ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DIPHENHYDRAMINE AND NAPROXEN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The proposed HPLC method was found to be simple specific precise accurate rapid and economical for simultaneous estimation of Diphenhydramine and Naproxen in tablet dosage form The developed method was validated in terms of accuracy precision linearity robustness and ruggedness and results will be validated statistically according to ICH guidelines The Sample recoveries in all formulations were in good agreement with their respective label claims. Mobile phase was Ortho phosphoric acid buffer and Methanol in the ratio of 65:35 were set.Kromosil C18 coloumn was selected with dimensions 250×4.6mm, 5µ and with a flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector and it showed maximum absorbance at 254 nm. As the methanol content was increased Diphenhydramine and Naproxen got eluted with good peak symmetric properties. The retention times for Diphenhydramine and Naproxen was found to be 2.589 min and 3.711 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to 150 % levels, R2 value was found to be as 0.999.

KEYWORDS : Diphenhydramine, Naproxen, RP-HPLC, detector and Linearity.

Introduction: Naproxen is a non steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties The mechanism of action of naproxen like that of other NSAIDs is believed to be associated with the inhibition of Cyclooxygenase activity Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti- inflammatory activity. Diphenhydramine is a first generation antihistamine a H1- receptor antagonist with anti-allergic properties and chemically is2-(diphenylmethoxy)-N, N- dimethylethanamine hydrochloride. It exhibits mechanism of action by reversing the action of histamine on capillaries. The aim of the present work is to develop an analytical method and validation for simultaneous estimation of Diphenhydramine and Naproxen in pure and pharmaceutical Tablet dosage form. In view of the need for a suitable cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Diphenhydramine and Naproxen in pure and pharmaceutical Tablet dosage form attempts were made to develop simple precise accurate and cost-effective analytical method for the estimation of Diphenhydramine and Naproxen. The proposed method will be validated as per ICH guidelines.

Materials and methods:

Instrumentation: System (Waters 2690**),** Pump (Analytical HPLC isocratic pump, gradient pump)**,** Detector (waters 996 diode array detector), Software (empower 2 software)**,** Column (Kromosil (250×4.6mm, 5µ) ODS C-18 RP-column)**,** Injector (Rheodyne injector with 20µ capacity)**,** Electronic balance (SHIMADZU electronic balance), Sonicator (Analytical Technologies Limited- Ultrasonic cleaner).

Chemicals and solvents: Methanol, Ortho phosphoric acid, Potassium dihydrogen ortho phosphate, Methanol, Tri ethyl amine and Water are obtained from Merck with grade HPLC.

Reference standards: Diphenhydramine and Naproxen- KP Laboratories Hyderabad, Midazole Tablet (Chemida lab Pvt Ltd) containing (From Local Pharmacy shop), Diphenhydramine – 500 Mg, Naproxen-250 mg.

Selection of detection Wavelength:. Standard solutions of Diphenhydramine and Naproxen were scanned in the UV range (200- 400nm) and the spectrums obtained were

overlaid and the overlain spectrum was recorded. From the overlain spectrum 254 nm was selected as the detection wavelength for the present study.

Selection of mobile phase: Initially the mobile phase tried was methanol and water, methanol and Methanol buffer and water in various proportions Finally the mobile phase was optimized to Buffer: Methanol in proportion 65:35 v/v respectively.

Preparation of Buffer: About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with Orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase: Mobile phase of Buffer and Methanol in proportion 65:35 v/v was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter.

Standard Preparation: Weigh accurately 10 mg Diphenhydramine Working Reference Standard and 15mg of Naproxen Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase (Stock solution). Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluents.

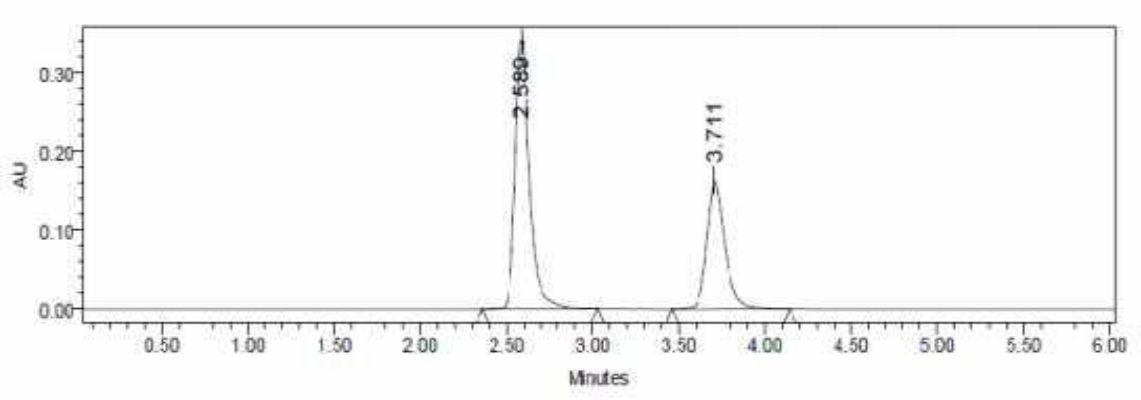

Figure 1: Chromatogram of Naproxen and Diphenhydramine

Assay

Preparation of samples for Assay Standard preparation: Weigh accurately 10mg Diphenhydramine Working Reference Standard and 15mg of Levomisol Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase (Stock solution)Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluents.

Sample preparation: 10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 10 tablets was transferred into a 100ml standard flask A volume of 70ml of mobile phase was added and sonicate for 30min Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter (Stock solution). Further pipette 0.25ml of Diphenhydramine and Naproxen of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluents.

Assay procedure: 20µL of the standard and sample solutions of Diphenhydramine and Naproxen were injected into the HPLC system and the chromatograms were recorded Amount of drug present in the capsules were calculated using the peak areas.

Validation Report:

System Suitability: All the System suitability parameters were satisfied thus the method passed the System suitability test.

Linearity: Serial dilutions of Diphenhydramine and Naproxen (20-60µg/ml and 10-30 µg/ml) were injected into the column and detected at a wavelength set at 254 nm The calibration curve was obtained by plotting the concentration vs peak area.

Accuracy: The accuracy study was performed for 50%, 100% and 150 % for Diphenhydramine & Naproxen. The mean % recovery of the Diphenhydramine and Naproxen at each level should be not less than 95.0% and not more than 105.0%.

Precision: The chromatograms of intra-day precision studies were shown Inter-day precision studies was done by injecting three (3) repeated injections for three consecutive days Peak area and %RSD were calculated and reported. The % RSD of the assay value for six determinations should not be more than 2.0%.

LOD & LOQ: The LOD was performed for Diphenhydramine and Naproxen was estimated to be 0.001µg/ml and 0.005 µg/ml respectively. The LOQ was performed for Diphenhydramine and Naproxen was estimated to be 0.004µg/ml and 0.015µg/ml respectively.

Specificity: ICH defines specificity as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Robustness: The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Diphenhydramine and Naproxen which can be resulted that the variation in flow rate affected the method significantly was shown in Table no 7. Diphenhydramine & Naproxen peaks in the chromatogram passed the system suitability criteria %RSD of peak areas of Diphenhydramine & Naproxen was not more than 2.0% for variation in mobile phase composition.

Results

Table 1. Results of System suitability Test for Naproxen and Diphenhydramine

Naproxen					Diphenhydramine				
Injection	**Retention time (t_R)**	**Peak Area**	**Plate count**	**Tailing factor**	**Injection**	**Retention time (t_R)**	**Peak Area**	**Plate count**	**Tailing Factor**
1	3.711	1185786	6389	1.3	1	2.589	2008408	5752	1.4
2	3.702	1184759	6455	1.3	2	2.570	2008412	5758	1.3
3	3.698	1187496	6234	1.6	3	2.572	2008357	5672	1.2
4	3.708	1190478	6478	1.3	4	2.578	2007478	5674	1.4
5	3.715	1183897	6502	1.30	5	2.582	2008475	5749	1.3
6	3.714	1184759	6384	1.2	6	2.584	2008364	5843	1.4
Mean	-	1186196	-	-	Mean	-	2008249	-	-
SD	-	2433.47	-	-	SD	-	380.0	-	-
% RSD	-	0.20	-	-	% RSD	-	0.01	--	-

Table 2. Preparation of Working standard solutions for Linearity

Sample ID	Diphenhydramine		Naproxen	
	Conc. (mcg/ml)	**Area**	**Conc. (mcg/ml)**	**Area**
20% of operating conc.	20	1224140	10	740046
40% of operating conc.	30	1595681	15	990204
60% of operating conc.	40	1992966	20	1183023
80% of operating conc.	50	2356546	25	1439886
100% of operating conc.	60	2797214	30	1682302

Table 3. Accuracy Study of Diphenhydramine and Naproxen

Sample Id	Conc found (µg/ml)	Concn Obtained(µg/ml)		% Recovery		Mean recovery		StatisticalAnalysis %RSD	
		DPH	NP	DPH	NP	DPH	NP	DPH	NP
50%	5	5.01	4.92	100.2	98.0	99.73	99.2	0.505	1.2
50%	5	4.96	4.96	99.2	99.2				
50%	5	4.99	5.02	99.8	100.4				
100%	10	9.95	9.95	99.5	99.5	98.8	99.5	0.66	0.2
100%	10	9.87	9.94	98.7	99.4				
100%	10	9.82	9.98	98.2	99.8				
150%	15	14.64	14.78	97.6	98.6	98.8	99.0	1.45	0.530
150%	15	14.76	14.94	98.4	99.6				
150%	15	15.06	14.83	100.4	98.8				

Table 4. Method Precision data for Diphenhydramine & Naproxen

S.No.	Conc. (µg/ml)	Diphenhydramine		Naproxen	
		Retention time(Rt)	Peak Area	Retention time(Rt)	Peak Area
1	40 & 20	2.586	2010800	3.713	1184689
2	40 & 20	2.588	2002956	3.714	1188199
3	40 & 20	2.590	2012800	3.734	1195842
4	40 & 20	2.590	2005243	3.737	1184210
5	40 & 20	2.591	2011092	3.741	1198327
Avg			2008998		1191598
SD			3920.9		6668.5
%RSD			**0.19**		**0.55**

Table 5. LOD and LOQ Data of Diphenhydramine and Naproxen

Diphenhydramine			Naproxen		
Conc.(x) (µg/ml)	**Peak Areas (y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Areas (y)**	**Statistical Analysis**
40	2004682	S = 39092 C=618048 LOD: 0.001µg/ml LOQ:0.004µg/ml	20	1184227	S =39092 c=369381 LOD:0.005 µg/ml LOQ: 0.015µg/ml

Table.6. Robustness data for Diphenhydramine and Naproxen

Std. Replicate	**Variation in flow rate**				**Variation in Mobile phase composition**			
	Flow Rate 0.8ml/min		**Flow Rate 1.2ml/min**		**Buffer: Methanol (40:60)**		**Buffer: Methanol (30:70)**	
	DPH	**NP**	**DPH**	**NP**	**DPH**	**NP**	**DPH**	**NP**
1	2492492	1500192	1676589	100524	1951632	1196996	1979168	1153397
2	2495874	1500426	1675428	100468	1954783	1198547	1967452	1154782
Mean	2494183	1500309	1676009	100496	1953208.0	1197772	1973310	1154090
SD	2391.4	165.5	820.9	39.59	2228.0	1096.2	8284.46	979.34
%RSD	0.09	0.01	0.04	0.03	0.11	0.09	0.4	0.08
RT	3.150	4.674	2.168	3.121	2.618	4.394	2.572	3.331
TF	1.4	1.2	1.3	1.2	1.3	1.2	1.3	1.2
TP	5752	7187	4207	5412	4577	6498	4476	6471

(RT = Retention time, TF = Tailing factor, TP = Theorectical plates)

Assay result: The % assays of Diphenhydramine and Naproxen were found to be 99.77% and 100.12%respectively Thus % Assay results were found to be within the limits i.e., 98-102% for both the drugs Hence the developed method can be routinely used for the simultaneous estimation of Diphenhydramine and Naproxen in the marketed formulations.

Table 7: Results of Assay

Parameters	Diphenhydramine	Naproxen
Standard peak area	2008408	1185786
Test peak area (mean)	2005829	1189695
Average Weight	694.2mg	694.2mg
Label claim	400 mg	150 mg
% Purity of Standard	99.50	99.58
Amt obtained	399.88 mg	150.10 mg
% Assay	99.77%	100.12%

Acknowledgements: We gratefully acknowledge ABIPER and RGUHS university for providing all needed things to prepare this manuscript

Conclusion: The newly developed RP-HPLC method for determination of Diphenhydramine & Naproxen in tablet dosage forms is specific, precise, accurate and rapid. The proposed method can be conveniently adopted for routine quality control analysis.

Bibliography

1. Dipti B. Patel, N. J. Patel, S. K. Patel, A. M. Prajapati, and S. A. Patel**,** Rp hplc method for the estimation of Dutasteride in tablet dosage form. Indian Journal of Pharmaceutical Sciences, 2010, 72(1): 113–116.
2. Chandan, M. Vasudevan World Academy of Science, Engineering and Technology International Journal of Medical, Health, Pharmaceutical and Biomedical Engineering, 2013, 7 (12).
3. Namrata Parikh1 , Drashti P. Shah1 , Shivangi J. Patel1 , Shreya R. Shah1, Prasanna K. Pradhan , Suddhasattya Dey , U. M.Upadhyay , Analytical Method Development and Validation for Simultaneous Estimation of Diphenhydramine Hydrochloride and Naproxen Sodium in its Combined Dosage Form , HumanJournals, 2016, 5 (2).
4. Kamepalli Sujana, D. Gowri Sankar, Konda Abbulu and O.Bala Souri, Simultaneous estimation of finasteride and tamsulosin hydrochloride by reverse phase HPLC in bulk and pharmaceutical dosage form, International Journal of Pharmacy & Life sciences, 2012, 3 (8), 1905-1908.
5. Mehta P, Sharma C S, Deepika N, Ranawat M S. Development and Validation of Related Substances Method by HPLC for Analysis of Naproxen in Naproxen Tablet and Formulations. Int. J. Pharm. Sci. Drug Res. 2012, 4(1), 63-69.
6. Riddhi G, Abhay D. Simultaneous Estimation of Sumatriptan Succcinate and Naproxen Sodium in Bulk Drug and Pharmaceutical Dosage Form by RP-HPLC Method.J. Drug Del. Ther.2013, 3(2), 93-97.
7. Vijayalakshmi R, Poorna Chandra Rao M, Kalyani P, Sandhya P, Dhanaraju M D. Simultaneous Estimation of Lansoprazole and Naproxen in Plasma by Validated RP HPLC Method. Indo Amer. J. Pharm. Res.2013, 3(10), 8317-8322
8. Elsinghorst P W, Kinzig M, Rodamer M, Holzgrabe U, Sorgel F. An LC-MS/MS Procedure for the Quantification of Naproxen in Human Plasma: Development,

Validation, Comparison with Other Methods, and Application to a Pharmacokinetic Study. J. Chromatogra. B.2011, 879(19), 1686-1696.

9. Gopinath S, Kumar R S, Shankar M B, Danabal P. Development and Validation of a Sensitive and High Throughput LC-MS/MS Method for the Simultaneous Determination of Esomeprazole and Naproxen in Human Plasma. Biomed. Chromatogr. 2013, 27(7), 894-899.