AJPRHC

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF THREE-COMPONENT IN TABLET DOSAGE FORMULATION.

RUPALI S JOSHI*, SAMEER S. KATIYAR, DEVENDRA B. ZOPE, AMOL T. SHINDE
For Author affiliations see end of the text
This paper is available online at www.jprhc.in

ABSTRACT:

An accurate, simple, reproducible and sensitive method for the determination of paracetamol, caffeine and chlorpheniramine maleate in tablet dosage form is developed and validated. The separation is achieved using HiQsilC18HS reverse-phase column (250 X 4.6 mm I.D., particle size 5µm) using a mixture of acetonitrile and water in the proportion 55:300 with final pH of 2.4 adjusted with o-phosphoric acid as a mobile phase. The flow rate is 1.0 mL/ min and effluents were monitored at 265 nm. Total run time is less than 12 min. and retention time of paracetamol, caffeine and chlorpheniramine maleate are 6.742, 9.417, and 3.683 min respectively. Validation of method is done as per ICH guideline for accuracy, precision, linearity, specificity, and sensitivity. The linearity for paracetamol is found to be 100-650 µg/mL where as for caffeine and chlorpheniramine maleate is found in the range of 15-100 µg/mL. Result of validation study is found statistically significant because all the statistical parameters were within the acceptance range (COV and S.D. <1.0 for both accuracy and precision). The limits of detection (LOD) values are 1.2014, 0.4587 and 0.8945 and limit of quantitation (LOQ) values are 0.5142, 0.4512 and 0.7845 µg/mL for paracetamol, caffeine and chlorpheniramine maleate respectively. High percentage recovery and low COV value revealed the reliability of the method for quantitative study of three drugs in Fevril tablets as a quality-control tool for routine quantitative determination of paracetamol, caffeine and chlorpheniramine maleate.

Keywords: Paracetamol, Caffeine, Chlorpheniramine maleate, ICH guidelines, RP-HPLC

INTRODUCTION

Without resolving mixtures of paracetamol, caffeine and chlorpheniramine maleate, simultaneous estimation has been successfully achieved by RP-HPLC. The validation is to validate methods by using same parameters as per ICH guidelines.[1] Chemically, paracetamol is *N*-acetyl-*p*-aminophenol, commonly used analgesic and antipyretic drug. Caffeine is 3, 7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or its monohydrate, used as central nervous system stimulant. Chlorpheniramine maleate is 3-(4-chlorophenyl)-N, N-dimethyl-3-pyridin-2-ylpropan-1-amine used as an antihistamine drug in pharmaceutical preparations for symptomatic relief of common cold and allergic diseases. Literature survey revealed that there are many methods reported for the determination of paracetamol by spectroscopy[2,3], HPLC[4]; caffeine by FTIR spectrophotometric[5], UV/vis spectrophotometric[6], and chlorpheniramine maleate by micellar electrokinetic chromatography[7], differential-derivative spectrophotometric[8], ion-pair liquid chromatography [9] methods in combination with other drugs in pharmaceutical dosage form. The same combination has been estimated and validated using conventional UV -spectrophotometric method.[10] The present work is the extension of previous work, with RP-HPLC method which is new modern technique with features including more accuracy, reproducible, sensitive, specific as compared to UV-visible spectrophotometric methods.

EXPERIMENTAL

Materials

Pure sample of paracetamol was obtained as gift samples from Zest Pharma, Indore, chlorpheniramine maleate from Auro Laboratories Ltd., Mumbai and caffeine from UCB Pharma. Vapi. The commercially available tablet, Fevril (Label claim: Paracetamol 650 mg, caffeine 30 mg and chlorpheniramine maleate 2 mg) was procured from local market. Analytical grade ortho-phosphoric acid (Merck), HPLC-grade water (Hi Media Laboratories Pvt. Ltd.) and HPLC-grade acetonitrile (Merck) were used.

Chromatographic System and Conditions

The method development was performed with a LC system consisting of a JASCO HPLC-2000 solvent delivery system with universal loop injector (Rheodyne 7725i) of injection capacity of 20 µL., JASCO UV-2075 Plus intelligent UV-Visible detector and JASCO PU-2080 isocratic HPLC pump. Separation was carried out on a HiQsilC18HS (250 X 4.6 mm I.D., particle size 5µm) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC installed properly with the chromatographic software. Whole work was carried

out in an air conditioned room maintained at temperature 25±2^{0}C. The detector was set at 265 nm and peak areas were integrated automatically by computer using the Borwin software program. The mobile phase, a mixture of acetonitrile and water in the proportion 55:300 was prepared, with pH adjusted to 2.4 using ortho phosphoric acid. The flow rate was 1.0 mL/ min and the analyte were monitored at 265 nm. The total run time was less than 12 min. Before analysis both the mobile phase and sample solutions were degassed by the use of a sonicator and filtered through a 0.2 µm filter paper. The identities of three compounds were established by comparing retention time of the sample solution with those of standard solutions.

PROCEDURES

Preparation of Standard Solution and Construction of Calibration Plots

The standard stock solutions of paracetamol, caffeine and chlorpheniramine maleate were prepared by dissolving 50 mg of each drug in 100 mL of mobile phase. From the stock solutions further dilutions were prepared by diluting required volume of solution with mobile phase and their area was noted by injecting 20 µL into the system. Then calibration curve was plotted, concentration against their respective area for paracetamol, caffeine and chlorpheniramine maleate separately. From the calibration curve it was found that paracetamol has linearity range between 100-650 µg/mL whereas for caffeine and chlorpheniramine maleate was 15-100 µg/mL. (Fig.2.)

Assay of tablet formulation

Twenty tablets of marketed formulation were accurately weighed individually and their average weight was determined after that they were crushed to fine powder. Standard addition method was used for analysis. A quantity of powder equivalent to 2 mg of chlorpheniramine maleate was weighed. An accurately weighed 98 mg of pure chlorpheniramine maleate and 70 mg of caffeine was added to finely powdered samples to get the concentration of chlorpheniramine maleate and caffeine in linearity range. With this addition, the ratio of paracetamol, caffeine and chlorpheniramine maleate in the samples was brought to 6.5:1:1. This sample powered dissolved in 50 mL of mobile phase in 100 mL volumetric flask and filtered through Whatman No.41 filter paper and the residue was washed with mobile phase. Then volume was made up to mark with mobile phase. Of this 0.5 mL of solution was diluted upto 10 mL with mobile phase to obtain final concentration of 325 µg/mL of paracetamol, 50 µg/mL of caffeine and chlorpheniramine maleate. The amounts of paracetamol, caffeine and chlorpheniramine maleate per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation was reported in Table I.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

The mobile phase is chosen after several trials with methanol, isopropyl alcohol, acetonitrile and water in various proportions and at different pH values. A mobile phase consisting of a mixture of acetonitrile and water in the proportion 55:300, with pH adjusted to 2.4 using ortho phosphoric acid is selected to achieve maximum separation and sensitivity. Flow rates between 0.5 to 1.5 per min are studied. A flow rate of 1.0 mL/min shows an optimal signal to noise ratio with a reasonable separation time. To determine the appropriate wavelength for simultaneous determination of paracetamol, caffeine and chlorpheniramine maleate solutions of these compounds in the mobile phase are scanned by UV–visible spectrophotometer in the range 200–400 nm. From the overlain UV spectra, suitable wavelength considered for monitoring the drugs is 265 nm. Solutions of each substance in the mobile phase are also injected directly for HPLC analysis and the responses (peak area) are recorded at 265 nm. It is observed that, no interference from the mobile phase or baseline disturbance and these three drugs absorbed well at 265 nm. It is, therefore, concluded that 265 nm is the most appropriate wavelength for analysis of both the drugs with suitable sensitivity (Fig.1.). Using a reversed-phase C18 column, the retention times for mixture of standard paracetamol, caffeine and chlorpheniramine maleate are observed to be 6.742, 9.417, and 3.683 min respectively. Total time of analysis is less than 12 min. The chromatogram at 265 nm showed a complete resolution of all peaks (Fig.3). The resolution (R_s) between three curves is 9.42 and 6.93. The result of capacity factor, tailing factor, theoretical plate's number are reported in Table 1.

The values obtained for these properties shows ($1 < k < 10$, $R_s > 2$) these chromatographic conditions are appropriate for separation and quantification of three compounds. The number of plates (N) is a measure of column efficiency; which shows the good separation efficiency of the column used.

Validation

The method is validated for linearity, accuracy, precision, repeatability, selectivity and specificity study. All the validation study is carried out by replicate injection of the sample and standard solutions.

Linearity

The linearity is determined for three drug, paracetamol, caffeine and chlorpheniramine maleate separately by plotting a calibration graph of peak area against their respective concentration. From the

calibration curve it is clear that paracetamol has linearity range between 100-650 µg/mL whereas for caffeine and chlorpheniramine maleates are in between 15-100 µg/mL. (Fig.2.)The linear regression equation for three drugs is as follows

$$y = 32525x + 66767$$ (R^2 =0.9964)……Paracetamol

$$y = 44974x + 95130$$ (R^2 =0.9951)……..Caffeine

$$y = 1188.2x - 459.63$$ (R^2 =0.9996)……..Chlorpheniramine maleate

Where y: peak area and x: concentration.

Percentage estimation of three drugs found in tablet dosage form are 101.50, 99.50 and 98.90 for paracetamol, caffeine and chlorpheniramine maleate respectively with standard deviation <1 (Tab. 2).

Accuracy

Accuracy of the developed method is confirmed by performing recovery study as per ICH norms at three different concentration levels- 80%, 100%, 120% by replicate analysis (n=3). Here to a preanalysed sample solution, standard drug solutions are added and then percentage of drug content is calculated. The result of accuracy study is reported in Table 3.

Recovery study reveals that the method is very accurate for quantitative estimation of paracetamol, chlorpheniramine maleate and caffeine in tablet dosage form as the statistical results are within the acceptance range i.e. COV and S.D. < 1.0.

Precision and LOD and LOQ study

Precision is determined by studying the repeatability and intermediate precision.

Repeatability

Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error are calculated for three drugs. Repeatability is performed for six times with tablets formulation. The results of statistical evaluation are given in Table 2.

Intermediate Precision (Inter-day and Intra-day precision)

An intermediate precision is carried out by intra and inter day precision study. In intra day study concentration of three drugs are calculated on the same day at an interval of one hour. In inter day study the concentration of drug contents is calculated on three different days. Study expresses within laboratory variation in different days. In both intra and inter-day precision study for the methods COV are not more than 1.0 indicates good intermediate precision.

LOD and LOQ study is carried out to evaluate the detection and quantification limits of the method to determine presence of any impurities by using following equation;

LOD = 3.3 σ / S

LOQ = 10 σ / S

Where σ is the standard deviation and S is the slope of the curve.

The result is reported in Table 4. The developed method is précised for quantitative study as the precision study found statistically significant as COV and S.D. < 1.0 for intra and inters day study.

Selectivity and Specificity

To check the selectivity of the developed method solutions of three the drugs are injected into the system and it is observed that three sharp peaks for paracetamol, caffeine and chlorpheniramine maleate are obtained at retention time of 6.742, 9.417, and 3.683 min. respectively in reference to placebo solution. Specificity of the method is assessed by comparing the chromatograms obtained from standard drugs (Fig.3.) with the chromatogram obtained from tablet (Fig.4.) solutions. As the retention time of standard drugs and the retention time of three drugs in sample solutions are same, so the method is specific. The developed method is found specific and selective as there is no interference of excipients found.

Table. 1. Result from system-suitability study

Property	PCM	CAF	CPM
Rt	6.742	9.417	3.683
T_f	1.25	1.15	1.38
k'	2.44	2.45	2.34
N	5466	7682	2743
Rs	9.42	6.93	-

PCM: Paracetamol, CAF: Caffeine, CPM: Chlorpheniramine maleate,
Rt : Retention time, T_f: Tailing factor, k' : Capacity factor,
N : Theoretical plates number Rs : Resolution

Table.2. Result of assay of tablet formulation

Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E*.
PCM	600	609	101.50	0.5874	0.5787	0.2398
CAF	30	29.85	99.50	0.9412	0.9459	0.3842
CPM	2	1.97	98.90	0.5402	0.5462	0.2205

PCM: Paracetamol, CAF: Caffeine, CPM: Chlorpheniramine maleate, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation.

Table. 3. Result of recovery study

Recovery level Amount Added	Mean % Recovery±S.D.#			COV		
	PCM	CAF	CPM	PCM	CAF	CPM
80%	98.70± 0.4512	100.40± 0.7812	100.30± 0.1542	0.4571	0.7780	0.1537
100%	99.50± 0.3652	101.20± 0.9874	101.50± 0.3256	0.3670	0.9756	0.3207
120%	98.80± 0.7812	98.60± 0.3210	98.90± 0.4512	0.7906	0.3255	0.4562

PCM: Paracetamol, CAF: Caffeine, CPM: Chlorpheniramine maleate,S.D.: Standard deviation, COV: Coefficient of variation, # Average of three estimation at each level of recovery

Table. 4. Result of intra day and inter day precision, LOD and LOQ study

Parameters	Values		
	PCM	CAF	CPM
LOD* (µg/mL)	1.2014	0.4587	0.8945
LOQ*(µg/mL)	0.5142	0.4512	0.7845
Intra-Day*Precision (COV)	0.9424	0.6495	0.5172
Inter-Day Precision (COV) n=3	0.5186	0.5190	0.4327

PCM: Paracetamol, CAF: Caffeine, CPM: Chlorpheniramine maleate, COV: Coefficient of variation, * Average of six determination.

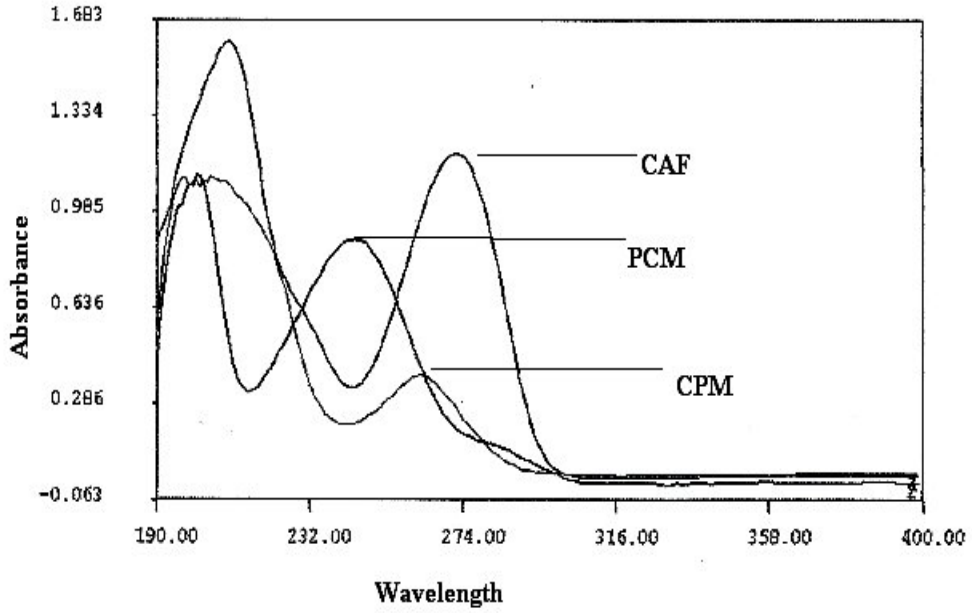

Figure 1. Overlain spectra of PCM- Paracetamol, CAF-Caffeine, and CPM- Chlorpheniramine maleate in mobile phase showing well absorption at 265 nm.

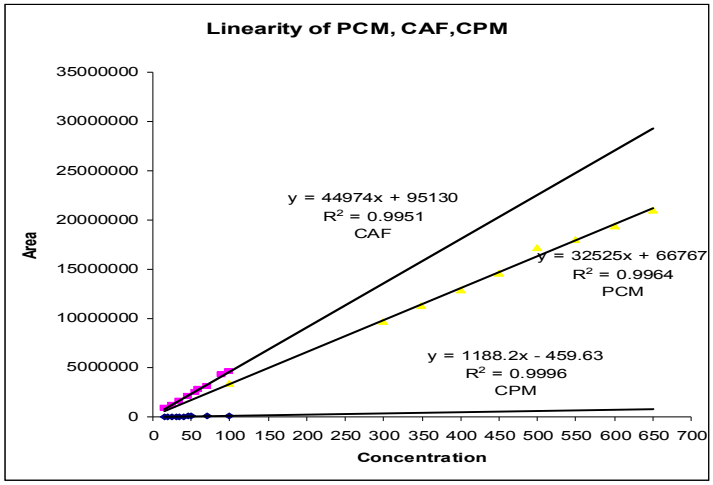

Figure 2. Linearity graph of PCM- Paracetamol, CAF-Caffeine, and CPM- Chlorpheniramine maleate.

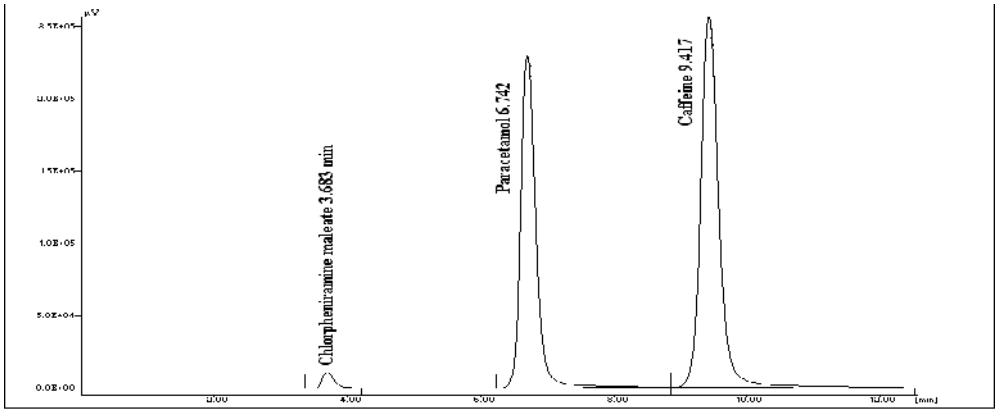

Figure 3. Chromatogram of Mixture of standard containing chlorpheniramine maleate, paracetamol and caffeine having concentration 100µg/ml.of each.

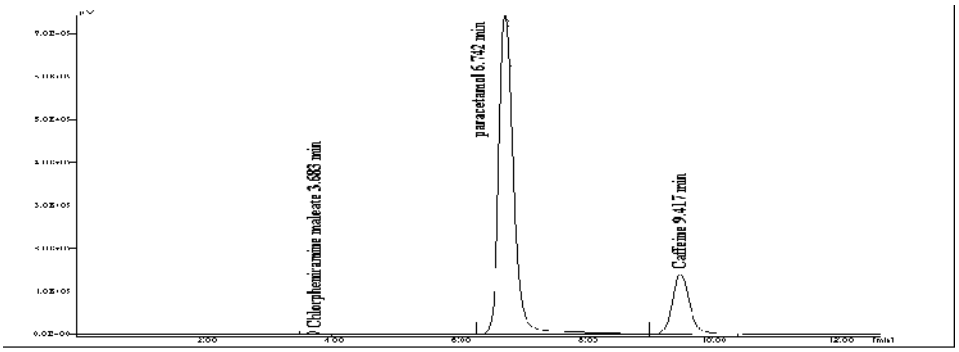

Figure 4. Chromatogram of Tablet sample containing chlorpheniramine maleate, paracetamol and caffeine having concentration 50,325, 50µg/ml.respectively.

CONCLUSION

The developed method is suitable for the identification and quantification of the combination of paracetamol, caffeine and chlorpheniramine maleate. A high percentage recovery and low COV value shows that the method can be successfully used on a routine basis. The COV and S.D. is less than 1 as compared to UV-visible spectrophotometric methods which ranges less than 2, makes the method more accurate, specific, reproducible and sensitive. The result shows that method could find practical application as a quality-control tool for the simultaneous estimation of three drugs from their combined dosage form in a quality-control laboratory.

REFERENCES

1. ICH, Validation of Analytical Procedure, Text and Methodology Q2(R1), International conference on Harmonization, IFPMA, Geneve, Switzerland, 2005.
2. Elsayed MA., Belal SF, Elwalily AF, Abdine H, Spectrophotometric determination of acetaminophen in tablets, syrups, and suppositories. Anal Chem., 62(3), 1979, 549-551. PMID: 479075.
3. Garg G, Saraf S, Saraf S, Simultaneous estimation of aceclofenac, paracetamol and chlorzoxazone in tablets. Indian J Pharm Sci., 69, 2007, 692-694.DOI: 10.4103/0250-474X.38481
4. Martin A, Garcia A, Barba C, Validation of a HPLC quantification of acetaminophen, phenylephrine and chlorphenitamine in pharmaceutical formulations: capsules and sachets. J. Pharm Bio Med Anal., 29(4), 2002, 701-714. PMID: 12093499
5. Paradkar MM, Irudayaraj J, A Rapid FTIR Spectroscopic method for estimation of caffeine in soft drinks and total methylxanthines in tea and coffee. J. food sci., 67(7), 2002, 2507–2511. DOI: 10.1111/j.1365-2621.2002.tb08767.x
6. Abebe B, Kassahun T, Mesfin R, Araya A, Measurement of caffeine in coffee beans with UV/vis spectrometer. Food Chemistry, 108(1), 2008, 310-315
7. Suntornsuk L, Pipitharome O, Wilairat P, Simultaneous determination of paracetamol and chlorpheniramine maleate by micellar electrokinetic Chromatography. J. Pharm. Biomed., 33(3), 2003, 441-449.

8. Erk N, Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods. J.Pharm. Biomed. Anal., 23, 2000, 1023. PMID: 11095304

9. De Beer JO., Vandenbroucke CV, Massart DL, .Experimental design for the rapid selectio of separation conditiones for methyl and propyl parahydroxybeyoate, phenylephrine hydrochloride and chlorphenamine maleate by ion-pair liquid chromatography. J. Pharm. Biomed. Anal., 12, 1994, 1379.PMID: 7849134

10. Joshi RS, Pawar NS, Sawant RL Validated spectrophotometric methods for simultaneous estimation of acetaminophen, chlorpheniramine maleateand cafeine in pure and tablet dosage form, Lat. Am. J. Pharm., 29 (7), 2010,1226-30.

AUTHORS AFFILIATIONS AND ADDRESS FOR CORRESPONDENCE

PDVVPF's College of Pharmacy, Post - MIDC,
Vilad Ghat, Ahmednagar 414111, (MS), India.
Ms. Rupali Sanjay Joshi
Lecturer, Pharmaceutical Chemistry Department,
P.D.V.V.P.F's College of Pharmacy,
Vilad – Ghat, Post MIDC,
Ahmednagar – 414 111 (M.S.) India.
Email :j.rupali2006@yahoo.in ☎ : +91901126440