

DEVELOPMENT AND VALIDATION OF RP–HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NIMESULIDE, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENIRAMINE MALEATE AND CAFFEINE ANHYDROUS IN PHARMACEUTICAL DOSAGE FORM

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Abstract: In this study, a simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of nimesulide (NS), phenylephrine hydrochloride (PE), chlorpheniramine maleate (CPM) and caffeine anhydrous (CF) in pharmaceutical dosage forms. A reversed phase Hypersil phenyl column (4.6 mm × 25 cm) with mobile phase having pH 5.5 consisting of methanol and buffer (55:45, v/v) was used. The flow rate was 1.0 mL per minute and the effluents were monitored at 214 nm. The retention times of all the drugs were found to be 7.47 min (NS), 3.944 min (PE), 4.55 min (CF) and 17.15 min (CPM), respectively. The linearity for all the drugs was obtained in the range of 300–800 µg/mL (NS), 15–32 µg/mL (PE), 16–32 µg/mL (CPM) and 30–180 µg/mL (CF), respectively. The results of analysis have been well validated according to guidelines of International Conference of Harmonisation of technical requirements for registration of pharmaceuticals for human use. The method was found to be simple, precise, economical, less time consuming and reproducible. Hence, the suggested procedure could be used for the determination of all the four drugs in commercial preparations.

Keywords: nimesulide, phenylephrine HCl, chlorpheniramine maleate, caffeine, RP–HPLC

Nimesulide (NS), 4-nitro-2-phenoxy-methanesulfonamide is a non-steroidal anti-inflammatory drug with antipyretic and analgesic properties. Various methods have been reported for the assay of NS in pharmaceutical formulations such as fluorimetry (1), spectrophotometry (2–6), HPLC (7–11), thin layer chromatography (12), voltametry (13) and capillary electrophoresis (14, 15).

Phenylephrine hydrochloride (PE), (R)-3-[1-hydroxy-2-(methylamino)ethyl]phenol hydrochloride is an α_1 adrenergic receptor agonist generally used as a decongestant, mydriatic, vasopressor and detumescent agent. Extensive literature survey has revealed various methods for the estimation of PE either alone or along with other drugs, such as capillary zone electrophoresis (15), fluorimetry (16), spectrophotometry (17, 18), spectrofluorometry (19) and HPLC method (20, 21).

Chlorpheniramine maleate (CPM), 3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-yl propan-

-amine (Z)-butenedioate is pharmacologically classified as an antihistaminic drug and is generally used in allergic conditions like rhinitis and urticaria. Various analytical procedures such as spectrophotometry (22) and HPLC (23–26) have been reported in the literature for the estimation of drug either alone or in combination with other drugs. Caffeine (CF), 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. Few estimation methods have been reported for the determination of this drug such as HPLC (26, 27).

Fixed dose combinations containing all the drugs are available in the tablet dosage form in the market, which are shown in Table 1. Till to date, no RP HPLC method for the simultaneous estimation of these drugs has been reported. To the best of our knowledge, present study is the first and best report for the purpose. The objective of the study is to

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Table 1. Marketed formulation containing estimated drugs.

No.	Marketed formulation	Manufactured by	Composition	Therapeutic action
1.	Nicip cold tablets	Cipla Pharma	Nimesulide – 100 mg Phenylephrine HCl – 12.5 mg Chlorpheniramine maleate – 4 mg Caffeine (anhydrous) – 30 mg	Anti-allergic and anti cold
2.	Nimumac cold tablets	Torque Pharma	Nimesulide – 100 mg Phenylephrine HCl – 5 mg Chlorpheniramine maleate – 4 mg Caffeine (anhydrous) – 30 mg	Anti-allergic and anti cold
3.	Synarin tablets	Embiotic Laboratories	Nimesulide – 100 mg Phenylephrine HCl – 10 mg Chlorpheniramine maleate – 4 mg Caffeine (anhydrous) – 30 mg	Anti-allergic and anti cold

develop a simple, sensitive, validated and best suitable routine analysis of all the four drugs in pharmaceutical dosage forms.

EXPERIMENTAL

Chemicals and reagents

Pharmaceutical grade (>99%) drugs NS, PE, CPM and CF were obtained from Acme Life Sciences Pvt. Ltd. Baddi, District Solan, Himachal Pradesh, India. Sodium lauryl sulfate, potassium dihydrogen phosphate, orthophosphoric acid and methanol were obtained from Rankem, Ranbaxy Fine Chemicals Ltd., Okhla, New Delhi, India. Triethylamine was obtained from Himeda Labs Pvt. Ltd., Mumbai, India. All dilutions were performed in standard volumetric glasswares which were soaked overnight in a mixture of chromic acid and sulfuric acid, rinsed thoroughly with distilled water and dried in hot air oven.

Instrumentation and chromatographic conditions

The instrument used was a Shimadzu chromatographic system (Japan), equipped with LC-2010 CHC binary gradient system having SPD-10A UV-Visible detector. Chromatographic separation was performed on reversed phase Hypersil phenyl column (4.6 mm × 25 cm) coupled with a guard column of the same material. Different mobile phases were tested in order to find the best conditions for separating the drugs simultaneously. The optimal composition of mobile phase was determined to be methanol and buffer in the ratio of 55 : 45 (v/v) having pH 5.5. Buffer solution was prepared by dissolving 0.383 g of potassium dihydrogen orthophosphate, 0.0562 g of triethylamine hydrochloride, 0.2812 g of sodium lauryl sulfate and 0.1125 mL of

orthophosphoric acid in 45 mL of distilled water. After addition of buffer, the pH of mobile phase was 5.5. The flow rate was maintained at 1.0 mL/min. The column effluents were monitored on UV-Visible detector set at 214 nm.

Preparation of working standard solutions

Stock solution of NS (1000 µg/mL, solution A) was prepared by transferring 50 mg of NS into a 50 mL volumetric flask and finally diluted up to the mark with mobile phase. Stock solution of PE (600 µg/mL, Solution B) was prepared by adding 30 mg of PE into a 50 mL volumetric flask and adding sufficient amount of mobile phase. The resulting solution was sonicated for 10 min and diluted up to the mark with mobile phase. Stock of CPM (500 µg/mL, Solution C) was prepared by adding 25 mg of CPM into a 50 mL volumetric flask. Then, a sufficient amount of mobile phase was added and further sonicated to dissolve, then cooled and finally diluted up to the mark with mobile phase. Stock solution of CF (1500 µg/mL, Solution D) was prepared by adding 75 mg CF into a 50 mL volumetric flask and sufficient amount of mobile phase was added, and further sonicated to dissolve, cooled and diluted up to the mark with mobile phase. Working standard solutions of NS (500 µg/mL), PE (24 µg/mL), CPM (20 µg/mL) and CF (150 µg/mL) were prepared by diluting 25 mL of solution A, 2 mL of Solution B and C, along with 5 mL of Solution D and 10 mL of methanol into 50 mL volumetric flask and diluting up to the mark with mobile phase.

Calibration curve

The calibration curves were plotted over a concentration range of 300–800 µg/mL for NS, 15–32 µg/mL for PE, 16–32 µg/mL for CPM and 30–180

$\mu\text{g/mL}$ for CF, respectively. The chromatogram of each solution was recorded at 214 nm. Calibration curves were constructed for all the four drugs by plotting peak areas *versus* concentrations. Each reading was the average of three determinations.

Estimation from pharmaceutical dosage form

The contents of 20 capsules were pooled and powder equivalent to about 50 mg of NS was weighed accurately and placed into 100 mL measuring flask. Ten milliliters of methanol was added and the solution was sonicated for 10 min and cooled. Then, sufficient amount of mobile phase was added and the solution was again sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with mobile phase. The filtrate and washings were com-

bined in a 100 mL volumetric flask and diluted up to the mark with mobile phase. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20 μL sample vial of the tray. The chromatogram was recorded. The injections were repeated five times and the peak areas were recorded.

Method validation

The method was validated for analytical procedures according to ICH guidelines in order to determine the linearity, sensitivity, precision and accuracy for the analyte. Regression characteristics, validation and system suitability parameters analysis of NS, PE, CPM and CF in pharmaceutical dosage form are shown in Table 2.

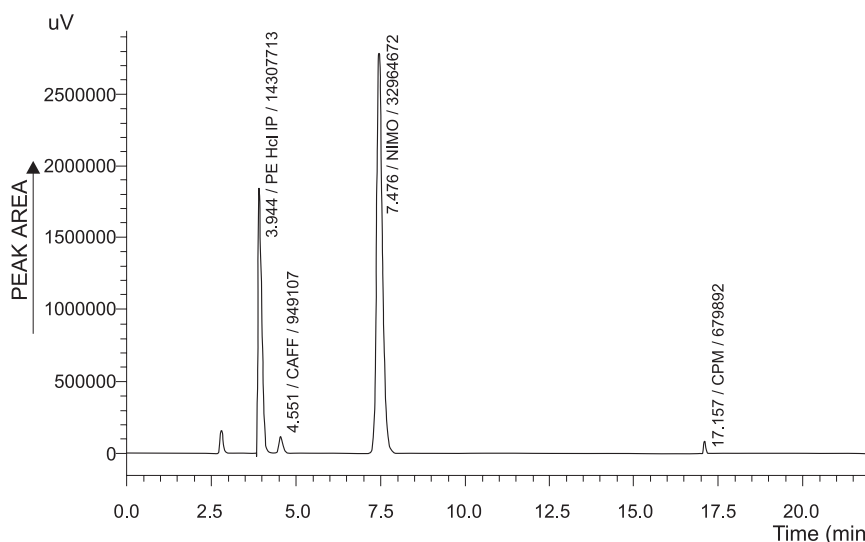


Figure 1. Chromatogram of sample solution measured at 214 nm by HPLC showing peaks of PE (retention time 3.944 min), CF (retention time 4.55 min), NS (retention time 7.47 min) and CPM (retention time 17.15 min)

Table 2. Regression characteristics, validation and system suitability parameters of HPLC method for analysis of NS, PE, CPM and CF in pharmaceutical dosage form.

	NS	PE	CPM	CF
Retention time (min)	7.47	3.94	17.15	4.55
Theoretical plate number	8913.782	5450.593	7618.717	6837.532
Tailing factor	1.108	1.391	1.421	1.307
Linearity range ($\mu\text{g/mL}$)	300–800	15–32	16–32	30–180
Correlation coefficient (r^2)	0.997	0.996	0.998	0.995
Slope	33115	25747	26567	49099
LOD ($\mu\text{g/mL}$)	3.08	0.84	1.14	4.55
LOQ ($\mu\text{g/mL}$)	9.34	2.54	3.45	0.45
Inter-day (%CV) n = 3	0.065–0.527	0.030–0.925	0.027–0.129	0.045–0.282
Intra-day (%CV) n = 3	0.052–0.596	0.240–1.567	0.017–0.518	0.008–0.0586

Linearity

The linear response of the drugs was determined by analyzing five independent levels of the calibration curve in the range of 300–800, 15–32, 16–32 and 30–180 µg/mL for NS, PE, CPM and CF, respectively, in triplicate.

Precision

Repeatability (Precision on replication)

These studies were performed by preparing the standard solutions of NS (500 µg/mL), PE (24 µg/mL), CPM (20 µg/mL) and CF (150 µg/mL) for three times and analyzing by proposed method, respectively.

Intermediate precision (Reproducibility)

The intra-day precision was determined for standard solution of all the drugs for three times on the same day. The inter-day precision was determined for standard solution of all the drugs for three separate days.

Recovery studies

To ascertain the accuracy of the proposed method, recovery studies were carried out in triplicate by spiking different concentrations of pure drug in the pre analyzed samples with three different concentrations of standards (2, 4, 6 µg/mL for all the drugs) within the analytical concentration range of the proposed method. Percent recoveries for all the drugs were found to be satisfactory.

Limit of detection

Limit of detection can be calculated using the following equation according to ICH guidelines:

$$\text{LOD} = 3.3 \times N/S$$

where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of quantification

Limit of quantification can be calculated using following equation according to ICH guidelines:

$$\text{LOQ} = 10 \times N/S$$

where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION**Method development**

Several mobile phase compositions were tried to resolve the peaks of NS, PE, CPM and CF. The optimum mobile phase having pH 5.5 containing methanol and buffer in the ratio of 55:45 (v/v) was selected. Buffer solution was made by dissolving potassium dihydrogen orthophosphate, triethylamine, sodium lauryl sulfate and orthophosphoric acid in distilled water. The retention time was found to be 7.47, 3.94, 4.55 and 17.15 min for NS, PE, CF and CPM, respectively. Quantification was achieved on UV-VIS detector at 214 nm and flow rate was 1.0 mL/min. A typical HPLC chromatogram was obtained as shown in Figure 1.

Table 3. Data of recovery study for NS, PE, CPM and CF by proposed method.

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	Total amount found (µg/mL)	% Recovery ± S.D. (n = 3)	% Mean recovery ± S.D. (n = 3)
NS	500	2	501.97	98.50 ± 0.25	99.03 ± 0.55
	500	4	503.96	99.00 ± 0.52	
	500	6	505.98	99.60 ± 0.45	
PE	024	2	25.97	98.50 ± 0.29	100.30 ± 0.40
	024	4	28.04	101.00 ± 0.75	
	024	6	30.1	101.60 ± 0.18	
CPM	020	2	21.98	99.20 ± 0.28	100.30 ± 1.33
	020	4	24.01	100.15 ± 0.59	
	020	6	26.11	101.83 ± 0.93	
CF	150	2	151.96	98.00 ± 0.22	99.97 ± 1.71
	150	4	154.03	100.80 ± 0.97	
	150	6	156.06	101.13 ± 1.30	

Table 4. Application of the proposed method to pharmaceutical dosage form.

Parameters	NS	PE	CPM	CF
Amount labelled (mg)	100	5	4	30
Amount found (mg)	97.35	4.80	4.06	29.73
% Amount found \pm S.D. (n = 3)	97.35 \pm 0.05	96.03 \pm 0.098	101.6 \pm 0.41	99.108 \pm 0.0748

Method validation

Linearity

The calibration curves were plotted over a concentration range of 300–800 $\mu\text{g/mL}$ for NS, 15–32 $\mu\text{g/mL}$ for PE, 16–32 $\mu\text{g/mL}$ for CPM and 30–180 $\mu\text{g/mL}$ for CF, respectively. The response was measured as peak area. The best fit for the calibration curve could be achieved by a linear regression equation, which was found to be $y = 33115x + 62494$, $y = 25747x + 17284$, $y = 26567x + 8463$, $y = 49099x + 35938$ for NS, PE, CPM and CF, respectively. The regression coefficient values (r^2) of all the drugs were found to be near 1 indicating high degree of linearity for all the drugs as shown in Table 2.

Precision

Percentage relative standard deviation (% RSD) or coefficient of variation (CV) was not more than 2% for all the drugs. The intra-day variation and inter-day variation were calculated as stated above.

Limit of detection and limit of quantitation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation and slope was adopted. The limit of detection for NS, PE, CPM and CF was 3.08, 0.84, 1.14 and 4.55 $\mu\text{g/mL}$. The limit of quantitation (LOQ) was found to be 9.34, 2.54, 3.45 and 0.455 $\mu\text{g/mL}$ for the drugs, respectively.

Accuracy

Accuracy of the method was assured by the use of standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for all the drugs were compared with those expected. The good recoveries with the standard addition method proved the accuracy of the proposed method as shown in Table 3.

Application to the pharmaceutical dosage form

The proposed validated method was successfully applied to determine all the drugs in bulk powder as well as in tablet dosage forms as shown in Table 4. No interference of the excipients with the peaks of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of NS, PE, CPM and CF in pharmaceutical dosage forms.

CONCLUSION

A successful attempt was made to develop a simple, precise RP-HPLC method for the simultaneous estimation of NS, PE, CPM and CF in pharmaceutical dosage forms. The amounts obtained by the proposed method were between 99.03% and 100.3%, i.e., within the acceptance level of 95% to 105%. The proposed method is rapid, accurate, selective, and reproducible, as shown by the results obtained. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations are analyzed.

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