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Simultaneous determination of dextromethorphan HBr and bromhexine HCl in tablets by first-derivative spectrophotometry

V. Tantishaiyakul *, C. Poeaknapo, P. Sribun, K. Sirisuppanon

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90110, Thailand

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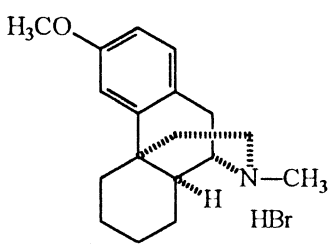
Abstract

A rapid, simple and direct assay procedure based on first-derivative spectrophotometry, using a zero-crossing and peak-to-base measurement at 234 and 324 nm, respectively, has been developed for the specific determination of dextromethorphan HBr and bromhexine HCl in tablets. Calibration graphs were linear with the correlation coefficients of 0.9999 for both analytes. The limit of detections were 0.033 and 0.103 μg ml^{-1} for dextromethorphan HBr and bromhexine HCl, respectively. A HPLC method has been developed as the reference method. The results obtained by the first-derivative spectrophotometry were in good agreement with those found by the HPLC method.

Keywords: Dextromethorphan HBr; Bromhexine HCl; First-derivative spectrophotometry; Reversed-phase chromatography

1. Introduction

Dextromethorphan HBr (**1**) is a cough suppressant which has a central action on the cough center in the medulla and bromhexine HCl (**2**) is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus [1]. The combination of these two drugs is used as antitussive and mucolytic in bronchitis and chronic pulmonary conditions.



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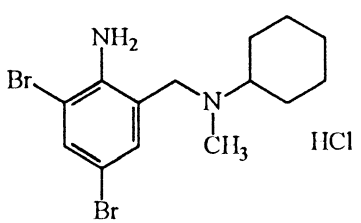

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* Corresponding author. Fax: +66 74 428239.

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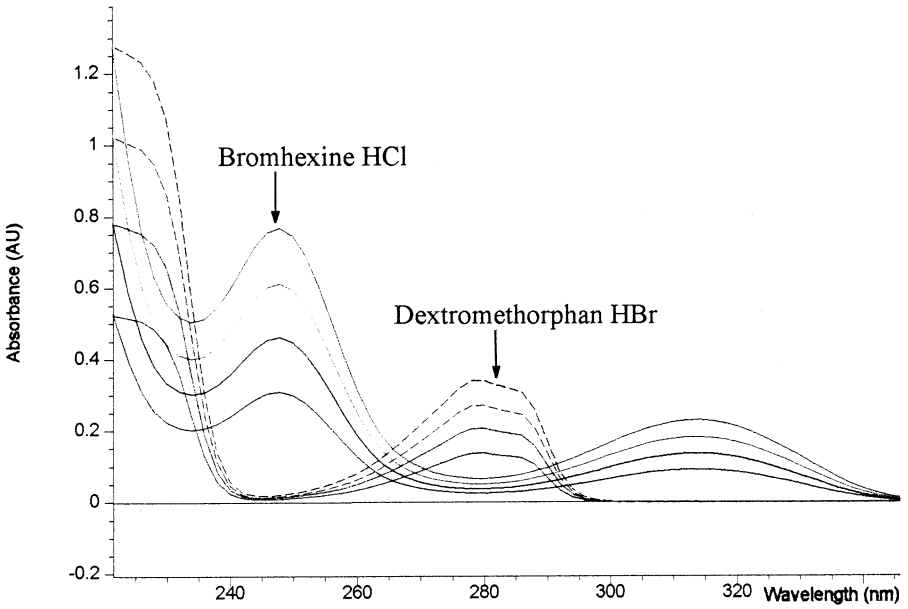

Fig. 1. Zero-order absorption spectra of: dextromethorphan HBr (24.0, 36.0, 48.0 and 60.0 μg ml^{-1}); bromhexine HCl (12.0, 18.0, 24.0 and 30.0 μg ml^{-1}) in 50% methanol in phosphate buffer (pH 6).

Official analytical methods in the British Pharmacopoeia [2] for the quantitation of bromhexine HCl or dextromethorphan HBr in the bulk drug uses potentiometric titrations with sodium hydroxide. According to the USP [3], dextromethorphan HBr in the bulk drug or in the dosage form is determined by high-performance liquid chromatography (HPLC). The determinations of bromhexine HCl or dextromethorphan HBr in compound formulations with other drugs in cough–cold products by HPLC have been reported [4–8]. Second-derivative spectrophotometry was studied for the determination of dextromethorphan HBr in the presence of guaiphenesin, however, the method was not applied successfully to the analysis of dextromethorphan in the commercial product which contains the high amount of guaiphenesin [9].

The derivative technique in UV spectrophotometry has been utilized successfully to overcome the problem of interference due to irrelevant spectral overlapping, which may be caused either by substances other than analytes or by excipient matrices commonly present in pharmaceutical formulations [10–12]. Besides, this technique offers a powerful enhancement of sensitivity and rapid determination without the requirement of extraction or separation. No spectrophotometric method or HPLC procedure has been reported for the simultaneous determination of dextromethorphan HBr and bromhexine HCl in pharmaceutical formulations or in tablets. It is desirable to develop methods of analysis for the simultaneous determination of both drugs for quality control laboratories. This work describes a simple and fast method for the determination of both drugs in tablets by first-derivative spectrophotometry.

2. Materials and methods

2.1. Materials

Dextromethorphan HBr and bromhexine HCl were obtained from Sigma (St. Louis, MO). Two brands of commercial tablets labeled to contain 15 mg of dextromethorphan HBr and 8 mg of

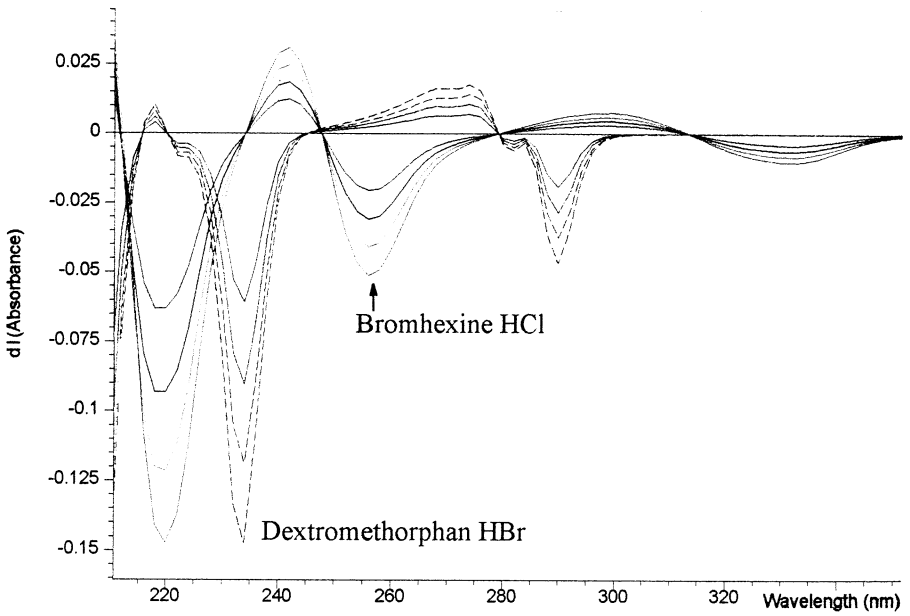

Fig. 2. First-derivative spectra of: dextromethorphan HBr (24.0, 36.0, 48.0 and 60.0 μg ml^{-1}); bromhexine HCl (12.0, 18.0, 24.0 and 30.0 μg ml^{-1}) in 50% methanol in phosphate buffer (pH 6).

bromhexine HCl were used. All other chemicals used were of analytical reagent grade.

2.2. Apparatus

Spectrophotometric analysis was performed on a Hewlett–Packard 8452A diode-array spectrophotometer, using a 1 cm quartz cell. Five data points were used for each point calculation in the derivative spectrum, and a Savitsky–Golay polynomial fitting with the polynomial degree of three were operated for the calculation of the derivative spectrum.

The high-performance liquid chromatograph was composed of a Waters 600 E pump controller, Waters 717 autosampler, Waters 486 UV detector, and Waters 746 Data Module (Waters, Milford, MA).

2.3. Chromatographic conditions

Chromatographic separation was carried out at ambient temperature on a 4 μ spherical, Nova-Pak C-8 column (3.9×150 mm i.d.) (Waters, Milford, MA). The compounds were separated isocratically with a mobile phase consisting of a mixture of methanol–KH_2PO_4 (10 mM) (55:45, v/v) with the pH of the water adjusted to 3.0 with phosphoric acid. The flow rate was 1 ml min^{-1}. The injection volume was 15 μl and the detector wavelength was set at 290 nm.

2.4. Preparation of standard solutions

The solutions of dextromethorphan HBr (600 μg ml^{-1}) and bromhexine HCl (300 μg ml^{-1}) were prepared by dissolving the compounds in methanol. For UV measurement, a 25.0 ml portion of each solution was transferred into a 50 ml volumetric flask and diluted to volume with phosphate buffer solution (pH 6), which consisted of KH_2PO_4 (0.5 M)–NaOH (0.5 M)–H_2O (12.5:1.4:36.1, v/v/v), to obtain the solutions of dextromethorphan HBr (300 μg ml^{-1}) and bromhexine HCl (150 μg ml^{-1}). The standard solutions of dextromethorphan HBr ($12.0 - 60.0$ μg ml^{-1}) and bromhexine HCl (6.0–30.0 μg ml^{-1}) were prepared by the appropriate dilution of

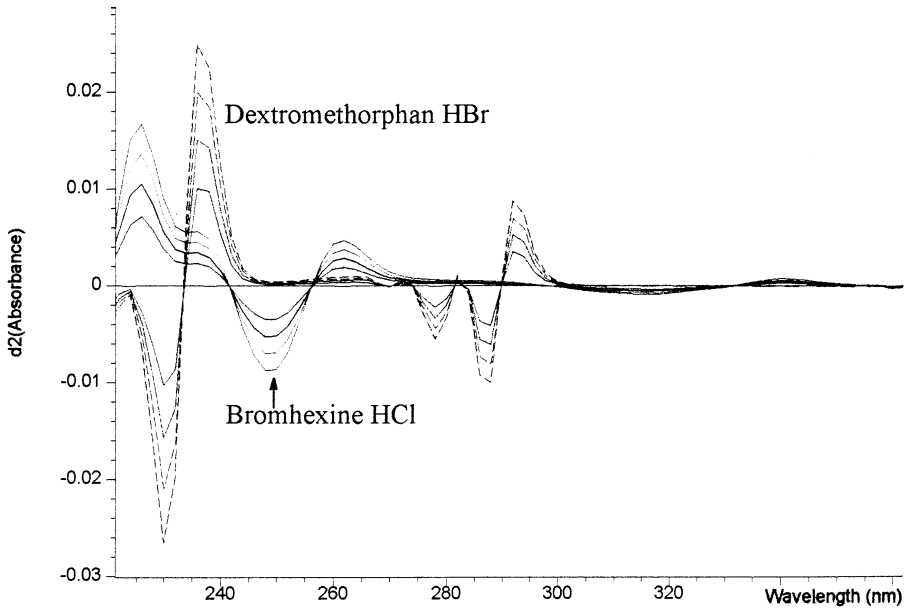

Fig. 3. Second-derivative spectra of: dextromethorphan HBr (24.0, 36.0, 48.0 and 60.0 μg ml^{-1}); bromhexine HCl (12.0, 18.0, 24.0 and 30.0 μg ml^{-1}) in 50% methanol in phosphate buffer (pH 6).

these solutions with 50% methanol in phosphate buffer solution (pH 6).

For HPLC procedure, the standard solutions of dextromethorphan HBr (24.0–120.0 μg ml^{-1}) and of bromhexien HCl (12.0–60.0 μg ml^{-1}) were prepared by appropriate dilution of the solutions of dextromethorphan HBr (600 μg ml^{-1}) and bromhexine HCl (300 μg ml^{-1}) in methanol with HPLC mobile phase.

2.5. Preparation of sample solutions

Ten tablets containing a nominal amount of 15 mg of dextromethorphan HBr and 8 mg of bromhexien HCl as active ingredients were weighed and finely powdered. A portion of the powder, equivalent to about 15 mg of bromhexine HCl, was weighed accurately and transferred into 50 ml volumetric flask and dispersed in 30 ml methanol by ultrasonic vibration for 5 min. The resulting suspension was diluted to volume with methanol and filtered. For UV measurement, 25 ml of the filtrate was transferred into a 50 ml volumetric flask and adjusted to volume with phosphate buffer solution (pH 6). A 3 ml aliquot was then diluted to 25 ml with 50% methanol in phosphate buffer solution (pH 6). For HPLC method, 3 ml of the filtrate was diluted to 25 ml with the HPLC mobile phase.

2.6. Preparation of calibration graphs

The UV spectra of working standard and sample solutions were recorded against 50% methanol in phosphate buffer solution (pH 6) as a blank. The concentrations of standard solutions versus their first-derivative (^{1}D) amplitudes at 234 (zero-crossing of bromhexine HCl) and 334 nm (peak-to-base) were plotted in order to obtain the calibration graphs.

A 15 μl aliquot of standard solutions for HPLC was injected onto the chromatographic column in triplicate. The concentrations of standard solutions versus their peak heights were plotted to obtain the calibration graph.

Table 1
Regression analysis of dextromethorphan HBr and bromhexine HCl standard solution ($n = 6$)

Sample (μg ml^{-1})[a]		Regression equation[b]	Correlation coefficient	SE[c]	
D	B			Slope	Intercept
12.0–60.0	—	$^1D_{234} = -2.40 \times 10^{-3}C - 2.30 \times 10^{-3}$	0.9999	1.02×10^{-5}	4.23×10^{-4}
12.0–60.0	6.0	$^1D_{234} = -2.39 \times 10^{-3}C - 3.06 \times 10^{-3}$	0.9996	2.86×10^{-5}	1.19×10^{-3}
—	6.0–30.0	$^1D_{334} = -3.30 \times 10^{-4}C - 2.70 \times 10^{-5}$	0.9999	4.78×10^{-7}	9.52×10^{-6}
12.0	6.0–30.0	$^1D_{334} = -3.30 \times 10^{-4}C - 1.20 \times 10^{-5}$	0.9999	8.60×10^{-7}	1.75×10^{-5}

[a] D, Dextromethorphan HBr; B, Bromhexine HCl.
[b] Amplitude of the first-derivative signal versus amount of drug in μg ml^{-1}.
[c] Standard error of slope (μg ml^{-1}) and intercept.

3. Results and discussion

Since both dextromethorphan HBr and bromhexine HCl dissolve much better in methanol than in water, methanol was used first as the solvent for spectrophtometric method. However, at the same concentration of drugs, the methanolic solutions of drugs containing tablet excipients showed a few spectral changes in the absorption spectra which gave unreliable results in the determination of the drugs. These spectral changes are reflected by the changes in pH of the solutions caused by tablet excipients. To control the pH change, the solution of 50% methanol in phosphate buffer pH 6, which consisted of KH_2PO_4 (0.5 M)–NaOH (0.5 M)–H_2O (12.5:1.4:36.1, v/v/v), was put to use and this mixture was used successfully as the diluent for spectrophotometric studies.

The zero-order absorption spectra of dextromethorphan HBr and bromhexine HCl solutions in 50% methanol in phosphate buffer (pH 6) are shown in Fig. 1. The spectra display overlapping in the region of 230–300 nm. This makes the determination of dextromethorphan HBr in the presence of bromhexine HCl by conventional UV spectrophotometry difficult, but the determination of bromhexine HCl from 315 to 325 nm might be possible without the interference from dextromethorphan HBr. The derivative spectrophtometry technique was, however, chosen for the determination of both drugs since it could remove broad-band contributions from excipients and might also overcome the interference from peak overlappings.

The selections of the suitable order of the derivative and optimum wavelengths are based on the fact that good linearity of the calibration plots are obtained, and the relative errors in the analyses of drugs in the presence of the other drug and tablet excipients are as low as possible. As seen in Figs. 2 and 3, the first- and second-derivative spectra of both dextromethorphan HBr and bromhexine HCl show characteristics which permit the determination of both drugs in the presence of each other. The zero-crossing wavelength points of bromhexine HCl at 324 and 241.5 nm in the first- and second-derivative, respectively, allow the direct determination of dextromethorphan HBr in the presence of bromhexine HCl. The amplitudes at these wavelenghts exhibit good linear responses to the dextromethorphan HBr concentrations. However, for the determination of bromhexine HCl, the first-derivative is preferred as it has the better signal-to-noise ratio. The first-derivative was, therefore, used for the determination of both drugs. The results obtained reveal that the best wavelengths for the

Table 2
Effect of tablet excipients on the first-derivative spectrophotometric determination of bromhexine HCl and dextromethorphan HBr mixtures ($n = 3$)

Additive	Concentration ratio		Recovery (%)	
	Additive/D[a]	Additive/B[a]	D[a] ± SD	B[a] ± SD
Lactose	6	10	98.42 ± 0.02	97.23 ± 0.22
Corn starch	6	10	99.56 ± 0.04	97.29 ± 0.36
Mg stearate	1	2	98.57 ± 0.04	97.63 ± 0.09
Talcum	1	2	99.00 ± 0.03	97.09 ± 0.22
HPMC[b]	1	2	99.04 ± 0.03	98.84 ± 0.22
Excipient mixture	25	25	98.68 ± 0.02	98.78 ± 0.18

[a] D, Dextromethorphan HBr; B, Bromhexine HCl.
[b] Hydroxypropyl methyl cellulose.

determination of dextromethorphan HBr and bromhexine HCl are at 234 (zero-crossing) and 324 (peak-to-baseline) nm, respectively.

The linear regression equations and the statistical evaluation of the calibration plots are given in Table 1. Under the experimental conditions described above, linear correlations with high values of correlation coefficients are obtained for the concentration ranges of 12.0–60.0 and 6.0–30.0 μg ml^{-1} for dextromethorphan HBr and bromhexine HCl, respectively.

A student's t-test was performed to determine whether the experimental intercepts of the regression lines were significantly different from the theoretical zero value. The test is based on the calculation of the quantities $t = i/S_i$, where i is the intercept of the regression lines, Si is the standard deviation of i. The values calculated for t are 0.32 for dextromethrophan HBr and 0.69 for bromhexine HCl. These values do not exceed the 95% criterion of $t = 2.78$ for six samples, so the intercepts are not significantly different from zero.

According to the IUPAC [13], the detection limits (C_L) was defined as $C_L = 3S_B/m$, where m is the slope of the corresponding calibration curve, and S_B is the standard deviation of the blank. These C_L values obtained can only be a true reflection of the limit of detections when m is well-defined or the good linearity of calibration curve obtained and intercept is essentially zero. From the experiment described, the limit of detections of dextromethorphan HBr and bromhexine HCl are 0.033 and 0.103 μg ml^{-1}, respectively.

To evaluate the specificity of the method in samples containing both dextromethorphan HBr and bromhexine HCl, two sets of solutions were examined. The first set contained the standard solution of 12.0–60.0 μg ml^{-1} of dextromethorphan HBr in the presence of 6.0 μg ml^{-1} of bromhexine HCl. The second set contained the standard solutions of 6.0–30.0 μg ml^{-1} of bromhexine HCl in the presence of 12.0 μg ml^{-1} of dextromethorphan HBr. The similarity of the regression equations in the solutions of the first set and the second set to those of the pure drugs solutions, as shown in Table 1, indicates the non-interference of one drug in the absorption measurements of the other at the chosen wavelengths.

In order to examine the effect of common excipients used in tablets on the determination of dextromethorphan HBr and bromhexine HCl, the recovery experiments were carried out on the solutions containing 36.06 μg ml^{-1} of dextromethorphan HBr and 18.12 μg ml^{-1} of bromhexine HCl in the excess of tablet excipients. The solutions of both drugs in each excipient and in a mixture of tablet excipients, which contained lactose, corn starch, PVP, magensium stearate, and talcum in the typical amounts used in tablet formulation, were recorded. The recoveries obtained, as shown in Table 2, indicate that the first-derivative spectrophotometric determination does not suffer from spectral interference of the excipients.

Table 3
Results for the determination of dextromethorphan HBr and bromhexine HCl in commercial tablets by first-derivative spectroscopy and HPLC ($n = 5$)

Sample	Dextromethorphan HBr			Bromhexine HCl		
	Recovery (%)		t	Recovery (%)		t
	^{1}D	HPLC		^{1}D	HPLC	
Brand 1	98.66	99.66	1.88	98.81	99.62	1.71
Brand 2	82.13	82.63	0.88	95.21	94.01	1.91

The reversed-phase HPLC method was developed as the reference method for the first-derivative technique. The mobile phase was chosen after several trials of methanol–water in various proportions and different pH values. The change in the wavelength of detection during the run was performed to achieve maximum detector response for both drugs. The chromatographic system described allows complete base line separation with the retention times of 3.55 and 4.84 min for dextromethorphan HBr and bromhexine HCl, respectively. The linearity of the detector responses for dextromethorphan HBr and bromhexine HCl was determined for the concentration ranges of 24.0–120.0 and 12.0–60.0 μg ml^{-1} for dextromethorphan HBr and bromhexine HCl, respectively. The following linear equations were obtained through regression analysis: $H = 150.31C + 1034.5$ ($r = 0.9980$) for dextromethorphan HBr; and $H = 175.34C + 459$ ($r = 0.9998$) for bromhexine HCl, where H is peak height and C is the drug concentration.

The accuracy of the first-derivative method was performed by analyzing the commercial tablets containing a mixture of dextromethorphan HBr and bromhexine HCl. The results obtained are summarized in Table 3 and compared to those with the HPLC method. The calculated t-test did not exceed the theoretical values ($t = 2.31$) at the 95% confidence level, indicating no significant difference between the two methods and demonstrating the utility of the purposed method for the simultaneous determination of both drugs.

4. Conclusions

The described first-derivative spectrophotometry permits the direct and accurate determination of dextromethorphan HBr and bromhexine HCl mixtures in tablets without the interference from each drug or tablet excipients. The first-derivative method has a potential for application in quality control laboratories as it is simple, rapid and shows good accuracy and precision.

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