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Journal of Advanced Scientific Research

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ISSN
0976-9595
Research Article

VALIDATED UV- SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF DEXTROMETHORPHAN AND CETRIZINE

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Received: 07-12-2021; Accepted: 09-04-2022; Published: 30-04-2022

 https://doi.org/10.55218/JASR.202213321

ABSTRACT

Using UV Spectrophotometric methods, a simple, precise and accurate approach is devised and verified for the simultaneous assessment of Dextromethorphan (DEX) and Cetrizine (CET) in combined dosage form. The approach 1 is the Dual wavelength method, which was developed, evaluates the difference in the absorbance of mixtures at wavelengths where single drugs have the same absorbance and vice versa. Approach 2 is a mean-centered ratio method that relies on the production of calibration by dividing the spectra of one substance by the spectra of the other. Approach 3 is a derivative spectrum method that relies on the derivative spectrum's zero crossing points to create calibration for two medications in the presence of the second. Simultaneous estimation of DEX and CET using the Q-absorption method and the simultaneous equation method has already been published. The two techniques Simultaneous equation method (Approach 4) and Q-Absorption method (Approach 5) are repeated to preserve the same circumstances throughout the experiment. To assess the accuracy and precision of all five approaches, six replication tests and recovery investigations utilizing recognized synthetic blends are used. The calibrations are used to analyze two pharmaceuticals contained in the tablet. The procedures have been validated in accordance with ICH recommendations.

Keywords: Dextromethorphan, Cetrizine, Simultaneous equation, Q-Absorption, Derivative spectrum, Dual wavelength, Mean centered ratio method.

1. INTRODUCTION

1.1. Cetrizine hydrochloride

Cetrizine hydrochloride, also known as 2–[2-[4-[(4-chlorophenyl) phenyl methyl]-1-piperazinyl]ethoxy] acetic acid, dihydrochloride, is a piperazine derivative. CET is nonsedating histamine (H-1) or antihistamine receptor blocker. CET's mode of action is to prevent histamine from promoting allergic responses in the body. Cetrizine is used to treat chronic urticaria, as well as perennial and seasonal allergetic rhinitis. For the quantitative determination of Cetrizine, only a few approaches have been employed. Fluorimetry [1], spectrophotometry [2-5], Titrimetry & conductometry [6], HPLC (High-Performance Liquid Chromatography) [7-8], GC (Gas Chromatography) [9], ion-selective electrodes [10] and liquid chromatography [11, 12] are some of the approaches.

1.2. Dextromethorphan hydrobromide

Dextromethorphan hydrobromide (DEX) is chemically [(+)-3-2-6 Methoxy-17-methyl-9α, 13α, 14α morphinan hydrobromide monohydrate]. DEX is a cough reliever that is used to treat coughs that are not productive. It operates on the cough centre in the medulla. DEX is absorbed fast from the stomach and intestines. Its demethylated metabolites, including DEX (a cough suppressant) are metabolized in the liver and excreted in the urine [13]. Various techniques for determining DEX in bulk medications, dose forms with other pharmaceuticals in cold and cough treatments and biological samples have been published. High performance Liquid Chromatography [14-16], UV spectrophotometry [17-20], capillary electrophoresis [21-22], Gas Chromatography [23-25], Liquid Chromatography [26-30] and Thin Layer Chromatography [31-32] are some of the procedures used.

Literature survey shows that different methods like UV Spectrophotometry [33-35], RP-HPLC [36-38] and Derivative spectrophotometry [39] are developed in combined dosage form of CET and DEX.

There is one reference accessible for the simultaneous estimation of DEX and CET in a combined formulation by UV spectroscopy; however, there are no Dual wavelengths, Mean centered ratio spectra or Derivative Spectrum Methods. Although sensitive, easy, and reliable approaches for simultaneous drug estimation such as the Dual wavelength method, Derivative Spectrum method and Mean centered ratio spectra exist, simultaneous determination utilizing the methods described above has yet to be reported. This motivated the writers to continue working in this direction. This paper presents the successful findings that were attained and reported. Structures of the two drugs were mentioned in Fig. 1.

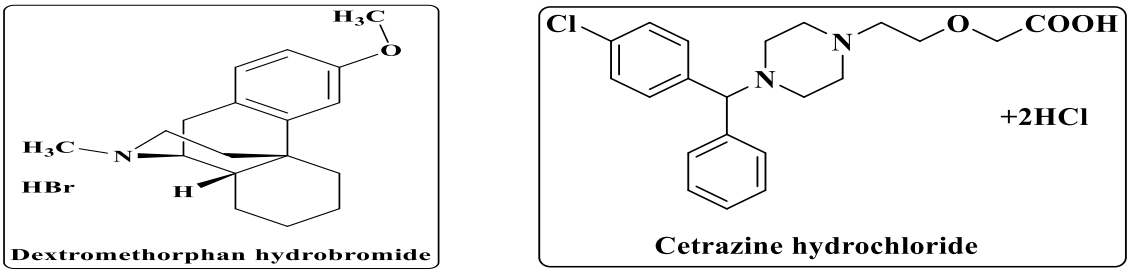

Fig. 1: Structure of Dextromethorphan hydrobromide and Cetrizine hydrochloride

2. MATERIAL AND METHODS

2.1. Instrumentation

The spectrophotometer utilized was an Elico SL 210 twin beam UV-Visible spectrophotometer with a pair of 1cm matched quartz cells and UV-PC software 4.01.01 version.

2.2. Pure samples

Hetero drugs pharmaceuticals provided Dextromethorphan hydrobromide and Cetrizine hydrochloride. All of the solutions were made with distilled water.

2.3. Working standard solution preparation

DEX and CET were weighed appropriately and put to a 100 mL volumetric flask, where they were dissolved in distilled water (10 mL) and brought up to the mark with distilled water to yield 300 µg mL^{-1} DEX and 300 µg mL^{-1} CET in separate volumetric flasks. The standard stock solutions (300µg mL^{-1}) were diluted separately with distilled water to yield working standards of 20 µg mL^{-1} DEX and 30 µg mL^{-1} CET. By scanning the solutions in the whole UV range, the absorbance maxima of the working standard solutions were found. Dextromethorphan and Cetrizine have maximum absorbances of 278 nm and 231 nm, respectively.

2.4. Calibration curves

Dextromethorphan standard solutions ranging in concentration from 2µg mL^{-1} to 20 µg mL^{-1} were added to a set of 10 mL standard flasks and Cetrizine standard solutions with concentrations range from 3 µg mL^{-1} to 30 µg mL^{-1}were added into the set of 10 mL standard flasks. Water was then added to each flask to get the solution up to 10 mL. All of the solutions were scanned in the UV region from 200 nm to 400 nm. To create calibration curves, graphs of absorbances versus corresponding concentrations were generated. Fig. 2 shows an overlain spectrum of Dextromethorphan and Cetrizine in water.

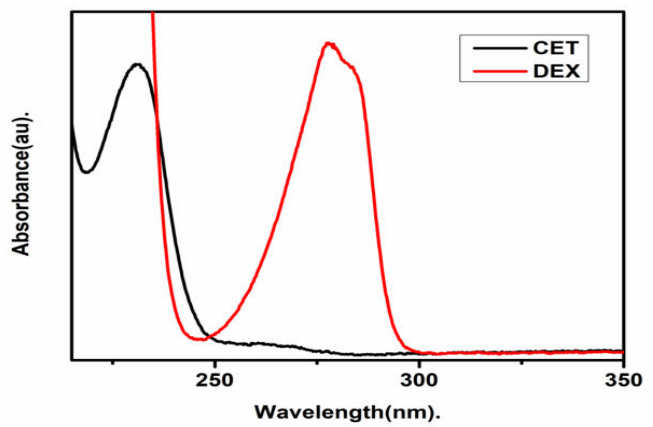

Fig. 2: Overlain spectra of DEX and CET

2.5. Recommended procedures

2.5.1. Approach 1- Dual wavelength method

Different aliquots of 2-20 µgmL^{-1} and 3-30 µgmL^{-1} of DEX and CET were taken independently from their respective working standard solutions into two distinct sets of 10 mL standard flasks and then the capacity was finished with distilled water. In the UV spectrophotometer, the aliquots were scanned at wavelengths between 200 and 300 nm. The overlain

spectra (Fig. 2) were utilized to choose four wavelengths for measurement of the two medications using the Spectrophotometric double frequency technique: 224.8 nm, 235.8 nm, 270.2 nm, and 287.6 nm. DEX is quantified by calculating the absorbance difference between 224.8 nm and 235.8 nm, whereas CET has similar absorbance values at both wavelengths. The difference in absorbance between 224.8 and 235.8 nm is related to the amount of DEX in the combination. Difference in the absorbance values at 270.2 nm and 287.6 nm is used to make quantitative measurements of the CET, whereas the DEX has the same absorbance values at both wavelengths. The absorbance difference between 270.2 nm and 287.6 nm is proportional to the amount of CET in the combination. The DEX and CET calibration curves are made by graphing the difference between a drug's absorbance value at a given wavelength and the drug's corresponding concentration. In the concentration ranges of 2-20 µg mL^{-1} and 3-30 µg mL^{-1}, DEX and CET followed the Beer-Lambert law with strong correlation coefficients.

2.5.2. Approach 2- Mean centered ratio method (MCR)

DEX aliquots equivalent to 2.0-20.0 µg mL^{-1} of their standard working solution were transferred exactly to a set of 10 ml standard flasks and the limit was made adequate with distilled water. Divide the absorbance spectra of the generated solution between 200 and 300 nm by the reference spectrum of 12 µg mL^{-1} CET and focus the spectrum on the average ratio obtained. CET standard solutions with doses ranging from 3.0 to 30.0 µg mL^{-1} have their spectra measured as well. To get ratio spectra, the recorded spectra were divided by the DEX 12 µg mL^{-1} reference spectra and condensed to the mean. The amplitude value of each centre average spectrum (peak to peak) of each concentration is used to plot the DEX and CET calibration curve.

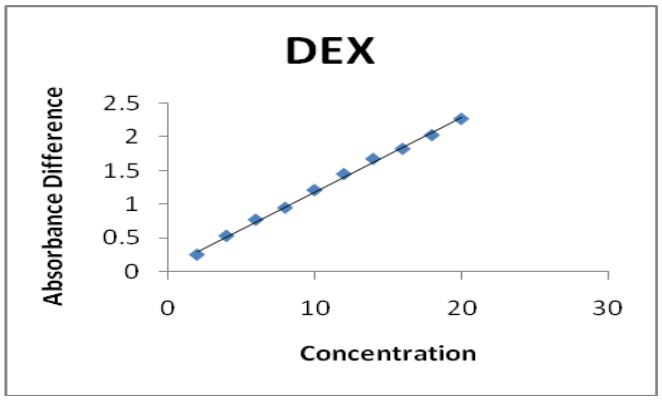

Fig. 3: Dual wavelength of DEX

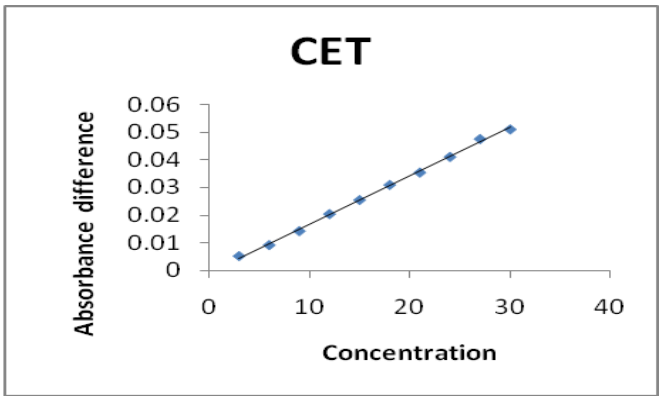

Fig. 4: Dual wavelength of CET

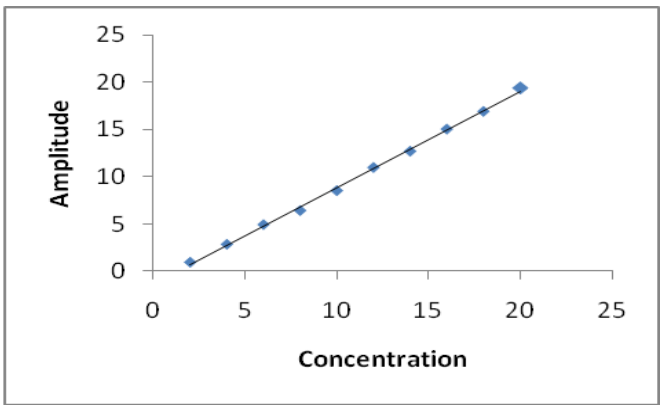

Fig. 5: MCR calibration curve for DEX

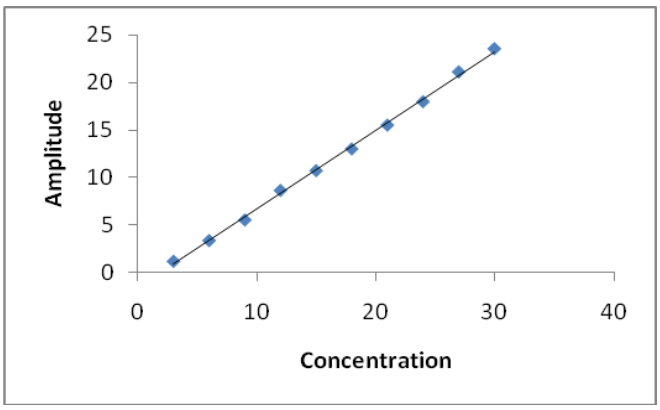

Fig. 6: MCR calibration curve for CET

2.5.3. Approach 3- Derivative spectrum method

Because it can be measured at the zero-crossing point of another medication, the derivative spectrum approach may be used to quantify analytes whose spectra overlap with low error, difficult extraction procedure and interference from the second drug or additives in the

formulation is minor. Different aliquots of 2-20 µg mL^{-1} DEX were transferred to a set of 10 mL standard flasks, and 3.0-30.0 µg mL^{-1} CET was added to the above series and the capacity was raised up to 10 mL using distilled water. Various 3-30 µgmL^{-1} aliquots of CET were put to separate set of 10 mL standard flasks, and then 2-20 g mL^{-1} DEX was added to the same set of 10 mL standard flasks, which were then filled with distilled water to the desired amount. The solution in the 200-300 nm wavelength range were scanned. Using the data, the difference in O.D (ΔO.D) was calculated and calibration curves were created by graphing the wavelength versus O.D. Then, for both DEX and CET, amplitude versus drug concentrations in combination plots was created.

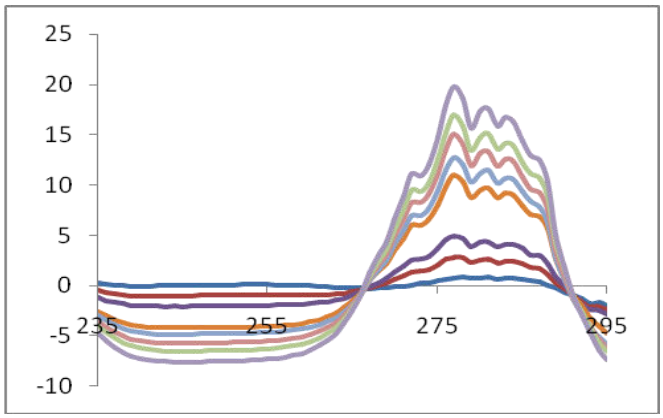

Fig. 7: MCR spectrum of DEX

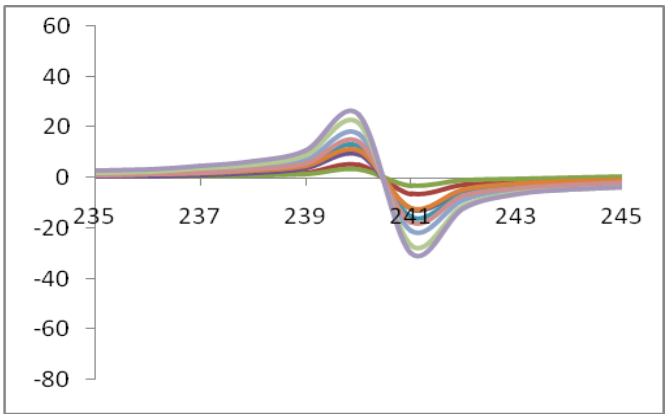

Fig. 8: MCR spectrum of CET

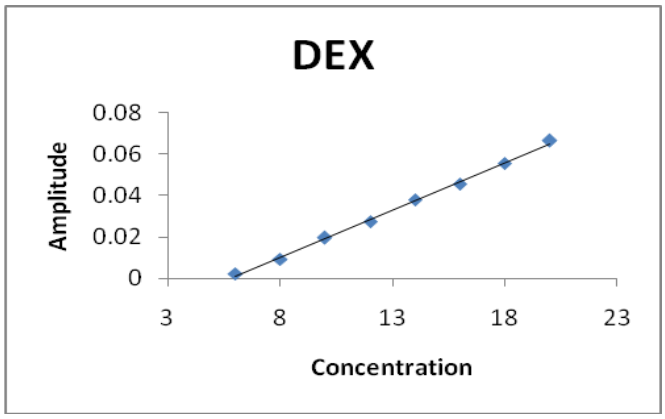

Fig. 9: Calibration curve for derivative Spectrum Method for DEX

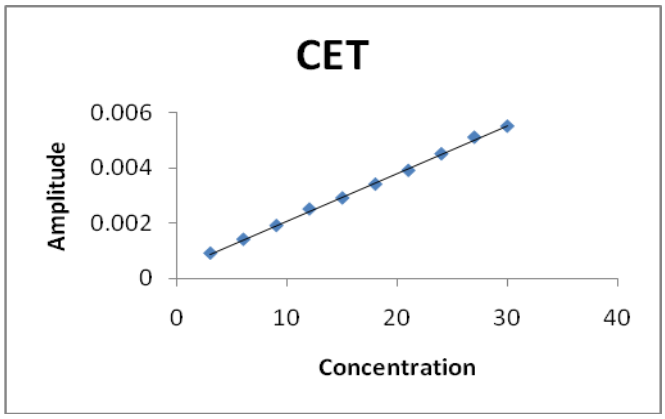

Fig. 10: Calibration curve for derivative Spectrum Method for CET

2.5.4. Approach 4- Simultaneous equations method

Both wavelengths of 278 nm, which is the maximum of DEX and 231 nm, which is the maximum of CET, were used for the construction of simultaneous equations. DEX produced linearity values in a range of 2-20 µg mL^{-1} for DEX and 3-30 µg mL^{-1} for CET when using a mixed standard solution. The approach may be used with a sample containing two pharmaceuticals, each of which absorbs at the same rate as the other. The two equations are based on the fact that the absorbance of a combination of Dextromethorphan and Cetrizine at 278 nm and 231 nm equals the sum of their individual absorbances. At two wavelengths, the absorptivity co-efficients of each medication were calculated.

Using the simultaneous equation with the equations (1) and (2), the concentrations of both the formulations present in the mixture were determined.

"$C_x = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$ .....(1)

$C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$ .....(2) "

The concentration of DEX in the working sample solution is denoted by Cx. The concentration of CET in the working sample solution is given by Cy (mixture).

A_1 = absorbance of mixture at 278 nm, A_2 = absorbance of the mixture at 231 nm

ax_1 = the absorptivity of DEX at 278 and ax_2 = the absorptivity of DEX at 231nm.
(ax_1 =501.2, ax_2 =122.5).
ay_1 is the absorptivity of CET at 278 nm and ay_2 are absorptivity of CET at 231 nm.
(ay_1 =22.7, ay_2=353).

2.5.5. Approach 5-Q-Absorption method

The ratio of the absorbance of two wavelengths, one of which is the Isoabsorption point and the other component's maximum absorbance value, is the subject of the Q-Absorption technique (Isoabsorption method). Both DEX and CET are reported to have iso-absorption sites at 236 nm in their overlay spectra. The second wavelength is DEX's absorbance maximum, which is 278 nm. In water, 2-20 µg mL^{-1} DEX and 3-30 µg mL^{-1} CET working standard solutions were produced, the absorbance values at 236 nm (Isoabsorption point) and 278 nm (max of DEX) were determined, the calibration curve was used to determine the absorption coefficients. The concentrations of both pharmaceuticals present in the mixed solution are determined using the formulae below.

"$C_X = [(QM - QY) / (QX - QY)] \times A_1/ax_1$..... (3)

$C_Y = [(QM - QX) / (QY - QX)] \times A_1/ay_1$ ..... (4)"

A_1 = absorbance of the drug mixture at 236 nm, A_2 = absorbance of the drug mixture at 278 nm, $ax_{1=}$ absorptivity of DEX at236 nm, ay_1 = absorptivity of CET at 236 nm, ax_2 = absorptivity of DEX at 278 nm, ay_2 = absorptivity of CET at 278 nm

$QM = A_2 / A_1$ (A_1=1.073, A_2= 0.2191)

$QX = ax_2 / ax_1$ (ax_1 =437.2, ay_1 =291.3; ax_2 = 501.2, ay_2 = 22.7)

$QY = ay_2 / ay_1$.

Table 1 compares the regression parameters of the simultaneous equation approach and the Q-absorption technique to the stated values. Table 2 lists the regression equation parameters for the other three approaches.

2.6. Analysis of mixtures prepared in the laboratory

Using distilled water as a blank, zero order absorption spectra of several DEX and CET mixes generated in the lab in varied amounts were recorded. the procedure was then repeated for each linearity technique. The generated regression equation is used to determine the levels of DEX and CET in the prepared sample.

Table 1: Comparison of Regression and analytical parameters of the proposed simultaneous equation method and Q-absorption method with reported one

Parameters	Simultaneous equation method (proposed method)		Q-Absorption ratio method (proposed method)		Simultaneous equation method* (reported method)		Q-Absorption ratio method* (reported method)	
	DEX	CET	DEX	CET	DEX	CET	DEX	CET
Range (µgmL^{-1})	2-20	3-30	2-20	3-30	10-30	10-30	10-30	10-30
slope	0.127	0.036	0.045	0.030	0.004	0.004	--	--
Intercept	-0.083	-0.046	-0.034	-0.034	0.2	0.04	--	--
correlation coefficient*	0.998	0.983	0.990	0.990	0.9999	0.9996	--	--
Sandell's sensitivity (µg cm^{-2})	0.007	0.0277	0.022	0.033	0.25	0.25	--	--
LOD	2.37	0.438	1.24	5.71	1.15	0.66	--	--
LOQ	7.1	1.32	3.46	17.33	3.5	2.0	--	--
Accuracy (mean± SD)	99.95± 0.432	99.90± 0.174	100.2± 0.512	99.95± 0.620	99.92± 0.650	100.03 ±0.550	100	100

**Six replicate samples for Dextromethorphan and Cetrizine*

2.7. Analysis of the drug formulations

To make a fine powder, 10 Kofrid tablets (10 mg-Medindian Medicare) were weighed and ground. Powdered tablets containing about 30 mg DEX and 30 mg CET were transferred to a standard 100 ml standard flask and correctly dissolved in 20 ml distilled water before sonicating for 35 minutes. Then, using pure water, reduced the amount to 100 mL. The resultant filtrate was properly diluted to obtain a concentration of approximately 20µg mL^{-1} DEX and 30µg mL^{-1} CET after filtering using Whatmann filter paper. The sample working solutions' extinctions were determined at 278 nm (DEX λmax) and 231 nm (CET λmax), and the amounts of two active compounds in the sample

solution were calculated using Eqs.1 and 2 (Approach 4). The Q- absorption technique was used to analyze the tablet solutions. The sample solutions' absorbances at 278 (DEX λmax) and 236 nm (iso-absorbance point) as well as the concentrations of both medications in the sample were measured. Equations 3 and 4 were used to determine them (Approach 5).

The DEX and CET dual wavelength procedures employed the identical tablet solutions to produce the graphs at 224.8, 235.8 nm (DEX) and 270.2, 287.6 nm (CET). The MCR approach and the derivative spectrum method are both calculated using the solutions. The tablet formulation test process was repeated 6 times. Table 3 shows the results of the tablet formulation analysis.

2.8. Studies on recovery

To see if the suggested methods are accurate, studies of recovery were conducted using the usual addition method at three distinct levels. After known quantities of the investigated pharmaceuticals were added to a given amount of pretested tablet powder, the recovery percentages were estimated. The recovery investigations, which are revealed in Table 3, were favorable.

Table 2: Regression and analytical parameters of the proposed Dual wavelength method, Mean centered ratio method and Derivative spectrum method for determination of Dextromethorphan hydrobromide, Cetrizine hydrochloride

Parameters	Dual wavelength method		Mean centered ratio method		Derivative spectrum method	
	DEX	CET	DEX	CET	DEX	CET
Range (μgmL^{-1})	2-20	3-30	2-20	3-30	2-20	3-30
Slope	1.036	0.834	0.174	1.083	0.005	0.04
Intercept	-1.558	-1.708	-0.030	9.184	0.071	0.006
Correlation co-efficient	0.995	0.981	0.982	0.969	0.997	0.991
Sandell's sensitivity ($\mu g\ cm^{-2}$)	0.0009	0.001	0.005	0.0009	0.2	0.166
LOD	11.0	7.623	1.73	2.52	0.065	6.9
LOQ	33.4	23.1	5.26	7.65	0.188	20.9
Accuracy (mean± SD)	100.4±0.56	99.7±0.632	99.89±0.9	100±0.827	99.82±0.67	99.8±0.54

Table 3: Quantitative determination of DEX and CET in Kofrid tablets by Simultaneous equation method, Q-absorption ratio method, Dual wavelength method, MCR method and Derivative spectroscopy method and application of standard addition technique

	Simultaneous equation method		Q-Absorbance ratio spectra		Dual wavelength method		Mean centered ratio method		Derivative spectrum method	
	DEX	CET	DEX	CET	DEX	CET	DEX	CET	DEX	CET
Taken μgmL^{-1}	12.75	18.5	12.75	18.5	12.75	18.5	12.75	18.5	12.75	18.5
	18.75	28.5	18.75	28.5	18.75	28.5	18.75	28.5	18.75	28.5
	30.0	33.0	30.0	33.0	30.0	33.0	30.0	33.0	30.0	33.0
Added μgmL-1	1.25	2.5	1.25	2.5	1.25	2.5	1.25	2.5	1.25	2.5
	3.75	4.5	3.75	4.5	3.75	4.5	3.75	4.5	3.75	4.5
	6.0	7.5	6.0	7.5	6.0	7.5	6.0	7.5	6.0	7.5
Found μgmL^{-1}	14.01	20.9	14.0	21.02	14.0	21.02	14.03	20.92	13.8	20.8
	22.4	32.9	22.6	32.9	22.48	32.09	22.48	33.0	22.4	32.9
	35.9	40.4	35.9	40.3	36.01	40.51	36.0	40.47	36.02	40.45
% recovery	100.07	99.52	100.0	100.0	100.0	100.0	100.2	99.61	98.57	99.04
	99.55	99.69	99.55	99.69	99.91	97.24	99.91	100.0	99.55	99.69
	99.72	99.75	99.72	99.50	100.0	100.0	100.0	99.92	100.0	99.87
RSD	0.2651	0.119	0.2272	0.301	0.058	1.625	0.148	0.205	0.752	0.436
Mean±SD	99.78±0.26	99.65±0.11	99.75±0.22	99.76±0.30	99.97±0.05	99.11±1.62	100±0.14	99.8±0.2	99.39±0.75	99.5±0.436

3. RESULT AND DISCUSSION

As a result, establishing analytical methods that are not only simple, precise and accurate, but also rapid and cost-effective, is crucial in discovering experimental drugs, which is the established method's major goal. When compared to HPLC technology, UV spectrophotometry saves money and time. This study validates five types of spectrophotometry, simultaneous

equations, absorption ratio, derived spectrum, dual wavelength, and methods that are accurate, sensitive, simple and fast for the simultaneous analysis of DEX and CET in their combined pharmaceutical dosage forms. Linearity was found in the range of 2-20 µg mL^{-1} for DEX and 3.0-30 µg $mL^{-1}$for CET for all five techniques. When analyzing a branded pill, the suggested method's estimated drug amount range is 99.6-100.2, as shown in Table 3.

3.1. Method validation

This approach has been validated in line with the recommendations of the International Conference on Harmonization (ICH).

3.1.1. Linearity

Linearity is linked to the proportionality of analyte concentration in samples. To produce accurate, precise and linear findings, the calibration range for DEX and CET was set by taking into account the practical range needed by the Lambert-Beer rule, as well as the concentrations of DEX and CET in the pharmaceutical dose form. Tables 1 and 2 show the linearity ranges for DEX and CET respectively.

3.1.2. Precision

The precision is established by statistically calculating a reliable estimate of the % RSD (relative standard deviation in percent) from a series of aliquots of a homogenous sample. Three repetitions of the working standards of the combination and the sample solution (14.0, 22.5, 36 µg mL^{-1} DEX and 5.25, 8.75, 12.25 µg mL^{-1} CET) concentrations were evaluated, and the relative standard deviation in percent (% RSD) was determined to be less than 2%.

3.1.3. Specificity

Because the findings of the related tablet solution indicated no influence from auxiliary components when compared to the standard working solution, the technique was labeled as specific.

3.1.4. Limit of detection

Limit of detection is the smallest quantity of analyte in a sample that can be determined under particular experimental circumstances. It is not necessarily quantified.

According to the ICH recommendations, the LOD may be computed using the formula below.

$$LOD = 3.3 \times \sigma/S$$

Here, σ is the intercept's standard deviation.; the slope of the related calibration curve is denoted by S.

3.1.5. Limit of quantification

The limit of quantification is the minimum concentration of analyte in the sample which can be detected under established experimental conditions with acceptable precision and accuracy. The LOQ can be calculated by the following formula based on the ICH guidelines.

$$LOQ = 10 \times \sigma/S$$

Here, σ is the intercept's standard deviation; the slope of the related calibration curve is denoted by S.

The values of Student's t-test and F-test are given in Table 4.

Table 4: Statistical analysis of the proposed Simultaneous equation method, Q-absorption ratio method, Dual wavelength method, MCR method and Derivative spectrum method and reported one for determination of DEX and CET in their pure forms

	Simultaneous equation method		Q-Absorption ratio Spectra		Dual wavelength method		Mean centered method		Derivative spectrum method		Reported method	
	DEX	**CET**	**DEX**	**CET**	**DEX**	**CET**	**DEX**	**CET**	**DEX**	**CET**	**DEX**	**CET**
Mean%	99.95	99.90	100.2	100.0	100.4	99.7	99.89	100	99.82	99.8	99.6	99.55
SD	0.432	0.174	0.51	0.620	0.564	0.632	0.902	0.82	0.678	0.546	1.08	0.56
N	6	6	6	6	6	6	6	6	6	6	6	6
Student t-test	0.386	0.202	2.61	0.134	0.156	0.044	0.616	0.54	0.564	0.062	---	---
F- test	**0.329**	**0.213**	**0.50**	**0.447**	**1.25**	**0.918**	**0.375**	**0.78**	0.469	0.453	--	---

4. CONCLUSION

The proposed procedures for determining Dextromethorphan and Cetrizine in pharmaceutical formulations were found to be precise, simple, accurate, low-cost and rapid. It was discovered that the devised approach had a percent RSD of less than 2. As a result, these procedures may be used to determine the concentrations of Dextromethorphan and Cetrizine in mixed formulations.

5. ACKNOWLEDGEMENT

The authors are thankful to the Head of the department, Department of Chemistry, Osmania University for providing the necessary instruments and infrastructure.

Conflict of interests

The authors declared no conflict

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