Validated Method for the Simultaneous Estimation of Orphenadrine Citrate and Paracetamol in Tablets by Simultaneous Equation Method

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Abstract : Orphenadrine Citrate and Paracetamol is a combination prescription drug used to relax muscles and relieve pain and discomfort caused by muscle injuries. Hence, an uncomplicated, specific, precise and economical method for the estimation of orphenadrine citrate and paracetamol in formulation by simultaneous equation method was developed and validated according to ICH guidelines. The simultaneous equation was formed and solved for two wavelengths 202.8 and 248 nm. The method obeys Lambert Beer's law at 2 to 12 µg/mL for both orphenadrine citrate and paracetamol. Results from the validation proved that the method is accurate, precise and robust. Recovery studies showed a satisfactory recovery of both the drugs.
Keywords : ICH guidelines, Lambert Beers equation, Orphenadrine citrate, Paracetamol, Simultaneous equation.

Introduction:

Orphenadrine Citrate:

Orphenadrine Citrate is a parasympathetic drug, a skeletal muscle relaxant belonging to ethanolamine antihistamine class with IUPAC name N,N- dimethyl- 2- [(2-methylphenyl) -phenylmethoxy] ethanamine; 2-hydroxypropane- 1,2,3- tricarboxylic acid [1]. It is used in the treatment of muscle injuries like strains and sprains which require physiotherapy and also used in the treatment of Parkinsons Disease in which it helps in motor control. It is also used in the treatment of Rheumatoid arthritis [2]. There are 309 patents issued for this drug. Its commercial band is Norflex, but its distribution is discontinued in the US market.
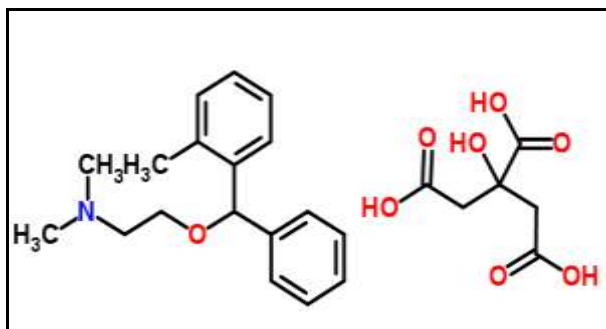
Figure 1: Structure of Orphenadrine Citrate

Paracetamol:

Paracetamol is also known as Acetaminophen belongs to the chemical class of p-amino phenol used mainly as Antipyretic and mild analgesic agent. It is chemically N-(4-hydroxyphenyl) acetamide. It has lot of uses which includes the treatment of fever, osteoarthritis, lower back pain, postoperative pains, headaches, tooth pain etc., as it is generally safe at recommended doses. It was discovered in 1877 [3].Around 31 thousand patents are issued for this drug [4]. Popular brand among them areCalpol, Dolo, Pacemol, Dispar etc.
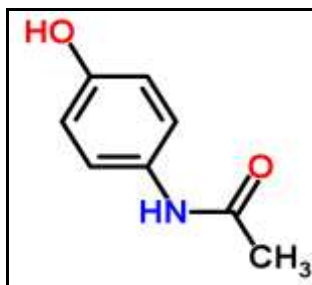

Figure 2: Structure of Paracetamol

Combination of Orphenadrine citrate and Paracetamol are used in the treatment of headaches caused due to tension and muscles spasm in the posterior parts of head and neck. The commonly available brands of these drugs are as follows, Norgesic by Valeant Pharmaceuticals, Myogesic by Dar Al Dawa, Orphamol by Torrent Pharmaceuticals etc. In most of the combination around 450 mg of Paracetamol and 35 mg of Orphenadine citrate was found.

The various analytical and bioanalytical methods are already developed for the simultaneous estimation of the combination of both the drugs. Few of the methods include, RP-HPLC [5], Derivative UV Spectroscopy [6], Capillary Electrophoresis [7], Thin Layer Chromatography [8] etc. From the review of literature it was found that all the methods were a bit complicated and were not cost effective methods. Most of the methods were of HPLC which required multiple steps in setting up the systems which was time consuming and also costly, but the method was sensitive. Whereas, other methods also were not that simple. As there were no simultaneous equation method in literature, we employed simultaneous equation method for the simultaneous estimation of both the drugs, as the method is simple, less time consuming and cost effective.

Materials & Method:

Equipment's used:

The instruments used in the study were as follows, UV/Visible Spectrophotometry 1800 series from Shimadzu, Japan. Digital analytical balance AX200 series was from Shimadzu, Japan. Sonicator (Vibra cell ultrasonicator) was also used in the study.

Reagents and materials:

Paracetamol was gift sample from Microlabs, Bangalore and Orphenadrine Citrate were gift samples from RL Fine Chem, Bangalore. Methanol was used as solvent which is of analytical grade procured from Merck (India). Volumetric flask of capacity 10, 50, 100 mL, Beakers, 2, 10 mL pipettes were used in the study made of borosilicate glass.

Preparation of Solutions:

Standard Stock Solutions Preparation:

Paracetamol (1000 μg/mL):

Accurately 100 mg of paracetamol was transferred into 100 mL volumetric flask. The drug was dissolved in 20 mL of methanol. The volume was made up to the mark using the same solvent.

Orphenadrine Citrate (1000 μg/mL):

Accurately 100 mg of orphenadrine citrate was transferred into 100 mL volumetric flask. The drug was dissolved in 20 mL of methanol. The volume was made up to the mark using the same solvent.

Working Standards Preparation:

Paracetamol (100 μg/mL):

About 5 mL of paracetamol standard stock solution was pipetted out into 50 mL volumetric flask. The volume was made up to the mark using methanol.

Orphenadrine Citrate (100 μg/mL):

About 5 mL of orphenadrine citrate standard stock solution was pipetted out into 50 mL volumetric flask. The volume was made up to the mark using methanol.

Calibration Curves Preparation:

Calibration curve for Paracetamol:

From the above paracetamol working standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL were pipetted out into a series of 10 mL volumetric flask. The volume were made up to the mark using methanol to obtain the concentration of 2 to 12 μg/mL. Absorbance of each solution was measured at 248 and 202.80 nm with methanol as blank. Concentration of paracetamol v/s obtained absorbance graph was plotted to obtain calibration curve. Regression equation was determined.

Calibration curve for Orphenadrine Citrate:

From the above orphenadrine citrate working standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL were pipetted out into a series of 10 mL volumetric flask. The volume were made up to the mark using methanol to obtain the concentration of 2 to 12 μg/mL. Absorbance of each solution was measured at 248 and 202.80 nm with methanol as blank. Concentration of orphenadrine citrate v/s obtained absorbance graph was plotted to obtain calibration curve. Regression equation was determined.

Simultaneous Equation Method:

After the preparation of working standard 100 μg/mL for both the drugs, each solutions were run for spectrum in order to determine the λ max of the drugs in the entire UV range with methanol as blank. The λ max for paracetamol and orphenadrine citrate were found to be 248 and 202.8 nm respectively. After knowing the λ max calibration curves were developed for both the drugs in the linearity range of 2 to 12 μg/mL. Three independent determination of the linearity ranges were individually measured for the absorbance's in both the wavelengths. Using the Lambert-Beers equation the absorptivity were determined for all the concentrations using absorbance. After taking the mean of absorptivity for all the sets, 4 absorptivity's are obtained that is ax1

and ax2 for Paracetamol and ay1 and ay2 for Orphenadrine citrate. Two simultaneous equations were developed using the absorptivity values. The equation was computed in such away that concentration of sample was able to be determined from the below equations.

CX= A2ay1-A1ay2/ax1ay2-ax2ay1

CY= A1ax2-A2ax1/ay2ax1-ax2ay1

Where CX and CY are concentrations of Paracetamol and Orphenadrine Citrate present in sample respectively. A1 and A2 are absorbance's of mixture at 248 and 202.8 nm respectively.

Sample Solution Preparation:

For the estimation of drug in tablet formulation, average weight of 20 tablets were taken. The tablets were crushed into fine powder. The amount of sample to be taken in such a way that about 10 mg of paracetamol and about 1.4 mg of orphenadrine citrate should be present in the sample weighed into 100 mL volumetric flask. About 8.6 mg of standard orphenadrine citrate should be weighed and transferred to the same volumetric flask. Sample was dissolved in 20 mL of methanol with the help of sonicator and the volume was made up to the mark with the same solvent. Further dilutions were made in such away that the final concentration of both the drug will be 8 µg/mL. The absorbance was measured at 248 and 202.8 nm and concentration was determined by using simultaneous equation.

Method validation:

The developed method was validated as per ICH guidelines for the consecutive parameters. Linearity and Range, Limit of detection (LOD), Limit of Quantitation (LOQ), Precision, Accuracy and Robustness.

Linearity & Range:

Six point linearity curve was developed for both paracetamol and orphenadrine citrate in the range of 2 to 12 µg/mL. Linearity was checked in terms of slope, intercept and correlation coefficient.

Precision:

Precision refers to the closeness of individual values to each other. Precision may be measured for three stages in the analytical method validation, which includes interday precision, intraday precision and repeatability.

i) Intraday precision:

Solutions of concentrations 2, 6 and 12 µg/mL of both the drugs absorbance measured at different time intervals in the same day and %RSD is determined.

ii) Interday precision:

Solutions of concentrations 2, 6 and 12 µg/mL of both the drugs absorbance measured on three successive days and %RSD is determined.

iii) Repeatability:

Concentration of 6 µg/mL were prepared for 6 times and the absorbances were measured to check the method precision. Co-efficient of variation was not more than 2%.

Accuracy:

Accuracy expresses the degree to which the results of sample comply with that of standard concentrations. In order to check the accuracy recovery studies are carried out at three different levels that is 80, 100 and 120%. This involves the spiking of standard drug with that of constant concentration of drug in the tablet formulation.

Limit of Detection:

Limit of detection is the minimum concentration of drug that can be detected by the developed method. It is calculated by using the below formula,

$$LOD = \frac{3.3 \text{ X Standard deviation from concentration}}{\text{Slope from caliberation curve}}$$

Limit of Quantitation:

Limit of Quantitation is the minimum concentration of drug that can be quantified by the proposed method. It can be calculated using the formula

$$LOQ = \frac{10 \text{ X Standard Deviation from Concentration}}{\text{Slope from calibration curve}}$$

Robustness:

Robustness of the method refers to a slight change in the method conditions like wavelength in case of UV Spectroscopy should not cause changes in the results. The robustness is measured for $\pm$ 2 nm change in wavelength. For Paracetamol, absorbance is measured at 246 and 250 nm respectively. Whereas, for orphenadrine citrate the absorbance is measured at 200.8 and 204.8 nm respectively.

Results & Discussion:

Selection of wavelength for simultaneous estimation of Paracetamol and Orphenadrine Citrate:

To determine the wavelength for the estimation the sample is exposed to UV radiations from 200 to 400 nm. Absorbance maxima obtained for paracetamol and orphenadrine citrate were yet 248 and 202.8 nm respectively. Overlay spectra of both the drug at same concentration is shown in **Figure 3.**
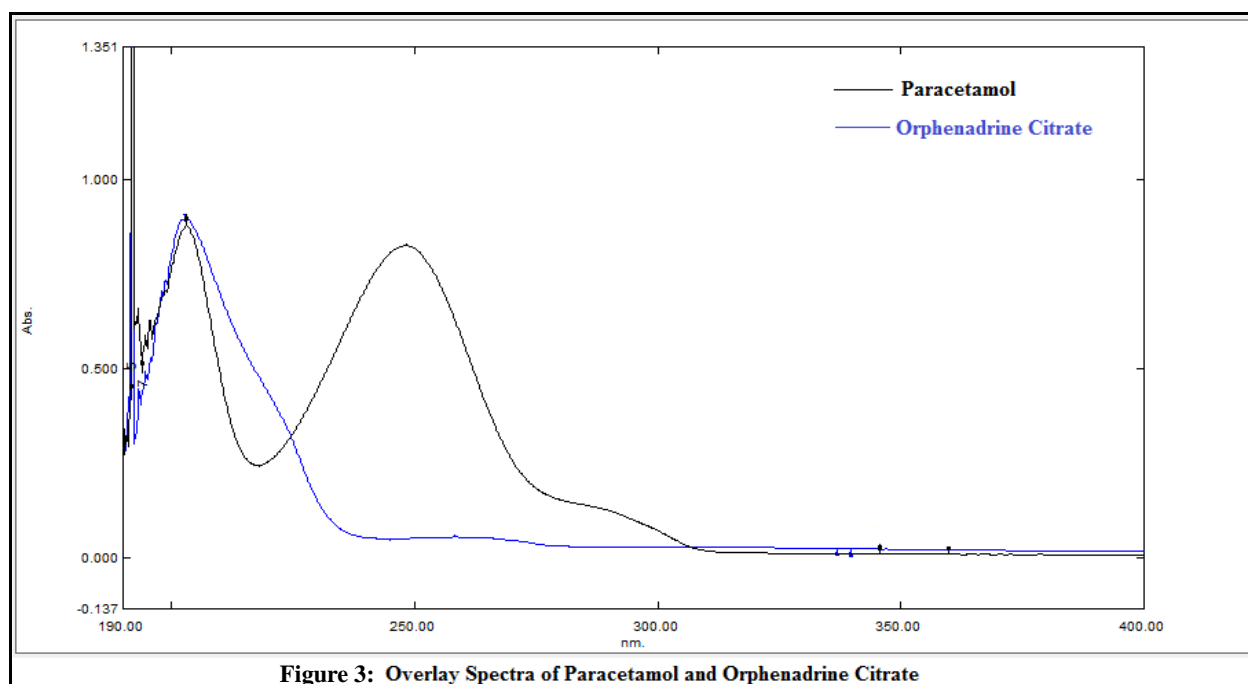

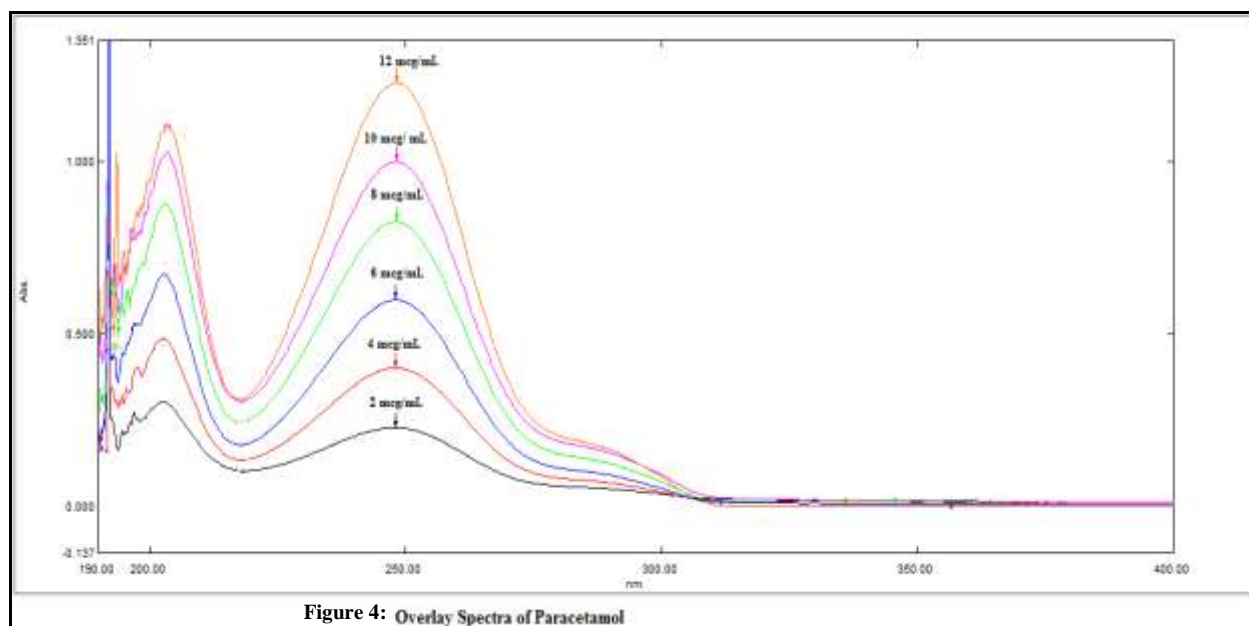**Figure 3: Overlay Spectra of Paracetamol and Orphenadrine Citrate**

Method Validation:

- **Linearity and range:**

The linearity for both the drugs i.e. paracetamol and orphenadrine citrate ranges from 2 to 12 µg/mL. Linearity data's are discussed in Table 1 for paracetamol and orphenadrine citrate.

Table 1: Linearity Data of Paracetamol and orphenadrine citrate

Drug	Concentration (µg/mL)	Absorbance mean ± S.D	
		248 nm	202.8 nm
Paracetamol	2	0.237±0.01	0.297 ± 0.002
	4	0.428± 0.012	0.481 ± 0.007
	6	0.627 ± 0.027	0.662 ± 0.003
	8	0.843 ± 0.023	0.862 ± 0.002
	10	1.057 ± 0.003	0.993 ± 0.002
	12	1.270 ± 0.033	1.185 ± 0.010
Orphenadrine Citrate	2	0.256 ± 0.018	0.052 ± 0.008
	4	0.462 ± 0.005	0.064 ± 0.002
	6	0.577 ± 0.023	0.075 ± 0.004
	8	0.748 ± 0.025	0.082 ± 0.008
	10	0.912 ± 0.008	0.093 ± 0.001
	12	1.026 ± 0.013	0.100 ± 0.002

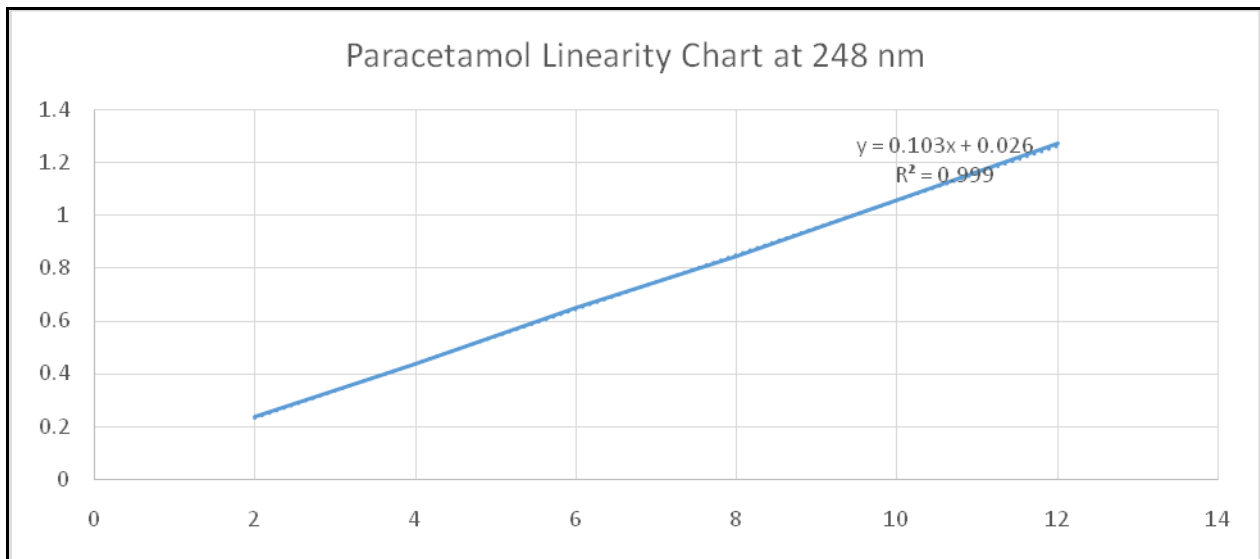**Figure 4:** Overlay Spectra of Paracetamol

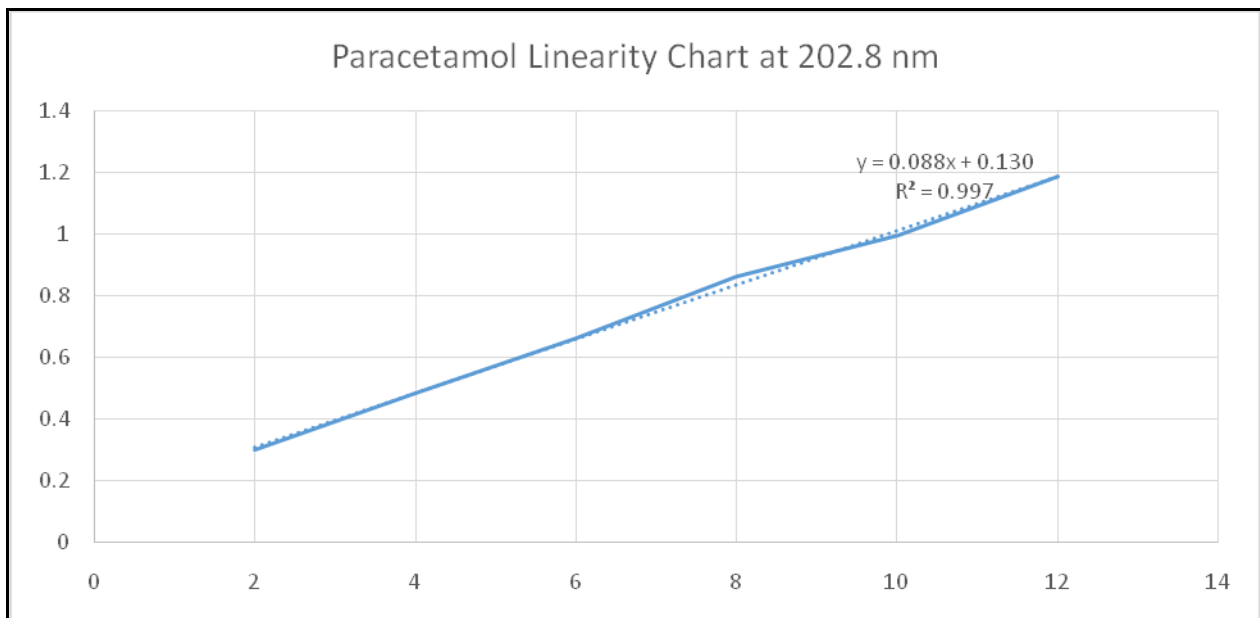**Figure 5: Linearity graph of paracetamol at 248 nm**

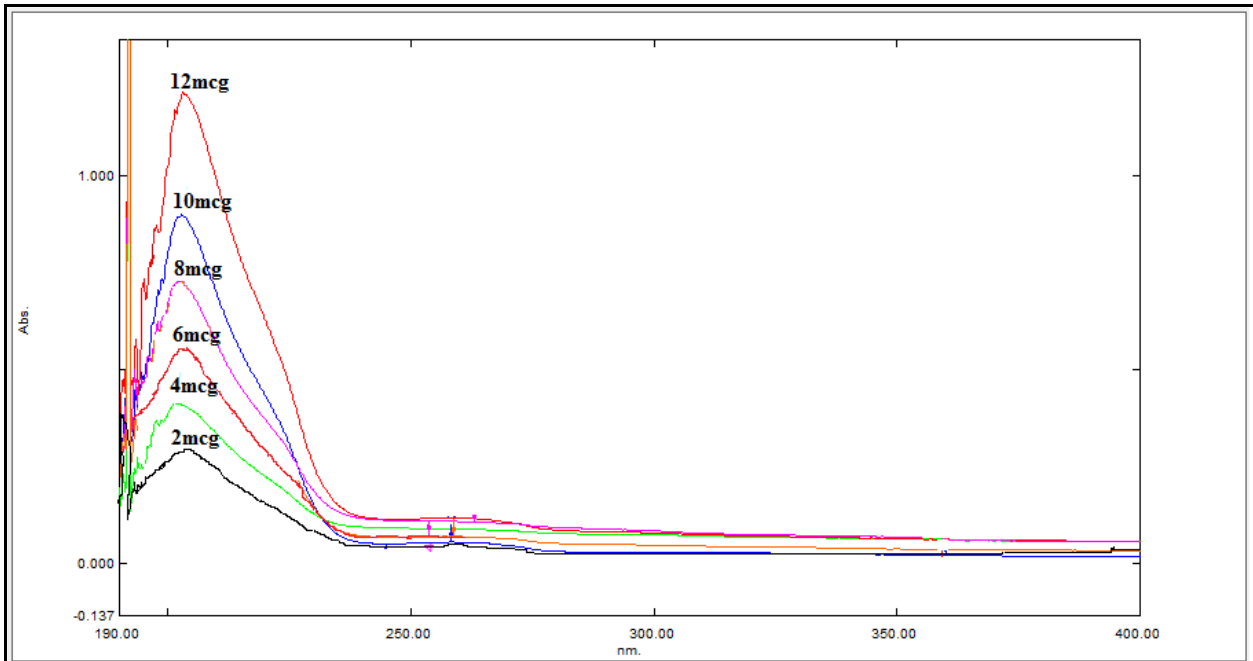**Figure 6: Linearity graph of paracetamol at 202.8 nm**

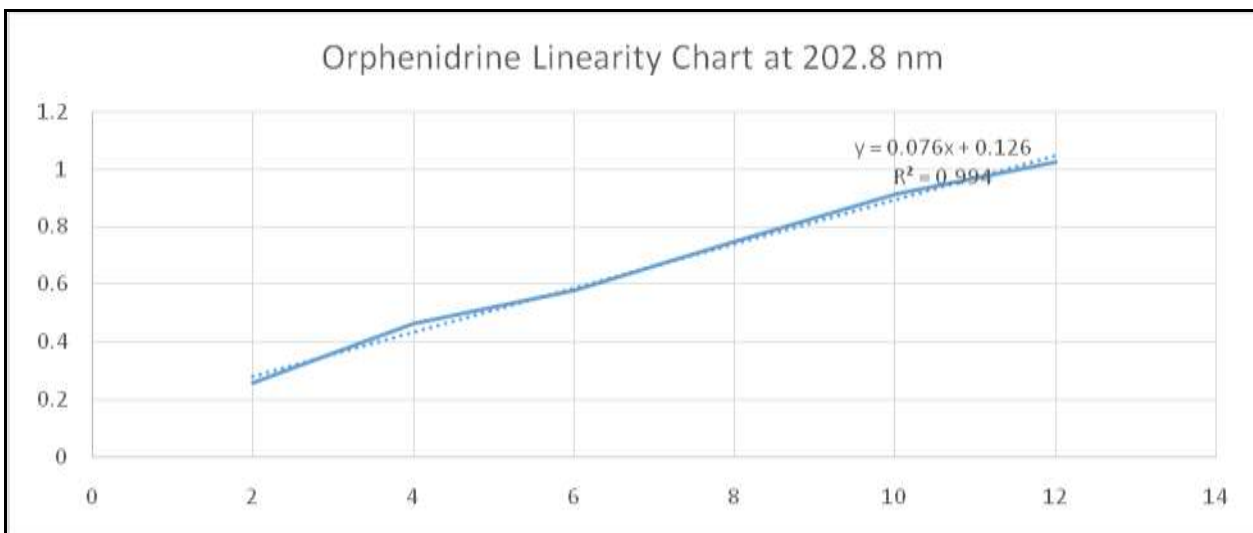**Figure 7:** Overlay Spectra of Orphenadrine Citrate

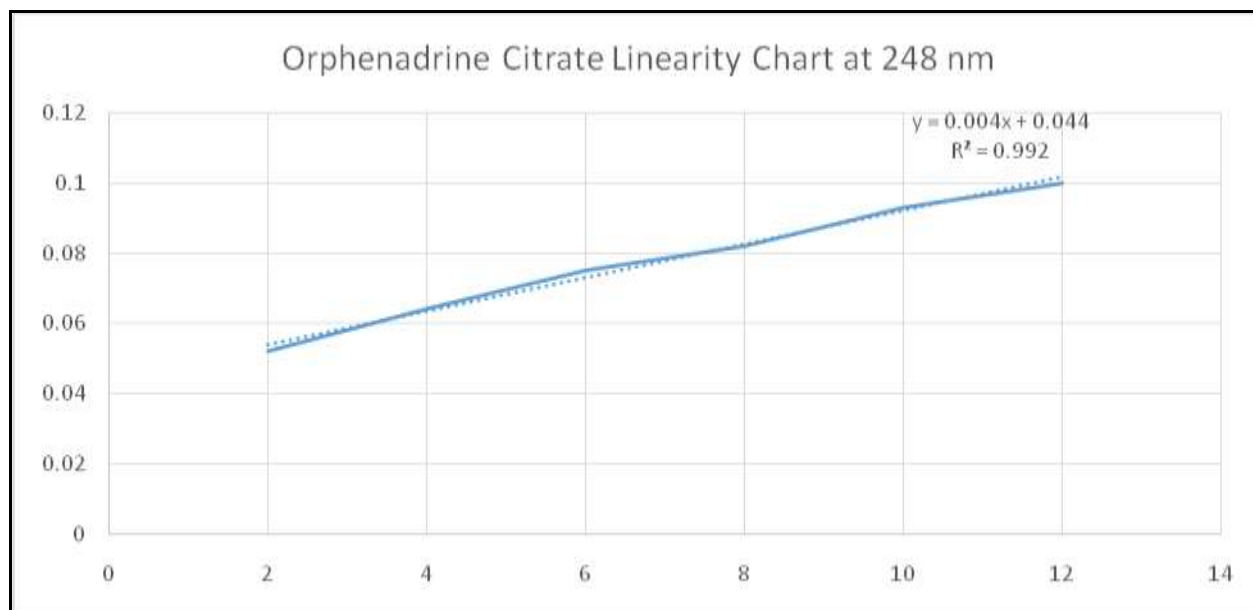**Figure 8: Linearity graph of orphenadrine citrate at 202.8 nm**

Figure 9: Linearity graph of orphenadrine citrate at 248 nm

- **Precision:**

1. **Intraday precision:**

The intraday precision for Paracetamol % RSD was found to be 0.14 to 0.42% for 248 nm and 0.025 to 0.15% for 202.8 nm respectively. Orphenadrine citrate showed %RSD of 0.12 to 0.58% and 0.023 to 0.54% at 202.8 and 248 nm respectively.

2. **Interday precision:**

The interday precision for Paracetamol % RSD was found to be 0.28 to 1.18 % for 248 nm and 0.096 to 1.01% for 202.8 nm respectively. Orphenadrine citrate showed %RSD of 0.52 to 1.86% and 0.35 to 1.64% at 202.8 and 248 nm respectively.

3. **Repeatability:**

The repeatability results for paracetamol was found to be 0.058 and 0.042% at 248 and 202.8 nm. For orphenadrine citrate % RSD was found to be 0.12 and 0.096% at 202.8 and 248 nm respectively.

- **LOD and LOQ:**

The Limit of detection value for Paracetamol and Orphenadrine Citrate was found to be 0.475 and 0.610 µg/mL. The Limit of Quantitation value for Paracetamol and Orphenadrine Citrate was found to be 1.44 and 1.85 µg/mL respectively.

- **Accuracy:**

Accuracy of the method was confirmed by the recovery studies in three levels that is 80, 100 and 120%. The % recovery was found to be in the range of 94.5 to 100.3% for Paracetamol and 99 to 100.5% for orphenadrine citrate. The recovery study data's are given in Table 2.

Table 2: Recovery study data's of Paracetamol and Orphenadrine Citrate

Drug	% level of Recovery	Amount of drug taken (µg/mL)	Amount of drug Spiked (µg/mL)	Total amount of drug found (µg/mL)	% Recovery
Paracetamol	80	4	2	6.02 ± 0.05	100.3%
	100	4	4	7.56 ± 0.23	94.5%
	120	4	6	9.98 ± 0.12	99.8
Orphenadrine Citrate	80	4	2	5.94 ± 0.18	99%
	100	4	4	8.04 ± 0.02	100.5%
	120	4	6	9.92 ± 0.03	99.2%

- **Analysis of marketed formulation:**

  The analysis of Myogesic tablets, which contained 450 mg of paracetamol and 35 mg of orphenadrine citrate was crossed checked with the developed simultaneous equation. The obtained results are shown in Table 3.

Table 3: Analysis of marketed formulation

Tablet	Drugs	Label Claim (mg)	Amount found (mg) (n=3)	% Label Claim
Myogesic	Paracetamol	450	448.93	98.42%
	Orphenadrine Citrate	35	34.82	99.48%

Table 4: Regression analysis report and summary of validation parameters for the proposed method

Parameters	Paracetamol		Orphenadrine Citrate	
Wavelength (nm)	248	202.8	202.8	248
Beer's law limit (µg/mL)	2-12	2-12	2-12	2-12
Regression equation (y=mx+c)	y= 0.1031x+ 0.0269	y= 0.0881x+ 0.1304	y= 0.0767x+ 0.1264	y= 0.0048x+ 0.0443
Slope (m)	0.1031	0.0881	0.0767	0.0048
Intercept (c)	0.0269	0.1304	0.1264	0.0443
Correlation Coefficient (R^2)	0.9998	0.9979	0.9944	0.9927
Repeatability (n=6) (%RSD)	0.058	0.042	0.12	0.096
Intraday (n=3) (%RSD)	0.14-0.42	0.025-0.15	0.12-0.58	0.023-0.542
Interday (n=3) (%RSD)	0.28-1.18	0.096-1.01	0.52-1.86	0.35-1.64
LOD (µg/mL)	0.475		0.610	
LOQ (µg/mL)	1.44		1.85	

Conclusion:

  A simple, precise, accurate, less time consuming and cost effective method was developed for the simultaneous estimation of multidrug component system of myogesic tablets. The method is suitable for multicomponent analysis as the interference of one drug with the estimation of other drug is nil in case of simultaneous equation spectrophotometric method. The method can be employed in the estimation of drug in both API and formulations. It can also be used as quality control tool in manufacturing. If extended the studies to minor concentrations, it can be employed in therapeutic drug monitoring.

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