Full Length Research Manuscript

Simultaneous Determination of Chlorpheniramine Maleate, Paracetamol and Phenylephrine Hydrochloride in Tablet Dosage Form by High Performance Liquid Chromatography

Sehrawat Renu*, Khatak Mamta, Maithani Mukesh[a], Khatak Sunil[b]
*International Institute of Pharmaceutical Sciences, Sonepat, Haryana.
[a]Analytical Research Zydus Research Centre, Ahmedabad, Gujrat.
[b]R,K.G.I.T. College of Pharmacy, Ghaziabad, Uttar Pradesh

Abstract

A new simple, rapid and sensitive HPLC method has been developed and validated for the simultaneous determination of chlorpheniramine maleate, paracetamol and phenylephrine hydrochloride in pharmaceutical preparation. Separation was achieved on a 250mm x 4.6mm, 5μm particle, C_8 column using an isocratic mobile phase consisting of 0.01M phosphate buffer: acetonitrile (70:30), pH of the mobile phase was adjusted at 3 with 50% orthophosphoric acid. The analysis was performed at flow rate of 1mLmin^{-1}. Detection of all compounds was carried by UV absorbance at 230nm and elution of the analytes was achieved in less than 8 minutes. The method was validated as per ICH guidelines. The linearity, accuracy and precision of the method were acceptable to good over the concentration range of 5-60μg mL^{-1} for all the three drugs. The lower limit of detection was found to be 0.36, 0.36 and 0.28μg mL^{-1} for chlorpheniramine maleate, paracetamol and phenylephrine hydrochloride respectively. The developed method is effectively used for the routine analysis of formulations for both quality control and assessment of stability.

*Corresponding author, Mailing address:
Renu Sehrawat
H.No. 206/1, Pkt: D-12, Sector-7,
Rohini, Delhi-110085
sunil_khatak@rediffmail.com

How to Cite this Paper:

Sehrawat Renu "Simultaneous Determination of Chlorpheniramine Maleate, Paracetamol and Phenylephrine Hydrochloride in Tablet Dosage Form by High Performance Liquid Chromatography" Int. J. Drug Dev. & Res., January-March 2013, 5(1): 258-263.

INTRODUCTION:

Combination of chlorpheniramine maleate (CPM), paracetamol (PCM) and phenylephrine hydrochloride (PE) is widely used for cough and cold

treatment as an antihistamine, analgesic and decongestant [1]. CPM [(RS)-3-(4-chlorophenyl)-3-(2-pyridyl) propylmethylamine hydrogen maleate] is a histamine H1 antagonist used in allergic reactions, hay fever, rhinitis, urticaria, and asthma. PCM [(N-(4-hydroxyphenyl) acetamide)] is a compound with analgesic and antipyretic properties [2, 3]. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding. PE [(3-{(1R)-1-hydroxy-2-methylaminoethyl} phenol hydrochloride)] is α-adrenergic agonist used as a mydriatic, nasal decongestant, and cardio tonic agent [4]. Structures of all three drugs depicted in Figure 1. Pharmaceutical formulations for the relief common cold symptoms usually contain a high proportion of acetaminophen and small amounts of phenylephrine hydrochloride and chlorpheniramine maleate this create a problem during simultaneous estimation of drugs.

Literature survey reveled that there have been numerous publications describing different HPLC methods for quantification of these three compounds both individually and in combination with other drugs but few HPLC methods have been used for simultaneous determination of CPM, PCM and PE in cold formulations. The method of Senyuva and Ozden [5] permits the rapid determination of the three actives in combined pharmaceutical dosage forms using a Bondapak CN column nevertheless acetaminophen is not separated from the solvent front, with the corresponding quantification problems, Kanumula *et al,*[6] that uses wavelength programming and pseudoephedrine hydrochloride as internal standard and the method developed by Krieger [7] for the separation of acetaminophen in analgesic preparations containing chlorpheniramine maleate, phenylephrine hydrochloride, and other active components by HPLC. Olmo *et al,* developed a method using two cyano column for the separation of these three drugs with impurities, but with high retention time [8].

Hence, aim of present study is to develop a simpler, accurate and selective validated isocratic HPLC method with simple C-8 column for the simultaneous estimation of the three actives in tablet pharmaceutical formulations.
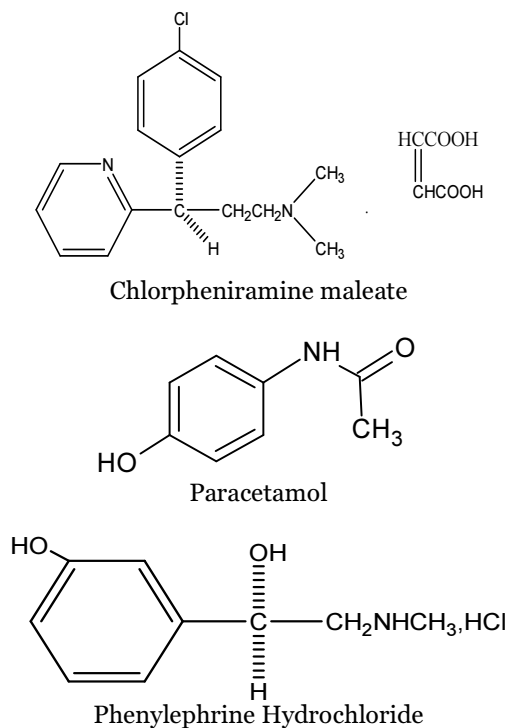

Figure 1: The structures of chlorpheniramine maleate, paracetamol and phenylephrine hydrochloride

EXPERIMENT:

Chemical and Reagents:

Working reference standard of chlorpheniramine Maleate, Paracetamol and Phenylephrine hydrochloride were kindly gifted by Sun Pharma, Mumbai, India. HPLC grade Acetonitrile, Methanol, Water, Orthophosphoric acid, Di-sodium hydrogen ortho-phosphate was purchased from Rankem, Ranbaxy Fine Chemical Limited, New Delhi, India. Mobile Phase and solution for the injection were filtered through 0.45μm membrane before use.

Chromatographic Conditions:

The HPLC (Perkin Elmer) instrument was equipped with a model series 200 pump, vacuum degasser (PerkinElmer series 200), Rheodyne injector with a 20μl loop and UV-Visible detector (PerkinElmer

Full Length Research Manuscript

series 200). Separation and quantitation was achieved on a reversed phase Princeton C_8 analytical column (250 x 4.6mm, 5μm particle size) using mobile phase 0.01M Na_2HPO_4 buffer: acetonitrile with Total Chrom Navigator S 200 Perkin Elmer software. The pH of mobile phase was adjusted at 3 with 50% orthophosphoric acid. The analysis was performed at flow rate of 1 mLmin^{-1}. The system was equilibrated with the mobile phase before injection. The column effluent was monitored on UV detector set at 230nm.

Preparation of Stock and Standard Solutions:

A stock solution of CPM, PCM and PE (1 mg mL^{-1}) were prepared by dissolving 100 mg CPM, PCM and PE in 100ml mobile phase (70:30, 0.01M Na_2HPO_4 buffer: acetonitrile) Appropriate volumes of the stock solution were transferred to 10mL volumetric flasks and the solutions were diluted with mobile phase to furnish final concentrations of all the three drugs in the range 5–60μg mL^{-1}.

Preparation of Pharmaceutical Formulations for Assay:

Twenty tablets were weighed, their mean weight determined and finely powdered. An equivalent weight to 500 mg Paracetamol, 10mg Phenylephrine hydrochloride and 4mg Chlorpheniramine Maleate weighed and transferred into a 10 ml volumetric flask and diluted to 10mL with mobile phase to get 5000 μgmL^{-1} PCM, 100 μgmL^{-1} PE and 400 μgmL^{-1} CPM stock solution. The resulting solution was centrifuged at 3000 rpm for 5 minute. Further dilution was performed with mobile phase to reach the calibration range for each compound.

Commercial Pharmaceutical Preparation:

Maxtra P (500mg paracetamol, 10mg phenylephrine hydrochloride and 4mg chlorpheniramine maleate) a tablet from Zuventus Healthcare Ltd., India were assayed.

RESULTS AND DISCUSSION:

Method Development and Optimization:

Chromatographic conditions were optimized with pH, mobile phase, wavelength and flow rate for the separation CPM, PCM and PE on a C_8 column. During the optimization of the method pH was tested from 6.7 to 2.75 (adjusted by addition of orthophosphoric acid) and two organic solvents (methanol and acetonitrile) were tested. Different percentage of acetonitrile (20 to 70%) was investigated which showed variation in retention of solutes with mobile phase composition follows the usual trends and showed faster elution of all studied compounds. But there was a problem of resolution between compounds. Therefore, a percentage of 30% was selected as an optimum as it offers a compromise between resolution and analysis time. A low pH was used to promote rapid elution and better peak shape of CPM and thus reduce total analysis time. The concomitant effects of optimum eluent composition, pH of the mobile phase and flow rate for the determination of CPM, CM and PE by HPLC were studied.

The conditions finally adopted were a C_8 column (250 x 4.6mm, 5μm particle size), mobile phase 0.01M Na_2HPO_4 buffer: acetonitrile (70: 30) with pH 3, flow rate 1mLmin^{-1}, 20 μl injection volume at ambient temperature. A typical chromatogram of standard drugs and marketed formulation are shown in figure 2 and figure 3 respectively.
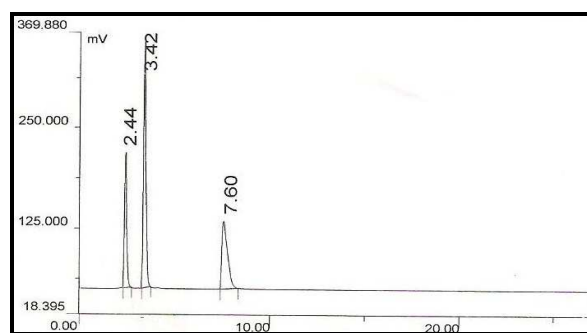

Figure 2: Typical chromatogram of standard solution of PE, PCM and CPM with RT 2.44, 3.42 and 7.60 respectively.
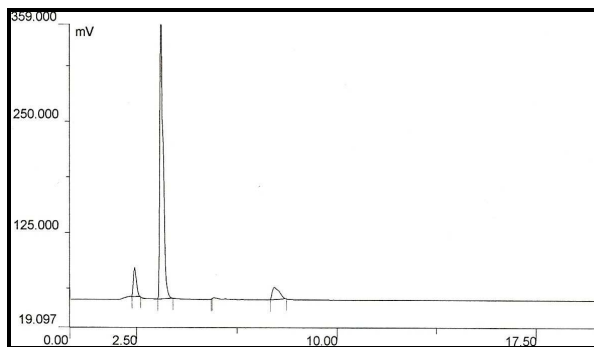
Figure 3: Chromatogram of sample solution of PE, PCM and CPM with RT 2.44, 3.41 and 7.58 respectively.

Linearity:

The calibration curves were established with seven different concentrations in the range 5-60µg for all the three drugs i.e. CPM, PCM and PE. Each solution was injected three times and a regression equation was established by plotting the peak mean area to concentration of drugs. The best fit for the calibration curve could be achieved by linear regression equation and regression coefficient values (r^2) of CPM, PCM and PE was found to be 0.9998, 0.9996 and 0.9996 respectively indicating a high degree of linearity for all drugs.

Table 1: Characteristic parameters of the proposed HPLC method for determination of CPM, PCM and PE.

Parameter	CPM	PCM	PE
Calibration range (µgmL^{-1})	5-60	5-60	5-60
LOD	0.36	0.36	0.28
LOQ	1.1	1.1	0.86
Regression equation	Y=42177x +97839	Y=82080x+ 219257	Y= 35003x + 75428
Intercept	97839	219257	75428
Correlation coefficient	0.9998	0.9996	0.9996

Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products [9]. The specificity studies revealed the absence of any other excipients interference, since none of the peaks appeared at the same retention time, as shown in figure 2. The interaction study of all drugs in standard solution was also carried out by comparing peak of each drug, individually Vs peaks of drug mixture.

Limit of Detection and Quantitation:

The limit of detection (LOD) was the lowest concentration of the analyte in a sample that could be detected under the stated experimental condition and limit of quantitation (LOQ), the lowest concentration of the active ingredients in a sample that could be determined with acceptable precision and accuracy. According to International Conference on Harmonization (ICH) recommendation, the approach based on the standard deviation (SD) of the response and slope (m) was used for determining the detection and quantitation limits [10]. LOD can be calculated according to formula: LOD = 3.3 (SD/m) and LOQ can be calculated according to formula: LOQ = 10 (SD/m). LOD values were found to be 0.36µgmL^{-1} for CPM, 0.36µgmL^{-1} for PCM and 0.28µgmL^{-1} for PE. LOQ values were found to be 1.1µgmL^{-1} for CPM, 1.1µgmL^{-1} for PCM and 0.86µgmL^{-1} for PE. Different statistical data are given in table 1.

Precision:

The precision of analytical procedure expresses degree of the agreement among individuals test when the procedure is applied repeatedly to multiple sampling of a homogeneous sample. Precision are considered at two levels: repeatability (intraday) and intermediate precision (inter-day). Intraday precision was determined by replicates analysis of the sample on one day. Inter-day precision was determined on three successive days and the corresponding results were expressed as the relative standard deviation (RSD) [11]. The RSD values for intra and inter-day precision were varied from 0.06% to 1.45% and from 0.05% to 1.75% respectively. The

data of repeatability and intermediate precision are presented in table 2.

Table 2: Results of the precision (intra- and inter-days) for standard solution.

Compound	Concentration (μgmL⁻¹)	Intraday mean RSD (%)	Inter-day mean RSD (%)
CPM	5-60	0.97	1.19
PCM	5-60	1.45	0.62
PE	5-60	0.83	0.83

Accuracy and Recovery:

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and value found. It was determined by applying the analytical method to synthetic mixture of excipients to which known quantities of the drugs, corresponding to 80%, 100% and 120% of the label claim had been added [12]. Each concentration was analyzed three times and recovery and RSD were calculated. According to the obtained results a good accuracy was observed from this method. The excipients in pharmaceutical formulation do not interfere in the analysis of these compounds in their pharmaceutical formulation. The data of recovery studies are compiled in table 3.

Table 3: Results of recovery tests for the drugs under study by proposed method.

Percentage of label claim	Chlorpheniramine maleate		Paracetamol		Phenylephrine hydrochloride	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
80%	99.36	0.06	99.24	0.19	99.36	0.06
100%	99.30	0.38	99.03	0.07	99.30	0.38
120%	100.50	1.20	100	0.10	98.80	1.20

Assay of Pharmaceutical Dosage Form:

The proposed method was successfully used to determine CPM, PCM and PE in their dosage forms. The amount of each compound was calculated using calibration curve method. Satisfactory results as listed in table 4 were obtained for each compound in good agreement with label claims. The results obtained indicate that the content of drug

corresponds to the drug label, which confirms good accuracy of the proposed method. Indeed, the assay percentage with respect to label claims were ranged 99.64 to 100.58% which showed that estimation of dosage form was accurate.

Table 4: Assay results of active ingredients in commercial sample

Drug	Conc. Claimed (mg/tab.)	Mean (μV.sec)	SD	RSD (%)	Conc. Found (mg/tab.)	% Assay
Paracetamol	500	2257226	21288	0.943143	502.9	100.58%
Phenylephrine Hydrochloride	10	459104.6	873.55	0.190274	9.96	99.64%
Chlorpheniramine Maleate	4	315916.7	2976.8	0.94229	3.99	99.75%

System Suitability:

System suitability parameters must be checked to ensure that the system is working correctly during the analysis [13]. System suitability data for the method were determined by analysis of standard working solutions. The resolution, capacity factor, tailing factor, theoretical plates, retention volume and asymmetric factor were determined and listed in table 5. From the table it is apparent that column efficiency is adequate, peaks well resolved from each other and from the void volume.

Table 5: System suitability data

Parameter	Phenylephrine Hydrochloride	Paracetamol	Chlorpheniramine Maleate
Resolution	-	2.45	8.36
Theoretical plate	3422	6561	12693
Capacity factor	0.15	0.76	1.8
Asymmetric factor	1.2	1.1	1.3
Tailing factor	1.3	1.2	1.5
Retention volume	2.44	2.42	7.60

CONCLUSION:

An isocratic HPLC method has been developed for the determination of chlorpheniramine maleate, paracetamol and phenylephrine hydrochloride in tablet dosage form, in less than 8 minute. The method is simpler and more accurate than the developed methods. Results from validation

experiments showed that the method is reliable and accurate and can therefore be used for routine analysis of the tablets for both quality control and assessment of stability.

REFERENCES:

1) Rang HP, Dale MM, Ritter JM, Moore PK. Test book of Pharmacology. Churchill Livingstone, 2007, pp253.

2) The Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, Delhi, Vol. I, II; 176, 177,556, 587, (1996).

3) The British Pharmacopoeia, The British Pharmacopoeia Commission, London, vol. 1; 498, 1653, 1654, 1714, 1715, (2008).

4) Lemke LT, Williams AD, Roche FT, Zito WS Foye's, Principle of Medicinal Chemistry, Lippincott Williams and Wilkines, 2008, pp 392-405.

5) Senyuva H, Ozden T. Simultanious HPLC determination of paracetamol, phenylephrine HCl and chlorpheniramine maleate in pharmaceutical dosage forms. *J. Chrom. Sc.* 2002; 40: 97-100.

6) Kanumula GV, Raman B, Bhoir I.C. Concurrent analysis of multi- component dosage formulation containing phenylpropanolamine HCl, cetrizine diHCl and acetaminophen by RP-HPLC with wavelength programming. *Indian Drugs.* 2001; 38: 203-206.

7) Garcıa A, Ruperez F.J., Marın A, A. Maza A, Barbas C. Poly (ethyleneglycol) column for the determination of acetaminophen chlorpheniramine and phenylephrine in pharmaceutical preparation. *J Chrom. B.* 2003; 785: 237-243.

8) Olmo B, Garcia A, Marin A, Barbas C. New approach with two cyno columns to the separation of acetaminophen, phenylephrine, chlorpheniramine and related compounds. *J Chrom. B.* 2009; 817: 159-165.

9) Ahuja S, Scypinski S. Handbook of Modern Pharmaceutical Analysis, California, Elsevier Publication, 2005, pp 96, 393.

10) ICH Harmonized Tripartite Guideline, Validation of analytical procedure text and methodology Q2 (R$_1$). In: International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, 2005.

11) Louhaichi MR, Jebali S, Loueslati MH, Adhoum N, Monser L. Simultaneous estimation of pseudoephedrine, pheniramine, guaifenesin, pyrilamine, chlorpheniramine and dextomethorphan in cough and cold medicines by HPLC. *Talanta*, 2009; 78: 991-997.

12) Suking S, Guarni L, Wang Y. Simultaneous determination of acetaminophen, caffeine, chlorpheniramine maleate in paracetamol and chlorphenamine maleate granules. *Chromatographia.* 2006; 64: 719-724.

13) Hadad GM, Emara S, Mahmoud W M M. Development and Validation of stability- indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetrizine and pseudoephedrine in pharmaceutical dosage forms. *Talanta*, 2009;79: 1360-1367.