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Simultaneous quantitative determination of salbutamol sulfate and bromhexine hydrochloride in drug preparations by difference spectrophotometry

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Summary

A difference spectrophotometric procedure has been developed for the simultaneous determination of salbutamol sulfate (SS) and bromhexine hydrochloride (BH) in tablet preparations. The method comprised the measurements of the absorbance of a solution of the drug mixture in pH 2.0 buffer solution relative to that of an equimolar solution in 0.1 N methanolic NaOH at wavelengths of 310 and 280 nm. The presence of identical isosbestic points for pure drug solutions and sample solutions indicated the non-interference of excipients in the absorption at these wavelengths. Compliance with Beer's law was achieved in the concentration range of 0–100 μg/ml for SS and 0–200 μg/ml for BH at these wavelengths.

Introduction

Difference spectrophotometry has proved particularly useful in the determination of medicinal substances by eliminating specific interference from degradation products and co-formulated drugs. It also eliminates the non-specific irrelevant absorption from the formulation matrix. Its advantages for selective analysis have been described by some workers (Doyle and Fezzari, 1974; Davidson, 1976; Davidson, 1984). The technique involves the reproducible alteration of the spectral properties of the analyte in equimolar solutions and the measurement of the absorbance difference (δA) between two solutions, provided the absorbances of the other absorbing interferents are not affected by the reagents used for the spectral property alteration. Simple aqueous acids, alkalis and buffers are most frequently used for inducing spectral alterations since many drugs are weak acids or bases whose state of ionisation and absorptivity depend on the pH of the solution. A combination of SS and BH in the form of a tablet preparation is widely used for bronchodilation in asthma, chronic bronchitis and emphysema.

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Chemically, salbutamol sulfate is the hemisulfate of 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino)ethanol while bromhexine hydrochloride is *N*-(2-amino-3,5-dibromobenzyl)-*N*-cyclohexylmethylamine hydrochloride (British Pharmacopoeia, 1980). Some methods have been reported for the assay of SS (Patel and Patanni, 1985; Miller and Greenblat, 1986; Emm and Lesko, 1988) and BH (Bechgaard and Nielsen, 1982; Emmanuel and Viera, 1986). The official monographs describe the procedure for individual assay of SS and BH (British Pharmacopoeia, 1980) but there are no reports about their simultaneous determination in the presence of each other. The present research describes the pH-induced difference spectrophotometric method for the simultaneous quantitation of SS and BH in the presence of each other as well as the excipients.

Experimental

Standard solutions

The stock solution of SS was prepared by dissolving 100 mg of pure SS in 100 ml of methanol. Appropriate volumes of aliquots of the stock solution were transferred to 50 ml volumetric flasks in duplicate. The volume was made up with pH 2.0 buffer and 0.1 N methanolic NaOH to give a series of equimolar solutions of 50 ml each in pH 2.0 buffer and 0.1 N methanolic NaOH containing 0–100 μg/ml of SS. The stock solution of BH was prepared by dissolving 100 mg of pure BH in 100 ml of methanol. Appropriate volumes of aliquots were used, as in SS, to prepare 50 ml each of a series of equimolar solutions of BH in pH 2.0 buffer and 0.1 N methanolic NaOH containing 0–200 μg/ml of BH. Similarly, two series of 50 ml each of equimolar solutions of mixtures of SS and BH in pH 2.0 and 0.1 N methanolic NaOH were prepared by using the stock solutions. The first series contained a constant concentration of BH (200 μg/ml) and a varying concentration of SS (0–100 μg/ml). The second series contained a constant concentration of SS (100 μg/ml) and a varying concentration of BH (0–200 μg/ml).

Sample preparation

20 tablets were accurately weighed, powdered and a weight of the powder equivalent to 50 mg of SS (and 100 or 200 mg of BH) was dissolved in 50 ml of methanol by thorough mixing and made up to volume in a 50 ml volumetric flask. The extract was filtered through Whatman No. 1 filter paper. The first and the last 5 ml of the filtrate were discarded. The sample solutions of 50 ml of each in pH 2.0 buffer and 0.1 N methanolic NaOH were prepared by using 10 ml aliquots of the filtrate so as to obtain equimolar solutions containing approx. 50 μg/ml of SS and 200 μg/ml of BH, respectively. In the case of liquid preparation, 10 ml of the syrup was diluted to 25 ml with methanol and appropriate aliquots were diluted with 0.1 N HCl and 0.1 N methanolic NaOH to obtain solutions containing approx. 100 μg/ml of SS and 200 μg/ml of BH.

The absorbance difference (δA) between the acidic solution and an equimolar 0.1 N methanolic NaOH solution of pure drugs and samples was measured from 230 to 350 nm with a Jasco 7800 UV-visible double-beam spectrophotometer by placing the acidic solutions in the reference compartment and 0.1 N methanolic NaOH solutions in the sample compartment. The absorbance difference of the analytes at 310 and 280 nm was corrected for the absorbance difference, if any, of 0.1 N methanolic NaOH solution relative to pH 2.0 buffer at these wavelengths.

Results and Discussion

The pK_a values of SS are 9.3 and 10.3 and that of BH is 8.5 (Paul, 1990). Hence, equimolar solutions of SS and BH in pH 2.0 buffer and 0.1 N methanolic sodium hydroxide (pH ~ 12) which were at pH values of about 2.0 pH units away from the pK_a values produced about 90% of the individual species and small changes in the pH did not produce any appreciable changes in the absorbance values (Doyle and Fezzari, 1974).

The difference absorption spectrum of solution of SS in pH 2.0 buffer in the reference cell and an equimolar solution of SS in 0.1 N methanolic NaOH in the sample cell compart-

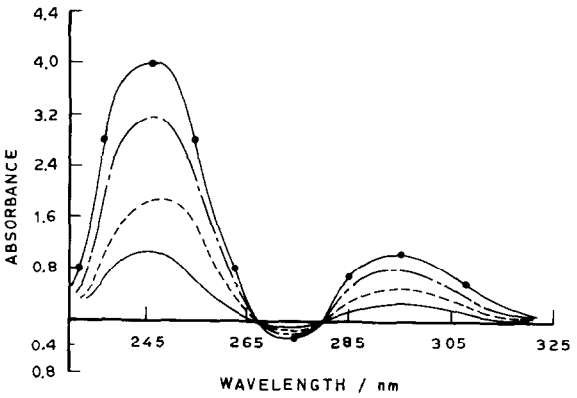

Fig. 1. Difference absorption spectra of salbutamol sulfate in pH 2.0 buffer vs. 0.1 M methanolic NaOH solutions. (———) 25 μg/ml, (- - - - -) 50 μg/ml, (— — —) 75 μg/ml, (●———●) 100 μg/ml.

ment showed a maximum value of δA at 246 and 297 nm and a minimum value of δA at 274 nm. Isosbestic points (a wavelength of zero δA due to equal absorptivities of the two species) occurred at 267 and 280 nm (Fig. 1). The difference absorption spectrum of solution of BH showed a maximum value of δA at 255 nm and a minimum value of δA at 327 nm. The isosbestic point of the BH spectrum was obtained at 310 nm (Fig. 2).

The wavelength of 310 nm at which the δA value of SS difference spectrum was about 0.210

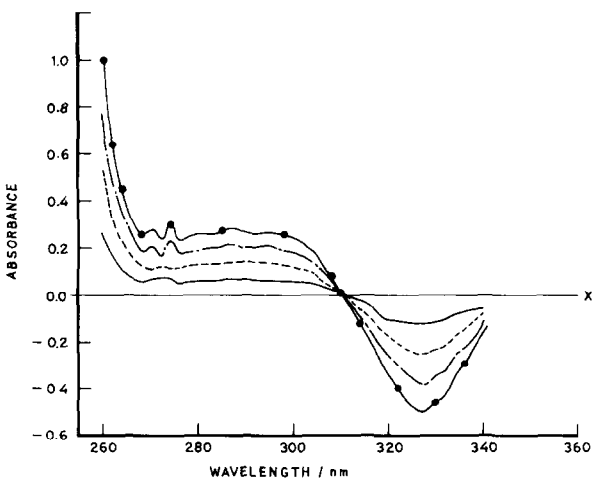

Fig. 2. Difference absorption spectra of bromhexine HCl in pH 2.0 vs 0.1 M methanolic NaOH solutions. (———) 50 μg/ml, (- - - - -) 100 μg/ml, (— — —) 150 μg/ml, (●———●) 200 μg/ml.

and 0.450 for the concentrations of 50 or 100 μg/ml of SS, respectively, was chosen for the estimation of SS. Similarly, the wavelength of 280 nm (isosbestic point of SS) was selected for the quantitation of BH (200 μg/ml) at which the δA value was about 0.250. These concentrations were chosen on the basis of the proportion of SS and BH in commercial formulations as well as to have absorbance values between 0.2 and 1.2 which would provide a minimum relative error (Connors, 1982).

The absorbance of the solutions of the pure SS, BH and their mixture in pH 2.0 and 0.1 N methanolic NaOH were monitored spectrophotometrically and were found to vary by ±0.003 AU over a period of 2 h.

The proportionality of the δA value and concentration of SS was found by measuring δA of the 10 pairs of solutions containing 0–100 μg/ml of SS at 310 nm. The linear regression equation calculated by using the method of least squares for these 10 pairs of solutions was

$$y = 0.0110x - 0.0037 \quad (1)$$

with a correlation coefficient of $r = 0.9999$. The proportionality of δA and the concentration of BH was found by measuring the δA values of solutions of BH containing 0–200 μg/ml of BH at 280 nm. The calculated linear regression equation was

$$y = 0.0012x - 0.0012 \quad (2)$$

with a correlation coefficient of $r = 0.9995$.

To evaluate further the specificity of the method in samples containing SS and BH, two series each of 10 solutions (mentioned under Standard solutions) were examined at the isosbestic wavelengths. The solutions of the first series gave a regression equation of

$$y = 0.0110x - 0.0006 \quad (3)$$

with a correlation coefficient of $r = 0.9999$ at 310 nm which was almost equal to that of Eqn 1, suggesting that the presence of BH did not affect the absorptivity of SS at 310 nm. The δA values

of the second series of solutions gave a regression equation of

$$y = 0.0011x - 0.0027 \quad (4)$$

with a correlation coefficient of $r = 0.9996$ at 280 nm. Its similarity to Eqn 2 suggested the non-interference of absorptivity of SS with that of BH at 280 nm. The identical isosbestic points of the two components in the standard and sample difference spectra confirmed the non-interference of the excipients in the measurement of the absorbance values at these wavelengths. The absorbance values of the two series of solutions are given in Table 1 and 2.

Results

The δA values of standard solutions of SS (100 μg/ml) and BH (200 μg/ml) relative to those of the sample solution were used for the determination of SS and BH in the commercial formulations.

The concentrations of SS (C_{SS}) and BH (C_{BH}) in the tablet contents of average weight (AW) as a percentage of the stated quantity of drug (C_t) were calculated using

$$C_{SS} = \frac{A_{310}^{sam} \times C_{SS}^{std} \times AW \times 100}{A_{310}^{std} \times WT_{sam} \times C_t}$$

$$C_{BH} = \frac{A_{280}^{sam} \times C_{BH}^{std} \times AW \times 100}{A_{280}^{std} \times WT_{sam} \times C_t}$$

TABLE 1

Selectivity of the method for the determination of salbutamol sulfate by difference spectroscopy

Composition of mixture (μg/ml)		Mean [a] (δA) (at 310 nm)	95% confidence [b] limit
SS	BH		
20	200	0.223 ± 0.002	± 0.002
40	200	0.442 ± 0.004	± 0.003
60	200	0.662 ± 0.003	± 0.002
80	200	0.880 ± 0.003	± 0.002
100	200	1.106 ± 0.004	± 0.003

[a] 10 replicate measurements.
[b] Based on Student's t-test distribution.

TABLE 2

Selectivity of the method for the determination of bromhexine hydrochloride by difference spectroscopy

Composition of mixture (μg/ml)		Mean [a] (δA) (at 280 nm)	95% confidence limit [b]
SS	BH		
100	40	0.050 ± 0.003	± 0.002
100	80	0.098 ± 0.004	± 0.003
100	120	0.142 ± 0.004	± 0.003
100	160	0.191 ± 0.004	± 0.003
100	200	0.239 ± 0.004	± 0.003

[a] 10 replicate measurements.
[b] Based on Student's t-test distribution.

where the concentrations of the standard solutions are expressed in mg/ml, WT_{sam} denotes the weight of tablet powder (mg/ml) taken for the preparation of sample solution and the weights are in mg. The results are listed in Table 3.

Conclusion

Thus far, difference spectroscopy had been used for the quantitation of only a few drug mixtures, mainly due to two reasons. Firstly, the application of difference spectroscopy for the analysis of the two-component formulations depends on the fortuitous juxtaposition of the isosbestic points. The positioning should be such that the isosbestic point of one component must be suitable for the measurement of the δA of the difference spectrum of the other component and

TABLE 3

Assay results of salbutamol sulfate and bromhexine hydrochloride in commercial formulations by difference spectroscopy

Sample	SS		BH	
	mg/ tablet	%w/w stated [a]	mg/ tablet	%w/w stated [a]
Brand A	1.99	99.58 ± 0.24	7.98	99.76 ± 0.22
Brand B	3.98	99.52 ± 0.19	8.00	100.04 ± 0.29
Brand C (liq.)	–	99.36 ± 0.38	–	99.14 ± 0.28

[a] Five replicate measurements.

vice versa. In addition, the δA values of the components at the isosbestic points must be from 0.2 to 1.2 for minimum relative error. The proposed method meets these requirements. The non-interference of the excipients in the determination is demonstrated by the identical isosbestic points in standard and sample solutions.

The rectilinearity of δA values at the isosbestic points is shown by the regression equations. The corresponding correlation coefficients and negligible intercepts confirm the proportional relationship between δA values and concentration at the isosbestic points and indicate the precision and reproducibility of the method. Hence, in the absence of an official method for the simultaneous determination of SS and BH, the proposed method is found to be suitable for the simultaneous determination of the components in drug formulations.

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