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Different spectrophotometric methods manipulating ratio spectra for simultaneous determination of salbutamol and bromhexine in binary mixture

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ABSTRACT

Four simple, specific, accurate and precise spectrophotometric methods manipulating ratio spectra were developed and validated for simultaneous determination of Salbutamol (SL) and Bromhexine (BH) namely; dual wavelength, ratio difference, ratio derivative, mean centering. The proposed spectrophotometric procedures do not require any preliminary separation step. The accuracy, precision and linearity ranges of the proposed methods were determined. The four methods were applied for the determination of the cited drugs in tablets and the obtained results were statistically compared with a reported spectrophotometric method. The comparison showed that there is no significant difference between the proposed methods and the reported method regarding both accuracy and precision.

KEYWORDS

Salbutamol;
Bromhexine;
Dual wavelength;
Ratio derivative;
Ratio difference;
Mean- centering.

INTRODUCTION

Salbutamol (SL), Synonym Albuterol, α-[[(1, 1-Dimethylethyl) amino] methyl]-4-hydroxy-1, 3-benzenedimethanol[1] (Figure 1a), is a sympathomimetic drug that stimulates beta adrenoceptors in the airways. It is used mainly as a bronchodilator, to relieve constriction in the airways during attacks of asthma and to alleviate the symptoms of chronic bronchitis and emphysema[2]. Bromhexine (BH), 2, 4-Dibromo-6-[[cyclohexyl (methyl) amino] methyl] aniline[1] (Figure 1b), is a mucolytic used in the treatment of respiratory disorders associated with productive cough[3]. Literature survey revealed that SL and BH are official in British Pharmacopoeia (BP)[4], there are many reported methods for analysis of both cited ingredients simultaneously, these were spectrophotometry[5-9], thin layer chromatography[10] and high-performance liquid-chromatography[11].

In this article, four different methods manipulating ratio spectra for the simultaneous determination of Salbutamol and Bromhexine in tablets are described. These methods show very simple and accurate way for the analysis of this binary mixture without the need of sophisticated instruments, expensive solvents, or large number of samples. The mathematical explanation of the procedures is illustrated.

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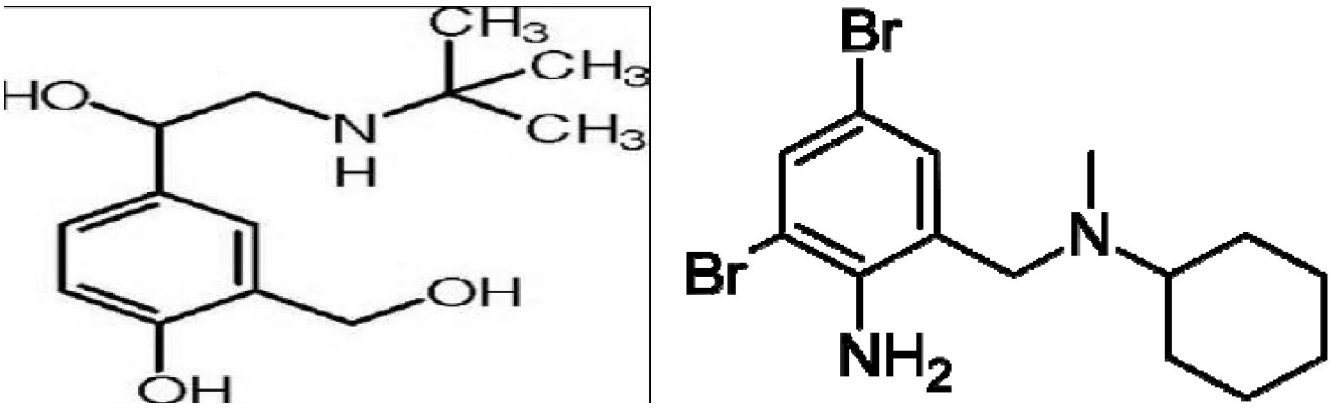

(a) (b)

Figure 1 : Structural formula for (a) Salbutamol and (b) bromhexine

EXPERIMENT

Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and a HP1020 LaserJet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 0.2 nm interval.

Softwares

Matlab 8.2.0.701 (R2013b), all calculations were performed using Intel(R) Core(TM) i3 CPU, 2.13 GHz, and 4.00 GB of RAM under Microsoft Windows 7 Home Premium™.

Chemicals and reagents

1. Salbutamol; kindly supplied by Egyptian International Pharmaceutical Industry company (EIPICO).
2. Bromhexine; kindly supplied by Arab Drug Company, Egypt.
3. Mucovent® Tablet dosage form; labeled to contain 1(SL)/4(BH) mg batch number 167044, 142053, Manufactured by Misr Company for Pharmaceutical Industries, Egypt.
4. Solvent: distilled water.

Standard solutions

1. Standard stock solutions of (SL and BH 1 mg/mL each) was prepared by transferring (0.1gm) of Salbutamol sulfate or bromhexine hydrochloride to 100 ml volumetric flask, dissolving in distilled water and the volume was then completed to the mark.
2. Standard working solutions of (SL and BH 100 µg/mL each) was prepared from stock solutions by appropriate dilutions with water.

Procedure

Spectral characteristics of SL and BH

The zero-order (D0) absorption spectra of SL and BH (10µg/mL for each) solutions were recorded against water as a blank over a range of 200–400 nm.

Construction of calibration curves

Aliquots equivalent to 40–400 µg/ml SL and 10–100 µg/ml BH are accurately transferred from their standard working solutions (100µg/ml) into two separate series of 10-ml volumetric flasks then completed to volume with water. The spectra of the prepared standard solutions are scanned from 200 to 400 nm and stored in the computer.

Dual wavelength method (DW)

By using the stored spectra of SL and BH, The difference in the absorbance for Salbutamol sulfate and bromhexine hydrochloride was measured at (226 and 247 nm), (207-224 nm), respectively.

Ratio difference method

For the determination of SL in presence of BH : the absorption spectra of salbutamol sulfate divided by the spectrum of bromhexine hydrochloride solution (7µg/ml). The difference in the peak amplitudes (ΔP) at the ratio spectra was measured at 274 and 283 nm (ΔP 274-283 nm).

For the determination of SL in presence of BH: the absorption spectra of bromhexine divided by the spectrum of salbutamol sulfate solution (20µg/ml). The difference in the peak amplitudes (ΔP) at the ratio spectra was measured at 244 and 255 nm (ΔP 244-255 nm).

Ratio derivative method (^{1}DD).

For the determination of SL in presence of BH: The stored spectra of SL are divided by the spectrum of 7µg/ml BH, then the first derivative of the ratio spectra (^{1}DD) with Δ=2 nm is obtained. The amplitude of the first derivative peak of (SL/BH) is measured at 224.0 nm. A calibration graph relating the peak amplitude at 224.0 nm to the corresponding concentrations in µg/ml of SL is constructed.

For the determination of BH in presence of SL, the stored spectra of BH are divided by the spectrum of 20 µg/ml SL, then the first derivative of the ratio spectra (^{1}DD) with Δ=2 nm is obtained. The amplitude of the first derivative peak of (BH/SL) is measured at 240.0 nm. A calibration graph relating the peak amplitude at 240.0 nm to the corresponding concentrations in µg/ml of BH is constructed.

For mean centering. The scanned spectra of SL are divided by the spectrum of 7µg/ml BH and the obtained ratio spectra are mean centered. The same is applied to BH spectra as they are divided by the spectrum of 20 µg /ml SL and are then mean centered. The calibration curves for both SL and BH are constructed by plotting the mean centered values at 278.0 nm and 244.0 nm for SL and BH, respectively, versus the corresponding concentration.

Analysis of SL and BH in mucovent® tablets by the proposed methods

Ten tablets of Mucovent® formulation were accurately weighed and finely powdered. An amount of the powder equivalent to one tablet was weighed, dissolved and Shacked for a while, then filtered through Whatman filter paper No.41 The filtered solutions transferred into 10 mL measuring flasks, Further dilution of the filtered solutions was prepared to obtain different concentrations. The spectra of these solutions were scanned from 200 to 400 nm, stored in the computer and analyzed by the proposed methods.

RESULTS AND DISCUSSION

SL is co-formulated with BH in Mucovent® tablets. It has wide application in the treatment of respiratory tract disorders. The aim of this work is to develop simple and accurate methods for the simultaneous determination of SL and BH in tablets. Molecular absorption spectroscopy has been extensively used for the determination of drugs in pharmaceutical preparations with a view to the development of analytical methods. The use of this technique for pharmaceutical analysis has the inherent constraint

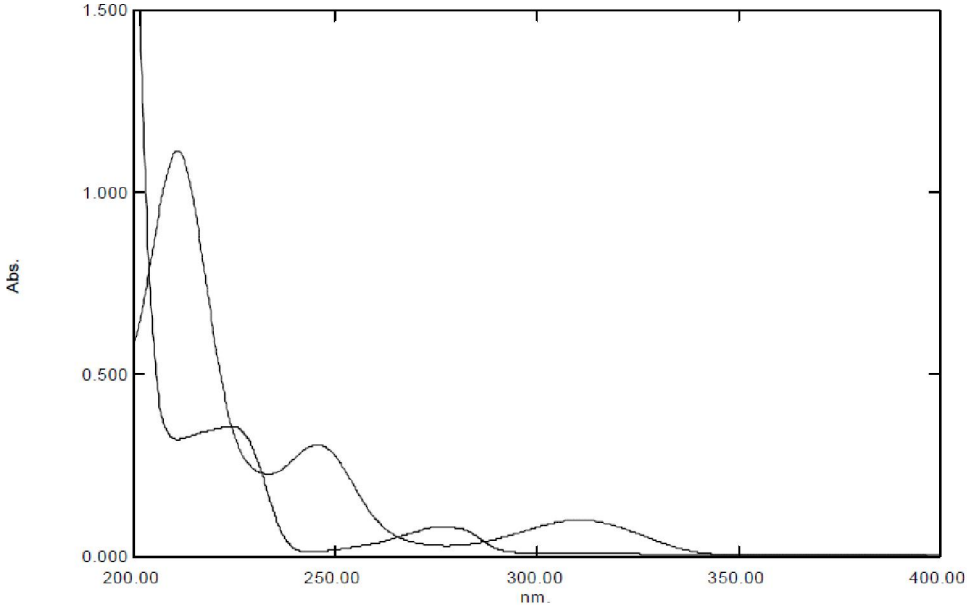

Figure 2 : Zero order absorption spectrum of 10µg/mL SL () and 10 µg/mL BH (——) using water as blank

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that most active drugs absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination. The absorption spectra of the two compounds, SL and BH show highly overlapped spectral band in the region 225–350 nm as shown in Figure 2.

Dual wavelength method[12]

Dual wavelength spectroscopy offers an efficient method for analyzing a component in presence of an interfering component. For elimination of interference, dual analytical wavelengths were selected in a way to make the absorbance difference zero for one drug in order to analyze the other drug as shown in Figure (2). In this method:

The interference from bromhexine hydrochloride can be removed by measuring the difference in absorbance at 226 and 247 nm. This difference is zero for bromhexine hydrochloride, while it is directly proportional to the concentration of Salbutamol sulfate.

The interference from Salbutamol sulfate can be removed by measuring the difference in absorbance at 207 and 224 nm. This difference is zero for Salbutamol sulfate, while it is directly proportional to the concentration of bromhexine hydrochloride.

Ratio difference method[13]

In this method, the absorption spectra of the drug were divided by a suitable absorption spectrum of the other drug (divisor) to get the ratio spectra. The difference in peak amplitudes between two selected

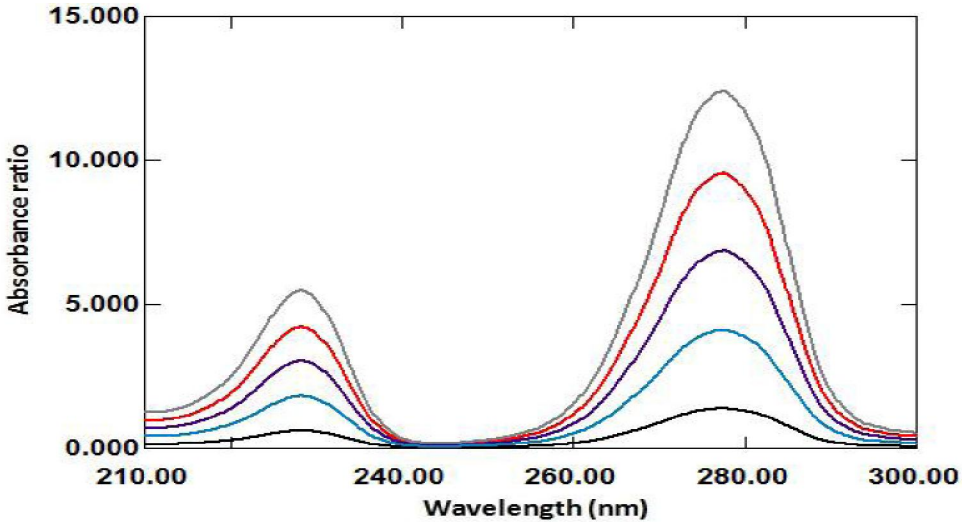

Figure 3 : Ratio spectra of salbutamol at various concentrations (4, 12, 20, 28 and 36 µg/ml) using 7µg/ml of bromhexine as a divisor

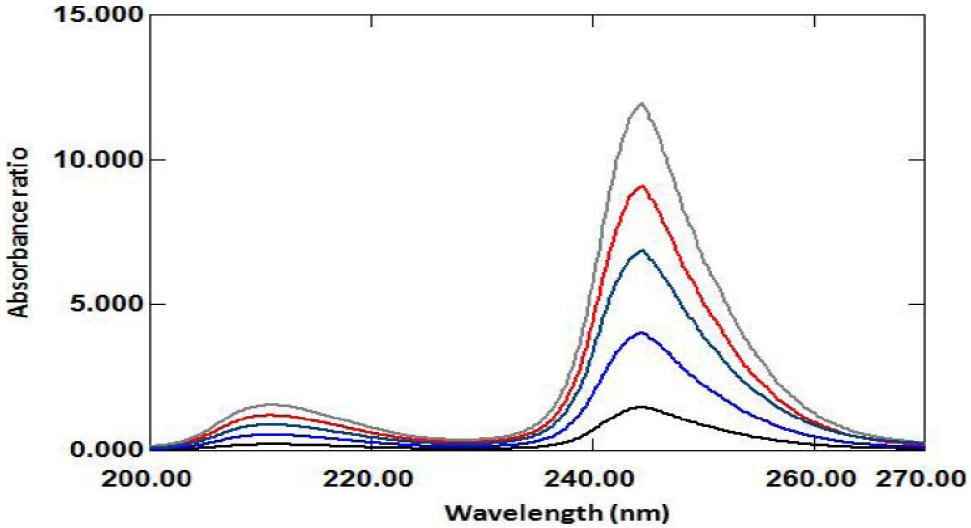

Figure 4 : Ratio spectra of bromhexine at various concentrations (1, 3, 5, 7 and 9 µg/ml) using 20µg/ml of salbutamol as a divisor

wavelengths in the ratio spectra is proportional to the concentration of the drug without interference from its divisor (Figure 3). The method comprises two critical steps, the first is the choice of the divisor. The selected divisor should compromise between minimal noise and maximum sensitivity. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and good linearity is present at each wavelength individually. In this method:

The absorption spectra of Salbutamol were divided by the absorption spectrum of bromhexine hydrochloride (7 µg /ml) as a divisor to get the ratio spectra, as shown in Figure (3). The difference in peak amplitudes between 274 and 283nm in the ratio spectra is proportional to the concentration of Salbutamol without interference from bromhexine.

The absorption spectra of Bromhexine were divided by the absorption spectrum of salbutamol sulfate (20 µg /ml) as a divisor to get the ratio spectra, as shown in Figure (4). The difference in peak amplitudes between 244 and 255 nm in the ratio spectra is proportional to the concentration of bromhexine without interference from salbutamol.

Ratio derivative method: (^{1}DD) [14]

Salinas et al. designed a spectrophotometric method, which is based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum)[15–17]. Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interfere the assay. In this method the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph. In this method,

The absorption spectra of Salbutamol were divided by the absorption spectrum of bromhexine hydrochloride (7µg /ml) as a divisor to get the ratio spectra, as shown in Figure (3). The amplitudes of the first derivative of the ratio spectra at 224 nm are proportional to the concentrations of Salbutamol without interference from bromhexine, as shown in

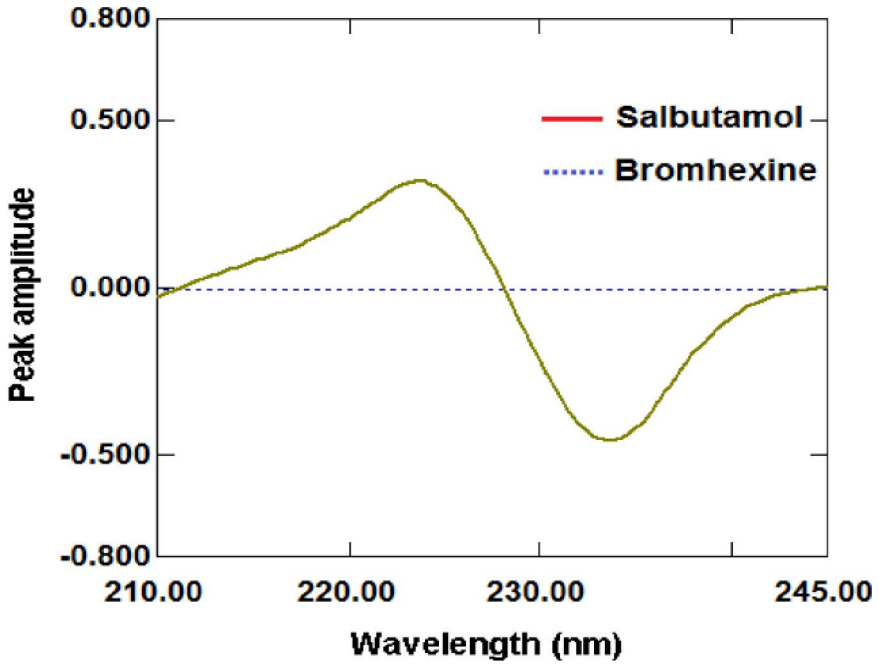

Figure 5 : First derivative of the ratio spectra of salbutamol (24µg/ml) and Bromhexine (24µg/ml) using 7 µg/ml of bromhexine as a divisor

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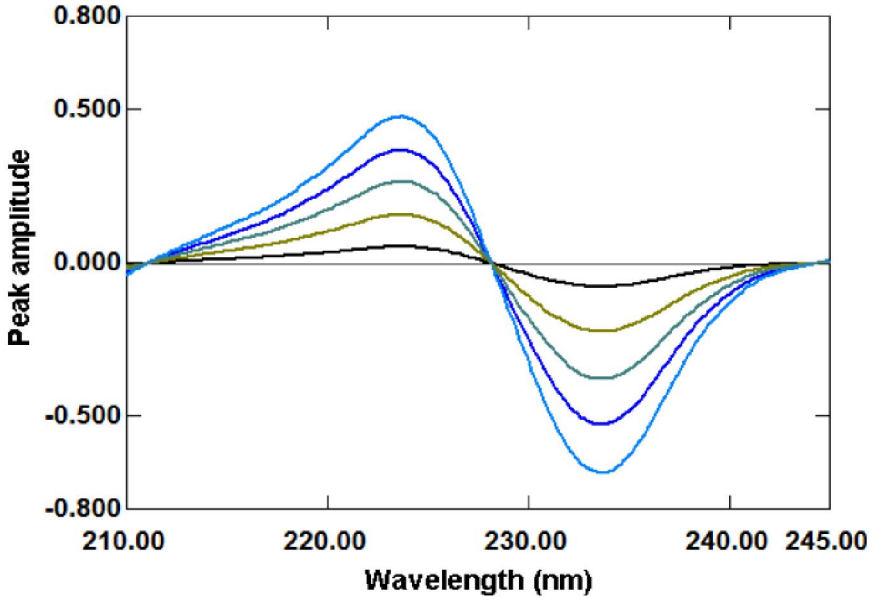

Figure 6 : First derivative of the ratio spectra of salbutamol at various concentrations (4, 12, 20, 28, and 36 µg/ml) using 7 µg/ml of bromhexine as a divisor

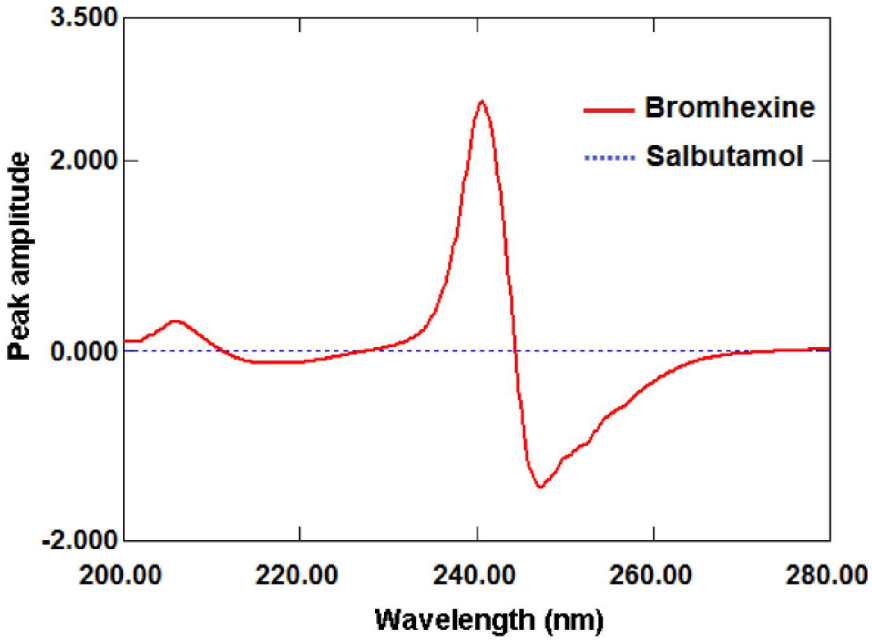

Figure 7 : First derivative of the ratio spectra of bromhexine (10µg/ml) and salbutamol (10µg/ml) using 20µg/ml of salbutamol as a divisor

Figures (5, 6).

The absorption spectra of bromhexine were divided by the absorption spectrum of salbutamol sulfate (20µg /ml) as a divisor to get the ratio spectra, as shown in Figure (4). The amplitudes of the first derivative of the ratio spectra at 240 nm are proportional to the concentrations of bromhexine without interference from salbutamol, as shown in Figures (7, 8).

Mean centering method [18]

For further improvement of the selectivity to resolve the overlap present between SL and BH, a simple method is applied; this method is based on the mean centering of ratio spectra. It eliminates the derivative step and therefore the signal-to-noise ratio is enhanced. In this method,

The absorption spectra of Salbutamol were divided by the absorption spectrum of bromhexine hydrochloride (7 µg /ml) as a divisor to get the ratio spectra as shown in Figure (3). The obtained ratio spectra (200-300 nm) were mean centered. The mean

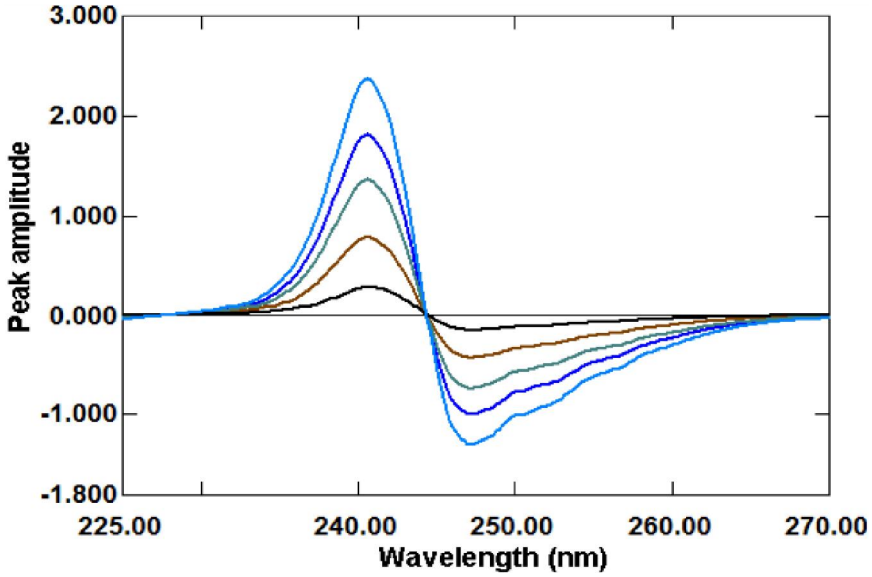

Figure 8 : First derivative of the ratio spectra of bromhexine at various concentrations (1, 3, 5, 7, and 9 µg/ml) using 20 µg/ml of salbutamol as a divisor

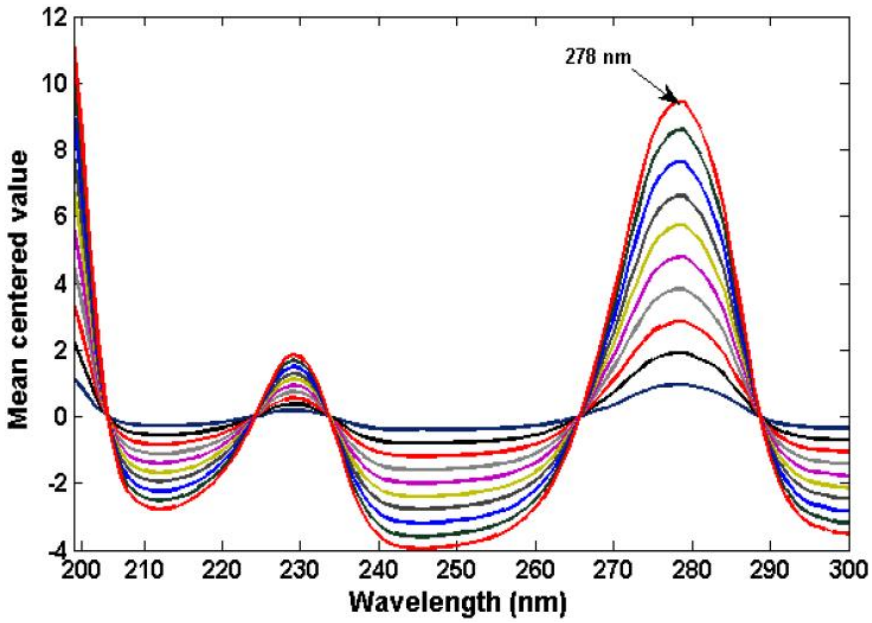

Figure 9 : Mean centering of the ratio spectra of Salbutamol at various concentrations (4, 8, 12, 16, 20, 24, 36 and 40 µg/ml) using 40 µg/ml of bromhexine hydrochloride as a divisor

centered values at 278 nm are proportional to the concentrations of Salbutamol without interference from bromhexine, as shown in Figure (9).

The absorption spectra of bromhexine were divided by the absorption spectrum of salbutamol sulfate (20 µg /ml) as a divisor to get the ratio spectra as shown in Figure (4). The obtained ratio spectra (200-270 nm) were mean centered. The mean centered values at 244 nm are proportional to the concentrations of bromhexine without interference from salbutamol, as shown in Figure (10).

VALIDATION OF THE METHODS

Linearity and rang

Dual wavelength method

The regression plot was found to be linear over the range of 4-40 µg/ml for Salbutamol sulfate and 1-10 µg/ml for bromhexine hydrochloride. The linear regression equations for the graphs are:

$$y_{Salb.} = 0.0282\ x_{Salb.} + 0.0015\ (r^2 = 0.9999)$$

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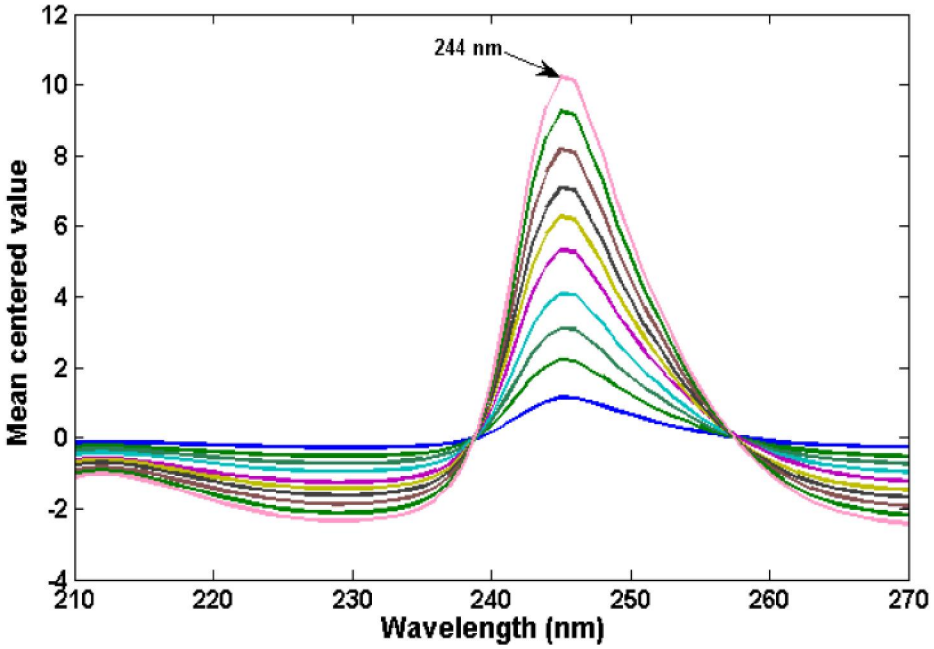

Figure 10 : Mean centering of the ratio spectra of Salbutamol at various concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml) using 20 µg/ml of salbutamol as a divisor

TABLE 1 : Spectral data for determination of Salbutamol sulfate and bromhexine hydrochloride by the proposed dual wavelength procedure

Parameters	Proposed method	
	Salbutamol sulfate	Bromhexine hydrochloride
Wavelength (nm)	226 and 247	207 and 224
Linearity range (µg/ml)	4- 40	1-10
LOD (µg/ml)	0.304	0.216
LOQ (µg/ml)	0.922	0.656
- Regression Equation	$y_{salb.} = b\ x_{salb.} + a$	$y_{brom.} = b\ x_{brom.} + a$
- Slope (b) ± S.D	0.0282 ± 0.0003	0.0607 ± 0.0008
- Intercept (a) ± S.D	+0.0015 ± 0.002	+0.0055 ± 0.003
Correlation coefficient (r^2)	0.9999	0.9998

$y_{salb.}$ and y_{brom} is the difference in absorbance at (226 and 247) nm,(207 and 224), $x_{salb.}$ and $x_{brom.}$ are concentration in mg/ml of Salbutamol sulfate and bromhexine hydrochloride respectively.

TABLE 2 : Spectral data for determination of Salbutamol sulfate and bromhexine hydrochloride by the proposed ratio difference procedure

Parameters	Proposed method	
	Salbutamol sulfate	Bromhexine hydrochloride
Wavelength (nm)	274 and 283	244 and 255
Linearity range (µg/ml)	4- 40	1-10
LOD (µg/ml)	0.505	0.143
LOQ (µg/ml)	1.530	0.434
- Regression Equation	ysalb.= b xsalb.+ a	ybrom.= b xbrom.+ a
- Slope (b) ± S.D	0.0542 ± 0.0009	0.9402 ± 0.011
- Intercept (a) ± S.D	+0.0053 ± 0.008	+0.0277 ± 0.040
Correlation coefficient (r^2)	0.9999	0.9998

$y_{salb.}$ and y_{brom} are the difference in peak amplitudes (ΔP274 -283 nm) and (ΔP244-255 nm) for Salbutamol sulfate and bromhexine hydrochloride respectively, x_{salb}. and x_{brom}. are concentration in µg/ml of Salbutamol sulfate and bromhexine hydrochloride respectively

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TABLE 3 : Spectral data for determination of salbutamol sulfate and bromhexine hydrochloride by the proposed ratio derivative procedure

Parameters	Proposed method	
	Salbutamol sulfate	Bromhexine hydrochloride
Wavelength (nm)	224	240
Linearity range (µg/ml)	4 - 40	1-10
LOD (µg/ml)	0.388	0.103
LOQ (µg/ml)	1.175	0.313
- Regression Equation	ysalb.= b xsalb.+ a	ybrom.= b xbrom.+ a
- Slope (b) ± S.D	0.0130± 0.0002	0.2060 ± 0.001
- Intercept (a) ± S.D	+0.0007± 0.001	+0.0413 ± 0.006
Correlation coefficient (r^2)	0.9999	0.9997

$y_{salb.}$ and y_{brom} are the peak amplitudes at 224 nm and 240 nm for Salbutamol sulfate and bromhexine hydrochloride respectively, $x_{salb.}$ and $x_{brom.}$ are concentration in mg/ml of Salbutamol sulfate and bromhexine hydrochloride respectively

TABLE 4 : Spectral data for determination of Salbutamol sulfate and bromhexine hydrochloride by the proposed mean centering procedure

Parameters	Proposed method	
	Salbutamol sulfate	Bromhexine hydrochloride
Wavelength (nm)	274	244
Linearity range (µg/ml)	4- 40	1-10
LOD (µg/ml)	0.383	0.195
LOQ (µg/ml)	1.161	0.592
- Regression Equation	ysalb.= b xsalb.+ a	ybrom.= b xbrom.+ a
- Slope (b) ± S.D	0.2371 ± 0.030	1.0034 ± 0.007
- Intercept (a) ± S.D	+0.0132 ± 0.027	+0.0921 ± 0.059
Correlation coefficient (r^2)	0.9999	0.9998

$y_{salb.}$ and y_{brom} are the mean centered value at 274 nm and 244 nm for Salbutamol sulfate and bromhexine hydrochloride respectively, $x_{salb.}$ and x_{brom}. are concentration in mg/ml of Salbutamol sulfate and bromhexine hydrochloride respectively

TABLE 5 : Intra-day and inter-day accuracy and precision for determination of Salbutamol sulfate by the proposed dual wavelength procedure

Drug	Conc. (µg/ml)	Intra-day			Inter-day		
		Found Conc.*± SD	Accuracy (R %)	Precision (RSD %)	Found Conc.* ± SD	Accuracy (R %)	Precision (RSD %)
Salbutamol	12	11.99 ±0.054	99.93	0.452	11.98 ±0.054	99.83	0.452
	16	16.06 ±0.089	100.36	0.556	16.11±0.054	100.66	0.336
	20	20.12 ±0.071	100.62	0.352	20.18 ±0.134	100.92	0.665
Bromhexine	4	3.96 ±0.012	99.11	0.315	3.95 ±0.008	98.65	0.214
	6	6.02 ±0.034	100.27	0.570	5.99 ±0.053	99.90	0.884
	8	8.02 ±0.053	100.26	0.661	8.00 ±0.005	100.05	0.063

*** Average of three determinations**

$y_{brom.}$ = 0.0607 $x_{brom.}$ + 0.0055 (r^2 = 0.9998)

Where ($y_{Salb.}$, $y_{brom.}$) is the difference in absorbance at (226 and 247 nm) & (207 and 224 nm) respectively, while($x_{Salb.}$, x_{brom}) are the concentration in µg/ml for Salbutamol sulfate and bromhexine hydrochloride respectively, and r^2 is the squared correlation coefficient for each corresponding drug.

Linearity range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in TABLE 1.

Ratio derivative method: (^{1}DD)

The regression plot was found to be linear over the range of 4-40 µg/ml for salbutamol and 1-10 µg/ml for bromhexine. The linear regression equations for the graphs are:

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TABLE 6 : Intra-day and inter-day accuracy and precision for determination of Salbutamol sulfate by the proposed ratio difference procedure

Drug	Conc. (µg/ml)	Intra-day			Inter-day		
		Found Conc.*± SD	Accuracy (R %)	Precision (RSD %)	Found Conc.* ± SD	Accuracy (R %)	Precision (RSD %)
Salbutamol	12	11.94 ±0.075	99.48	0.625	11.91 ±0.075	99.23	0.626
	16	16.02 ±0.113	100.10	0.704	16.04±0.085	100.25	0.531
	20	20.04 ±0.196	100.19	0.976	20.09±0.085	100.43	0.421
Bromhexine	4	3.99 ±0.010	99.86	0.252	4.01 ±0.016	100.33	0.405
	6	5.94 ±0.051	99.04	0.853	5.97 ±0.048	99.43	0.803
	8	7.96 ±0.033	99.45	0.417	7.95 ±0.030	99.38	0.372

*** Average of three determinations.**

TABLE 7 : Intra-day and inter-day accuracy and precision for determination of Salbutamol sulfate by the proposed ratio derivative procedure

Drug	Conc. (µg/ml)	Intra-day			Inter-day		
		Found Conc.*± SD	Accuracy (R %)	Precision (RSD %)	Found Conc.* ± SD	Accuracy (R %)	Precision (RSD %)
Salbutamol	12	12.00 ±0.089	99.98	0.740	12.00 ±0.089	99.98	0.740
	16	16.13 ±0.118	100.79	0.729	16.15±0.089	100.95	0.550
	20	20.18 ±0.154	100.88	0.762	20.20±0.118	101.01	0.582
Bromhexine	4	3.99 ±0.010	99.74	0.255	3.98 ±0.005	99.38	0.136
	6	5.97 ±0.040	99.49	0.666	5.98 ±0.050	99.62	0.835
	8	7.97 ±0.029	99.60	0.364	7.98 ±0.052	99.77	0.656

*** Average of three determinations.**

TABLE 8 : Intra-day and inter-day accuracy and precision for determination of Salbutamol sulfate by the proposed mean centering procedure

Drug	Conc. (µg/ml)	Intra-day			Inter-day		
		Found Conc.*± SD	Accuracy (R %)	Precision (RSD %)	Found Conc.* ± SD	Accuracy (R %)	Precision (RSD %)
Salbutamol	12	11.98 ±0.069	99.86	0.579	11.96 ±0.076	99.65	0.634
	16	16.07 ±0.115	100.45	0.713	16.09±0.077	100.56	0.480
	20	20.13 ±0.121	100.66	0.599	20.14±0.084	100.72	0.419
Bromhexine	4	3.96 ±0.020	99.10	0.503	3.97 ±0.006	99.18	0.144
	6	5.98 ±0.042	99.68	0.695	5.97 ±0.053	99.48	0.886
	8	8.00 ±0.005	99.97	0.062	8.03 ±0.032	100.36	0.396

*** Average of three determinations.**

$y_{salb} = 0.0130\ x_{salb} + 0.0007\ (r^2 = 0.9999)$

$y_{brom} = 0.2060\ x_{brom} + 0.0413\ (r^2 = 0.9997)$

Where ($y_{Salb.}$, $y_{brom.}$) are the amplitude of the first derivative of the ratio spectra of salbutamol and bromhexine at 224 nm and 240 nm respectively, (x_{Salb}., x_{brom}) are the concentration in µg/ml for Salbutamol sulfate and bromhexine hydrochloride respectively and r^2 is the squared correlation coefficient.

Linearity range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in TABLE 2.

Ratio difference method

The regression plot was found to be linear over the range of 4-40 µg/ml for salbutamol and 1-10 µg/ml for bromhexine. The linear regression equations

TABLE 9 : Determination of Salbutamol sulfate and Bromhexine hydrochloride in synthetic laboratory mixtures by the proposed dual wavelength procedure

Determination of Salbutamol				Determination of Bromhexine			
Salbutamol (µg/ml)	Bromhexine (µg/ml)	Salbutamol found (µg/ml)	Recovery % of Salbutamol	Bromhexine (µg/ml)	Salbutamol (µg/ml)	Bromhexine found (µg/ml)	Recovery % of Bromhexine
4	16	3.99	99.73	2	0.5	1.99	99.26
8	32	8.03	100.40	4	1	4.03	100.70
12	48	12.00	100.03	6	1.5	5.97	99.53
16	64	16.05	100.29	8	2	8.07	100.82
20	80	20.09	100.44	10	2.5	9.90	99.01
Mean			100.18				99.87
RSD%			0.295				0.841

TABLE 10 : Determination of salbutamol sulfate and bromhexine hydrochloride in synthetic laboratory mixtures by the proposed ratio difference procedure

Determination of Salbutamol				Determination of Bromhexine			
Salbutamol (µg/ml)	Bromhexine (µg/ml)	Salbutamol found (µg/ml)	Recovery % of Salbutamol	Bromhexine (µg/ml)	Salbutamol (µg/ml)	Bromhexine found (µg/ml)	Recovery % of Bromhexine
4	16	3.96	99.03	2	0.5	2.02	100.78
8	32	8.04	100.48	4	1	3.96	99.06
12	48	12.10	100.81	6	1.5	6.05	100.79
16	64	16.18	101.10	8	2	8.06	100.71
20	80	20.23	101.17	10	2.5	9.99	99.90
Mean			100.52				100.25
RSD%			0.870				0.762

TABLE 11 : Determination of salbutamol sulfate and bromhexine hydrochloride in synthetic laboratory mixtures by the proposed ratio derivative procedure

Determination of Salbutamol				Determination of Bromhexine			
Salbutamol (µg/ml)	Bromhexine (µg/ml)	Salbutamol found (µg/ml)	Recovery % of Salbutamol	Bromhexine (µg/ml)	Salbutamol (µg/ml)	Bromhexine found (µg/ml)	Recovery % of Bromhexine
4	16	4.02	100.58	2	0.5	1.98	98.96
8	32	8.10	101.25	4	1	4.03	100.69
12	48	12.10	100.83	6	1.5	5.97	99.49
16	64	16.18	101.11	8	2	7.93	99.13
20	80	20.25	101.27	10	2.5	10.06	100.62
Mean			101.01				99.78
RSD%			0.294				0.825

for the graphs are:

$y_{salb} = 0.0542\ x_{salb} + 0.0053$ ($r^2 = 0.9999$)

$y_{brom} = 0.9402\ x_{brom} + 0.0277$ ($r^2 = 0.9998$)

Where ($y_{Salb.}$, $y_{brom.}$) are the difference in peak amplitudes between the two selected wavelengths in the ratio spectra of salbutamol and bromhexine (ΔP274 -283 nm) and (ΔP244-255 nm) respectively, ($x_{Salb.}$, x_{brom}) are the concentration in µg/ml for Salbutamol sulfate and bromhexine hydrochloride respectively and r^2 is the squared correlation coefficient.

Linearity range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in TABLE 3.

Mean centering method

The regression plot was found to be linear over

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TABLE 12 : Determination of salbutamol sulfate and bromhexine hydrochloride in synthetic laboratory mixtures by the proposed mean centering procedure

Determination of Salbutamol				Determination of Bromhexine			
Salbutamol (µg/ml)	Bromhexine (µg/ml)	Salbutamol found (µg/ml)	Recovery % of Salbutamol	Bromhexine (µg/ml)	Salbutamol (µg/ml)	Bromhexine found (µg/ml)	Recovery % of Bromhexine
4	16	4.03	100.86	2	0.5	2.00	99.83
8	32	8.12	101.56	4	1	3.95	98.85
12	48	12.21	101.76	6	1.5	5.99	99.78
16	64	16.30	101.88	8	2	8.05	100.63
20	80	20.39	101.95	10	2.5	10.00	99.98
Mean			101.60				99.81
RSD%			0.432				0.639

TABLE 13 : Application of standard addition technique for analysis of Mucovent® tablet by applying the proposed dual wavelength methods

Mucovent® tablets							
Salbutamol sulfate				Bromhexine hydrochloride			
Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %	Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %
	16	16.17	101.04		6	5.99	99.86
8	24	24.15	100.61	4	8	7.94	99.20
	32	31.80	99.39		10	9.98	99.77
Mean			100.35				99.61
RSD%			0.853				0.362

TABLE 14 : Application of standard addition technique for analysis of Mucovent® tablet by applying the proposed ratio difference methods

Mucovent® tablets							
Salbutamol sulfate				Bromhexine hydrochloride			
Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %	Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %
	16	16.09	100.57		6	5.99	99.75
8	24	24.10	100.41	4	8	8.03	100.44
	32	31.70	99.06		10	9.96	99.63
Mean			100.01				99.94
RSD%			0.827				0.434

the range of 4-40 µg/ml for salbutamol and 1-10 µg/ml for bromhexine. The linear regression equations for the graphs are:

$y_{salb} = 0.2371\ x_{salb} + 0.0132\ (r^2 = 0.9999)$

$y_{brom} = 1.0034\ x_{brom} + 0.0921\ (r^2 = 0.9998)$

Where ($y_{Salb.}$, $y_{brom.}$) are the mean centered value of the ratio spectra at 278 nm and 244 nm respectively, ($x_{Salb.}$, x_{brom}) are the concentration in ìg/ml for Salbutamol sulfate and bromhexine hydrochloride respectively, r^2 is the squared correlation coefficient.

Linearity range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in TABLE 4.

Limit of detection and quantitation

For determination of Salbutamol sulfate and bromhexine hydrochloride, LOD and LOQ were calculated according to ICH guidelines from the following equations:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

TABLE 15 : Application of standard addition technique for analysis of Mucovent® tablet by applying the proposed ratio derivative methods

Mucovent® tablets							
Salbutamol sulfate				Bromhexine hydrochloride			
Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %	Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %
	16	16.18	101.11		6	6.00	99.98
8	24	24.25	101.06	4	8	7.99	99.92
	32	31.87	99.59		10	9.87	98.72
Mean			100.58				99.54
RSD%			0.856				0.711

TABLE 16 : Application of standard addition technique for analysis of Mucovent® tablet by applying the proposed mean centering methods

Mucovent® tablets							
Salbutamol sulfate				Bromhexine hydrochloride			
Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %	Taken (µg/ml)	Pure added (µg/ml)	Pure found (µg/ml)	Recovery %
	16	16.14	100.90		6	5.96	99.31
8	24	24.15	100.64	4	8	8.05	100.65
	32	31.76	99.26		10	9.97	99.73
Mean			100.27				99.90
RSD%			0.878				0.683

TABLE 17 : Determination of salbutamol sulfate and bromhexine hydrochloride in mucovent® tablet by the proposed dual wavelength and reported methods

Parameters	Proposed method		Reported method[5]	
	Salbutamol	Bromhexine	Salbutamol	Bromhexine
N*	5	5	5	5
$X^{?}$**	99.09	99.11	100.01	99.49
SD	0.511	0.668	1.383	0.690
RSD%	0.515	0.674	1.383	0.694
t***	1.97 (2.31)	1.11 (2.31)	____	____
F***	2.35 (6.39)	1.07 (6.39)	____	____

*** Number of experiments, ** The mean of percent recovery of pharmaceutical preparation, *** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)**

Accuracy and precision

According to the ICH guidelines,[19,20] three replicate determinations of three different concentrations of the studied drugs in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. Accuracy as recovery percent (R %) and precision as percentage relative standard deviation (RSD %) were calculated and results are listed in TABLES (5, 6, 7 and 8).

Specificity

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the studied drugs. The results are listed in TABLES (9, 10, 11 and12).

Recovery study using standard addition technique

Recovery study was performed by adopting standard addition technique, different concentration (16, 24 and 32 µg / ml) of standard salbutamol sulfate and (6, 8, 10 µg / ml) of standard bromhexine hydrochloride solution were added to already analyzed pharmaceutical preparation, results are presented in

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TABLE 18 : Determination of salbutamol sulfate and bromhexine hydrochloride in mucovent® tablet by the proposed ratio difference and reported methods

Parameters	Proposed method		Reported method[5]	
	Salbutamol	Bromhexine	Salbutamol	Bromhexine
N*	5	5	5	5
X?**	99.89	99.50	100.01	99.49
SD	0.581	0.698	1.383	0.690
RSD%	0.583	0.696	1.383	0.694
t***	1.27 (2.31)	1.81 (2.31)	____	____
F***	2.55 (6.39)	1.37 (6.39)	____	____

*** Number of experiments, ** The mean of percent recovery of pharmaceutical preparation, *** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)**

TABLE 19 : Determination of salbutamol sulfate and bromhexine hydrochloride in mucovent® tablet by the proposed ratio derivative and reported methods

Parameters	Proposed method		Reported method[5]	
	Salbutamol	Bromhexine	Salbutamol	Bromhexine
N*	5	5	5	5
X?**	100.03	100.05	100.01	99.49
SD	0.411	0.448	1.383	0.690
RSD%	0.425	0.447	1.383	0.694
t***	0.658 (2.31)	0.587 (2.31)	____	____
F***	2.25 (6.39)	1.17 (6.39)	____	____

*** Number of experiments, ** The mean of percent recovery of pharmaceutical preparation, *** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)**

TABLE 20 : Determination of salbutamol sulfate and bromhexine hydrochloride in mucovent® tablet by the proposed mean centering and reported methods

Parameters	Proposed method		Reported method[5]	
	Salbutamol	Bromhexine	Salbutamol	Bromhexine
N*	5	5	5	5
X?**	100.07	99.61	100.01	99.49
SD	0.521	0.625	1.383	0.690
RSD%	0.525	0.624	1.383	0.694
t***	1.87 (2.31)	1.81 (2.31)	____	____
F***	2.35 (6.39)	1.27 (6.39)	____	____

*** Number of experiments, ** The mean of percent recovery of pharmaceutical preparation, *** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)**

as shown in TABLE (13,14,15 and16).

Pharmaceutical applications

The proposed procedure was applied to the simultaneous determination of Salbutamol sulfate and bromhexine hydrochloride in Mucovent® Tablet. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method[5]. No significant differences were found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the stud-

ied drug in its pharmaceutical dosage form, as shown in TABLES (17,18,19,20).

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