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

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Abstract

The aim of the present work is to develop a new simple sensitive accurate and economical analytical method and validation for the Simultaneous estimation of Diphenhydramine and Naproxen in pure and pharmaceutical Tablet dosage form by using RP-HPLC and validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. And also to apply the developed method for the simultaneous estimation of Diphenhydramine and Naproxen in pure and pharmaceutical tablet dosage form. System (waters 2690 usa), 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). 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. 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.

Keywords: RP-HPLC, Naproxen, Diphenhydramine, Economical analytical method.

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 [1].

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

Instrumentation: System (waters 2690 usa), 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) [2].

Selection of chromatographic condition: Proper selection of the method depends upon the nature of the sample its molecular weight and solubility The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used The reversed phase HPLC was selected for the separation because of its simplicity and suitability" [3].

Selection of detection wavelength: The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. "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. [4].

Optimization of flowrate: The method was performed with flow rates 0.8ml 1.5ml and 1ml/min Flowrate of 1ml/min was found to be ideal as it gave sharp peak.

Observation: The separation of two analytical peaks was good The plate count also above 2000

tailing factor below 2 and the resolution is above 2 The condition is taken as optimized method"

OPTIMIZED METHOD

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 [5].

Preparation of mobile phase: Mobile phase consist of buffer: Methanol of P 2.5 (35:65) 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.

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: 20□L 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".

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

VALIDATION:

SYSTEM SUITABILITY: All the System suitability parameters were satisfied thus the method passed the System suitability test and results are shown in table 1.

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 and shown in the table 2.

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% and shown in the table 3.

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% and shown in the table 4.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION: 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 was shown in table 5.

SPECIFICITY: ICH defines specificity as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present Typically this might include impurities degradants matrix etc and shown in Figure 6.

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

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
Correlation Coefficient			0.999	

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
40	2004587		20	1186425	

Figure 1. Chromatogram of Diphenhydramine and Naproxen

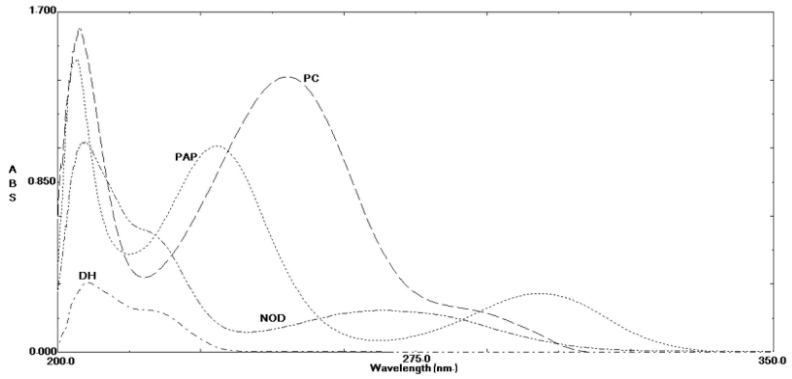

Table.7. Robustness data for Diphenhydramine and Naproxen

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)

CONSENT AND ETHICAL APPROVAL: It is not applicable

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COMPETING INTEREST: Authors have declared that no completing interests exists.

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.

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