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Simple Spectrophotometric Methods for the Simultaneous Determination of Pseudoephedrine and Loratadine in Bulk and Tablet Forms

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Two simple, accurate and precise spectrophotometric methods were developed for the simultaneous determination of pseudoephedrine sulphate and loratadine in bulk and tablets. Absorbance factor and absorbance correction methods are proposed in this study. The two methods are principally exploit the spectral characteristics of the two compounds to affect mathematical manipulation of Beer's law. The approach in the two methods enabled removal of the spectral interference from loratadine at the wavelength for the determination of pseudoephedrine, hence it was possible to the determine pseudoephedrine in the presence of loratadine using two wavelengths (257 nm and 280 nm). Beer's law was obeyed in the concentration ranges of 140-860 µg/ml and 6-36 µg/ml with correlation coefficients (R^2) 0.9998 and 0.9999 for pseudoephedrine and loratadine, respectively. The accuracy and the precision of the proposed methods were very good with percent relative standard deviation (%RSD) < 2% being in agreement with International Council on Harmonization guidelines. The analysis of variance (ANOVA) test confirmed the validity of the proposed methods and their comparability with a reported method, as indicated by p-values greater than 0.05. The p-values obtained were 0.385 for loratadine and 0.692 for pseudoephedrine sulfate, demonstrating no significant difference between the methods and supporting their accuracy and reliability. The low limits of detection (LOD) of 0.6891 µg/ml for loratadine and 46.7172 µg/ml for pseudoephedrine sulfate confirm the high sensitivity of the proposed methods, making them suitable for precise quantitative analysis.

Keywords: UV Spectrophotometry, absorbance subtraction, loratadine, pseudoephedrine, tablets

INTRODUCTION

Loratadine (LOR) is a long-acting, non-sedative second-generation H1 receptor blocker utilized for relieving symptoms of allergic conditions like rhinitis and chronic urticaria. On the other hand, pseudoephedrine (PSE) acts as both a direct and indirect sympathomimetic agent, commonly administered orally to alleviate nasal congestion. Often, it is combined with other ingredients in formulations intended for cough and cold symptom relief. [1] The chemical structures of LOR an PSE are given in Figure 1.

Since the combination of LOR and PSE in tablet form is not official in any compendia, no direct spectrophotometric method has been established for their simultaneous determination. Chromatography has been employed for this purpose, with two reversed-phase HPLC methods described in literature. However, in these methods, PSE tends to elute early, sometimes with the solvent front, indicating inadequate retention.[2,3] To address this challenge, an alternative method utilizing C18 and cyanopropyl columns in series was developed, successfully resolving the two analytes and preventing early elution of PSE. [4]

However, the suitability of the method for routine analysis remains uncertain. Subsequently, a method employing a cation exchange column was introduced, offering a more practical solution to the elution issue [5,6].

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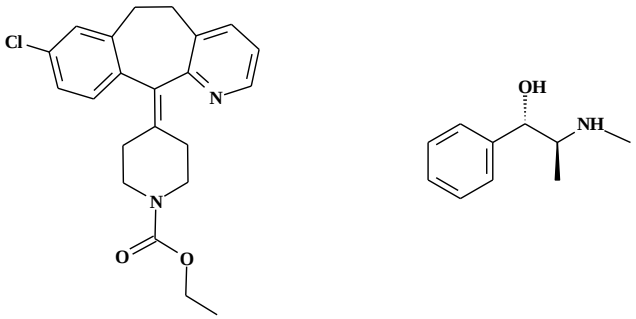

Loratadine Pseudoephedrine

Figure 1. Chemical structure of Loratadine Pseudoephedrine

High-performance thin-layer chromatography has been explored as alternative analytical technique [7]. Moreover, various spectroscopic methods have been investigated, including first derivative spectroscopy [2], chemometric analysis of spectrophotometric data [8], and examination of non-linear second-order spectrophotometric data. [9]

The aim of this study is to establish and validate straightforward spectrophotometric methods for the simultaneous determination of LOR and PSE; in both bulk and tablet formulations which are suitable for quick in process quality control and in countries where acquisition of sophisticated spectrophotometric or chromatographic equipment.

Absorbance factor spectrophotometric method[10,11] enables the analysis of a mixture containing two drugs, X and Y, even when their spectra overlap at one wavelength (λ_1) with Y extending over X, while X does not contribute significantly at another wavelength (λ_2). In this approach, λ_1, where the overlap occurs, is utilized for separate quantitative estimation of both X and Y in the mixture (X + Y). Through the utilization of mathematically calculated factors for one of the components, determination can be achieved. By following simple manipulation steps, absorbance values corresponding to X and Y can be isolated. Consequently, the concentration of each component can be determined via regression equations established at the overlapping wavelength, eliminating the necessity for a supplementary method.

The absorbance values corresponding to X and Y at λ_1 are computed using an absorbance factor, a constant for pure Y representing the average ratio between the absorbance values of different concentrations of pure Y at λ_1 to those at λ_2 (i.e., abs λ_1/abs λ_2) as shown in equations 1 and 2.

$$\text{Absorbance of Y in the mixture at } \lambda_1 = \text{abs}_{\lambda1}/\text{abs}_{\lambda2} * \text{abs}_{\lambda2}\ (X + Y) \quad \ldots\ldots\ldots\ldots\ldots(1)$$

$$\text{Absorbance of X in the mixture at } \lambda_1 = \text{abs}_{\lambda1} - (\text{abs}_{\lambda1}/\text{abs}_{\lambda2} * \text{abs}_{\lambda2}\ (X + Y)) \ldots. (2)$$

Subsequently, the corrected absorbance values at λ_1 are utilized to calculate the concentration of each analyte from its respective calibration curve.

Consequently, since at 280 nm the absorbance is due to loratadine alone (A_{LOR280}), then the concentration of LOR in the mixture can be calculated using equation 3.

$$C_{LOR} = \frac{A_{LOR280}}{a_{LOR280}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The contribution of LOR absorbance to the total absorbance (A_m) at 257 nm; can be calculated from the knowledge of concentration (C_{LOR}) and absorptivity ($a_{LOR\ 257}$), using equation 4.

$$A_{LOR\ 257} = C_{LOR} * a_{LOR257} \quad \ldots\ldots\ldots\ldots (4)$$

The absorbance due to PSE at 257 nm ($A_{PSE\ 257}$) is given by equation 5 and the concentration as depicted by equation 6.

$$A_{PSE\ 257} = A_m - A_{LOR\ 257} \quad \ldots\ldots\ldots\ldots..(5)$$

$$C_{PSE} = (A_m - A_{LOR\ 257})/\ a_{PSE257} \quad \ldots..(6)$$

EXPERIMENTAL

Chemicals and Materials

Loratadine (99.5%) bulk powder was obtained from (Blue Nile Pharmaceuticals, Khartoum North, Sudan) while pseudoephedrine sulphate (98.8%), was obtained from (SPIMACO, Al-Qassim, Kingdom of Saudi Arabia). Hydrochloric acid (AR) was purchased from Scharlau Chemie, Spain. Clarinase Tablets (Schering-Plough-Belgium) labeled to contain 5mg Loratadine and 120 mg pseudoephedrine sulphate per tablet was purchased from Saudi Arabia.

UV spectrophotometric measurements were carried out on Shimadzu UV-1800 (Kyoto, Japan) equipped with a 1-cm pair of quartz cuvettes. Data analysis was performed using Microsoft Excel Spreadsheet 2013. [12]

Preparation of solutions

Stock standard solutions

Stock solutions of loratadine (150 µg/ml) and pseudoephedrine sulfate (3600 µg/ml) were prepared by accurately weighing 15 mg of loratadine and 180 mg of pseudoephedrine sulfate into 100 ml and 50 ml volumetric flasks, respectively. The substances were dissolved in 0.1 M HCl, and the volume was adjusted to the mark with the same solvent to ensure complete dissolution.

Calibration standards

Separate linearity standards of each analyte was prepared by diluting different aliquot volumes from its corresponding stock standard solutions using 0.1 HCl to give concentration in the range of (6-36 µg/ml) and (140-860 µg/ml) for LOR and PSE, respectively.

Laboratory synthetic mixtures

Into six separate 25 ml volumetric flasks, laboratory synthetic mixtures containing different concentrations of LOR and PSE were prepared by diluting different aliquot volumes from their corresponding stock standard solutions using 0.1 N HCl as a diluent.

Sample preparation

Ten tablets were accurately weighed and finely powdered; a quantity of the powdered tablet equivalent to the average mass of one tablet was weighed and transferred into 50 ml volumetric flask, approximately 30 ml diluent were added and the mixture was sonicated for 15 minutes. The mixture was then diluted to volume with 0.1M HCl and the resulting solution was centrifuged at 30000 rpm for 15 minutes. The clear supernatant solution was filtered through 0.45µ nylon filter. Five millilitres of the filtrate were diluted to 25 ml with 0.1M HCl.

Method validation

The proposed method was validated according to the ICH guideline requirements [12] for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The absorbance values of these solutions were measured at the pre-selected wavelengths, and regression analysis data was obtained using the method of least squares. The slope, intercept, the standard deviation of the slope and intercept and standard deviation of the residual were reported.

Limit of detection and limit of quantification

The following equations were used to calculate; the limit of detection (LOD) and limit of quantification (LOQ) of the two analytes from the regression analysis data[12].

$$LOD = 3 \times Sx.y / S$$

$$LOQ = 10 \times Sx.y / S$$

where Sx.y = standard deviation of the residuals, S = slope of the calibration curve

Precision

To access the method's repeatability precision (intra-day), six independent sample solutions containing analytes at 100% concentrations were analysed within the same day. The intermediate precision (inter-day) was evaluated by repeating the same procedure on a different day using fresh reagents and samples. The results were expressed

as percentage content and relative standard deviation (%RSD).

Accuracy

The accuracy was accessed by analyzing the nine synthetic mixtures, the results were expressed as percentage content and relative standard deviation (%RSD).

RESULTS AND DISCUSSION

Spectral Characteristics

The overlain ultraviolet spectra of the (Fig. 2) shown severe overlapping of the two analytes spectral bands over the range of 225-275 nm, hence direct determination of PSE from its wavelength of maximum absorbance at 257 nm is not possible as LOR also absorbs at the same wavelength. In addition to the absorbance at 257 nm, LOR also absorbs at 280 nm showing overlapping-free spectral band, hence these two wavelengths (257 nm and 280 nm) were chosen as the analytical wavelength for the application of the proposed methods.

Linearity

Calibration curves were constructed for LOR and PSE by measuring absorbance at specified wavelengths, resulting in correlation coefficients (r^2) exceeding 0.990, indicating a strong correlation between concentration and absorbance within the concentration ranges of 6-36 µg/ml for LOR and 140-860 µg/ml for PSE see Table 1.

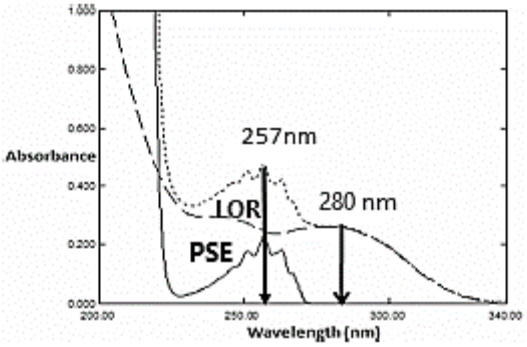

Figure 2. Over lay spectra of LOR and PSE at working concentrations in 0.1 M HCl

Table 1. Regression analysis results

Parameter	Analyte		
	LOR		PSE
wavelength (nm)	257	280	280
concentration (µ/ml)	6.12 - 36.72		144 - 864
slope (b)	0.0225	0.0236	0.00092
intercept (a)	0.0082	0.0101	0.0059
correlation coefficient (r2)	1.0000	1.0000	0.9999
standard deviation of the slope (Sb)	0.0001	0.0001	0.0000
standard deviation of the intercept (S_a)	0.0016	0.0018	0.0047
limit of detection (LOD) µg/ml	0.2110	0.2274	15.4167
limit of quantitation (LOQ) µg/ml	0.6394	0.6891	46.7172
residuals standard deviation ($S_{y.x}$)	0.0014	0.0016	0.0043

The average absorption factor of 0.947 was calculated from the calibration series; dividing the absorbance values of LOR at 256.6 nm by their corresponding ones at 280 nm. The average absorptivity values determined as the slopes of the regression lines at 257 nm were 2.25 $x10^{-2}$ and 9.24 $x10^{-4}$ ml µg $^{-1}cm^{-1}$for LOR and PSE, respectively and 2.36 $x10^{-2}$ ml µg $^{-1}cm^{-1}$ for LOR at 280 nm.

Statistical analysis of the calibration data was undertaken to determine the Limit of Detection (LOD) and Limit of Quantification (LOQ) for both substances. These values, expressed in µg/ml, denote the minimum concentrations at which LOR and PSE can be reliably detected and quantified using the proposed UV spectrophotometric methods.

Precision

The precision of the proposed method was evaluated by analyzing the six replicate samples on two different days using fresh chemicals and reagents.

For the absorbance factor method; the concentrations of the PSE and LOR in the samples were calculated using equations 1 and 2, while equations 3 to 6 were used to calculate the concentrations of the two analytes based on absorbance correction method. The percent relative standard deviations (%RSD) of the assay repeatability on two different days (repeatability) were less than 2% and the % RSD of the combined assay values (intermediate precision) from both days were less than 3.0%; confirming good precision of the proposed method. The precision of both methods was confirmed by the low %RSD values obtained. For the absorbance factor method, the %RSD was 0.87 for loratadine (LOR) and 0.89 for pseudoephedrine sulfate (PSE), while for the absorbance correction method, both analytes exhibited similarly low %RSD values, demonstrating the methods' reliability and reproducibility. [13]

Accuracy (recovery)

The accuracy of the methods was assessed by analyzing six laboratory-prepared synthetic mixtures containing varying concentrations of the two analytes. The actual content of both drugs were very close to the label claim, additionally; the two methods produced relative standard deviation (RSD) values below 2% confirming the high precision of the proposed methods. The average recoveries of loratadine (LOR) and pseudoephedrine sulfate (PSE) using the absorbance factor method were 101.23% ± 1.08% and 102.37% ± 0.96%**,** respectively. Similarly, the recoveries obtained using the absorbance correction method were 101.23% ± 1.08% for LOR and 101.18% ± 0.77% for PSE. To further validate the method, a statistical comparison was conducted between the obtained results by the proposed methods and those from a previously reported high-performance liquid chromatographic method [5]. The analysis showed that there is no significant difference between the three methods. The calculated F-values were less than the critical F-values ($p > 0.05$ at 95% confidence level); hence the developed method can be considered as accurate and precise as the reported HPLC method the determination of the two analytes. Statistical analysis results are depicted in Table 2.

CONCLUSION

The proposed methods proved to be simple, accurate and precise for the determination of loratadine and pseudoephedrine in bulk form and tablet forms. Statistical and analytical validation of the results confirmed that the proposed methods can be used for the routine analysis in quality control laboratories, for quick in-process control check and in countries where acquisition of sophisticated spectrophotometric or chromatographic equipment.

Conflict of interest: The authors do not have any conflict of interest with the commercial identities mentioned in this article.

Table 2. Statistical comparison of the proposed methods with the reported method (PSE and LOR Determination)

SUMMARY						
PSE						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Absorbance factor	6	607.15	101.1917	1.080257		
Corrected absorbance	6	603.02	100.503	1.087627		
Reported	6	607.0977	101.183	0.587364		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	1.871524485	2	0.935762	1.018887	0.385	3.68232
Within Groups	13.77623751	15	0.918416			
Total	15.64776199	17				
LOR						
Absorbance factor	6	604.99	100.8317	0.682697		
Corrected absorbance	6	604.99	100.8317	0.682697		
ANOVA						
Between Groups	0.63440104	2	0.317201	0.376687	0.692	3.68232
Within Groups	12.63120388	15	0.84208			
Total	13.26560492	17				

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