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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CHLORPHENIRAMINE MALEATE AND DEXTROMETHOPHAN HYDROBROMIDE IN PURE AND COMBINATION BY UV SPECTROSCOPY

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Keywords:

Simultaneous estimation, Chlorpheniramine maleate, Dextromethorphan hydrobromide, Spectrometric method

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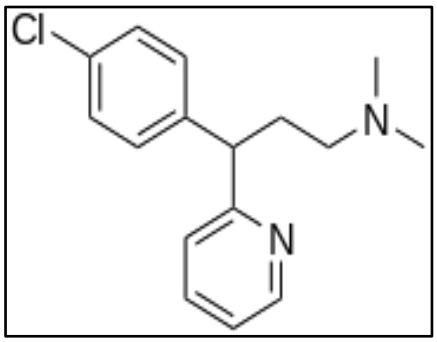
ABSTRACT

A simple, accurate, precise, rapid and economical Vierodt's method or simultaneous equation method was developed and validated for simultaneous estimation of chlorpheniramine maleate and dextromethorphan hydrobromide in pure and combined dosage form. Distilled water was used as an economical solvent and all spectroscopic parameters were optimized. The method was based on the measurement of absorbance at two wavelengths 259nm and 276nm, λmax of chlorpheniramine maleate and dextromethorphan hydrobromide. Calibration curves of chlorpheniramine maleate and dextromethorphan hydrobromide were found to be linear in the concentration range of 10-50ug/ml respectively. The method has been validated statistically and by recovery studies. The results of analysis have been validated statically.[1-10]

INTRODUCTION

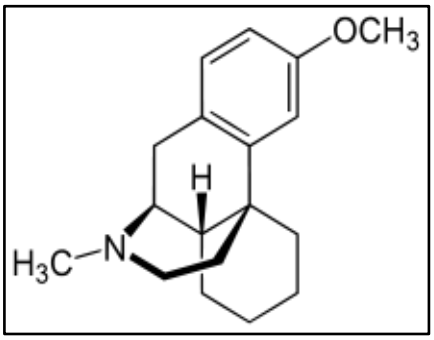
Chemically chlorpheniramine maleate (CPM) is (*RS*)-3-(4-Chlorophenyl)-3-(Pyrid-2-yl) propyldimethylamine hydrogen maleate. Chlorpheniramine maleate is alkylamine derivative with the actions and uses of the antihistamines. It is one of the most potent antihistaminic and cause a moderate degree of sedation. For estimation of chlorpheniramine maleate HPLC method have been reported. It is used as an antihistaminic in allergic reactions, prevents muscular response of histamine and thereby reducing cough receptors lining the respiratory mucous membrane for symptomatic treatment of common cold.[1-10]

Figure 1: Chlorpheniramine maleate



Chemically Dextromethorphan hydrobromide is ent-3-methoxy-9a-methylmorphinan hydrobromide monohydrate. It is a cough suppressant used for the relief of nonproductive cough. It has a central action on the cough center in the medulla. Different methods have been reported to estimate the DEX in bulk forms with other drugs in cough cold products and in biological samples which includes HPLC, first and second derivative technique UV spectrometry, capillary electrophoresis, GC, LC, TLC etc.[1-10]

Figure 2: Dextromethorphan hydrobromide



Some procedures have been described for the assay of either chlorpheniramine maleate or Dextromethorphan hydrobromide in pharmaceutical preparations, such as Spectrometry and HPLC. Numerous UV, HPLC and HPTLC based methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. But no method had yet been reported for simultaneous estimation of these two drugs using UV bulk drug and syrup dosage forms using water as solvent. This paper describes simple, accurate, precise, rapid and economical method for simultaneous determination of Chlorpheniramine maleate and Dextromethorphan hydrobromide form syrup formulation.[1-10]

Spectrometry deals with instruments based on the absorption or emission of electromagnetic radiation as a result of its interaction with matter. Absorption spectrometry is the quantification of electromagnetic radiation absorbed by atoms, molecules or ions of specific wavelength. The amount of absorption depends depends on the wavelength of radiation and structure of compound. The absorption of radiation is due to subtraction of

energy from the radiation beam when electrons in orbital of lower energy are excited into orbital of higher energy. Since this is a electron transition phenomenon, UV is sometime called electronic spectroscopy. The technique of UV visible spectrometry is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380$_{nm}$)or visible (380-800$_{nm}$) radiation absorbed by a substance in solution by an instrument which measures the ratio or function of the ratio of the intensity of two beams of light in UV-visible region. The basis of all spectrometric methods for multicomponent sample analysis is the property that (a) The absorbance of a solution is the sum of absorbances of individual component or (b) The measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference cell. The various spectrometric methods which are used for estimation of drug in combine dosage form include simultaneous equation method, absorbance ratio method, geometric correction method, orthogonal polynomial method, differencespectrophotometery derivative spectrometery absorption correction method, multicomponent method of analysis and two wavelength quantation method.[1-3]

SIMULTANIOUS EQUATION METHOD OR VIERODT'S METHOD-

If a sample contains two absorbing drugs (x and Y) each of which absorbs at the λmax different from the other, it may be possible to determine both drugs by the technique of simultaneous equations (vierodt's method), provided certain criterias apply. The information required is,[1-3]

(a) The absorbtivities of X at and λ_1 and λ_2 are ax_1 and ax_2 respectively.

(b) The absorptivities of Y at and λ_1 and λ_2 are ay_1 and ay_2 respectively.

(c) The absorbance of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively.

Let, C_x and C_y be the concentration of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that λ_1 and λ_2, the absorbance of X and Y.

Atλ_1,

$A_1 = ax_1bc_x + ay_1bc_y$----------(1)

Atλ_2,

$A2 = ax_2bc_x + ay_2bc_y$----------(2)

For measurement in 1cm cell b=1

Rearrange equation.2

$$Cy = \frac{A2 - ax2bcx}{ay2}$$

Substituting for Cy in eq. 1 and rearranging,

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$----------(3)

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$----------(4)

As an exercise one needs to drive modified equation containing a symbol b for path length for application in situation where A_1 and A_2 are measured in cells other than 1cm path length. Criteria for obtaining maximum precision based upon absorbance ratios have been suggested that place limits on the relative concentration of the components of the mixture. The criteria are that the ratios[1-3]

$$\frac{A2/A1}{ax2/ax1} \text{ and } \frac{A2/A1}{ay2/ay1}$$

Should lie outside the range 0.1-2.0 for the precise determination of X and Y respectively. These criteria are satisfied only when the λmax

of two component are reasonably dissimilar. An additional criterion is that the two components don't interact chemically thereby negating the initial assumption that the total absorbance is the sum of individual absorbance. The additiviy of the absorbance should always be confirmed in the development of a new application of this techniques.[1-3]

MATERIALS USED-

Chlorpheniramine maleate and Dextromethorphan hydrobromide in crude form and in syrup form.

APPARATUS AND CONDITIONS-

A double beam shimadzu-1900 UV/VIS spectrophotometer with data processing capacity was used. Absorption and overlein spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell at a scanned speed of 100nm/min and fixed slit width of 3n.[1-7]

SIMULTANEOUS EQUATION METHOD-

Selection of analytical wavelength-

Pure drug sample of chlorpheniramine maleate and dextromethorphan hydrobromide were dissolved separately in distilled water so as get several different dilutions of standard in the concentration range 10-50ug/ml for both the sample drugs. All the dilutions were scanned in the range of 200-400nm. Figure no 3 and 4 represents the absorbance spectra of Chlorpheniramine maleate and Dextromethorphan hydrobromide respectively. Figure 5 represents the overlein spectra of both the drugs. Two wavelengths selected for formation of simultaneous equation are 259nm and 276nm for Chlorpheniramine maleate and Dextromethorphan hydrobromide respectively.[1-7]

Determination of E(1%, 1cm) of drugs at selected wavelengths-

Aliquot portion of Chlorpheniramine maleate and Dextromethrophan hydrobromide solution were diluted separately with distilled water to obtain a concentration of 10ug/ml for both drugs. The absorbance of each resulting solution were measured at 259nm and 276nm. The E (1%, 1cm) values (ax1,ax2, ay1 and ay2) were determined from five different concentrations of 10ug/ml of Chlorpheniramine maleate and Dextromethorphan hydrobromideusing following equation.[1-7]

$$E(1\%, 1cm) = \frac{Absorbance}{Concentration(\frac{g}{100ml})}$$

The contents of Chlorpheniramine maleate and Dextromethorphan hydrobromide in syrup dosage form were calculated using two form of simultaneous equations.

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Where, A_1 and A_2 are the absorbance of mixture at 259nm and 276nm respectively,ax_1 and ax_2 are absorptivities of Chlorpheniramine maleate at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of Dextromethorphan hydrobromide at λ_1 and λ_2 respectively. C_x and $C_y$are the concentrations of Chlorpheniramine maleate, Dextromethorphan hydrobromide respectively. The result of analysis are given in table 3.[1-7]

Figure 3: Absorbance spectra of Chlorpheniramine maleate

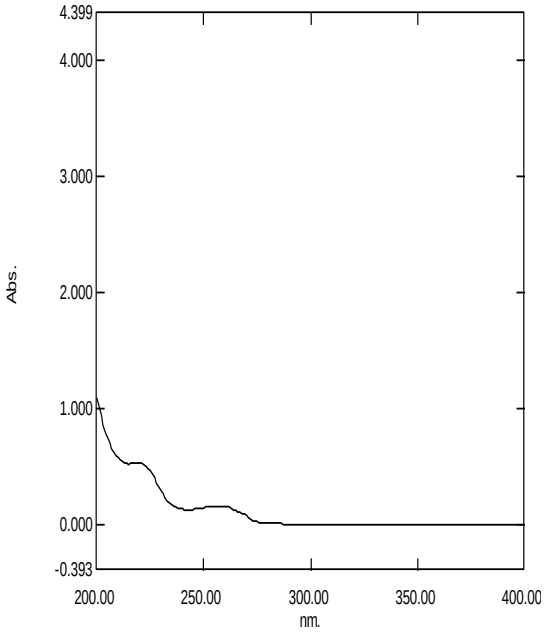


Figure 4: Absorbance spectra of Dextromethorphan hydrobromide

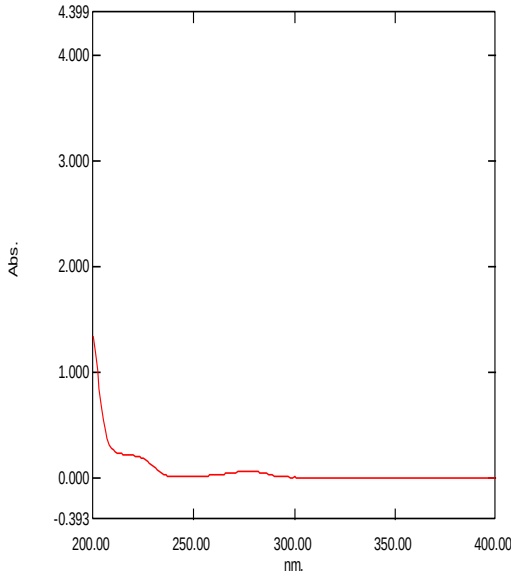


Figure 5: Overlein spectrum of Chlorpheniramine maleate and Dextromethorphan hydrobromide

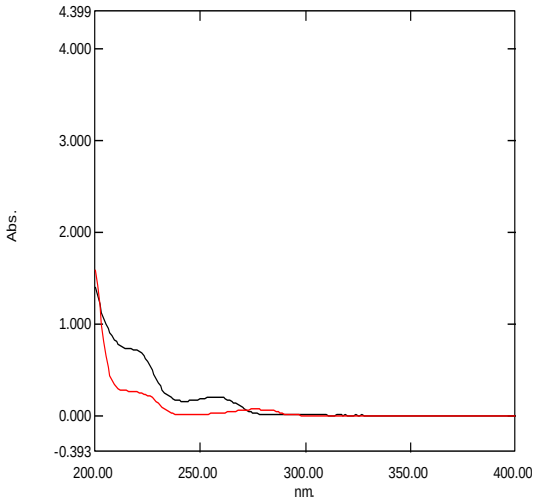


Table 1: Absorptivity values for Chlorphenirmine maleate at 259nm and 276nm

SR.NO	Concentration of CPM (gm/100ml)	Absorbance at 259nm	Absorbance at 276nm	Absorptivity at 259nm	Absorptivity at 276nm
1	0.003	0.583	0.105	194.33	35
2	0.003	0.579	0.104	193.33	34.66
3	0.003	0.586	0.105	195.33	35
4	0.003	0.586	0.105	195.33	35
5	0.003	0.585	0.103	195	34.33
	MEAN	0.583	0.104	194.6	34.79

Table 2: Absorptivity values for Dextromethorphan hydrobromide at 259nm and 276nm

SR.NO	Concentration of DEX (gm/100ml)	Absorbance at 259nm	Absorbance at 276nm	Absorptivity at 259nm	Absorptivity at 276nm
1	0.006	0.109	0.382	18.16	63.66
2	0.006	0.108	0.382	18	63.66
3	0.006	0.111	0.384	18.5	64
4	0.006	0.11	0.384	18.33	64
5	0.006	0.108	0.382	18	63.66
	MEAN	0.109	0.382	18.19	63.66

METHOD VALIDATION-

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for intended analytical application. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The proposed method was validated for various parameters such as linearity, precision, accuracy, limit of detection (LOD), Limit of quantification (LOQ), according to ICH guidelines.[6-10]

Table 3: Result of analysis of syrup formulation

Drug	Lable claim (mg)	Amount found (mg)	Mean % drug recovered
Chlorpheniramine Maleate	4mg	3.97mg	99.25%
Dextromethorphan Hydrobromide	10mg	3.30mg	33%

CALIBRATION CURVE FOR CHLORPHENIRAMINE MALEATE-

Table 4: Absorbance of Chlorpheniramine maleate at different concentrations

Wavelength	Concentration	Absorbance
259nm	10ug/ml	0.2
	20ug/ml	0.38
	30ug/ml	0.58
	40ug/ml	0.74
	50ug/ml	0.95

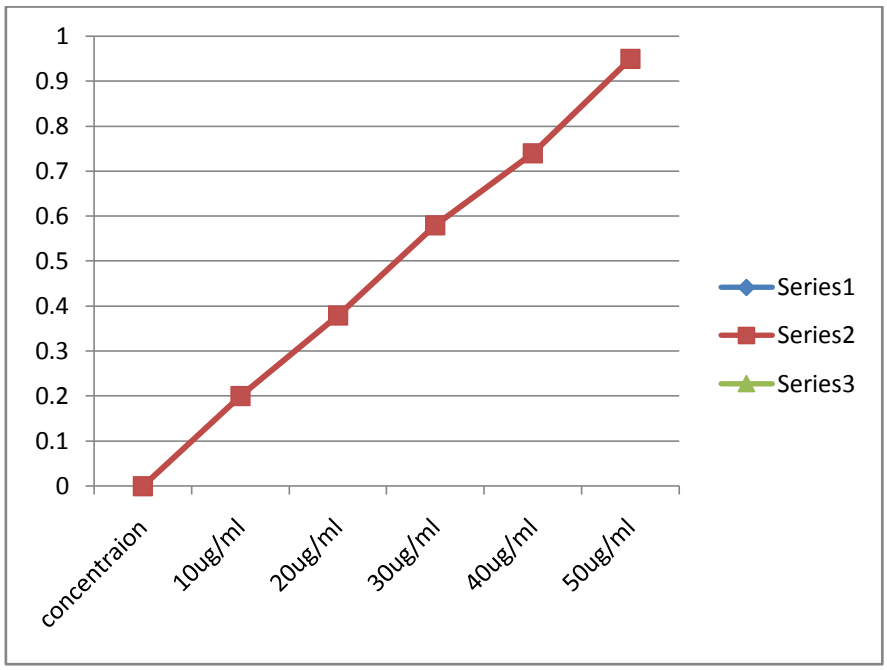
LINEARITY-

Linearity was studied by preparing solutions at different concentration levels. Calibration curve of Absorbance Vs. Concentration was plotted using standard solutions of 10-50ug/ml of chlorpheniramine maleate and 10-50ug/ml of dextromethorphan hydrobromide and regression line equation and correlation coefficient was determined.[6-10]

PRECISION-

The Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of the method was studied by intraday and interday variation in the method of Chlorpheniramine maleate and dextromethorphan hydromide. Method repeatability was evaluated by assaying six samples, prepared as described under sample preparation. Inter day precision was performed by assaying six samples in different days as described in the sample preparation. The results are represented in table 6 and 7.[6-10]

Figure 6: Linearity graph of Chlorpheniramine maleate at 259nm



CALIBRATION CURVE FOR DEXTROMETHORPHAN HYDROBROMIDE –

Table 5: Absorbance of Dextromethorphan hydrobromide at different concentrations-

Wavelength	Concentration	Absorbance
276nm	10ug/ml	0.07
	20ug/ml	0.13
	30ug/ml	0.19
	40ug/ml	0.25
	50ug/ml	0.31
	60ug/ml	0.38

Figure 5: Linearity graph of Dextromethorphan hydrobromide at 276nm

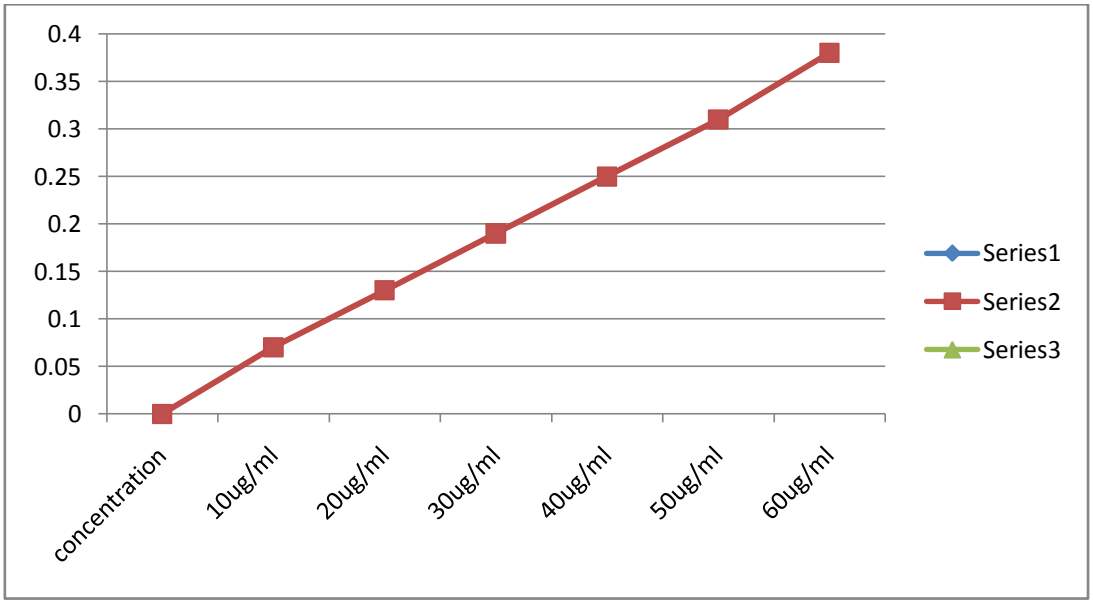


Table 6: Intraday precision

Drug	Label claim(mg)	Amount found (mg)	Mean %drug recovered
Chlorpheniramine Maleate	4mg	3.97mg	99.25%
Dextromethorphan Hydrobromide	10mg	3.30mg	33%

Table 7: Interday precision

Drug	Label claim(mg)	Amount found (mg)	Mean % drug recovered
Chlorpheniramine Maleate	4mg	3.97mg	99.25%
Dextromethorphan Hydrobromide	10mg	3.30mg	33%

ACCURACY-

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy of the method was determined.[6-10]

LIMIT OF DETECTION (LOD) –

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample can be detected but not necessarily quantitated as an exact value. The detection limit was determined by using six sets of calibration curves and estimating the standard deviation of the response and slope of the calibration curve. The results were calculated using following equation and the result are presented.[6-10]

$$LOD = 3.3\frac{\sigma}{s}$$

Where,

σ = Standard deviation of Y-intercept of the calibration curves

S = slope of calibration curve.

LIMIT OF QUANTITATION (LOQ)-

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit was calculated using six sets of calibration curves and estimating the standard deviation of the response and slope of the calibration curve. Results were calculated using following equation and results are presented.[6-10]

$$LOQ = 10\frac{\sigma}{s}$$

Where,

σ = Standard deviation of Y-intercept of the calibration curves.

S= Slope of calibration curve.

Table 8: Analytical method validation results

SR. NO	Parameter	Chlorpheniramine Maleate	Dextromethorphan Hydrobromide
1	Wavelength	259nm	276nm
2	Regeration equation	Y=0.01853X +0.0183	Y=0.0061X +0.0066
3	slope	0.01853	0.0061
4	Y-intecept	0.0183	0.0066
5	%recovery	99.25%	33%
6	LOD(ug/ml)	3.25	3.57
7	LOQ(ug/ml)	9.87	10.81

RESULT AND CONCLUSION-

The developed new simple simultaneous equation method was applied successfully to the syrup formulation and the assay results were indicating that this method can be effectively used for the estimation of both drug in combined dosage form.

The developed simultaneous equation method is simple, precise, specific and accurate. Statistical analysis proved that the method was repeatable and selective for the simultaneous estimation of Chlorpheniramine maleate and Dextromethorphan hydrobromide in pure and dosage form without any intereference from the excipients. This new simple method can be used routinely for the estimation of these drugs.[1-10]

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