ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Simultaneous Estimation Of Paracetamol And Phenylephrine Hydrochloride In Pharmaceutical Dosage Form By High Performance Liquid Chromatography.

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ABSTRACT

A simultaneous method of phenylephrine hydrochloride and paracetamol was developed. The separation was achieved by kromasil C18 column having dimensions of 250 mm x 4.6mm internal diameter, with column oven temperature of 30^{0C}. Mobile phase was selected acetonitrile: 0.1% ortho phosphoric acid in the ratio of 45:55 with flow rate of 1ml/min. Detection was carried out at 211nm. Retention time of phenylephrine hydrochloride and paracetamol were found respectively on 1.8 and 2.5. The method was validated as per ICH guidelines with linearity in the range of 0.2-3µg/mL and 20- 300 µg/mL respectively of Phenylephrine hydrochloride and Paracetamol. The developed method was found to be precise, accurate and sensitive for simultaneous estimation of phenylephrine hydrochloride and paracetamol in pharmaceutical dosage form.

Keywords: acetonitrile, accuracy, precision, paracetamol, phenylephrine hydrochloride.

https://doi.org/10.33887/rjpbcs/2022.13.4.8

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ISSN: 0975-8585

INTRODUCTION

Phenylephrine hydrochloride (PHE) is chemically designed as 3-(1-hydroxy-2-methyl-amino-ethyl) phenol hydrochloride and have molecular formula of $C_9H_{13}NO_2$.HCl.[1] (fig-1). It is alpha-adrenergic sympathomimetic agents used as a decongestants[2-3]. Extensive literature survey revealed various methods available for the estimation of PHE in single and combined dosage form, such as simultaneous method development of ebastine and phenylephrine hydrochloride by RP-HPLC [3], Nimesulide, phenylephrine hydrochloride, chlorpheniramine maleate and caffeine by RP-HPLC [2], RP-HPLC method development of five drug combination[4]. Spectrophotometric method for phenylephrine hydrochloride [1].

Paracetamol (PCM), is chemically N-(4-hydroxyphenyl) acetamide[5-6] (fig-2) and it is used as antipyretic and analgesic [7]. This drug is officially present in Indian pharmacopoeia [8], British pharmacopoeia [9] and United state pharmacopoeia [10]. Various methods are available with single and combination of two and three drugs [11-12] but no methods available for simultaneous study of Phenylephrine hydrochloride and paracetamol.

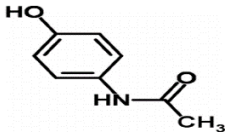

Figure 1: Structure of paracetamol

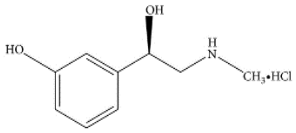

Figure 2: Structure of phenylephrine hydrochloride

MATERIAL AND METHODS

Instrumentation

Agilent infinity-II HPLC with UV detector and enable kromasil C18 column (250 x4.6 mm, 5µm) was used for chromatographic study of target analyte. The acquisition and integration of data was performed by using openlab ezchrom workstation software.

Materials and Reagents

Pure Phenylephrine hydrochloride and paracetamol were obtained as gift sample from Deccan pharma Uttrakhand India, HPLC grade methanol, acetonitrile was purchased from Qualigens India.

Experimental

Solution Preparation

Preparation of standard stock solution

Accurately weighed 50mg of paracetamol and 10 mg of phenylephrine hydrochloride transferred to 25 mL volumetric flask separately. It was dissolved in 20mL, 0.01N sodium hydroxide and sonicated to dissolve it, further volume was made up through 0.01 N sodium hydroxide to obtained the concentration of2000µg/mL and 400 µg/mL respectively, after that10 ml of paracetamol and 0.5mL of phenylephrine hydrochloride was pipetted out from stock solution and transfer it to 100 mL volumetric flask and make the volume up to the mark with mobile phase and prepare the concentration of 200 µg/mL and 2 µg/mL respectively.

ISSN: 0975-8585

Preparation of sample solution

Paracetamol and phenylephrine hydrochloride tablet in ratio of 500and5mg (label claimed of Sanizof, Geno pharmaceuticals Ltd) was prepared in laboratory. Twenty tablets were weighed and finely grounded to fine powder. From this powdered tablet, weigh the powder material equivalent to 500 mg of paracetamol and 5 mg of phenylephrine hydrochloride. Transfer it in a clean and dry 100 mL of volumetric flask and diluted it with 0.01 N sodium hydroxide followed by sonicate it for 15 minutes. Filter the solution through suitable 0.45 µ syringe filter and filtrate was diluted with mobile phase and prepare the concentration of 200µg/mL of paracetamol and 2µg/mL of phenylephrine hydrochloride.

RESULTS AND DISCUSSION

Method optimization

HPLC system was used to analyze both compound with sufficient separation and fine peak shapes with in a short analysis time there for all experiments were carried out on kromasil C18 column (250 x4.6 mm, 5µm) by trying various mobile phase condition. After the initial experimental trials, the optimum condition was found to be the mobile phase of acetonitrile: 0.1% ortho phosphoric acid (45:55 v/v) with 1mL/min flow rate at 30^{0c} oven temperature and 211nm UV detection length (Figure-3). At optimized conditions PCM and PHE were eluted at retention time of 2.5 and 1.8 respectively. (Figure-4)

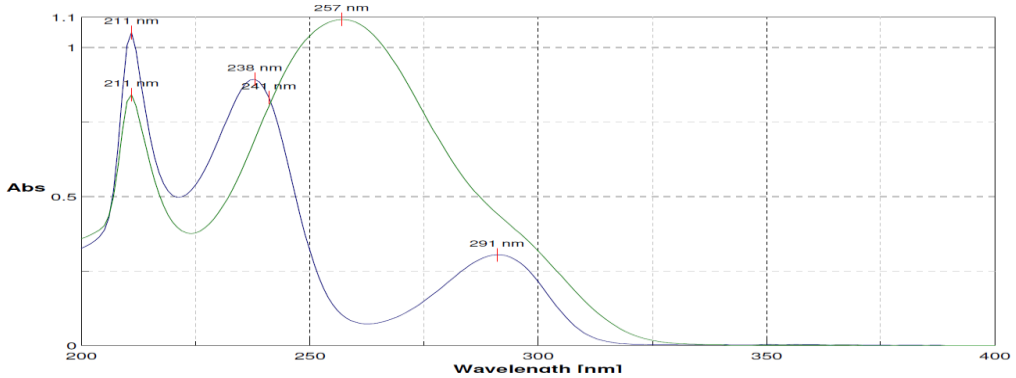

Figure 3: Overlay Q point spectra for selection of wavelength

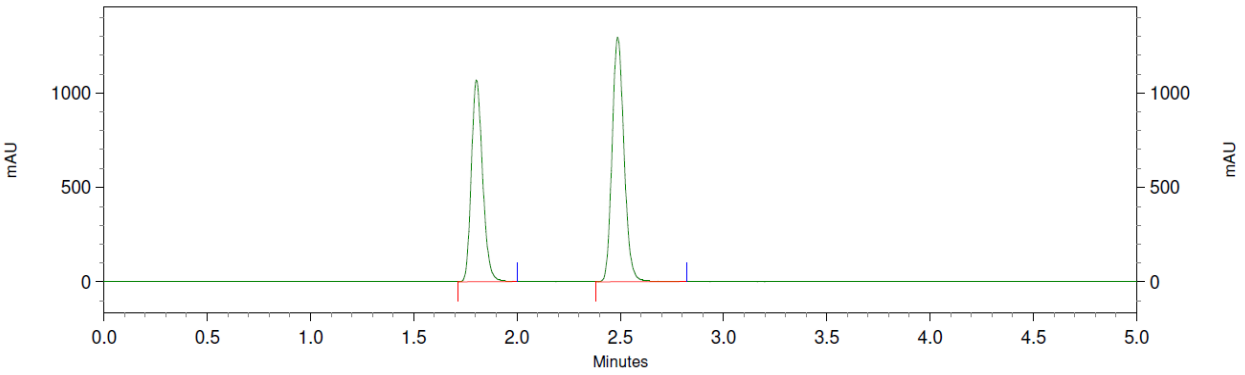

Figure 4: Determination of retention factor of Paracetamol and Phenylephrine hydrochloride

Absorption maxima of Phenylephrine hydrochloride appear at 211 nm, 238 nm & 291 nm. and absorption maxima of Paracetamol were appeared at 211 nm ,257 nm. but overlay Q point at 211 nm, 241 nm. Therefore, both drugs exhibit significant absorption at 211 nm and 241 nm wavelength. Hence 211 or 241 nm wavelength can be used for chromatography development but peak area of phenylephrine hydrochloride was not under acceptable limit at 241nm.Hence 211 nm wavelength finalized for optimization of proposed method development and validation.

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System suitability

Suitability test was performed to the chromatograms and to check the various parameter like column efficiency not less than 2000 theoretical plates, asymmetry not more than 2 and analysis time was not more than 10 minutes for both drugs.

Method validation

The method was validated accordingly ICH guideline as to linearity, range, limit of detection, limit of quantification, accuracy, precision and specificity.

Linearity

The solution of PCM and PHE were analysed at different concentration range of 20-300µg/mL and 0.2- 3 µg/mL. (Figure-5) The graph of the peak area against concentration proved linear. Five different concentration of phenylephrine hydrochloride and paracetamol within the linear range were analysed. Each concentration solution was prepared in triplicates. (Table-1)

Table 1: Linearity data (n=5)

Parameters	PCM	PHE
Regression equation	y=7,61,136.06x+32,54,429.93	y = 743862x - 4226.6
Correlation coefficient	0.9993	0.9997
Linearity range (µg/mL)	20-300	0.2-3
LOD(µg/mL)	4.57	0.015
LOQ(µg/mL)	13.84	0.044

*Where y is the peak area and x is the concentration in µg

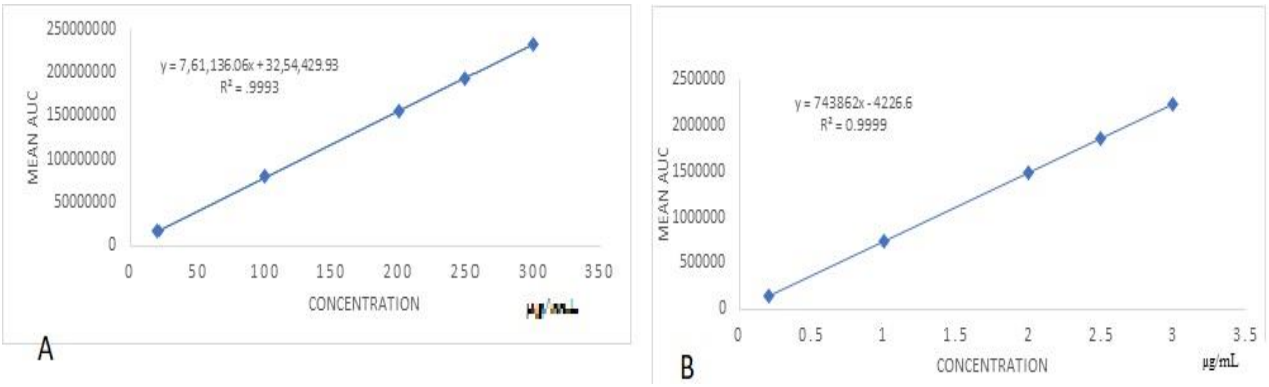

Figure 5: Linearity graph of Paracetamol(A) and Phenylephrine hydrochloride(B)

Limit of detection and Limit of quantification

Limit of detection and limit of quantification were identified by the use of linearity regression equation and standard deviation of residuals. (Table-1)

LOD= 3.3*standard deviation of residuals/slope

LOQ= 10* standard deviation of residuals/slope

Accuracy

Standard addition method was used to determine the accuracy. In this method known amounts of PCM and PHE were added in the sample solution previously analyzed and then compared experimental and true value. Three level of solution were made corresponding to 50,100, and 150 %. Each concentration solution was added in triplicates and shown less than % RSD less than 2 on each level. (Table-2)

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Table 2: Accuracy of proposed method

Drug	Level	Spiked concentration (µg/mL)	Recovered concentration	% Recovery	%RSD
PCM	50%	100	99.63	99.63	0.072111
	100%	200	199.01	99.50	0.319531
	150%	300	298.45	99.48	0.295541
PHE	50%	1	0.996	99.6	0.57735
	100%	2	2.003	100.16	0.76376
	150%	3	3.003	100.11	0.69388

Precision

Instrumental repeatability was checked by repeatedly injecting six solutions containing paracetamol(200µg/mL) phenylephrine hydrochloride (2 µg/mL) at day one for intraday precision and apply six injections of same concentration on day two by another analyst for inter-day precision. (Table-3)

Table 3: Result of precision

Drugs	Parameters	Intraday precision	Inter-day precision
PCM	%RSD	0.307	0.411
	Mean % assay	99.41	99.94
PHE	%RSD	0.414	0.352
	Mean % assay	100.01	99.85

Robustness

Influence of small variation in chromatographic conditions such as flow rate (±10%), detection wavelength(±3nm), change in column oven temperature(±2^{0c}) was studied to determine the robustness of the method by HPLC for simultaneous estimation of PCM and PHE. The % RSD was found to be less than 2.[Table-4]

Table 4: Result of Robustness

Parameters	PCM (%RSD)	PHE (%RSD)
Flow rate (±10%)		
1.1mL/min	0.574	0.44
0.9 mL/min	0.825	0.024
Wavelength (±3nm),		
214nm	0.448	0.109
208nm	0.521	0.066
column oven temperature(±2^{0c})		
32^{0c}	0.629	0.084
28^{0c}	0.296	0.039

System suitability

The solution of paracetamol containing 200µg/mL and phenylephrine hydrochloride concentration of 2 µg/mL was injected five times and chromatogram were recorded. The number of theoretical plates, asymmetry and % RSD were calculated and found to recommended limit.[Table-5]

Table 5: Result of system suitability

Parameters	PCM	PHE
% RSD (n=5)	0.72	0.74
Theoretical plates	8470	5673
Asymmetry	1.15	1.18
Retention time	2.48	1.80

Specificity

The determination of proposed method was specific, injected blank, placebo was not having interference at retention time of paracetamol and phenylephrine. Sample solution, standard solution both exhibit same retention time. Hence developed chromatographic condition pass the criteria of specificity.

CONCLUSION

The proposed HPLC method was used for the simultaneous determination of paracetamol and phenylephrine hydrochloride was found to be specific, accurate, precise, and rapid. Hence the current HPLC method may be used for routine analysis of the raw materials and combinational dosage formulation containing paracetamol and phenylephrine hydrochloride.

ACKNOWLEDGEMENT

The authors express their gratitude to Director Dr. Sanjaya kumar panda of Sherwood college of pharmacy to providing support and Vidisha lab for providing research facility to complete this work.

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