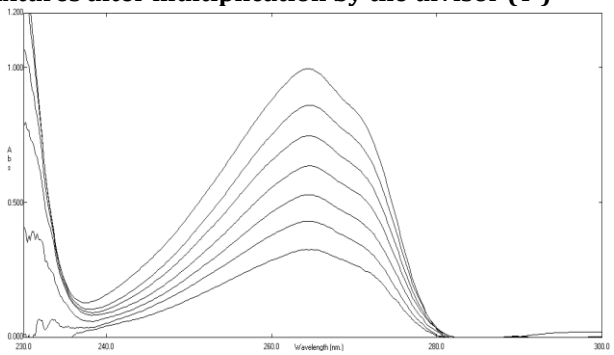
Figure 8. Division spectra of laboratory prepared mixtures of bambuterol HCl (X) and its degradation product (Y) using 600µg/ml of degradation product (Y') as a divisor and methanol as a blank after subtraction of the constant



The constant can be determined directly from the curve by the straight line which is parallel to the wavelength axis in the region where BHD is extended. The correct choice of the divisor is fundamental, as if the concentration of the divisor increases or decreases, the resulting constant value will be proportionally decreased or increased.[8] A linear correlation was obtained between the absorbance and the corresponding concentration of BH at its corresponding wavelength: the regression equation was:

A= 0.0011C- 0.0017 r =0.9998

Figure 9. Zero order absorption spectra of bambuterol HCl obtained by the proposed ratio subtraction method for the analysis of laboratory prepared mixtures after multiplication by the divisor (Y')



Bivariate method

The bivariate calibration method may be competitive and in some cases even superior to commonly use derivative spectrophotometric methods as applied for the resolution of binary mixtures. The advantage of bivariate calibration method is its simplicity and the fact that derivatization procedures are not necessary. Unlike other chemometric techniques, there is no need for full spectrum information and no data processing is required. Calibration function was calculated (r > 0.9990), m_i- and e_i-values were taken for the bivariate algorithm. In order to apply the bivariate method to the resolution of binary mixture of BH and its degradate, signals of the two components located at eight wavelengths were selected: 250, 255, 260,265, 270,275, 278 and 280 nm. The calibration curve equations and their respective linear regression coefficients are obtained with the aim of ensuring that there is a linear relationship between the absorbance values and the concentrations. All the calibration curves at the selected wavelengths showed satisfactory linear regression coefficients (r > 0.9990). The slope of the linear regression were estimated for both components at the selected wavelengths and used for determination of the sensitivity matrices K, proposed by Kaiser's method.[9] The determinants of these matrices were calculated as shown in Table **1**.

Table 1. Application of Kaiser's method in the selection of wavelength pair for the mixture of bambuterol HCl and its degradate: the absolute values of determinants of sensitivity matrices ($K \times 10^{-7}$).

λ/λ	250	255	260	265	270	275	278	280
250	0	0.142	2.59	2.13	-0.64	-5.18	-7.68	-8.35
255		0	0.69	-0.41	9.28	-9.43	-12.05	-12.65
260			0	-1.63	-6.41	-13.28	-16.49	-17.19
265				0	-5.67	-14.80	-18.90	**-19.71**
270					0	-10.8	-14.92	-16.41
275						0	-4.68	-6.75
278							0	-2.21
280								0

The wavelength set was selected for which the highest matrix determinant value was obtained. For the bivariate determination of BH and its degradate the wavelengths 265 and 280 nm were used. At these selected wavelengths, the one-component calibration curves were obtained in the range of 100–1000µgmL^{-1} for both components. The linear regression calibration formulae used for the bivariate algorithm are presented in Table **2**.

Table 2. Linear regression calibration formulae used for the bivariate algorithm for bambuterol HCl.

Component	Calibration Equation	
	λ=265nm	λ=280nm
Bambuterol hydrochloride	A =0.0011x+0.007 (r =0.9999)	A=0.00009x-0.0008 (r =0.9996)
Degradate	A =0.001x +0.0001 (r =0.9997)	A =0.0018x+0.028 (r =0.9999)

The mean percentage recoveries were 99.68±1.009 and 101.17±0.843, for BH and its degradate, respectively. The advantage of this method over the other spectrophotometric methods is the ability for simultaneous determination of the intact drug and it's degradates in mixtures.

Stability-indication

To assess the stability-indicating efficiency of the proposed methods, the degradation product of BH was mixed with its pure sample at different ratios and the mixtures were analyzed by the proposed methods. Table **3** illustrates good selectivity in the determination of BH in the presence of up to ~70% (w/w) of its degradate by the proposed methods.

Table 3. Determination of bambuterol hydrochloride in laboratory prepared mixtures by the proposed spectrophotometric methods.

Methods	^{2}D at 270 nm	^{1}DD at 250 nm	Ratio subtraction at 265 nm	Bivariate method
Mean± SD	99.97±0.818	100.24±0.758	99.87±0.741	100.03±0.491

Application of the proposed methods to the pharmaceutical formulations

The suggested methods were successfully applied for the determination of BH in tablets showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique (Table **4**), and the precision was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis results (Table **5**).

Table 4. Quantitative determination of bambuterol hydrochloride in pharmaceutical formulations by the proposed spectrophotometric method

Preparation	^{2}D at 270 nm	^{1}DD at 250 nm	Ratio subtraction at 265 nm	Bivariate method
Bambedil 10 tablets, Batch No.09018 Mean± S.D.	100.36±0.996	100.04±1.149	99.67±1.421	99.79±0.425
Bambec 10 tablets, Batch No.740 Mean± S.D.	100.16±0.351	99.73±0.359	99.68±0.591	100.33±0.851
Lela Free 20 tablets, Batch No.MT1070409 Mean± S.D.	100.37±0.929	100.60±0.794	100.27±0.452	99.80±0.818

Table 5. Assay validation parameters of the proposed spectrophotometric methods for the determination of pure samples of bambuterol hydrochloride.

Parameter	Drug at λ= 272nm	DD_1 method at 250 nm	Ratio subtraction at 265 nm	Bivariate method
Accuracy (mean ± S.D.)	101.17±0.807	100.06±1.971	99.91±1.141	99.68±1.009
Specificity	99.97±0.818	100.24±0.758	99.87±0.741	100.03±0.491
Precision Repeatability* Intermediate precision**	 99.78±0.756 98.85±0.981	 100.48±0.781 100.81±1.016	 100.05±0.935 100.35±0.981	 100.09±0.551 100.22±0.630
Linear range (µg/ml) Slope Standard error of the Slope Intercept Standard error of the intercept Correlation coefficient (r)	100-1000 -0.0002 8.9×10^{-7} -0.0005 0.000552 0.9999	100-1000 0.0029 2.74×10^{-5} 0.0153 0.01703 0.9996	100-1000 0.0011 7.36×10^{-6} - 0.0071 0.004565 0.9998	100-1000 0.0011 5.97×10^{-6} 0.007 0.003707 0.9999

**the intraday and **the inter-day mean values ± standard deviations of samples of concentration of 200, 500, 600 µg/ml of bambuterol hydrochloride and its degradation product.*

Statistical analysis

Results of the suggested methods for determination of BH were statistically compared with those obtained by applying pharmacopoeial non aqueous titration method.[3] The calculated *t*- and *F*-values[10] were found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision (Table **6**).

Table 6. Statistical analysis of the results obtained by the proposed spectrophotometric methods and the compendial method for the determination of bambuterol hydrochloride in pure powder form.

Item	^{2}D method	^{1}DD method	Ratio subtraction	Bivariate method	Pharmacopoeial method*[(3)]
Mean	101.17	100.06	99.91	99.68	100.15
S.D.	0.807	1.971	1.141	1.009	1.258
Variance	0.651	3.885	1.302	1.018	1.583
n	10	10	10	10	5
Student's t test	1.914 (2.160)**	0.124 (2.160)	0.403 (2.160)**	0.823 (2.160)**	
F value	2.432 (3.630)**	2.454 (6.000) **	1.216 (3.630)**	1.555 (3.630)**	

**non aqueous titration method .*
***the values in parenthesis are the corresponding tabulated t and f values at p=0.05.*

CONCLUSION

Unlike the mostly recommended HPLC-procedures, the proposed spectrophotometric methods are simple and not expensive. The reagents used in the proposed methods are cheap and readily available. The procedures applied in each method do not involve any critical reactions or tedious sample preparations. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility of assaying BH in its pharmaceutical formulation without interference due to the excipient or the degradation product.

The suggested methods are found to be simple, accurate, selective and equally sensitive with no significant

difference of the precision compared with the reference method.[3] They could be applied for routine analysis of pure drug or in its pharmaceutical formulation.

REFERENCES

1. Sweetman S C, editor. Martindale. The Complete Drug Reference. 33rd ed. London: Pharmaceutical Press. 2002; 761.
2. O'Neil M J, Smith A, Heckelman P E, Budavari S; Merck index. 13th ed. John Wiley and Sons. 2001; 166-167.
3. The British Pharmacopoeia. 3rd ed. Vol.1. London: Stationary Office Books. 2001; 211-212.
4. Zhang Y; Determination of bambuterol hydrochloride tablets. *Jiangsu Pharm Clin Res*. 2001; 9:13-4.
5. Bartolincic A, Druskovic V, Sporec A, Vinkovic V; Development and validation of HPLC methods for the enantioselective analysis of bambuterol and albuterol. *J Pharm Biomed Anal*. 2005; 36:1003–10.
6. Wannerberg O, Persson B, Chromatogr J A; Liquid chromatographic methods for the determination of bambuterol hydrochloride and related compounds. *J Chromatogr A*. 1988; 435: 199-203.
7. Harris R, Hodgkinson P, Larsson T, Muruganatham A; Quantification of bambuterol hydrochloride in a formulated product using solid-state NMR. *J Pharm Biomed Anal*. 2005; 38:8:58-64.
8. El-Bardicy M G, Lotfy H M, El Sayed M A, El-Tarras M F; Smart stability-indicating spectrophotometric methods for determination of binary mixtures without prior separation. *J AOAC Int*. 2008; 91(2):299–310.
9. Massart D L, Vandeginste B G M, Deming S N, Michotte Y, Kaufman L (Eds.); Chemometrics: A Textbook, Elsevier, Amsterdam. 1988; 124.
10. Spiegel M R, Stephens L J (Eds.); S chaum: Outline of Theory and Problems of Statistics, Schaum Outline Series, New York, 1999.