Research Article

Method Development and Validation by RP-HPLC for Simultaneous Estimation of Paracetamol, Caffeine, Phenylephrine and Chlorpheniramine in Tablet Dosage Form

Madhusudan T. Bachute[1], S.V.Shanbhag[2*]

1 .Department of Chemistry, KBP Mahavidyalaya, Pandharpur, Solapur University, Solapur, MS, 413304,India.

2 Department of Chemistry, R & D Centre, Bharathiar University, Coimbatore, TN, 641046, India.

***Corresponding author's E-mail:** svshanbag@gmail.com

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ABSTRACT

A new and simple reverse phase high performance liquid chromatographic method has been developed and validated for determination of Phenylephrine hydrochloride, Chlorpheniramine Maleate, Paracetamol and Caffeine in tablet dosage form. The HPLC method includes use of Cosmosil C-18 (250×4.6mm, 5.0µm) column, a mobile phase consisting of buffer and Methanol at 1.00 ml/minute flow rate and gradient determination with UV detector at 220 nm. Retention time was found to be 4.33, 10.36, 13.85 and 17.35 minutes for Phenylephrine HCL, Paracetamol, Caffeine and Chlorpheniramine Maleate respectively. The method was validated as per ICH guidelines and applied to tablet dosage form without any interference from excipients. The validation characteristics included accuracy, precision, linearity, specificity, limit of quantitation, limit of detection and robustness. Validation acceptance criteria were met in all cases. This method can be used successfully for the quality assessment of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine in tablet dosage form.

Keywords: Caffeine, Chlorpheniramine, Paracetamol, Phenylephrine, RP-HPLC, Tablet.

INTRODUCTION

A Combination of Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine is widely used for analgesic, antipyretic, antihistamine and antitussive activity.

Chlorpheniramine maleate (CPM) is used as an antihistaminic agent in allergic reactions, which prevents muscular response of histamine and thereby reducing cough and common cold (Fig. 1)[13] is chemically 2-[p-chloro-[2-dimethylamino) ethyl] benzyl] pyridine maleate. Various analytical methods such as Spectroscopy [10, 12], HPLC[8,9,12] have been reported for estimation of Chlorpheniramine maleate from various dosage form. Paracetamol (PARA) is a centrally and peripherally acting non-opioid analgesic and antipyretic (Fig. 2)[13] is chemically N-(4-hydroxyphenyl) acetamide. Various analytical methods such as spectroscopy[10,14], HPLC[2,3,4,,6,8,13] have been reported for estimation of Paracetamol from various dosage form. Phenylephrine HCl (PEH) acts as nasal and sinus decongestant (Fig. 3) [13] is chemically 3-[1-hydroxy-2-(methyl amino) ethyl] phenol hydrochloride. Various analytical methods such as spectroscopy [8, 12, 15], HPLC [7, 11, 12, 14, 16] has been reported for estimation of Phenylephrine HCl from dosage form. Caffeine (CAF) is an addictive stimulant (Fig. 4)[13] is chemically 1, 3, 7-trimethyl-3,7-dihydro-1H -purine -2,6-dione. In humans it stimulates the central nervous system, heart rate, and respiration and has psychotropic properties and acts as mild diuretic. Various analytical methods such as spectroscopy [10], HPLC[2, 6, 13] are used for its estimation.

Paracetamol, Phenylephrine hydrochloride, Chlorpheniramine maleate and Caffeine are commercially available in tablet dosage form. Literature survey revealed that one HPLC method is available for estimation of these four actives in combination, but it needs improvement. So efforts were taken to make available simultaneously evaluating, optimized, simple and cost effective HPLC method for estimation of these four actives in pure and in tablet dosage form as per International Conference on Harmonisation (ICH) guidelines[1].

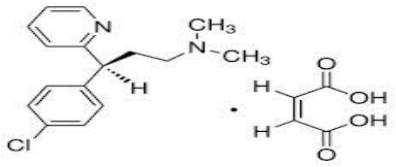


Figure 1: Chlorpheniramine Maleate (CPM)

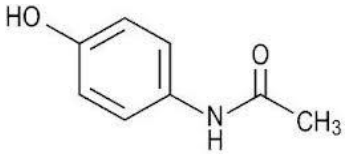


Figure 2: Paracetamol (PARA)

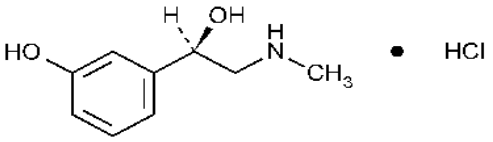


Figure 3: Phenylephrine HCL (PEH)

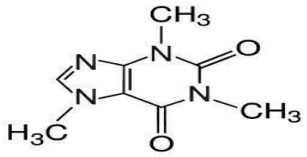


Figure 4: Caffeine (CAF)

MATERIALS AND METHODS

Apparatus

The system consisted of Dionex Gradient System with UV Detector 730D and pump SP930 D. Chromatographic separation was carried by gradient method at room temperature with Cosmosil C-18 (250×4.6mm, 5.0µm) column. Instrumental settings were at flow rate of 1.00 ml/min, at column temperature of 30°C and detector wavelength of 220 nm.

Chemicals and reagents

Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine were supplied from Wallace Pharmaceuticals ,Goa, India ,Methanol (HPLC grade) and Sodium dihydrogen phosphate dihydrate, Tetrabutyl ammonium hydrogen sulphate (A. R. grade) were from Merck, Mumbai, India. Water used was deionised and double distilled. Pharmaceutical dosage form containing Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine was obtained commercially. Each tablet was labelled to contain 10 mg Phenylephrine HCl, 2 mg Chlorpheniramine Maleate, 500 mg Paracetamol and 30 mg Caffeine.

Mobile phase

Mobile phase A

Transfer 1.56 g sodium dihydrogen phosphate dihydrate and 1.70 g of tetrabutyl ammonium hydrogen sulphate into a 1000 mL volumetric flask. Dissolve and dilute to volume with water and mix. Filter the solution through 0.45 µ filter and degas.

Mobile phase B

Methanol.

Gradient program is as given below

Time (minutes)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0	95	5
18	55	45
23	55	45
25	95	5
30	95	5

Preparation of solutions

A working standard solution contains 10 µg/ml Phenylephrine HCl, 2µg/ml Chlorpheniramine Maleate, 500 µg/ml Paracetamol and 30 µg/ml Caffeine was prepared by dissolving Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine standard in diluents. The mixture was sonicated for 30 minutes or until standard dissolved completely. Sample solutions prepared by taking 20 tablets of Helpex Anticold, powdered finely and weighed accurately and taken equivalent amount of powder to contain 10 mg Phenylephrine, 2 mg Chlorpheniramine, 500 mg Paracetamol and 30 mg caffeine in 100 ml volumetric flask and added 70 ml diluent and sonicated for 30 minutes, made up volume with diluents. Taken 2.5 ml from above solution in 25 ml volumetric flask and made volume with diluent.

Method Validation

The proposed HPLC method was subjected to validation for various parameters like system suitability, specificity, range and linearity, accuracy, precision and robustness in accordance with International Conference on Harmonization (ICH) guidelines.

Linearity

The mixed standard stock solution (10 µg/mL of Phenylephrine HCl, 2 µg/mL of Chlorpheniramine Maleate, 500 µg/mL of Paracetamol and 30µg/mL of Caffeine) was further diluted to get Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine concentration in the range of 10-50µg/mL, 2-10 µg/mL, 500-2500 µg/mL and 30-150 µg/mL respectively.

Precision

Intra-day precision study

Accurately weighed tablet powder, equivalent to 10 mg Phenylephrine HCL, 2 mg Chlorpheniramine Maleate, 500 mg Paracetamol and 30 mg Caffeine was transferred into a 100 ml volumetric flask. An amount of diluent (50 ml) was added, sonicated for 30 minutes and diluted to the 100 ml mark with same diluent. Aliquots (2.5ml) of this solution was taken and diluted to 25 ml with the diluent to obtain concentrations of 10, 2 and 500, 30µg/ml of Phenylephrine, Chlorpheniramine Maleate, Paracetamol and Caffeine respectively. Samples were injected in HPLC and the mean, standard deviation and relative standard deviation were calculated for each sample.

Inter-day precision study

The above samples were analysed again on the following day for inter-day precision study and the mean, standard deviation and relative standard deviation were calculated.

Limit of detection and limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal to noise ratios of 3 for LOD and 10 for LOQ, respectively. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine was made from the standard stock solution. The samples were injected in LC system and measured signal

to noise ratio from the samples were compared with those of blank samples.

Robustness of the method

To evaluate robustness of a HPLC method, few parameters were deliberately varied like flow rate, wavelength.

Specificity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. The peak purity of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine was determined by comparing the spectrum at three different regions of the spot. Effect of excipients of formulation was studied to check, whether it interfered with the assay.

Accuracy

The accuracy of the method was determined by the method of standard addition. The known quantities of standard were added to the placebo at three different levels in triplicate, so as to obtain final concentration at the level of 70 %, 100 %, and 130% of the target concentration. All individual recoveries and overall recovery was calculated as per ICH guideline.

Analysis of a marketed formulation

To determine the content of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine in conventional tablet (Brand name: Helpex Anticold ,Label claim: 500 mg Paracetamol, Phenylephrine HCl 10 mg, Chlorpheniramine Maleate 2 mg and 30 mg caffeine per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 10 mg of phenylephrine, 2 mg of Chlorpheniramine Maleate, and 500 mg of Paracetamol and 30 mg of Caffeine was transferred into a 100 ml volumetric flask containing 70 ml diluent, sonicated for 30 minutes and diluted up to 100 ml with diluent. Taken 2.5 ml from above solution in 25 ml volumetric flask and made up volume to 25 ml with diluent and made concentrations of 10, 2 and 500, 30 µg/ml of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol, Caffeine respectively. A 20-µL volume of sample solution was injected into HPLC system, under the conditions described above.

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop a simultaneous assay method for Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine respectively. For method optimization different ratios of methanol, water and ammonium buffer solution were tried but it was found that gradient method of sodium dihydrogen phosphate dihydrate and tetrabutyl ammonium hydrogen sulphate buffer solution and methanol, at flow rate 1.00 ml/min gives acceptable retention time (Rt), plates and good resolution for Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and caffeine as shown in Fig 5.

Linearity

Linearity of the method was studied by injecting five different concentrations of the drug prepared in the mobile phase in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. The Linearity range for Phenylephrine, Chlorpheniramine, Paracetamol and caffeine were found to be 10-50 µg/ml, 2-10 µg/ml, 500-2500 µg/ml and 30-150 µg/ml respectively. The slopes and its correlation for Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine are shown in Table No.1.

Precision

Precision was measured in terms of repeatability of measurement, performed by injecting the standard solution six times (n=6) and measuring the peak areas. The RSD was found to be 0.071, 0.099, 0.030, and 0.706 for Phenylephrine HCl (PHE), Chlorpheniramine Maleate (CPM), Paracetamol (PARA) and Caffeine (CAF) (Table No.2). This shows that the precision of the method is satisfactory as relative standard deviation is not more than 2.0%.

Limit of detection and limit of quantitation

The limit of quantification (LOQ) and limit of detection (LOD) were evaluated based on signal-to-noise ratios by serial dilution of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine solution. The LODs and LOQs values are mentioned in following Table No.3

Robustness of the method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effects of change in flow rate were studied. The method was found to be unaffected by small changes like ±0.1 change in flow rate (Table No.4).

Specificity

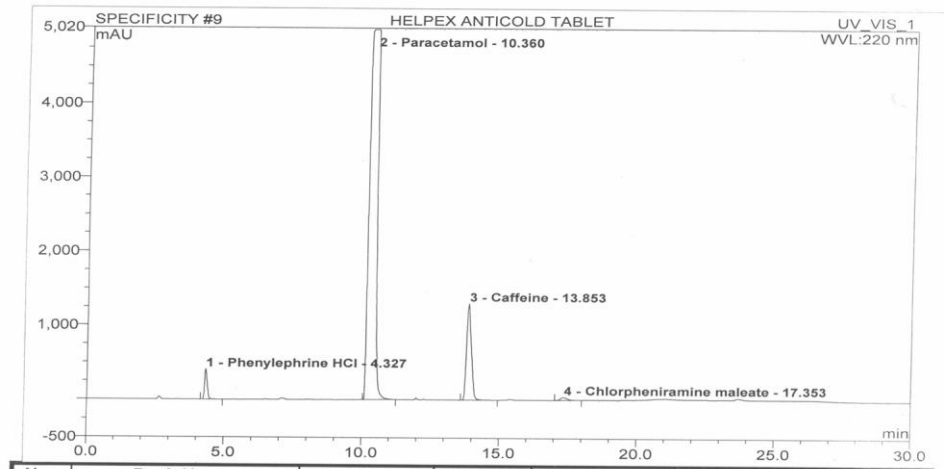
The specificity of the method was evaluated to ensure separation of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol, Caffeine and was demonstrated by assaying samples of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine. The method demonstrated good resolution between the peaks and was found to be free of interference. For demonstrating the specificity of the method for drug formulation, the drug was spiked, wherein the excipients used in different formulation products did not interfere with the drug peak and thus the method was specific for Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol, Caffeine.

Recovery studies

As shown from the data in (Table No.6), good recoveries of the Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine from tablet samples ranged from 98.5-101.3%.

Assay of marketed tablet formulation

Experimental results of the amount of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine in tablets, expressed as a percentage of label claims were in good agreement with the label claims, thereby suggesting that there is no interference from any of the excipients, which are normally present. The drug content was found to be 98.84% for Phenylephrine HCl, 100.90 % for Chlorpheniramine Maleate, 99.21% for Paracetamol and 99.39 % for Caffeine (Table No.5)



No.	Peak Name	Ret.Time min	Area mAU*min	Resolution(EP)	Asymmetry(EP)	Plates(EP)
1	Phenylephrine HCl	4.33	44.695	16.67	1.40	9713
2	Paracetamol	10.36	1705.494	8.02	0.87	5670
3	Caffeine	13.85	261.053	9.21	1.47	29349
4	Chlorpheniramine maleate	17.35	10.584	n.a.	1.23	25063
Total:			2021.826		4.980	69795.000

Figure 5: HPLC chromatogram of standard Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and caffeine (10µg/mL, 2µg/mL, 500µg/mL and 30µg/mL)

Table 1: Linearity slope and correlation for PHE, CPM, PARA and CAF

No.	Name of Drug	Slope	Correlation
1	Phenylephrine HCl	1.001	0.999
2	Chlorpheniramine Maleate	1.019	1.000
3	Paracetamol	1.018	0.999
4	Caffeine	0.990	1.000

Table 2: LOD and LOQ of PHE, CPM. PARA and CAF

Parameter	PHE	CPM	PARA	CAF
LOD (µg/ ml)	3.89	1.29	317.15	14.51
LOQ (µg/ ml)	11.80	3.88	961.08	43.98

Table 3: Robustness studies for PHE, CPM, PARA, and CAF

Factor	Level	PHE	PARA	CAF	CPM
Flow rate (ml/min)		Rt	Rt	Rt	Rt
0.9	-0.1	4.45	10.50	13.91	17.52
1.0	0	4.33	10.36	13.85	17.35
1.1	+0.1	4.12	10.09	13.62	17.24

Table 4: Interday & Intraday Precision studies for PHE, CPM, PARA, CAF

S. No.	Parameters	Data obtained			
		PHE	CPM	PARA	CAF
	Interday				
1.	Drug area found	51.1551	11.4132	58.3147	6.6934
2.	SD	0.0441	0.0125	0.0193	0.0175
3.	% RSD	0.0861	0.1093	0.0330	0.2621
	Intraday				
4.	Drug area found	51.1317	11.4153	58.3147	6.6934
5.	SD	0.0396	0.0138	0.0193	0.0175
6.	% RSD	0.0775	0.1207	0.0330	0.2621

Table 5: HPLC Assay of Tablet formulation

No	Name of drug	Label clam	% Drug obtained	% RSD *
1	PHE	10 mg	98.84	0.04
2	CPM	2 mg	100.90	0.27
3	PARA	500 mg	99.21	0.01
4	CAF	30 mg	99.39	0.45

*Average of six determinations

Table 6: Recovery Studies of PHE, CPM, PARA & CAF

Recovery Level (%)	Drug	Concentration of drug (µg/ml)		% Recovery	SD	% RSD
		Drug taken	Std drug added			
70	**PHE**	10	7	100.43	0.02	0.07
100		10	10	98.50	0.04	0.08
130		10	13	100.61	0.05	0.09
70	**CPM**	2	1.4	99.29	0.01	0.08
100		2	2	101.3	0.02	0.16
130		2	2.6	98.61	0.02	0.17
70	**PARA**	500	350	100.86	0.04	0.09
100		500	500	99.30	0.20	0.33
130		500	650	99.61	0.16	0.20
70	**CAF**	30	21	99.43	0.02	0.44
100		30	24	99.50	0.03	0.52
130		30	39	100.15	0.04	0.53

*Average of six determinations

CONCLUSION

In present work, RP-HPLC method for the simultaneous estimation of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine was developed and validated as per ICH guidelines. The standard deviation and % RSD (<2 %) are within limit, indicating high degree of precision of the method.

The results of the recovery studies performed show the high degree of accuracy of the proposed method. Hence, it can be concluded that the developed method is simple, accurate, precise, reproducible and economic and hence can be employed successfully for the estimation of Phenylephrine HCl, Chlorpheniramine Maleate, Paracetamol and Caffeine in bulk and tablet dosage form.

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