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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DEXTROMETHORPHAN HYDROBROMIDE AND PHENYLEPHRINE HYDROCHLORIDE

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ABSTRACT

The combination of Dextromethorphan hydrobromide and phenyl ephrine hydrochloride is used to treat cough, stuffynose and sinus congestion caused by allergies, the common cold or the flu. Five simple, accurate, rapid and precise uv spectrophotometric methods have been developed for simultaneous estimation of Dextromethorphan hydrobromide and phenylephrine hydrochloride in combined formulation, as a syrup.. First method employs solutions of simultaneous equations using, while the second method called Q-ratio or iso-absorptive method based on measurement at iso-absorptive point as well λmax of other drug. The third method *viz.* Derivative spectrum method, depends on zero crossing points of the derivative spectrum, which enables the construction of calibration for both the drugs in the presence of second one. Dual wavelength, the fourth method developed measures the difference in the absorbance of the mixtures at wavelengths whereas single drug has the same absorbance and viceversa. Mean centered ratio method which depends on construction of calibration by dividing spectra of the one compound with the other is reported as fifth method. All the five methods are tested for accuracy, precision by six replicate experiments and recovery studies using known synthetic mixtures. The calibrations are applied for the analysis of two drugs in the syrup. The methods are validated in terms of ICH guidelines.

Keywords: Dextromethorphan hydrobromide, Phenyl ephrine hydrochloride, Simultaneous- equations method, Q-analysis, Derivative spectrophotometry, Mean centered ratio method, dual wavelength, Validation.

INTRODUCTION

Dextromethorphan hydrobromide (DEX), [(+) -3-2-6 Methoxy- 17-methyl-9α, 13α, 14α- morphinan hydrobromide monohydrate] (Fig. 1a) is a cough suppressant used for the relief of nonproductive cough. It has a central action on the cough centre in the medulla. DEX is rapidly adsorbed from the gastro intestinal tract. It is metabolized in the liver and excreted in the urine as unchanged DEX and demethylated metabolites including DEX, which has some cough suppressant activity [1]. Different methods have been reported for the determination of DEX in the bulk drug, in the dosage forms with other drugs in cough cold products and in biological samples which include HPLC [2-6], the first and second-derivative technique uv spectrophotometry[7-10], capillary electrophoresis [11-13], GC[14-16], LC[17-20] and TL[C21-22] methods. Phenyl ephrine hydrochloride (PHE),[(R)-1-(3-hydroxy phenyl) 2-(methylamino) ethanol hydrochloride] (Fig.1b), is a white crystalline powder and belongs to the group sympathomimetics. It acts in stimulating the alpha receptors in certain areas of the body. It is used locally, as decongestant, for non-specific and allergic conjunctivitis, sinusitis and nasopharyngitis. PHE nasal drops are used for treating symptoms such as runny nose, sneezing, itching of the nose and throat. Various methods have been reported for analysis of PHE *viz.*Spectrophotometry [23,24], High performance liquid chromatography[25], micellar liquid chromatography[26] and Capillary zone electrophoresis [27].

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Since no spectrophotometric method is reported for simultaneous estimation of PHE and DEX in combined dosage form therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by the simultaneous equation, Q-analysis, derivative method, Dual wavelength method and Mean centered ratio method.

MATERIALS AND METHOD

A double-beam Elico UV-Visible spectrophotometer model SL 210 with a pair of 1-cm matched quartz cells and UV-PC personal software version 4.01.01 was used.

Pure samples

Dextromethorphan hydrobromide and Phenylephrine hydrochloride and were obtained as a gift sample from Hetero drugs Pvt Ltd. Distilled water was used to prepare all solutions.

Preparation of standard solution

Accurately weighed about 30 mg of DEX and PHE were transferred to 100.0 ml volumetric flask and 50.0 ml of distilled water was added to dissolve the drug and diluted up to the mark with distilled water, to get 300 µg mL^{-1} of DEX and 400 µg ml^{-1} of PHE in separate volumetric flask. The standard stock solutions (400 µg ml^{-1}) were further diluted separately to obtain working standard of concentration 185µg mL^{-1} of DEX and 285µg ml^{-1} of PHE.

Working standard solutions were scanned in the entire UV range to determine the λmax. The λmax of Dextromethorphan and Phenylephrine were found to be 278 nm and 273 nm.

Calibration curves

Ten standard dilutions of each drug were prepared separately having concentrations of 18.5 µg ml^{-1}- 185µg ml^{-1} of DEX and 28.5µg ml^{-1} - 285 µg ml^{-1} of PHE. The absorbances of standard solutions were measured at 278 nm and 273nm and calibrated curves were plotted at these wavelengths.

Method 1

Simultaneous equations method

Two wavelengths selected for the formation of simultaneous equations were 278 nm (λmax of DEX) and 273 nm (λmax of PHE). Similarly mixed standard solutions were also used and the drugs showed linearity in the range of 18.5-185.0µg mL^{-1} for DEX and 28.5-285.0 µg mL^{-1} for PHE. The method was based on simultaneous equation method of vierodt. The method is applicable in the case of sample containing two drugs, each of which absorbs at the λmax of the other(Beckett et al,1997).Two equations were constructed based upon the fact that the absorbance of mixture of Dextromethorphan and Phenylephrine at278 nm and 273 nm is the sum of the absorbances at respective wavelengths. The absorptivity co-efficients of each drug at both wavelengths were determined. The concentrations of two drugs in the mixture were calculated using equations (1), (2).

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

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

Where C_x and C_y are concentrations of PHE and DEX respectively in the sample solution (mixture). A_1 and A_2 are the absorbances of the mixtures at 273 nm and 278 nm respectively. $ax_1$and ax_2 are absorptivities of PHE at 273 nm and 278 nm respectively.(ax_1 =38.24, ax_2 =34.92). $ay_1$and ay_2 are absorptivities of DEX at 273 nm and 278 nm respectively.(ay_1 =51.75, ay_2=62.11).

Method 2

Absorbance ratio or Q-analysis method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlain spectrum of DEX and PHE, two wavelengths were selected, one at 275.8 nm, isoabsorptive point for both the drugs and the other 273 nm (λmax of PHE). The absorbances of the standard and sample solutions were prepared in the same manner as in the previous method. The method employs Q values; the concentrations of drugs in the sample solution were determined by using the following formula.

For PHE

$C_X = [(Q_M - Q_Y)/Q_Y - Q_X)A_1/ax_1$

For DEX

$C_Y = [(Q_M - Q_X)/Q_Y - Q_X)A_1/ay_1$

$Q_X = ax_2/ax_1 Q_M = A_2/A_1$

$Q_Y = ay_2/ay_1$

$A_{1=}$ Absorbance of mixture at 273 nm.

A_2=Absorbance of mixture at 275.8 nm.

ax_1, ay_1 are absorptivities of PHE and DEX at 273 nm; ax_2, ay_2 are absorptivities of PHE and DEX at 275.8 nm respectively.

Method 3

Derivative spectrum method

The technique of derivative spectroscopy can be used with minimum error for the quantification of analytes whose spectra are overlapping by one another. It is a simple and cost effective analytical method for the simultaneous estimation of drugs in multicomponent samples that do not require the separation of the individual drugs nor any complicated extraction procedure. Since the quantitation can be done at the zero crossing point of the other drug there is little interference either from the second drug or from formulation additives. Different aliquots equivalent to 18.5-185.0 µg mL^{-1} and 28.5-285.0 µg mL^{-1} of DEX and PHE were separately transferred from their respective standard working solutions into two separate series of 10 mL volumetric flasks and then the volume was completed using distilled water. They were scanned in the

wavelength range of 200 - 300 nm. From the data Difference in O.D (Δ O.D) was calculated and the calibration curves were constructed by plotting wavelength versus Δ O.D. Then amplitude versus concentration of the drug in the mixture graphs was constructed for both DEX and PHE.

Method 4

Dual wavelength method

Different aliquots equivalent to 18.5-185.0 μg mL^{-1} and 28.5-285.0 μg mL^{-1} of DEX and PHE were separately transferred from their respective standard working solutions into two separate series of 10 mL volumetric flasks and then the volume was completed using distilled water. They were scanned in the wavelength range of 200 - 300 nm. From the overlain spectra (Figure.2), four wavelengths 267.1nm, 269.4 nm, 281.0 nm and 285.6 nm were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of PHE is carried out by measuring the absorbance difference value at between 274.2 nm and 285.6 nm where DEX has same absorbance at both the wavelength. The difference between 274.2 nm and 285.6 nm is directly proportional to concentration of PHE in the mixture. The quantitative determination of DEX is carried out by measuring the absorbance difference value at 267.1 nm and 281.0nm where PHE has same absorbance at both the wavelength. The difference between 267.1 nm and281.0 nm is directly proportional to concentration of DEX in the mixture.

Method 5

Mean centered ratio method (MCR)

Aliquots of DEX equivalent to 18.5-185.0μg mL^{-1} were accurately transferred from its standard working solution into a set of 10mL measuring flasks and the volume was adjusted using water. The absorption spectra of the prepared solutions were recorded in the range of 200–300 nm, were divided by the standard spectrum of 142.5μg mL^{-1} of PHE and then the obtained ratio spectra mean centered. By the same way the spectra of different concentrations of standard solutions of PHE in the range of 28.5-285 μg mL^{-1} were recorded. The stored spectra were divided by the standard spectrum of 111 μg mL^{-1} of DEX to obtain the ratio spectra which were then mean centered. Calibration curves for both DEX and PHE were constructed by plotting the amplitude values of their respective mean centered ratio spectra (peak to peak) against their corresponding concentrations.

Analysis of laboratory prepared mixtures

Zero order absorption spectra of different laboratory prepared mixtures containing different ratios of DEX and PHE were recorded using distilled water as blank and the procedure under linearity for each method was then followed. Concentrations of DEX and PHE in the prepared samples were calculated from the computed regression equations.

Analysis of the pharmaceutical dosage form

As per label claim (Trigenic CS) syrup contains 5 mg/5ml PHEand 10 mg/5ml DEX, So 5ml syrup was pipette out and taken in a 100 ml volumetric flask and dissolved by using small amount of1:1 methanol and water solution and sonicated for 10 min. Then made the solution upto the mark with water. Then different concentrations of syrup samples were prepared by serial dilution method and used for analysis. Prepared syrup samples were analysed on UV spectrophotometer.

Recovery studies

To study the accuracy of the proposed methods, recovery studies were carried out by application of the standard addition technique (Table.2). Known amounts of the studied drugs were separately added to a definite amount of the powdered tablet; the prepared samples were then analyzed as under linearity and the percentage recoveries were then calculated.

RESULTS AND DISCUSSION

DEX and PHE drugs act as anti-allergic and anti-tussive. Hence it is very important to develop analytical methods which are not only accurate, precise, and rapid but also simple and economic for determination of the studied drugs in their pharmaceutical dosage form and this is the main task of the developed spectrophotometric methods. Since UV-spectrophotometric methods have the advantages of saving time and cost when compared to the HPLC technique, this work concerns with the development and validation of five spectrophotometric methods, Simultaneous equation, Ratio absorption, Derivative spectrum ,dual wave- length and mean centered ratio methods for determination of the suggested drugs. Moreover, the suggested methods provide a simple, rapid, sensitive and accurate way for simultaneous analysis of DEX and PHE in their combined dosage forms.

Method validation

Validation of the method has been carried out according to ICH recommendations

Linearity and range

The calibration range for DEX and PHE was established through considerations of the practical range necessary according to adherence to Beer–Lambert's law and the concentrations of DEX and PHE present in the pharmaceutical dosage form to give accurate, precise and linear results. Linearity ranges of both DEX and PHE are shown in Table1.

Accuracy

The accuracy of the results was checked by

applying the proposed methods for determination of different blind samples of DEX and PHE and the concentrations were obtained from the corresponding regression equations. Good percentage recoveries were obtained and are presented in Table1. Accuracy of the methods was further assured by applying the standard addition technique where good percentage recoveries were obtained, confirming the accuracy of the proposed methods (Table2)

Precision

Repeatability

Three concentrations of DEX and PHE (12, 16, 20 µg/mL) were analyzed three times intra-daily using the proposed methods. Good percentage recoveries were obtained, confirming the repeatability of the methods (Table 1).

Specificity

Specificity of the methods was achieved by analysis of different laboratory prepared mixtures of DEX and PHE within their linearity ranges. Satisfactory results are shown in Table 1.

Table 1. Regression and analytical parameters of the proposed simultaneous equation method, Q-absorption method, Derivative spectrum method, dual wavelength method and Mean centered ratio method for determination of Dextromethorphan hydrobromide and Phenylephrine hydrochloride

Parameters	Simultaneous equation method		Q-Absorption ratio method		Derivative spectrum method	
	DEX	PHE	DEX	PHE	DEX	PHE
Range	18.5-185 µg ml^{-1}	28.5-285 µg ml^{-1}	18.5-185 µg ml^{-1}	28.5-285 µg ml^{-1}	18.5-185 µg ml^{-1}	28.5-285 µg ml^{-1}
Slope	0.005	0.003	0.003	0.003	0.002	0.003
Intercept	-0.095	0.059	0.066	0.074	-0.003	-0.0028
Correlation co-efficient	0.996	0.989	0.993	0.990	0.982	0.996
Sandell's sensitivity (µg cm^{-2})	0.2	0.33	0.33	0.33	0.5	0.33
LOD	1.386	1.54	4.598	0.77	1.15	0.66
LOQ	4.5	4.66	13.9	2.33	3.5	2.0

Parameters	Dual wavelength method		MCR method	
	DEX	PHE	DEX	PHE
Range	18.5-185 µg ml^{-1}	28.5-285 µg ml^{-1}	18.5-185 µg ml^{-1}	28.5-285 µg ml^{-1}
Slope	0.036	0.031	0.174	1.083
Intercept	-0.062	-0.02	-0.030	9.184
Correlation co-efficient	0.994	0.998	0.982	0.969
Sandell's sensitivity (µg cm^{-2})	0.027	0.032	0.005	0.0009
LOD	0.129	0.0745	0.026	0.004
LOQ	0.391	0.225	0.081	0.013

Table 2. Quantitative determination of PHE and DEX in Trigenic CS syrup by Simultaneous equation method, Q-absorption ratio method, Derivative spectroscopy method, dual wavelength method and MCR method

	Simultaneous equation method		Q-Absorbance ratio spectra		Derivative Spectrum method	
	DEX	PHE	DEX	PHE	DEX	PHE
Taken µg mL-1	27.5 46.0 64.5	42.5 71.0 99.5	27.5 46.0 64.5	42.5 71.0 99.5	27.5 46.0 64.5	42.5 71.0 99.5
Found µg mL-1	27.49 45.59 64.51	42.51 70.99 99.51	27.51 45.59 64.49	42.51 71.02 99.51	27.48 46.01 64.51	42.48 70.99 99.52
%recovery	99.96 99.91 100.01	100.02 99.98 100.01	100.03 100.19 99.98	100.02 100.02 100.01	99.92 100.02 100.01	99.95 99.98 100.02

RSD	0.050	0.02	0.1096	0.0057	0.0055	0.0351
Mean±SD	99.96±0.05	100.00±0.020	100.0±0.109	100.01±0.005	99.98±0.055	99.98±0.035

	dual wavelength method		**Mean centering of ratio spectra**	
	DEX	PHE	DEX	PHE
Taken µg mL-1	27.5 46.0 64.5	42.5 71.0 99.5	27.5 46.0 64.5	42.5 71.0 99.5
Found µg mL-1	27.49 46.02 64.51	42.51 71.01 99.48	27.52 46.01 64.49	42.49 70.98 99.51
%recovery	99.96 100.04 100.01	100.02 100.01 99.97	100.07 100.02 99.98	99.97 99.97 100.01
RSD	0.0404	0.0264	0.0450	0.0230
Mean±SD	100.0±0.040	100.0±0.026	100.02±0.045	99.98±0.023

Table 3. Statistical analysis of the proposed Simultaneous equation method, Q-absorption ratio method, Derivative spectroscopy method, dual wavelength method and MCR method and reported one for determination of DEX and PHE in their pure forms

	Simultaneous equation method		**Q-Absorption ratio spectra**		**Derivative Spectrum method**		**Reported method**	
	DEX	**PHE**	**DEX**	**PHE**	**DEX**	**PHE**	**DEX**	**PHE**
Mean %	99.91	99.98	100.19	100.01	100.02	99.98	99.74	99.95
SD	0.543	0.142	0.418	0.709	0.785	0.645	0.932	0.983
N	6	6	6	6	6	6	6	6
Studentt-test	0.386	0.202	2.61	0.134	0.564	0.062		
F- value	0.339	0.023	0.201	0.577	0.709	0.430		

	dual wavelength method		**Mean centering of ratio spectra**		**Reported method**	
	DEX	**PHE**	**DEX**	**PHE**	**DEX**	**PHE**
Mean %	100.04	99.97	99.98	100.01	99.74	99.95
SD	0.956	0.932	1.02	0.927	0.932	0.983
N	6	6	6	6	6	6
Studentt-test	0.055	0.0344	0.426	0.49		
F- value	1.05	0.898	0.276	0.882		

Fig 1. Structures of drugs

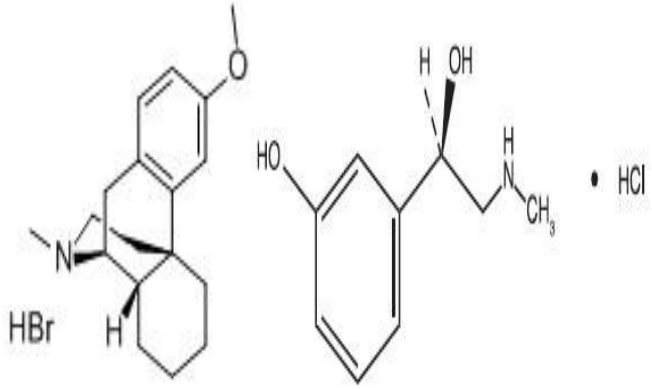


Fig 2. Overlain spectra

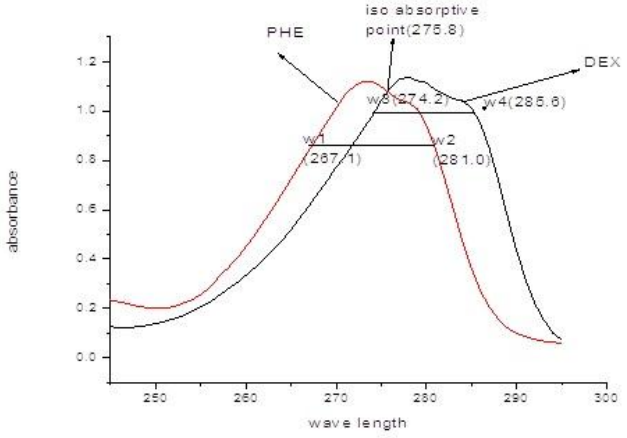
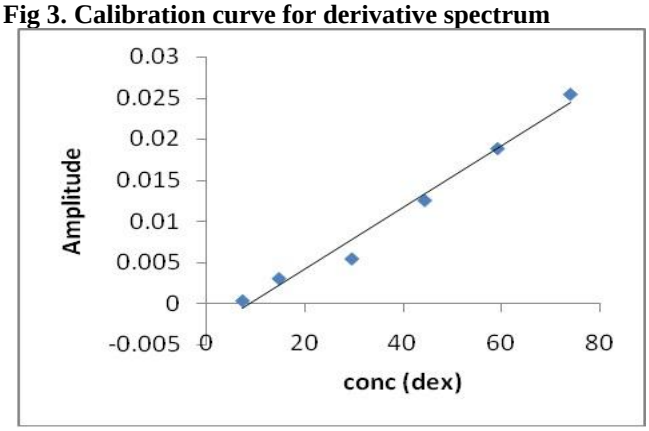
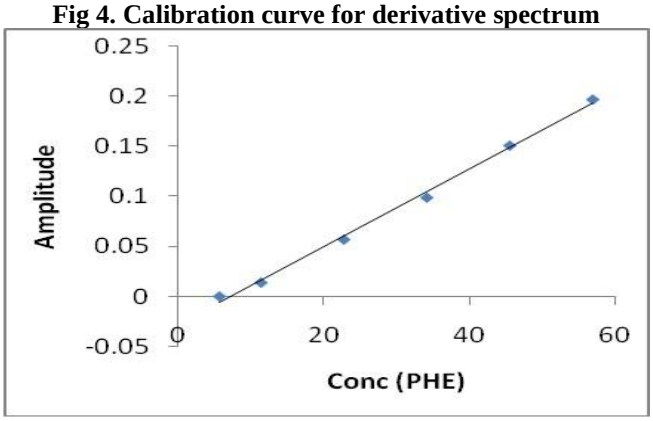


Fig 3. Calibration curve for derivative spectrum



Method for DEX

Fig 4. Calibration curve for derivative spectrum



Method for PHE

Fig 5. Dual wavelength of DEX

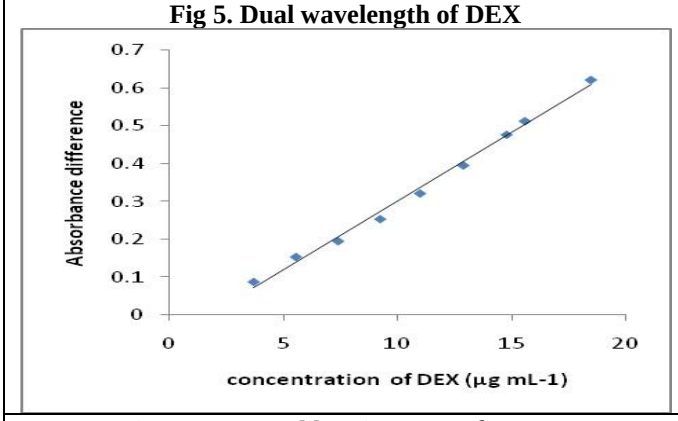


Fig 6. Dual wavelength of PHE

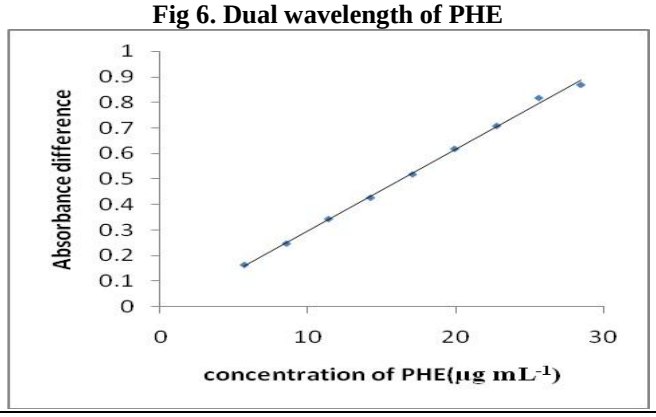


Fig 7. MCR Calibration curve for DEX

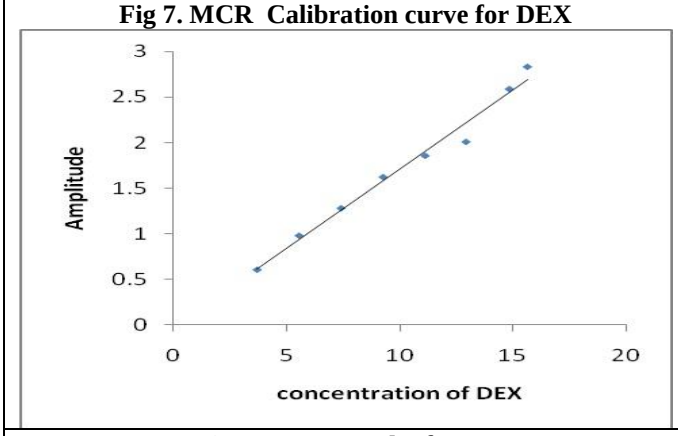


Fig 8. MCR Calibration curve for PHE

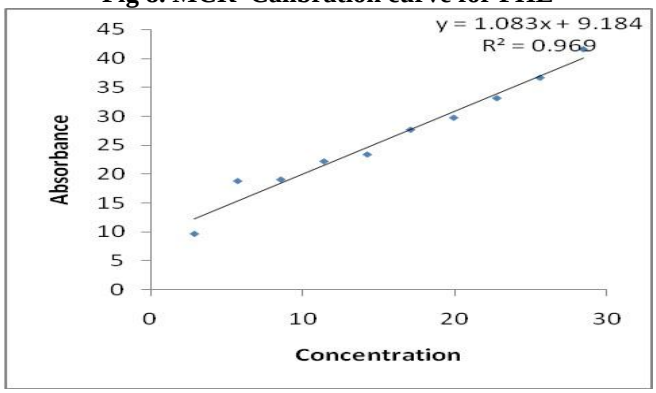


Fig 9. MCR graph of DEX

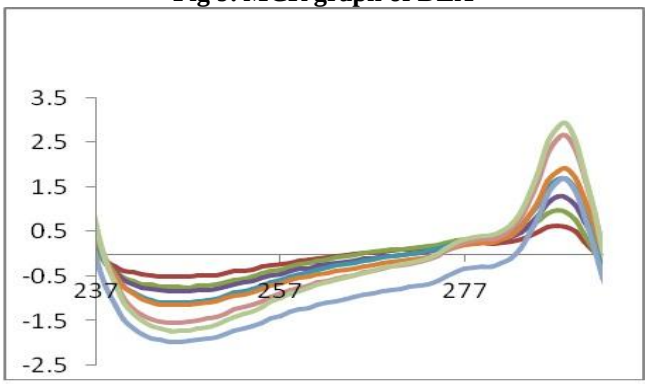
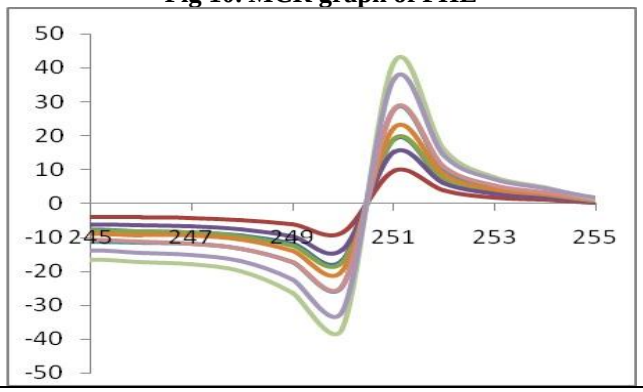


Fig 10. MCR graph of PHE



CONCLUSION

The proposed methods for simultaneous estimation of Dextromethorphan and Phenylephrine in combined dosage form were found to be simple accurate economical and rapid. The % RSD was found to be less than 2% in the developed method. Hence proposed method may be used for routine analysis of these drugs in combined dosage forms.

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