Hygeia.J.D.Med. January 2018 – June 2018 ISSN 2229 3590
Hygeia:: journal for drugs and medicines
January 2018
Open Access www.hygeiajournal.com
Research article section: Pharmaceutical analysis
A Half Yearly Scientific, International, Open Access Journal for Drugs and Medicines
DOI:10.15254/H.J.D.Med.9.2018.171 Research Article

DEVELOPMENT AND VALIDATION OF AN RP- UFLC METHOD FOR THE ESTIMATION OF DEXCHLORPHENIRAMINE MALEATE

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Key words: Dexchlorpheniramine maleate, Method development, Validation, UFLC

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Received: 9 September 2017,
Revised: 25 September 2017
Accepted: 11 October 2017,
Available online: 4 January 2018

ABSTRACT

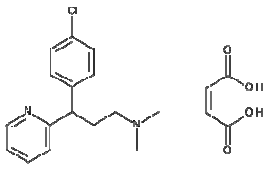
Plan: *Dexchlorpheniramine maleate is a Chlorpheniramine dextrorotatory isomer, which is twice active than Chlorpheniramine. It's widely used in the coughs, cold and allergic conditions including angioedema, urticaria, conjunctivitis, rhinitis, and pruritic skin disorders. The main objective of the present research work deals with the development of simple, rapid and sensitive RP- UFLC analytical method for estimation of Dexchlorpheniramine maleate in bulk and finished formulation.*

Methodology: *simple, rapid and sensitive RP- UFLC analytical method was developed. Optimized chromatographic separation condition was done by using an isocratic mode with the mobile phase of 25 mM of potassium dihydrogen ortho phosphate (pH 4) and acetonitrile at the ratio of 80:20v/v with a flow rate of 1ml/min. Stationary phase used was C18 column (250 x 4.6 mm). Dexchlorpheniramine maleate detection was carried at 260 nm. The developed method was evaluated for the validation parameters as per the ICH guidelines.*

Outcome*: The developed optimized chromatographic condition was achieved and results showed good peak resolution. The developed method for the Dexchlorpheniramine maleate can be used for the qualitative and quantitative estimation of finished and bulk formulations.*

1. INTRODUCTION

Dexchlorphenirarnine Maleate is a Chlorpheniramine dextrorotatory isomer, chemically known (3S)-3-(4-chlorophenyl)-*N*,N-dimethyl-3-(pyridin-2 yl) propan-l-arnine maleate[1]as shown in fig 1.It's widely used in the coughs, cold and allergic conditions including angioedema, urticaria, conjunctivitis, rhinitis, and pruritic skin disorders.



Dexchlorphenirarnine maleate

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Hygeia.J.D.Med. Vol.9 (2), January 2018

The European Pharmacopoeia III describes the HPLC determination of the enantiomeric purity with the limit of 2% (m/m) of R-enantiomer in the tested sample for dexchlorpheniramine maleate[2] In Indian Pharmacopoeia the chromatogram obtained with the test solution peak corresponding to the (R)-enantiomer should not be more than the area of the principal peak of the reference solution (2.0 per cent)[1].

There are various analytical methods reported for the estimation of Chlorpheniramine inspectroscopic[3-5] and chromatographic[6-11], however, there are very less number of papers are reported for the estimation of Dexchlorphenirarnine Maleate in bulk, formulations and biological fluids[2].

This paper describes the development and validation of a simple, sensitive, and rapid, UFLC[12-13] (Ultra Fast Liquid Chromatography) method for the determination of Dexchlorphenirarnine Maleate as per ICH guidelines [14].The developed method also finds advantage, economical, reliable when compared with existing methods.

2. MATERIALS AND METHODS

2.1. Materials

Acetonitrile of HPLC grade, Ortho-Phosphoric acid, *potassium di-hydrogen phosphate* and Triethylamine AR grade were obtained from Qualigens Fine Chemicals and S.D. Fine chemicals. Triple distilled water HPLC grade obtained from Milli-Q RO system. Working Standards of Dexchlorpheniramine Maleate were obtained from Sigma Aldrich.

2.2. Identification studies

Dexchlorpheniramine Maleate identification studies were carried out by melting point study by placing 2 mg of the drug in a capillary tube and the reading where noted for the melting point in melting point apparatus.

2.3. Solubility

Dexchlorpheniramine Maleate solubility study was carried at different solvents such as organic and inorganic solvent respectively. About 10 mg of drug was accurately weighed in 25 ml a standard flask, in that about 10-15 ml of organic and inorganic solvent were added and kept in isothermal shaker at 25 ± 0.5^0C for 48 hours to reach equilibrium. After the equilibrium is reached the sample are withdrawn from the isothermal shaker and the sample are centrifuged at 4000 rpm for 4 min. The supernatant liquid was then taken and filtered through Whatman filter paper. The different Dexchlorpheniramine Maleate solvent sample was then measured at UV visible spectrometer.

2.4. Selection of wavelength for detection of components

About 10 mg of the Dexchlorpheniramine Maleate was accurately weighed in 10ml standard flask, for that 5-6 ml of acetonitrile was added and kept in a sonicated for 2-5 min, then the solution is made up to the mark by using same solvent.

From the above solution 100µg/ml of the standard Dexchlorpheniramine Maleate solution were prepared and the spectrum was recorded in the UV visible spectrometer at the region from 200-400.

2.5. Preparation of standard stock solution

About 10 mg Dexchlorpheniramine Maleate was accurately weighed taken in a 10ml standard flask in 10ml standard flask, to that 6-7 ml of acetonitrile was added and kept in a sonicated for 2-5 min, then the solution is made up to the mark by using the same solvent to produce 1000µg/ml. This solution is then refrigerator for further use.

2.6. Preparation of Buffer (25mm)

Weigh accurately about 1.701 g of potassium dihydrogen ortho phosphate in to a 500ml beaker, to that add 300ml of triple distilled water and kept in a sonicator for 15 min or until dissolve. To that final volume was adjusted with triple distilled water to produce 500ml. The above solution was adjusted to the pH of 4 by using ortho phosphoric acid.

2.7. Preparation of Calibration curve

From the above standard stock solution, suitable dilutions were prepared in the range of 0, 10, 100, 200, 400 and 500µg/ml using acetonitrile as solvent. The standard calibration curve was plotted against concentration vs. peak area and the intercept and slope values were recorded.

2.8. Chromatographic conditions

UFLC system used was Shimadzu (Japan) with gradient solvent delivery system pump (LC-20 AD), rheodyne injector (7725i) with 20 µL loop and photo diode array (PDA) detector. Software LC solution was used as a data station for interpreting the chromatograms. The separation of the analyties were carried out on Phenomenex C_{18} column (250 x 4.6 mm, i.d. 5µm) using potassium dihydrogen ortho phosphate buffer (25mM, pH-4.0) and acetonitrile with the ratio of 20:80 v/v at the flow rate of 1ml/min. The detection of the analyte was carried out at 260nm.

2.9. Method validation

The developed method for Dexchlorpheniramine Maleate was validated for specificity, selectivity, linearity, sensitivity, accuracy, precision, robustness and ruggedness as per the ICH guidance.

2.10. Specificity and selectivity

The specificity of the developed method was carried by injecting six injections of standard and the sample retention time, and the interference peak was studied. The selected chromatographic condition of the standard retention time and peak should be ideal and should be free from degradants, matrix, impurities, preservatives, recipients and related impurities.

2.11. Linearity and Sensitivity

Linearity of the Dexchlorpheniramine Maleate was carried by plotting a calibration graph of standard peak area in y axis versus concentration in x axis at the ranging of 0.0-500.0 µg/ ml as shown in table 1 and fig 2. From the calibration graph correlation of coefficient (r2) was measured. Limit of detection (LOD) and limit of quantitation (LOQ) were reported as 3 and 10 times the noise level obtained from three replicate injections of standard respectively.

2.12. Accuracy

Accuracy of the developed method was carried out by recovery study. Mean percentage (%) recovery of analytes was used as a measure of accuracy. Six injections of sample concentration were spiked with the standard concentration and the percentage recovery, mean and standard deviation (SD) was calculated.

2.13. Precision and repeatability

Six injections of three different concentrations of low, middle and high concentration were injected into the optimized chromatograph and mean, standard deviation (SD), percentage relative standard deviation (% RSD) were calculated. Precision was carried at two levels of intra and interlay precision and repeatability were carried out on the analytic sample.

2.14. Robustness and Ruggedness

The robustness and ruggedness for the developed method was carried out by slightly changing the parameters such as slight change in the optimized chromatographic conditions like change in column temperature, change in column, flow rate, pH and instruments and operators.

3. RESULTS & DISCUSSION

Dexchlorpheniramine Maleate is also called as D-Chlorpheniramine maleate. The melting point for the Dexchlorpheniramine Maleate was carried out by fisher scientific apparatus and the results were found to be 115^{o} c, which conforms the purity of the compounds[15]. The optimization of the chromatographic condition was carried out on the base of separation, sharp peak, repeatability of the peak and economical.

Method development was carried by changing the mobile phase and standard phase parameters such as buffer strength, buffer concentration, pH ratio, organic concentration and column as C8 and C18. Based on the trial and error method Dexchlorpheniramine Maleate optimized UFLC conditions was achieved at 25mM of potassium dihydrogen ortho phosphate (pH 4) and acetonitrile as the mobile phase at the ration of 20:80 v/v.

The good separation was achieved at C18 column (250 x 4.6 mm, i.d. 5µm) with the detection of 260 nm. With the optimized condition, standard and sample solutions showed good separation at the retention time of 6.1 min, respectively at the flow rate of 1ml/min.

The real goal of the validation process is to challenge the method and determine the limits of allowed variability for the conditions needed to run the method. Validation of the developed method was carried as per the ICH guidelines.

The specificity of the developed Dexchlorpheniramine Maleate method was carried out by comparing the standard and the sample retention time. Six injections of the standard and the sample solution were injected into the UFLC and the peak interference was studied. The developed optimized chromatographic condition peak was free from the matrix, excipients, impurities, degradants, related impurities and preservatives effects, hence the selected method was specific and results are shown in fig 3-5.

Linearity range was carried from the concentration range from 0-500 µg/mL, which was found to be linear in range. The calibration was plotted by taking peak area in y axis and concentration in x axis. Linear regression was followed and the correlation of coefficient (r2) was used as measure of linearity. The regression equation for the linearity and the range was found to be 0.995 respectively, and the results are shown in table 1 and fig 2.

The accuracy of the optimized methods was determined by spiking the known standard concentration to the sample solution. Accuracy was carried out by injecting six injections of known standard concentration of 200 µg/mL in to sample solution and percentage Nominal were calculated and founded to be 97.72 % respectively, and the level was consistent at all the levels and the results are shown in table 2.

Precision study of the developed method was carried out at two different levels of intra- and interday levels at three different concentrations. Six injections of three different concentration of 10,200 and 500 µg/mL of low, middle and high concentrations of linearity were injected and the percentage RSD and standard deviation were calculated as shown in the table 3 & 4 and the results are well within the limits.

The developed method was rugged and robust when evaluated for change in the chromatographic condition for robustness and ruggedness. Limit of detection and limit of quantification for Dexchlorpheniramine Maleate were found to be 100ng/mL and 300ng/mL based on the signal to noise ratio.

Table 1: Calibration and linearity studies

S.No	*Concentration (µg/mL)*	*Peak Area*
1	0	0
2	10	32898
3	100	309874
4	200	632532
5	400	1076547
6	500	1356798

n=6

Table 2: Accuracy studies

S.No	*Measured Concentration (µg/mL)*	*Actual Concentration (µg/mL)*	*% Nominal*
1	195.74	200	97.87
2	194.34	200	97.17
3	195.01	200	97.505
4	196.21	200	98.105
5	193.98	200	96.99
6	197.45	200	98.725
Mean	195.46	200	97.72
SD	1.2844	0	0.64
%RSD	0.6571	0	0.66

n=6

Table 3: Precision studies: intra-day precision

	Concentration ((µg/mL) (n=6)		
S.No	*10*	*200*	*500*
1	9.54	194.85	487.04
2	9.41	192.63	491.73
3	8.99	195.54	489.54
4	9.34	195.28	492.61
5	9.73	196.44	492.05
6	9.41	194.07	491.44
Mean	9.40	194.80	490.735
SD	0.25	1.32	2.09
%RSD	2.61	0.68	0.43

n=6

Table 4: Precision studies: inter-day precision

S.No	*Inter-day precision - 1 Concentration (µg/mL)*			*Inter-day precision - 2 Concentration (µg/mL)*		
	10	*200*	*500*	*10*	*200*	*500*
1	9.21	195.52	487.87	9.11	192.65	489.54
2	9.41	194.02	489.05	9.43	190.54	490.83
3	8.95	190.72	490.6	9.09	194.36	492.65
4	9.28	192.43	492.54	8.99	192.37	493.9
5	9.73	192.95	490.42	9.13	193.67	494.8
6	9.49	192.76	492.2	9.25	190.44	492.32
Mean	9.35	193.07	490.45	9.17	192.34	492.34
SD	0.27	1.61	1.79	0.15	1.60	1.94
%RSD	2.84	0.83	0.37	1.68	0.83	0.39

n=6

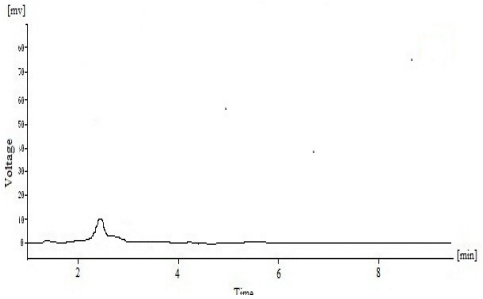

Fig.1

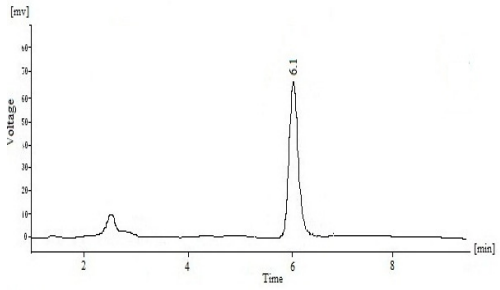

Fig.2

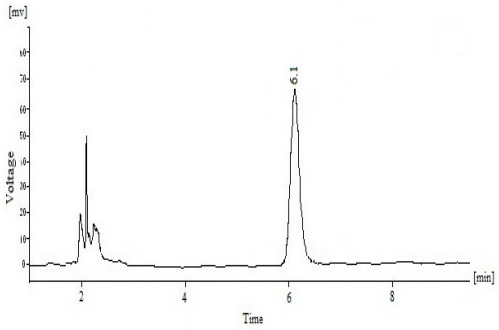

Fig.3

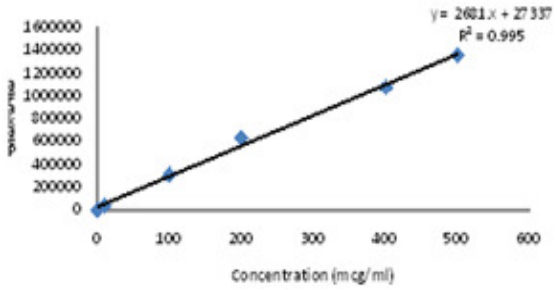

Fig. 4

Fig.1: Typical HPLC chromatogram for Blank, Fig.2: Typical HPLC chromatogram of Standard, Fig.3: Typical HPLC chromatogram of Sample, Fig.4: Calibration Curve of Dexchlorpheniramine maleate.

4. CONCLUSION

The developed method is simple, rapid and sensitive and validated as per the ICH Q2R1 guidelines such as specific, sensitive, accurate, precise, reproducible, robust, LOD and LOQ. This method for the Dexchlorpheniramine Maleate can be used for the quantitative and qualitative estimation of bulk and finished formulations.

Conflict of interest: None

ACKNOWLEDGEMENTS

Authors wish to thank Sree Vidyanikethan College of pharmacy, Tirupati for providing necessary facilities for carrying the research work.

REFERENCES

1. Indian pharmacopeia, **2010**; Vol 2: 177-1179.
2. Aravindaraj Joghee. R, Gopinath .R, Rajan. S, Mahesh Kumar. S, Nanjan Moola J, Suresh. B. Development and application of a validated liquid chromatography-mass spectrometry method for the determination of dexchlorpheniramine maleate in human plasma. *Iranian Journal of Pharmaceutical Sciences* **2007**; 3(3): pp 161-170.
3. Sawant R Joshi. R, Lanke P, Bhangale L. Simultaneous estimation & validation of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in tablets by spectrophotometric method. *Journal of Pharmaceutical Research and Health Care* **2011**; 3 (2):23–28.
4. Praveen B, Padmaja. M, Krishna.RK. Development and validation of UV spectrophotometric method for simultaneous estimation of codeine phosphate and chlorpheniramine maleate in combined liquid dosage form. *International Journal of Pharmacy and Technology* **2011**; 3(3): 3390–3400.
5. Biswas.A. Spectrophotometric method for determination of chlorpheniramine maleate in pharmaceutical preparations in the presence of codeine phosphate and ephedrine hydrochloride. *Analyst* **1980**; 105 (1249): 353–358. CrossRef
6. Maithani.M, Raturi. R, Gautam.V. Development and validation of a RP-HPLC method for the determination of chlorpheniramine maleate and phenylephrine in pharmaceutical dosage form. *International Journal of Comprehensive Pharmacy***2010**; 1(5):1–4.
7. Hood DJ, Cheung.HY. A chromatographic method for rapid and simultaneous analysis of codeine phosphate, ephedrine HCl and chlorpheniramine maleate in cough-cold syrup formulation. *Journal of Pharmaceutical and Biomedical Analysis***2003**; 30(5):1595–1601. CrossRef
8. ErkN, Kartal. M. Simultaneous high performance liquid chromatographic and derivative ratio spectra spectrophotometry determination of chlorpheniramine maleate and phenylephrine hydrochloride. *Farmaco***1998;** 53 (8-9): 617–622. CrossRef
9. Chittrakarn. S, Penjamras. P, Keawpradub. N. Quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in a kratom (*Mitragyna speciosa* Korth.) cocktail using high-performance liquid chromatography. *Forensic Science International* **2012**; 217 (1–3):81–86. CrossRef PMid:22018854
10. Hu. Z, Zou. Q, Tian. J, Sun. L, Zhang.Z. Simultaneous determination of codeine, ephedrine, guaiphenesin and chlorpheniramine in beagle dog plasma using high performance liquid chromatography coupled with tandem mass spectrometric detection: application to a bioequivalence study. *Journal of Chromatography* B **2011**; 879 (32): 937–3942.CrossRef PMid:22119507
11. Hood. D. J, Cheung H. Y. A chromatographic method for rapid and simultaneous analysis of codeine phosphate, ephedrine HCl and Chlorpheniramine maleate in cough-cold syrup formulation. *Journal of Pharmaceutical and Biomedical Analysis* **2003**; 30 (5): 1595–1601. CrossRef
12. Shanmugam R , Gowthamarajan KK , Priyanka DL, Madhuri K, Hemnath E, Dhanabal SP, Development and validation of a UFLC method for simultaneous estimation of Quercetin and Rutin, *Hygeia.J.D.Med* **2013**; 5:113-120.
13. Shanmugam R, Gowthamarajan.K K, Priyanka DL, Madhuri K, Elango K. Development and validation of reverse phase - Ultra Fast Liquid Chromatography (RP-UFLC) method for simultaneous estimation of Curcumin and Piperine, *Pak. J. Pharma. Sci.* **2013**; 27: 901-906.
14. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology **2005**, Available at: www.ich.org/products/guidelines/quality/article/qualityguidelines.html (accessed Jan 18, 2011).
15. Safety Data Sheet, Dexchlorpheniramine Maleate : Catalog number -1179005 Available at: www.cymitquimica.com/uploads/products/45/pdf/1179005-msds.pdf

Shanmugam R, Murali P*, Lalitha Priyanka D, Madhuri K, Ashok Kumar CK. Development and validation of an RP- UFLC method for the estimation of dexchlorpheniramine maleate. *Hygeia.J.D.Med* **2018**; 9(2)26-33:.Available from http://www.hygeiajournal.com, DOI: 10.15254/H.J.D.Med.9.2018.171