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ORIGINAL ARTICLE

RP-HPLC-DAD method for the determination of phenylepherine, paracetamol, caffeine and chlorpheniramine in bulk and marketed formulation

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Tablet dosage

Abstract A simple, specific and accurate isocratic RP-HPLC-DAD method was developed for the simultaneous determination of phenylephrine, paracetamol, caffeine and chlorpheniramine in bulk and tablet dosage form. The four contents are present in variable concentrations and have variable chromatographic behavior making the process of analysis very difficult. For present studies a reversed-phase C-18 column (150 mm × 4.5 mm i.d., particle size 5 μm) with mobile phase consisting of acetonitrile, methanol and 10 Mm phosphate buffer 16:22:62 (v/v) (pH of buffer 2.5 ± 0.02, adjusted with *ortho* phosphoric acid) was used. The flow rate was 1.0 ml/min and eluents were monitored at 280 nm. The mean retention times of phenylephrine, paracetamol, caffeine and chlorpheniramine were found to be 1.8, 3.1, 5.2 and 10.9 min, respectively. The method was validated in terms of linearity, range, specificity, accuracy, precision and robustness. The proposed method was successfully applied to the estimation of phenylephrine, paracetamol, caffeine and chlorpheniramine in combined tablet dosage form.

1. Introduction

Paracetamol is analgesic and antipyretic chemically it is *N*-(4-hydroxyphenyl) acetamide. Phenylephrine chemically is (1*R*)-1-(3hydroxy-phenyl)-2-(methylamino) ethanol hydrochloride and is used as sympathomimetic (descongestants), chlorpheniramine maleate chemically is 3-(4-chlorophenyl)-*N*, *N*-dimethyl-3-pyridin-2-ylpropan-1-amine and is used as an H1-receptor antagonist (antihistaminic). Caffeine chemically is (1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione and acts as a central nervous system stimulant. Structural formulas of

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Table 1 Precision studies.

Concentration µg/ml	Mean measured concentration ± %RSD	
	Repeatability (n = 6)	Intermediate precession (n = 3)
PARA		
250	251.60 ± 3.2	250.30 ± 2.5
375	376.60 ± 3.9	374.50 ± 3.2
500	501.40 ± 2.2	502.60 ± 3.3
PHE		
7.50	7.40 ± 1.5	7.45 ± 2.5
11.25	11.30 ± 2.3	11.20 ± 2.6
15	15.10 ± 2.5	14.80 ± 3.2
CAF		
15	14.90 ± 2.3	14.95 ± 2.8
22.5	22.40 ± 2.8	22.55 ± 3.2
30	30.10 ± 3.2	30.10 ± 2.8
CPM		
1	0.95 ± 2.2	1.01 ± 3.5
1.5	1.45 ± 3.2	1.50 ± 3.6
2	1.95 ± 3.8	1.95 ± 3.8

PARA, PHE, CAF and CPM are given in Fig. 1. These substances are frequently associated in pharmaceutical formulations against common cold, but are present in formulations with an important imbalance between the quantities of different active ingredients in the dosage forms. Moreover, the active compounds have very different polarity and, therefore chromatographic behavior. The mentioned combination is available in tablet dosage form as a single unit dose with concentration of these active ingredients in varying concentrations which are as 500 mg of PARA, 15 mg of PHE, 30 mg of CAF and 2 mg of CPM. The variation of these active ingredients makes the process of analysis difficult. So far no single HPLC method is reported to determine the mentioned ingredients quantitatively in this combination.

The literature reveals number of analytical methods published for PARA, PHE, CAF and CPM with some other drug combinations.

Methods for paracetamol and its combinations in pharmaceuticals or in biological fluids have been reported. Paracetamol has been determined in combination with other drugs using titrimetry (British Pharmacopoeia, 1998; European Pharmacopoeia, 1997), voltammetry (Saeed and Reyhaneh-Sadat, 2011), fluorimetry (Hossein and Yahya, 2011), colorimetry (Shihana et al., 2010), UV-spectrophotometry (Ghulam et al., 2011), quantitative thin-layer chromatography (TLC) Atul et al., 2008, high-performance liquid chromatography (HPLC) Prasanna and Reddy, 2009; Godse et al., 2009; Franeta et al., 2002; Godse et al., 2001; Pattan et al., 2009; Gopinath et al., 2007; Olmo et al., 2005 and gas chromatography (GC) Belal et al., 2009 in pharmaceutical preparations.

An HPLC method for phenylepherine in combination with chlorpheniramine maleate have been reported (Mukesh et al., 2010).

Chlorpheniramine maleate has been reported to be quantified in combination with other active agents by spectrophotometry (Khoshayand et al., 2010; Maryam and Mehdi, 2005), HPLC (Sandeep, 2011), LC–MS–MS (Qiongfeng et al., 2008) and Voltammetric (Shekappa et al., 2011).

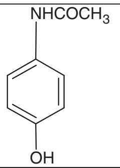
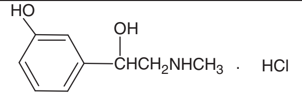
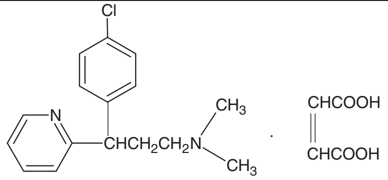
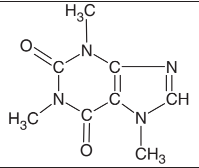
	Structural formula
Paracetamol (PARA)	
Phenylephrine Hydrochloride (PHE)	
Chlorpheniramine maleate (CPM)	
Caffeine (CAF)	

Figure 1 The structures of paracetamol (PARA), phenylephrine hydrochloride (PHE), chlorpheniramine maleate (CPM) and caffeine (CAF).

Caffeine has been quantified in combination with some other active agents by variety of analytical methods such as spectrophotometry (Kuldeep et al., 2011), HPLC (Viswanath et al., 2011) and HPTLC (Misra et al., 2009).

To our knowledge, the methods described in the literature do not cover the analysis of the combination PARA, PHE, CAF and CPM in pharmaceutical formulations. Therefore, the main objective of this work was to develop a single separation method for analyzing these four analytes which are present in variable concentrations in tablet dosage form.

Within this context, a simple alternative methodology for determination of these drugs in tablets using an isocratic chromatographic mode in analysis time of 15 min was proposed. After validation of method for various parameters, the method proved to be successful and was applied to the analysis of commercial products containing these active ingredients.

2. Materials and methods

2.1. Chemicals and reagents

Working standards of pharmaceutical grade phenylepherine hydrochloride, paracetamol, caffeine and chlorpheniramine maleate were obtained as generous gifts from Leben pharmaceuticals (Akola Maharashtra, India). They were used without further purification. Fixed dose combination tablet Sinarest (Centaur pharma. ltd) containing 15 mg phenylepherine hydrochloride, 500 mg Paracetamol, 30 mg caffeine and 2 mg chlorpheniramine maleate was purchased from local market, Yavatmal, Maharashtra, India. All the chemicals were of HPLC grade, purchased from Merck Chemicals, India. Water used was double distilled and filtered through a 0.45 µm filter.

2.2. Instrumentation

The HPLC system consisted of waters series 600E pump quaternary gradient, waters online degasser module a 996 photodiode array (PDA) detector, a 515 autoinjector; data were acquired and processed by use of EMPOWER software (all equipments from Waters, Milford). The chromatographic separations were carried out on a C-18 Phenomenex column (150 mm × 4.5 mm i.d., particle size 5 μm) with isocratic conditions.

2.3. Preparation of standard stock and sample solution

Preliminarily sample preparation was done in acetonitrile but analates demonstrated signs of instability behaving variably with time after injecting them in various chromatographic conditions. Further studies were carried by preparing stock solution of analates in mixture of phosphate buffer 10 Mm pH 2.5 and acetonitrile (95:5) taking accurately weighed quantity of PARA, CAF, PHE and CPM transferred to 25 ml volumetric flasks separately to give stock solutions of 250 μg/ml each of PARA, CAF, PHE and CPM.

For preparation of sample solution of tablets twenty tablets (Sinarest Tab, Centaur) were weighed and powdered finely. Tablet powder equivalent to 2 mg of CPM, 15 mg of PHE, 500 mg of PARA 30 mg of CAF was transferred to a 25 ml volumetric flask and dissolved in 20 ml of Phosphate buffer 10 Mm pH 2.5 and acetonitrile 95:05. The solution was ultra sonicated for 15 min and filtered through 0.45 micron membrane filter. The solutions were further diluted to obtain concentration of 60 μg/ml of PHE, 20 μg/ml of PARA, 120 μg/ml of CAF and 8 μg/ml of CPM. This mixture was subjected to HPLC analysis in developed chromatographic conditions.

2.4. Chromatographic conditions

The mobile phase consisted of a mixture of acetonitrile: methanol and 10 Mm phosphate buffer pH 2.5 adjusted with *ortho* phosphoric acid in the ratio 16:22:62 (v/v) flow rate of 1 ml/min at isocratic mode the eluants were monitored at 280 nm. The mobile phase was filtered through 0.45 micron membrane filter and degassed before use. The injection volume was 20 μl and all analyses were performed at ambient temperature.

3. Results and discussion

3.1. Method development and optimization of chromatographic conditions

In order to optimize the method for drug analysis in pharmaceutical formulations, preliminary tests were performed to select optimal conditions. Parameters such as ideal mobile phase and their proportions at optimum pH were exhaustively studied so as to achieve a reasonable degree of separation of analates. Several binary or ternary eluents were tested using different proportions of solvent, such as acetonitrile, methanol, water and buffer at different pH conditions. However, satisfactory results were achieved by 16:22:62% v/v/v of acetonitrile:methanol:phosphate buffer (10 Mm) pH 2.5 adjusted with *ortho* phosphoric acid at flow rate of 1 ml/min followed by detection at 280 nm. Fig. 2 shows the chromatogram for standard mixture obtained through the optimized variables in accordance with the features described above. Table 3 shows analytical parameters such as retention time, asymmetry, tailing and theoretical plates obtained for the optimal chromatographic conditions.

3.2. Method validation

3.2.1. Selectivity and linearity

Method selectivity was assessed by the peak purity test (comparison between analyte peak and auto threshold in the purity plot) using diode array detector. The analyte chromatographic peak was not found to be attributable to more than one component indicating the method to be selective (International Conference Harmonisation, 1995).

For linearity, an external method was used for the simultaneous determination of four ingredients. Five concentrations were chosen ranging from 50% to 150% of the target analyte concentrations in formulations. So the concentrations were PARA 250 to 750 μg/ml, phenylephrine hydrochloride 7.5 to 22.5 μg/ml, caffeine 10 to 45 μg/ml and chlorpheniramine maleate 1.0 to 3 μg/ml. All the solutions were prepared in Phosphate buffer (pH 2.5): acetonitrile 95:05. Each point was analyzed three times ($n = 3$). Each concentration of standard mixture solutions was injected in triplicate and the mean value of peak area was taken for the calibration curve. Calibration graph was obtained by plotting peak area *versus* concentration of standard drugs [Fig. 3 (A), (B), (C) and (D)]. The linear regression equations for PHE, PARA, CAF and CPM were found to be $y = 527.33x + 99$, $y = 84197x + 92339$, $y = 14367x + 31023$ and $y = 9011x + 745$, respectively. The regression coefficient values (R^2) were found to be 0.999, 0.999, 0.980 and 0.984, respectively indicating an acceptable degree of linearity.

3.2.2. Specificity

The specificity of method was accessed from the chromatogram where complete separation of PARA, PHE, CAF and CPM was achieved and against no potential interferences in the presence of placebo. The peaks obtained were sharp and well separated at the baseline. Also excipients from formulation were not interfering with assay. No interferences were detected at retention times of PARA, PHE, CAF and CPM in sample solution proving the method to be specific.

3.2.3. Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision is determined through the estimate of the relative standard deviation (RSD) values. The precision in the validation of this optimized method was performed at two levels: repeatability and intermediate precision.

Repeatability ($n = 6$) in sample area was carried out for 100.0% of the test concentration. In the present case, concentrations at 500, 15, 30 and 2 μg/ml for PARA, PHE, CAF and CPM, respectively were used. Intermediate precision ($n = 6$) was performed on different days. All results presented acceptable precision values (not exceeding 5.00%) as shown in Table 1.

3.2.4. Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is

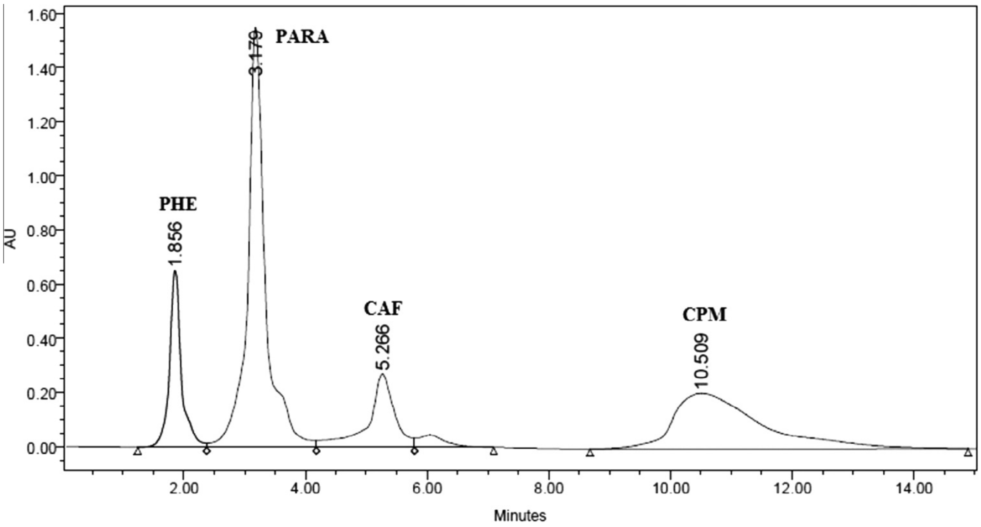


Figure 2 HPLC chromatogram obtained during simultaneous determination of PHE, PARA, CAF and CPM.

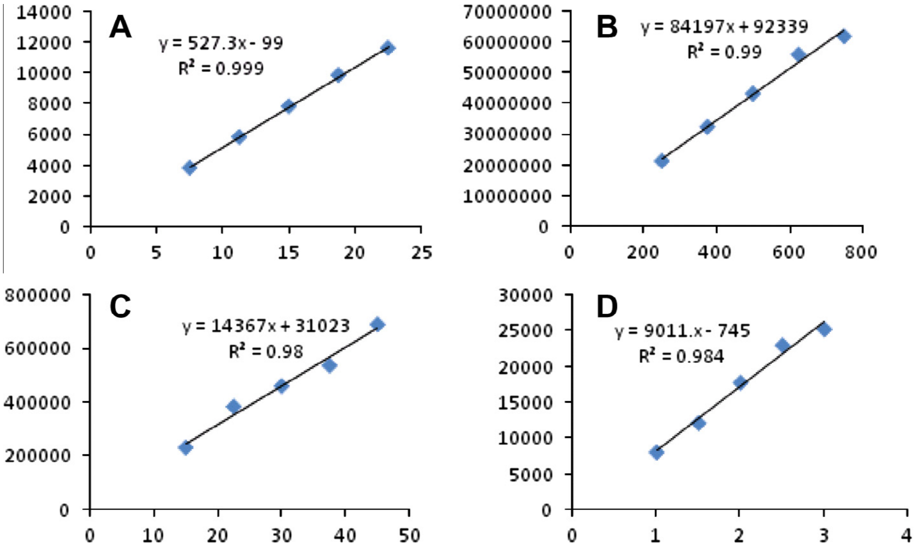


Figure 3 Calibration curves of (A) PHE, (B) PARA, (C) CAF, (D) CPM.

expressed as % recovery determined by standard addition method. Accuracy was assessed by spiking the active ingredients into the placebo at different concentrations 50%, 100%, and 150% each of the labeled claim and injected in developed chromatographic conditions in triplicate. The recovery data for accuracy studies is shown in Table 2.

Table 2 Accuracy studies of PHE, PARA, CAF and CPM.

Recovery level (%)	Std. added to placebo	Amount added (mg)	Mean recovery (mg) ± %RSD ($n = 3$)	Mean% recovery
50	PHE	7.5	7.45 ± 2.4	99.33
	PARA	250	249.50 ± 2.6	99.80
	CAF	15.0	14.95 ± 2.2	99.66
	CPM	1.0	0.97 ± 2.6	97.00
100	PHE	15	14.90 ± 2.5	99.33
	PARA	500	501.50 ± 2.9	100.30
	CAF	30	30.20 ± 2.4	100.66
	CPM	2	1.95 ± 2.7	97.50
150	PHE	22.5	22.70 ± 2.6	100.88
	PARA	750	753.50 ± 2.5	100.46
	CAF	45	45.30 ± 2.3	100.66
	CPM	3	3.05 ± 2.4	101.66

Table 3 System suitability studies.

STD. sol.	Parameters (*mean values) $n = 7$			
	RT*	Asymmetry*	Tailing*	Theoretical plates*
PHE	1.8	1.214	1.107	14942
PARA	3.1	0.841	0.9	13052
CAF	5.2	1.952	1.432	8790
CPM	10.9	2.055	2.528	24214

* = mean value (n = 7).

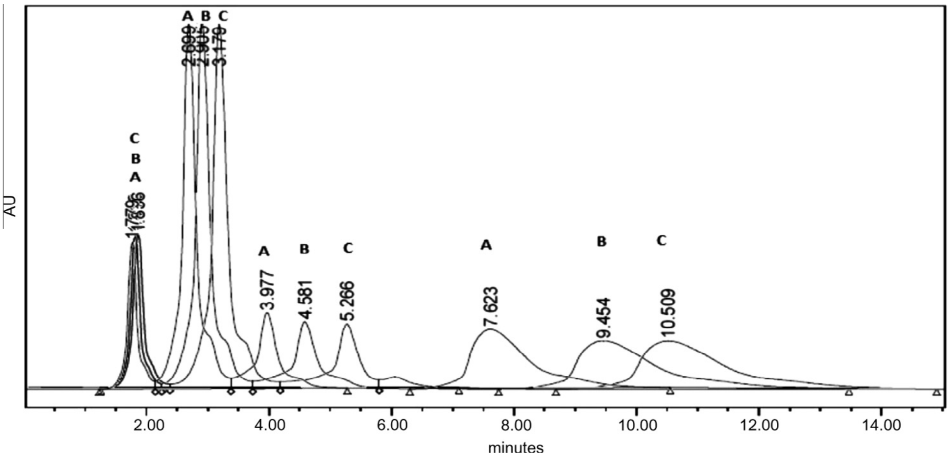


Figure 4 Robustness studies: for change in pH of buffer. (A) pH 2.4, (B) pH 2.6, (C) pH 2.5.

Table 4 Robustness studies for PHE, PARA, CAF and CPM.

System suitability parameters		%RSD of peak area response (n = 3)				Mean tailing factor (n = 3)				Mean retention time in min (n = 3)			
Variations		PHE	PARA	CAF	CPM	PHE	PARA	CAF	CPM	PHE	PARA	CAF	CPM
Change in flow	+10	2.8	2.5	2.4	2.6	1.64	1.56	0.95	0.89	2.1	2.82	3.64	7.95
	0	2.5	3.2	2.5	2.9	1.1	0.9	1.4	1.5	1.8	2.52	3.34	7.18
	−10	2.6	3.1	2.4	2.1	1.61	1.61	1.08	0.98	1.52	2.15	3.10	6.60
Change in% Organic phase (Acetonitrile)	+10	2.70	2.4	3.2	2.9	1.52	1.66	1.34	1.22	2.10	2.75	3.55	7.50
	0	2.8	2.3	2.2	2.6	1.2	1.2	1.6	1.3	1.88	2.55	3.40	7.20
	−10	2.90	2.6	2.8	2.9	1.8	1.8	1.4	1.6	1.90	2.60	3.60	7.10
Change in pH	+0.1	2.6	2.9	2.4	2.6	1.5	1.6	1.7	1.2	1.85	2.55	3.36	7.20
	0	2.5	2.6	2.4	1.3	1.6	1.4	1.5	1.6	1.90	2.60	3.50	7.20
	−0.1	2.6	2.4	1.8	1.9	1.5	1.4	1.6	1.5	1.90	2.65	3.30	7.60

3.2.5. System suitability parameters

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the retention time, asymmetry factor, tailing factor and theoretical plates of the peaks were calculated. The results are shown in Table 3.

3.2.6. Robustness studies

Robustness of the developed method was evaluated by deliberate minor modifications in chromatographic conditions. The parameters included variation of percentage of organic phase, for present studies the change in concentration of acetonitrile in the mobile phase was varied as it was organic solvent in minor concentrations in developed chromatographic conditions. Second parameter was flow rate and third was pH (Fig. 4). Robustness of the method was checked at concentration levels 500 μg/ml for PARA, 15 μg/ml for PHE, 30 μg/ml for CAF and 2 μg/ml for CPM. The results of robustness studies are presented in Table 4. The system suitability parameters considered for deliberate changes were %RSD of peak areas, mean tailing factor and mean retention time.

Table 5 Analysis of marketed formulation by proposed method.

Commercial formulation	Ingredients	Labeled amount (mg)	Amount found (mg)	Found%
Sinarest tab®	PHE	15	14.95	99.67
	PARA	500	502.60	100.52
	CAF	30	30.80	102.66
	CPM	2	2.05	102.50

3.2.7. Analysis of formulation

The proposed HPLC method was applied to simultaneous determination of PHE, PARA, CAF and CPM in Sinarest Tablet®. The quantitative results of these assays are summarized in Table 5. Satisfactory results were obtained for each compound in good agreement with labeled claims. No interferences of excipients were seen in chromatogram.

4. Conclusion

A novel RP-HPLC-DAD method has been developed for the simultaneous estimation of PHE, PARA, CAF and CPM in bulk and tablet in which the active agents are present in variable concentrations. The problem associated with the present combination was the variability in chromatographic behavior of these analates also the variation in concentrations in dosage forms. The method gave good resolution for all the four drugs with a short analysis time below 10 min. The developed method was validated. It was found to be simple, precise, accurate, and sensitive. The proposed method was specific as the excipients present in the dosage forms have no interference in the determination of the active constituents. The proposed method can be used for routine analysis of PHE, PARA, CAF and CPM in combined dosage form which are present in variable concentrations. It can be also applied in the quality control of bulk manufacturing of presented API's.

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