Open Access
Full Length Article

ORIGNAL ARTICLE

A RP-HPLC Method for Simultaneous estimation of Chlorpheniramine Maleate, Paracetamoland Phenylephrine Hydrochloride in Bulk

Aqib Zahoor[1], Hira Munir[1], Raja Junaid[1],Shafiquat Hussain[1], Safila Naveed[2] , Muhammad Osama Alam[1], Kashifa Khanum[1], Khan Usmanghani[1,2], Fatima Qamar[2], Sidra khan[2]

[1] Herbion Pakistan (Pvt.) Limited, Karachi,

[2] Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

Keywords: RP-HPLC, chlorpheniramine maleate CM, phenylephrine hydrochloride PH, paracetamol PM

Author`s Contribution
All the authors contributed significantly to the research that resulted in the submitted manuscripts

Article info.
Received: Jan 02, 2018
Accepted: Mar 26, 2018

Funding Source: *Nil*
Conflict of Interest: *Nil*
Cite this article: *Zahoor A, Munir H, Junaid R, Hussain S, Naveed S, Alam O, Khanum K, Usmanghani k,QamarF,Khan S. A RP-HPLC method for simultaneous estimaxtion of chlorpheniramine maleate, paracetamoland phenylephrine hydrochloride in bulk. RADS J. pharm. pharm. sci. 2018;6(1):53-58.*

Address of Correspondence
Author: *zaidisy3@ngha.med.sa*

ABSTRACT

Background: Chlorpheniramine maleate (CM) is used to treat sneezing, itching, watery eyes, and runny nose. It is an antihistamine drug. In combination with an analgesic paracetamol (PM) and a decongestant phenylephrine hydrochloride (PH) is commonly sold to treat cough and cold. The aim of study is to develop simple and less time consuming RP-HPLC method was determination for these three drugs in tablet dosage form.

Methodology: The parting of these drugs was carried out using phenomenexluna 5µ CN 100A (250×4.6mm×5µ) analytical column using phosphate buffer (pH 6.2): Acetonitrile (70:30 v/v) mobile phase. The elution rate was set at 1 ml/min and 45^{O}C is the column temperature. The UV detector set at 215nm.

Results All three drugs were separated effectively, 2.43 min is the retention time of CM, 3.75 for PM and 7.84 min for PH at 215 nm. 0.999, 0.998 and 0.999 is the correlation coefficient for CM, PM and PH respectively. The relative standard deviation was found to be below 2% for six replicates. The method parameters were validated according to ICH guidelines.

Conclusion: it is concluded that the proposed method can be effectively applicable to the pharmaceutical dosage forms having these drugs in combination without any interference of excipients.

INTRODUCTION

Chlorpheniramine maleate (CM) is used to treat sneezing, itching, watery eyes, and runny nose. It is an antihistamine drug.In combination withan analgesic paracetamol (PM) and a decongestant phenylephrine hydrochloride (PH) is commonlysold to treat cough and cold [1]. Chemically CM is chlorophenylpyridylpropylmethylamine hydrogen maleate. It is a histamine H1 receptor antagonist.However PM isanalgesic and antipyretic drug and is chemically hydroxyphenyl acetamide. It is known as a weak inhibitor of

prostaglandins (PGs) synthesis. Though, the in vivo effects of paracetamol are much like same to those of the selective COX-2 inhibitors [2,4]. PE is α - adrenergic agonist and chemically it is hydroxymethylaminoethylphenol hydrochloride, it induces dilation of the pupil, decongestant for nasal passage and cardio protective agent [5]. A combination of Acetaminophen, phenylephrine hydrochloride and chlorpheniramine maleate is broadly used in Pharmaceutical formulations for the relief common cold symptoms. Simultaneous estimation of these drugs is problematic. A numerous studies describing HPLC methods for individual and combined estimation of these three compounds separately and in combination with other drugs but only limited chromatographic methods have reported for simultaneous determination of CM, PM and PH in cold formulations. Ali, A., Ahmed reported estimation of Aminophylline and Chlorpheniramine Maleate in combined Formulation [6]. Senyuva and Ozden [7] reported method for determination of combined pharmaceutical dosage form having all three active however acetaminophen was not separated properly.A method reported used pseudoephedrine hydrochloride as internal standard [8]. Another study reported for the separation of acetaminophen containing CM, PE and other active components in analgesic preparations by HPLC [9]. A method used cyano column for the quantification of these three drugs but retention time was high [10-12].Hence,the aim of our study is to formulate a validated isocratic RP-HPLC method for the estimation of these three agents in bulk.

METHODOLOGY

Chemical and Reagents:

CM PM and PH was gifted from a Pharmaceutical industry. All reagents such as Orthophosphoric acid, Acetonitrile, Methanol, and Di-sodium hydrogen ortho-phosphate were of HPLC grade and analytical grade. All the solutions weresonicated and filtered through 0.45µm membrane.

Chromatographic Conditions:

High performance liquid chromatography (model shimadzu) was equipped with a 20µl loop and UV-Visible detector. A reversed phasephenomenexluna 5µ CN 100A (250×4.6mm×5µ) analytical column was used forSeparation and quantitation using mobile phase phosphate buffer (having pH 6.2): Acetonitrile (70:30 v/v). The elution rate was set at 1 mLmin-1. UV detector set at 215nm.

Preparation of phosphate buffer pH 6.2

Phosphate buffer was prepared in 1L water by dissolving 1.36 mL orthophosphoric acid. PH was set to 6.2 by the addition of triethylamine in buffer solution.

Preparation of Standard Stock Solutions:

Stock solution was prepared by dissolving 26 mg paracetamol (0.26mg/ml) , 8 mg chlorphenirmine maleate (0.08mg/ml) and 20 mg of phenylphrineHcl (0.2mg/ml) working standard into 100 ml volumetric flask using diluents (mobile phase) .Flaskwas shaken for 5 min. Solution wasfilteredthrough HPLC filter (0.45µm) and proceed for analysis.

Sample solutions Preparation:

Take randomly twenty tablets, weighed and crushed them in to fine powder. An equivalent amount of powder to 4mg CM and 10mg PHtransferred into a 10 ml volumetric flask to get 40 µgmL-1 CM and 100 µgmL-1 PH (Solution 1). 650 mg ParacetamolMaleate weighed and 6500 µgmL-1 PM of the stock solution was prepared (solution 2). 1 ml of both solutions was mixed and Additional dilutions were performed to attain the calibration range for each compound.

RESULTS AND DISCUSSION

Method Development and Optimization:

Chromatographic conditions were optimized with wavelength, flow rate, pH and mobile phase for the separation of CM, PM and PH on phase phenomenexluna 5µ CN 100A (250×4.6mm×5µ) analytical column using mobile phase phosphate buffer (pH 6.2): Acetonitrile. pH was tested from 6.7 to 3. Different percentage of phosphate buffer (pH 6.2) and Acetonitrile was observed that showed retention time variation of the drugs. Therefore, a mobile phase phosphate buffer having pH 6.2: Acetonitrile (70:30 v/v) ,flow rate 1mLmin-1 and 20 µl injection volume was selected as it gives a good resolution and analysis time.

Specificity:

There was no excipients interference in the analysis. Moreover there was no other peak appeared at the same retention time. There was no interaction between all drugs in standard solution. A chromatogram of combined formulation is shown in figure 1.

Linearity:

The calibration curves of six samples having different concentrations in the range 10-60µg were established for all three drugs i.e. CM, PM and PH. Rregression equation was formed by plotting the peak Height against concentration of drugs by injecting every drugs thrice. The result was tabulated in table 1showing an excellent linear relationship for all drugs.

Precision:

Intraday and Inter-day precision was done by repeating the performance for one day and for three successive days respectively and obtained results are expressed as RSD. The RSD or relative standard deviation for intra and interday precision were little bit deviate from 0.06% to 1.45% and from 0.05% to 1.75% respectively. Data are shown in table 2.

Accuracy:

The accuracy was determined by spiking method at 80%, 100% and 120%. Each concentration was investigatedthrice.Results showed a good accuracy, percent recovery and RSD. The excipients in combined formulation do not hinder in the analysis of these compounds. The results of recovery iscompiled in table 3.

System Suitability:

The resolution, tailing factor, capacity factor, theoretical plates and retention volume were shown in table 4. The results proved to be an efficient column and good peaks which were efficiently resolved.

Limit of Detection and Quantitation:

Limit of Detection (LOD) and Quantitation (LOQ) were calculated as specified by ICH recommendations using Standard deviation and slope for quantification and detection. Following formula is used to calculate LOD = 3.3 (SD/m) and LOQ = 10 (SD/m) respectively. LOD values were found to be 0.36µgmL-1 for CM, 0.36µgmL-1 for PM and 0.28µgmL-1 for PH. LOQ values were found to be 1.1µgmL-1 for CM, 1.1µgmL-1 for PM and 0.86µgmL-1 for PH also shown in table 5.

Assay of Pharmaceutical Dosage Form:

The analytical method was proposed to determine CM, PM and PH in their dosage forms. Calibration curve method was used for amount calculation. Results are tabulated in table 4. The results indicated that the drug content corresponds to the label claim, representing a good accuracy. The assay percentage was found to be 99.64 to 100.58% which showed of dosage form estimation was accurate.

CONCLUSION

An isocratic HPLC method has been validated for the determination of CM, PH and PM in bulk.

Analysis time was less than 8 minute. Results from experiments showed that the method is reliable, accurate and can be employed for routine analysis of the tablets for both quality control and stability testings.

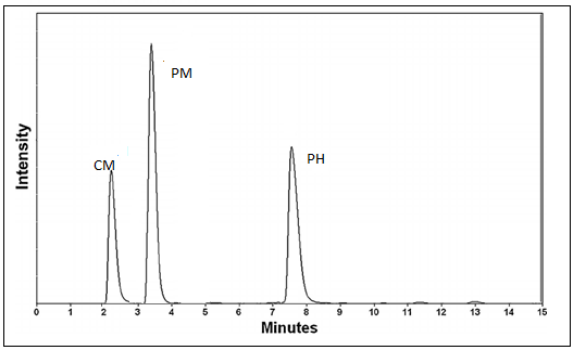

Figure 1: Chromatogram for simultaneous determination of CPM PCM and PE

Table 1: Regression Equation

Drugs	Regression equations	r^2
CM	y = 585.5x + 2.091	0.999
PM	y = 9.9714x + 491.67	0.998
PH	y = 10.114x + 288.33	0.999

Table 2: Precision

Drugs	Inter-day	Intra-day
	%RSD	%RSD
CM	0.58	0.88
	0.57	0.79
	0.37	0.89
	0.11	1.12
	0.9	1.01
	0.67	1.02
PM	0.58	1.34
	0.57	0.97
	0.37	0.98
	0.11	1.05
	0.19	0.86
	0.67	0.96
PH	1.75	0.87
	99	99
	1.11	1.11
	0.95	0.95
	1.15	1.15
	1.11	1.45

Table 3: Accuracy

Drugs	Conc μgmL^{-1}	%RSD	% Recovery
CM	80%	0.046	99.99
	100%	0.096	97.377
	120%	0.048	98
PM	80%	0.047	97.36
	100%	0.049	97.36
	120%	0.051	97.99
PH	80%	0.121	101.7
	100%	0.118	101.7
	120%	0.121	100.9

Table 4: System Suitability

Parameter	CM	PM	PH
Ret.Time	2.43	3.75	7.84
Height	475.88	1017.97	455.25
Percent relative area	14.97	44.42	40.61
Theoritical Plates (N)	9350	10381	8356

Table 5: LOD and LOQ

µgmL-1	CPM	PCM	PE
LOD	0.36	0.36	0.28
LOQ	1.1	1.1	0.86

Each tablet contains

S.No	Ingredients	Quantity/Tablet
	ACTIVES	
01.	Paracetamol	650 mg
02.	Phenylephrine HCL	10 mg
03.	Chlorphenirmine maleate	4 mg
	EXCIPIENTS	
04.	Maize starch	57.40 mg
05.	Lactose monohydrates	92.06 mg
06.	Crosscarmellose sodium	26.40 mg
07.	Pvp K-30	22 mg
08.	Aerosil-200	2.64 mg
07.	Magnesium Stearate	5.50 mg
08.	Purified water	200 mg
	Avg.wt	870 mg
	COATING MATERIAL	
09.	Coat dry 7500 As Greenish yellow	10 mg
10.	Purified Water	0.20 ml
	Avg.wt ofter Coating	880 mg

Quantitative Analysis Of Chlorpheiramine maleate, Paracetamol & Phenylephrine Hcl In Novex Extra (Flurex)Tablet By Hplc

Working parameter :

Stationary phase	phenomenex luna 5µ CN 100A (250×4.6mm×5µ)
Flowrate	1.0ml/min
Wavelength	215 nm
Temperature	45ºC
Injection volume	20µL
System	isocratic system
Mobile phase	phosphate buffer (pH 6.2) : Acetonitrile (70:30 v/v).

Preparation of phosphate buffer pH 6.2 :

The phosphate buffer was prepared by dissolving 1.36 mL orthophosphoric acid in 1 L water. Triethylamine was added to the phosphate buffer solution in order to adjust the pH to 6.2.

Method of Analysis :

Chromatograph 20 µL of test solution and standard Chloropheniramine maleate ,paracetamol and phenylphrine Hcl solutions alternately on liquid chromatograph with UV detector or DAD detector obtaining not less than 6 chromatograms of standard and 3 chromatograms of test solution .

Preparation of standard solution :

Weigh accurately about 26 mg paracetamol (0.26mg/ml) , 8 mg chlorphenirmine maleate (0.08mg/ml) and 20 mg of phenylphrine Hcl (0.2mg/ml) working standard into 100 ml volumetric flask. Add diluents (mobile phase) up to the mark .Shake the flask for 5 min. Filter the solution through HPLC filter (0.45µm) and proceed for analysis.

Preparation of sample solution:

Take 20 tablets and determine the average weight of the tablet then crush into fine powder. Transfer a quantity of the powder equivalent to two tablet into 100 ml volumetric flask .Add diluent up to the mark and sonicate the flask for 20 min . Filter the solution through whatman filter paper 41. (Solution 1).

For Chlorpheneramine maleate and phenylphrine Hcl :

Use solution 1 for the chlorpheneramine maleate and phenylphrine Hcl .

For Paracetamol :

Take 2 ml of solution 1 in 100 ml volumetric flask add diluents (mobile phase) up to the mark .Shake the flask for 2 min . Filter the solution through HPLC filter (0.45µm) and proceed for analysis of paracetamol .

Calculation:

Chloropheniramine maleate ,paracetamol and phenylphrine Hcl content in flurex tablet is calculated by the following formula .

$$X = \frac{A_{SMP} \times Wt_{STD} \times D_{SPL} \times \%\ purity \times avg.wt}{A_{STD} \times D_{STD} \times Wt_{SMP} \times 100 \times label\ claim} \times 100$$

= ……%.

Where,

ASMP Mean value of peak area of tested solution samples

ASTD Mean value of peak area of standard solution samples

WSMP Weight of sample,

WSTD Standard weight,

DSPL Dilution of sample .

DSTD Dilution of standard .

NOTE :

%purity of

Chloropheniramine maleate = 99.7%

Paracetamol =99.8 %

Phenylphrine Hcl =99.8 %

The retention times of the standards were approximately between ,

2-3 min for chlorpheramine maleate .

3.5-5 min for paracetamol .

7-9 min for phenylphrine Hcl .

REFERENCES

1. *Brostoff J, Lockhart JD. Controlled trial of terfenadine and chlorpheniramine maleate in perennial rhinitis. Postgraduate medical journal. 1982;58(681):422-3.*
2. *Hamid F, Qamar F, Naveed S, Saleem S, Basheer S, Khan S, Sadia H, Usmanghani K. A Novel RP-HPLC Method for Simultaneous Determination of Moxifloxacin and Atorvastatin. RADS Journal of Pharmacy and Pharmaceutical Sciences. 2017;5(4):50-6.*
3. *Acheampong A, Gyasi WO, Darko G, Apau J, Addai-Arhin S. Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation. SpringerPlus. 2016;5(1):625.*
4. *Asif S, Alam T, Nawab A, Naveed S. Simple UV Spectrophotometric Method Development for Determination of Meropenem in Bulk Form. RADS Journal of Pharmacy and Pharmaceutical Sciences. 2017;5(1):45-9.*
5. *Hayward KL, Powell EE, Irvine KM, Martin JH. Can paracetamol (acetaminophen) be administered to patients with liver impairment?. British journal of clinical pharmacology. 2016;81(2):210-22.*
6. *Ali A, Ahmed M, Mahmud T, Qadir MA, Nadeem K, Saleem A. Stability-indicating High-performance liquid chromatography method for simultaneous determination of aminophylline and chlorpheniramine maleate in pharmaceutical formulations. Indian journal of pharmaceutical sciences. 2015;77(5):515.*
7. *Şenyuva H, Özden T. Simultaneous high-performance liquid chromatographic determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in pharmaceutical dosage forms. Journal of chromatographic science. 2002;40(2):97-100.*
8. *Kanumula Gangaram V, Raman B, Bhoir IC. Concurrent analysis of a multicomponent dosage formulation containing chlorpheniramine maleate, phenylephrine HCi, caffeine and acetaminophen by RP-HPLC with wavelength programming. INDIAN DRUGS-BOMBAY-. 2001;38(4):203-7.*
9. *Kanumula GV, Raman B. Bhoir IC Concurrent analysis of multi-component dosage formulation containing phenylpropanolamine HCl, cetrizine diHCl and acetaminophen by RP-HPLC with wavelength programming. Indian Drugs. 2001;38:203-6.*
10. *Olmo B, García A, Marín A, Barbas C. New approaches with two cyano columns to the separation of acetaminophen, phenylephrine, chlorpheniramine and related compounds. Journal of Chromatography B. 2005;817(2):159-65.*
11. *Khalid F, Gul S, Khan MN, Alam MT. UV Spectrophotometric Method for Estimation of Moxifloxacin HCl in Tablet Dosage Form and Comparative Study of its Different Brands. RADS Journal of Pharmacy and Pharmaceutical Sciences. 2015;3(2):41-9.*
12. *Shakeel S, Naveed S, Usmanghani K, Sarwar G, Alam MT. RP-HPLC Simultaneous Analysis of Glimepride and NSAIDs in Active Pharmaceutical Ingredient, Formulations and Human Serum. RADS Journal of Pharmacy and Pharmaceutical Sciences. 2015;3(2):58-64.*