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Original Research Article

Simultaneous determination of four active pharmaceuticals in tablet dosage form by reversed-phase high performance liquid chromatography

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Abstract

Purpose: *To develop a single, low-cost and rapid analytical method for the simultaneous determination of four active components - chlorpheniramine maleate, paracetamol, phenylephrine hydrochloride and caffeine – in a tablet dosage form.*
Method: *This method was based on reverse-phase high performance liquid chromatography (RP-HPLC) and involved the use of a C-18 column (250 × 4.6 mm, 5.0 µm), a mobile phase consisting of buffer solution and methanol at a flow rate of 1.00 mL/min, and gradient determination with UV detection at 220 nm.*
Results: *Retention time was 4.33, 10.36, 13.85, and 17.35 min for phenylephrine hydrochloride, paracetamol, caffeine, and chlorpheniramine maleate, respectively. Specificity data showed no interference from the excipients, and accuracy of the method was close to 100 %. The method was validated as per the guidelines of International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), and all the results met the acceptance criteria for accuracy, precision, linearity, specificity, limit of quantitation, limit of detection, and robustness.*
Conclusion: *This method can successfully perform quantitative assessment of phenylephrine HCl, chlorpheniramine maleate, paracetamol, and caffeine in tablet combination dosage forms faster and more cost-effectively than conventional methods.*

Keywords: *Caffeine, Chlorpheniramine, Paracetamol, Phenylephrine, RP-HPLC, Tablets, Simultaneous quantitation, Combination dosage*

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INTRODUCTION

Pharmaceutical companies are increasingly pursuing combination dosage forms that can treat multiple symptoms simultaneously. A widely-used combination dosage form for the treatment of upper respiratory infections is the combination of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol, and caffeine, which is utilized for its analgesic, antipyretic, antihistamine, and antitussive activity.

Chlorpheniramine maleate (CPM; 2-[p-chloro-[2-dimethylamino) ethyl] benzyl] pyridine maleate) is used as an antihistaminic agent in allergic reactions. Paracetamol (PARA; N-[4-hydroxyphenyl] acetamide) is a centrally- and peripherally-acting non-opioid analgesic and antipyretic. Phenylephrine hydrochloride (PHE; 3-[1-hydroxy-2-(methyl amino) ethyl] phenol hydrochloride) acts as nasal and sinus decongestant. Caffeine (CAF; 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione) is an addictive stimulant that elevates heart rate and respiration, confers psychotropic properties, and acts as a mild diuretic.

Various analytical methods, including spectroscopy [1] and high performance liquid chromatography (HPLC) [2-3], have been utilized for the estimation of CPM alone [1-3] in various dosage forms. Similarly, diverse analytical methods are available for the determination of PARA [4-7], PHE [8-11], and CAF [12-15] from different dosage forms. However, no single method is available for the simultaneous determination of all four active components in a combination form.

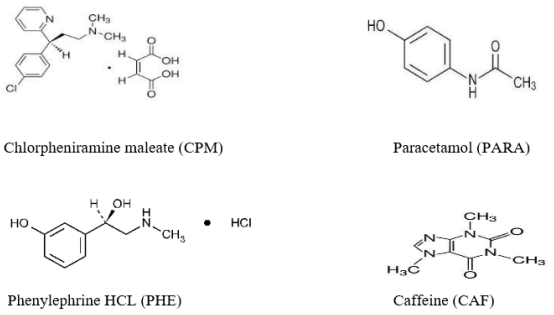


Figure 1: Chemical structure of active ingredients determined from novel reverse-phase high performance liquid chromatography (RP-HPLC) characterization of a combination dosage form.

A survey of existing literature survey suggested that a single procedure capable of simultaneously determining multiple active components present in commercial drug dosage forms would be a useful alternative to existing methods, reducing both time and costs spent on individual, serialized determinations of combination drug forms.

EXPERIMENTAL

Apparatus

Prior to this study, we optimized our protocol using different ratios of methanol, water, and ammonium buffer solution. Our apparatus consisted of Dionex Gradient System with UV Detector 730D and pump SP930 D. (ThermoFischerScientific, USA). Chromatographic separation was carried out at room temperature with a Cosmosil C-18 column (250 × 4.6 mm, 5.0 µm). (Nacalai Tesque ,Japan). Instrumental settings included a flow rate of 1.00 mL/min, column temperature of 30°C, and detector wavelength of 220 nm.

Chemicals and reagents

PHE, CPM, PARA, and CAF were supplied from Wallace Pharmaceuticals, Goa, India; HPLC-grade methanol, sodium dihydrogen phosphate dihydrate, and analytical research-grade tetra butyl ammonium hydrogen sulfate were from Merck, Mumbai, India. The water used was deionized and double-distilled. Tablets containing PHE, CPM, PARA, and CAF were obtained commercially (Helpex Anticold tablets). (Sava Healthcare ,India). Each tablet contained 10 mg PHE, 2 mg CPM, 500 mg PARA, and 30 mg CAF.

Mobile phase

Mobile phase A*:*

For mobile phase A, 1.56 g sodium dihydrogen phosphate dihydrate and 1.70 g tetra butyl ammonium hydrogen sulfate were transferred to a 1,000-mL volumetric flask and dissolved in water to reach a volume of 1,000 mL, then mixed. The solution was then filtered through a 0.45-µm filter and degassed.

Mobile phase B**:** Methanol

Table 1: Gradient profile of Mobile Phase A and B

Time (min)	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

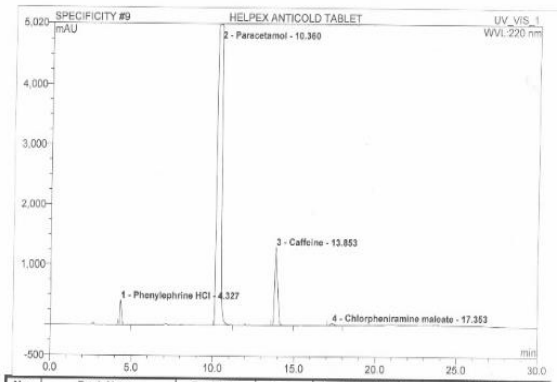
Preparation of solutions

A working standard solution containing 10 µg/mL PHE, 2 µg/mL CPM, 500 µg/mL PARA, and 30 µg/mL CAF was prepared by dissolving PHE, CPM, PARA, and CAF standards in 1 L 50% methanol. The mixture was sonicated for 30 min or until the standard dissolved completely. Sample solutions were prepared by finely powdering and weighing 20 tablets of the retail combination dosage. An amount of powder containing 10 mg PHE, 2 mg CPM, 500 mg PARA, and 30 mg CAF was added to a 100-mL

volumetric flask and diluted with 70 mL 50% methanol diluent, mixed well and sonicated for 30 min, cooled to room temperature, and made up to the mark with additional diluent. A 2.5-mL aliquot of this solution was added to a 25-mL volumetric flask and made up to the mark with additional diluent.

RESULTS

The HPLC procedure was optimized by developing a simultaneous assay method for PHE, CPM, PARA, and CAF. At a flow rate 1.00 mL/min, the gradient method of sodium dihydrogen phosphate dihydrate and tetra butyl ammonium hydrogen sulfate buffer solution with methanol gave acceptable retention time, number of theoretical plates, and good resolution for PHE, CPM, PARA, and CAF standards (Figure 2). The system suitability tests revealed that numbers of theoretical plates were above 2,000 and the tailing factor was less than 2.



No.	Peak Name	Ret.Time	Area	Resolution(EP)	Asymmetry(EP)	Plates(EP)
		min	mAU*min			
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 2: HPLC chromatogram of phenylephrine hydrochloride (PHE), chlorpheniramine maleate (CPM), paracetamol (PARA), and caffeine (CAF) standards

Method development and validation parameters

The reverse-phase HPLC (RP-HPLC) method was developed for simultaneous estimation of PHE, PARA, CAF and CPM in tablet dosage forms, with RTs of 4.33, 10.36, 13.85, and 17.35 min, respectively. All validation parameters were quantified as detailed below.

Linearity

Linearity was assessed by injecting five different concentrations of each standard in the mobile phase in triplicate into the chromatographic system, keeping the injection volume constant. The peak values of area were plotted against the corresponding concentrations to obtain the calibration graphs.

The linearity ranges for PHE, CPM, PARA, and CAF were 10–50 µg/mL, 2–10 µg/mL, 500–2,500 µg/mL, and 30–150 µg/mL, respectively. The slopes and their correlations with PHE, CPM, PARA, and CAF are shown in Table 2. PHE, CPM, PARA, and CAF showed a linear response between 10–50 µg/mL, 2–10 µg/mL, 500–2,500 µg/mL, and 30–150 µg/mL.

Table 2: Linearity slopes and correlation coefficients for PHE, CPM, PARA, and CAF

Name of drug	Slope	Correlation coefficient
Phenylephrine HCl	1.001	0.999
CP maleate	1.019	1.000
Paracetamol	1.018	0.999
Caffeine	0.990	1.000

Precision

Precision parameters assessed the repeatability of measurement and were calculated by injecting each standard solution six times, then measuring the peak areas. The RSD were 0.0861, 0.1093, 0.0330 and 0.2621 for PHE, CPM, PARA, and CAF respectively (Table 3).

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 PHE, CPM, PARA, and CAF solutions. The LOD and LOQ values are tabulated in Table 4.

Robustness

The robustness of the developed RP-HPLC method was assessed by making small, deliberate variations in the optimized method parameters and monitoring flow rate. The method was found to be unaffected when flow rate was changed ±0.1 mL/min (Table 5).

Specificity

The specificity of this method was assessed by examining the chromatogram and identifying where complete separation of PARA, PHE, CAF and CPM occurred. We observed no potential interference from the presence of excipients. The chromatographic peaks obtained were well-separated at the baseline and were sharp in nature.

Table 3: Inter- and intra-day precision studies for PHE, CPM, PARA, and CAF

Parameter	PHE	CPM	PARA	CAF
Inter-day				
Peak mean area (µg/mL)	51.1551	11.4132	58.3147	6.6934
SD	0.0441	0.0125	0.0193	0.0175
RSD (%)	0.0861	0.1093	0.0330	0.2621
Intra-day				
Peak mean area (µg/mL)	51.1317	11.4153	58.3147	6.6934
SD	0.0396	0.0138	0.0193	0.0175
RSD (%)	0.0775	0.1207	0.0330	0.2621

SD: standard deviation; RSD: relative standard deviation

Table 4: 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 5: Robustness study for PHE, CPM, PARA, and CAF

Factor	Level	PHE	PARA	CAF	CPM
Flow rate(mL/min)		RT (min)	RT (min)	RT (min)	RT (min)
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 6: Recovery data for PHE, CPM, PARA, and CAF (mean, n = 6)

Recovery level (%)	Name of drug	Recovery (%)	SD	RSD (%)
70	PHE	100.43	0.02	0.07
100		98.50	0.04	0.08
130		100.61	0.05	0.09
70	CPM	99.29	0.01	0.08
100		101.3	0.02	0.16
130		98.61	0.02	0.17
70	PARA	100.86	0.04	0.09
100		99.30	0.20	0.33
130		99.61	0.16	0.20
70	CAF	99.43	0.02	0.44
100		99.50	0.03	0.52
130		100.15	0.04	0.53

Mean of six determinations

Recovery

Recoveries of PHE, CPM, PARA, and CAF from tablet samples ranged from 98.5–101.3 % (Table 6).

Application of developed method to a commercial tablet formulation

The developed RP-HPLC method identified similar quantities of PHE, CPM, PARA, and CAF in tablets as that listed on the product's label, ranging from 98.84–100.90% of the label amounts (Table 7). These findings confirmed our specificity results, indicating that excipients did not result in interference.

Table 7: Content of PHE, CPM, PARA and CAF in tablet formulation by HPLC technique

Drug	Label claim (mg)	Content found (%)	RSD (%)
PHE	10	98.84	0.04
CPM	2	100.90	0.27
PARA	500	99.21	0.01
CAF	30	99.39	0.45

DISCUSSION

Here, we describe a RP-HPLC method developed and validated for the simultaneous determination of PHE, CPM, PARA, and CAF in tablet combination dosage form. The total run time required for the method was less than 20 min for eluting all four active drugs (PHE, CPM, PARA, and CAF), a dramatic reduction in run time per sample. Recovery of PHE, CPM, PARA, and CAF ranged from 98.84–100.90% of reported label contents, and the slope and correlation coefficient nearly equaled one, indicating the high accuracy of this method. These results indicate that this method can be used for the routine analysis of combinations of four active pharmaceuticals, such as PHE, CPM, PARA and CAF, in tablet dosage form.

This method produced well-differentiated determinations, with resolutions consistently greater than 2. The mean peak area of the chromatograms was plotted against the concentration of PHE, CPM, PARA, and CAF to obtain the calibration curve. Repeatability and intermediate precision values were within the acceptable limits set by ICH, indicating that the method is precise. Specificity experiments indicated no interference; the peaks of excipients and diluents did not overlap with the main peaks of PHE, CPM, PARA, and CAF. The LOD and LOQ values obtained by the proposed method were low, which indicated the sensitivity of the method. The stability studies indicate that both standard and sample drugs were stable up to 24 h, and change in flow rate, temperature and mobile phase composition did not cause any significant changes in the results. RSD for precision was < 2 %, confirming that this method is sufficiently precise.

CONCLUSION

The developed method has a significant advantage over other methods of analysis because it is capable of simultaneous determination of multiple active components present in combination dosage form, resulting in a faster, more cost-effective analysis. The time required for sample analysis is a few hours. The method has been validated extensively as per ICH guidelines, and it is suitable for use in pharmaceutical firms and laboratories.

DECLARATIONS

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Conflict of interest

The authors confirm that no conflict of interest is associated with this study.

Contributions of authors

We confirm that this work was done by the author(s) named in this manuscript, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. SVS conceived the study and participated in the design as well as analysis and interpretation of data and in the drafting of the manuscript. SLT was involved in the acquisition of data, its analysis and interpretation, and drafting of the manuscript. MTB was involved in critically revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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