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Simultaneous Estimation and Validation of Paracetamol and Phenylephrine Hydrochloride in Powder Form by Using U.V. Spectrometric Method

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Abstract-A simple, rapid, accurate, precise and economic spectrophotometric method based on simultaneous equation for simultaneous estimation of Paracetamol and Phenylephrine HCl in pure powder form has been developed. Method is based on solving simultaneous equation. Paracetamol and Phenylephrine HCl showed maximum absorbance at 240 and 270 nm respectively, so absorbance was measured at 240 and 270 nm wavelengths for estimation of Paracetamol and Phenylephrine HCl respectively. Paracetamol and Phenylephrine HCl both the drugs obeys beer Lambert's law in the concentration range of 1-10µg/ml with correlation coefficient of 0.9971 and 0.9966 respectively. Method was developed and validated as per ICH guidelines and can be applied for the routine estimation of the Paracetamol and Phenylephrine HCl in pure powder form.

Keywords-Paracetamol, Phenylephrine HCl, U.V. Spectrophotometer, Simultaneous equation, Validation.

1. INTRODUCTION

Paracetamol (PARA) is chemically N-(4-hydroxyphenyl) acetamide. Paracetamol freely soluble in ethanol (95%) and in acetone, sparingly soluble in water, very slightly soluble in dichloromethane and in ether. Paracetamol has been recrystallisedfrom water and dried at pressure of 2 kPa at 70 degree. Paracetamol contain not less than 99.0% and not more than 101.0% of C8H9NO2 calculated on dried basis.[1] Various analytical methods, such as, spectrophotometry, High performance liquid chromatography(HPLC), High performance thin layer chromatography (HPTLC) have been reported for the estimation of Paracetamol from its formulation.[2]Paracetamol or acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics. The onset of analgesia is approximately 11 minutes after oral administration of Paracetamol, and its half-life is 1-4 hours. Though acetaminophen is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity.[3]Hepatic necrosis and death have been observed following over dosage; hepatic damage is likely in an adult who takes more than 10 g in a single dose or if a 2-year old child takes more than 3 gm. Usual oral adult dose is 500 mg to 1 g for three or four times a day.[4]
Phenylephrine hydrochloride (PHE) is chemically (R)-1-(3-hydroxyphenyl)-2 methyl amino ethanol hydrochloride is a direct sympathomimetic agent, a selective α1 agonist, causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations. Phenylephrine hydrochloride is widely used as a decongestant drugs and available as an oral medicine or as a nasal spray. Phenylephrine is rarely used as a vasopressor to increase the blood pressure in unstable patients with hypotension.[5]
Phenylephrine hydrochloride syrup is used for relieving congestion, cough and preventing or treating symptoms such as runny nose, sneezing, itching of the nose and throat, watery eyes due to colds, flu, or hay fever. Various analytical techniques have been reported in the literature for the analysis of Phenylephrine hydrochloride including, titrimetry fluorometry, ion pair chromatography, High-performance liquid chromatography, micellar liquid chromatography, micellar electrokinetic chromatography, capillary zone electrophoresis and flow Injection analysis with chemiluminescence detection.[6]

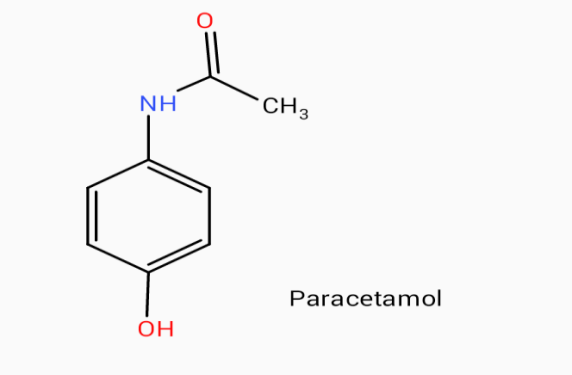

Figure 1: Chemical Structure of Paracetamol

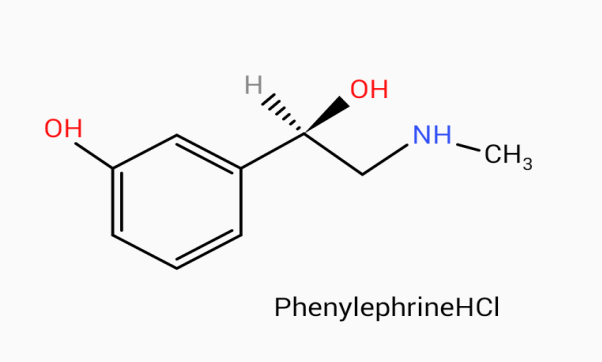

Figure 2: Chemical Structure of Phenylephrine Hydrochloride.

2. METHOD AND MATERIAL

Preparation Of Standard Stock Solution

About 100 mg of Paracetamol and Phenylephrine HCl pure drug was weighed accurately and transferred in to 100 ml of volumetric flask respectively. The volume was made up to 100 ml using distilled water to obtain a solution that has a concentration 1000 µg/ml. 1 ml of above solution was taken and diluted up to 10 ml using distilled water to obtain a solution that has a concentration 100 µg/ml. The above 10 ml solution was taken and diluted up to 100 ml using distilled water to obtain a solution that has a concentration 10 µg/ml. From above stock solution 1ml, 2 ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml solution were taken in separate volumetric flask and diluted using distilled water up to 10 ml to each to obtain solution that has concentration 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml and 10µg/ml respectively.

Calibration Curve

The standard solutions of PARA and PHE were diluted with methanol individually to get the concentration of 10µg/ml and 10µg/ml respectively and were scanned in the UV range 400-200nm. The λmax of both the drugs were found to be 240nm and 270 nm respectively. The spectral data was processed to obtain first order derivative spectrum at wavelength interval of 2 nm and scaling factor 10 for the range of 400-200nm with scanning speed of 400nm/min. It was observed that PARA shows zero crossing at 240nm while PHE shows zero crossing at 270nm. At zero crossing point of PARA (240nm), PHE showed a measurable dA/dλ whereas at zero crossingpoint of PHE (270nm), PARA showed a measurable dA/dλ. Hence the wavelengths 240nm and 270nm were selected as analytical wavelengths for determination of PARA and PHE first order derivative method respectively.[7]

Preparation Of Sample Solution

About 100 mg of Paracetamol and Phenylephrine HCl pure drug was weighed accurately and transferred in to 100 ml of volumetric flask respectively. The volume was made up to 100 ml using distilled water to obtain a solution that has a concentration 1000 µg/ml. 1 ml of above solution was taken and diluted up to 10 ml using distilled water to obtain a solution that has a concentration 100 µg/ml. The above 10 ml solution was taken and diluted up to 100 ml using distilled water to obtain a solution that has a concentration 10 µg/ml. From above stock solution 1ml, 2ml, 3ml, 4ml and 5ml of Paracetamol and Phenylephrine HCl in combination were taken and diluted up to 10ml to obtain 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml respectively.

Simultaneous Equation Method

From the overlain spectra of Paracetamol (10µg/mL) and Phenylephrine HCl (10µg/mL), two wavelengths i.e. 240 nm as λ max of Paracetamol and 270 nm as λ max of Phenylephrine HCl were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 240 nm and 270 nm. A set of two simultaneous equations were formed using absorptivity values as given below, at selected wavelength. The concentrations of two drugs in mixture were calculated using set of two simultaneous equations.

$$Cx = \frac{A2\ aY1 + A1\ aY2}{aX2\ aY1 + a\ X1\ aY2} \quad (1)$$

$$Cy = \frac{A1\ aX2 - A2\ aX1}{aX2\ aY1 + aX1\ aY2} \quad (2)$$

Where, Cx and Cy are concentrations of Paracetamol and Phenylephrine HCl in µg/mL respectively in known sample solution. A1 and A2 absorbances of sample solutions at 240 nm and 270nm respectively. ax1 and ax2 are absorptivity of Paracetamol at 240 nm and 270 nm, ay1 and ay2 are absorptivity of Phenylephrine HCl at 240 nm and 270 nm. The concentration of Cx and Cy can be obtained by solving equation (1) and (2). Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.[8]

3. VALIDATION

Linearity:

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range was found to be 1-10 µg/ml for Paracetamol and 1-10 µg/ml for Phenylephrine HCl.[9]the linearity data for method is presented in **Table**

Accuracy:
To check the accuracy of the proposed method recovery studies were carried out at 80, 100,120 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level.[10] The results of the recovery studies are reported in Table –

Precision
Precision was determined by studying the repeatability and intermediate precision.
Interday and Intraday precision
The interday and intraday precision was determined by assay of the sample solution on the interday precision was determined by assay of the sample solution on the same day and on different days at different time interval respectively.[11]

Limit of detection (LOD) and Limit of Quantification (LOQ)
The Limit of Detection (LOD) is the smallest concentration of analyte that give the measurable response. LOD was calculated using the following formula and shown in Table no…..

LOD = 3.3(σ / s)
Where,
S = Slope of calibration curve
σ= standard deviation of the response.
The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table no.

LOQ = 10 (σ / s)

Where,

S = slope of calibration curve
σ= standard deviation of the response.[12]

4. RESULT

Linearity range for Paracetamol and Phenylephrine are 1-10 µg/mL and 1-10 µg/mL at respective selected wavelengths. The coefficient of correlation for Paracetamol at 240 nm and for Phenylephrine at 270 nm is 0.9971 and 0.9966 respectively. Both drugs shows good regression values at their respective wavelengths and the results of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

Table 1: Calibration curve of Paracetamol and Phenylephrine

Sr. No.	Concentration	PARA	PHE
1	1 µg/ml	0.093	0.029
2	2 µg/ml	0.163	0.035
3	3 µg/ml	0.243	0.048
4	4 µg/ml	0.315	0.056
5	5 µg/ml	0.393	0.071
6	6 µg/ml	0.466	0.083
7	7 µg/ml	0.546	0.094
8	8 µg/ml	0.630	0.103
9	9 µg/ml	0.698	0.113
10	10 µg/ml	0.772	0.128

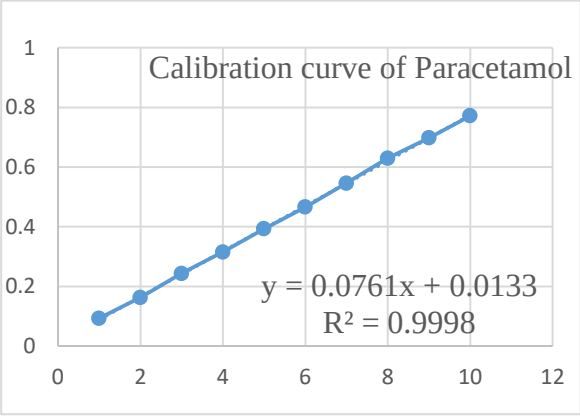

Fig 3: Calibration curve of Paracetamol

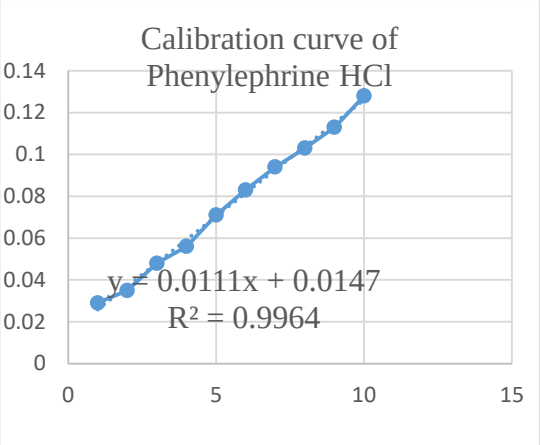

Fig 4: Calibration curve of Phenylephrine HCl

Table 2: Optical Characteristics and Validation Parameter

Sr. No.	Parameter		
	Drugs	PARA	PHE
1	Working nm	240	270
2	Beers Law Limit µg/ml	1-5	1-5
3	Correlation Coefficient	0.9971	0.9966
4	Regression equation (y =a + bc)	Y=0.0825x +0.0069	Y=0.033x+0.0063
5	Slop	0.0825	0.0333
6	LOD	0.101	0.199
7	LOQ	0.306	0.606

Table 3: Precision data for developed method

	Day 1		Day 2		Day 3	
	PARA	**PHE**	**PARA**	**PHE**	**PARA**	**PHE**
1 µg/ml	79%	61%	99%	161%	108%	199%
2 µg/ml	80%	65%	89%	114%	88%	103%
3 µg/ml	81%	76%	87%	107%	94%	134%
4 µg/ml	76%	73%	82%	111%	81%	93%
5 µg/ml	76%	69%	81%	90%	81%	83%

5. CONCLUSION

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for simultaneous estimation of Paracetamol and Phenylephrine in powder for as well as formulation.

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