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Development and Validation of Related Substances Method by HPLC for Analysis of Naproxen in Naproxen Tablet Formulations

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ABSTRACT
A simple, selective, rapid, precise and isocratic reversed phase high-pressure liquid chromatography method has been developed and validated for estimation of related substances of Naproxen in pharmaceutical dosage form. It was performed on a YMC-ODS A Pack (5µ particles size) (250mm × 4.6mm) column using mobile phase containing Acetonitrile and 10 mM Ammonium acetate buffer pH 3.8 in ratio 550:450 v/v (pH 3.8 adjusted with acetic acid) at the flow rate 0.8 ml/min. Detection was performed at 254 nm and a sharp peak was obtained for Naproxen at a retention time at about 5.9 ± 0.01 min. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 0.25-3µg/ml; the regression coefficient was 1.000. The detection (LOD) and quantification (LOQ) limits were 0.13 and 0.25µg/ml respectively. The method was validated for accuracy, precision, specificity, robustness, and detection and quantification limits, in accordance with ICH guidelines. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of Naproxen and its impurities. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase showed that the method is suitable for routine quantification of impurities in Naproxen in pharmaceutical dosage forms with high precision and accuracy.

Keywords: Naproxen, RP-HPLC, YMC-ODS A, validation.

INTRODUCTION

Naproxen [(+)-2-(6-methoxy-2-naphthyl) propionic acid or (NAP), is a non-steroidal anti-inflammatory drug with anti-inflammatory, analgesic and antipyretic properties often preferred to acetylsalicylic acid (aspirin) because of its better absorption following oral administration and fewer adverse effects. Anti-inflammatory effects of naproxen are generally thought to be related to its inhibition of cyclo-oxygenase and consequent decrease in prostaglandin concentrations in various fluids and tissues. [1] Formulated in tablets or suppositories, it is used in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrhea and acute gout. Naproxen in commercial formulations has been determined by coulometry [2], UV spectrophotometry [3-6], heavy atom-induced room temperature phosphorescence [7] and high-performance liquid chromatography (HPLC). [8-13] There was no HPLC Related substances method specified for Naproxen API and tablet formulations in United States Pharmacopoeia. Hence, there was a need to develop it which became the purpose of the further study. [14]

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Present work describes the development of simple, selective, rapid, accurate and precise RP-HPLC method for the determination of Related Substances of Naproxen in pharmaceutical dosage forms.

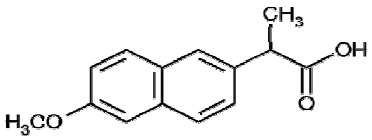

Fig. 1: Structure of Naproxen

EXPERIMENTAL

Apparatus and chromatographic condition

Chromatographic separation was performed on an Agilent 1100 Series HPLC system. YMC-ODS A Pack (5µ particles size) (250 mm × 4.6 mm) column was used for separation. Analysis was performed at ambient temperature.

Materials

Standard gift sample of Naproxen and impurities were provided by Sandoz Pvt. Ltd., Mumbai. Tablet formulations of Naproxen (500 mg) (Brand: Naprosyn) were procured from a local pharmacy. All the chemicals and reagents used were of analytical grade. High-purity water available from Millipore purification system was used.

Method Development

A detailed literature search was conducted to check the available methods for quantification of Naproxen impurities in tablet formulation and very few information was available. Hence, it is decided to start the method development using reversed phase chromatography; as reversed phase chromatography is most commonly used and ample columns are available for reversed phase chromatography. Further, considering the pKa of Naproxen 4.2, decided to select mobile phase pH range near to pKa. Based on this information, selected mobile phases were investigated in the development of an HPLC method suitable for analysis of Naproxen tablet formulation. These included Acetonitrile-Water-Acetic Acid, 550:450:1(v/v), Acetonitrile-Water-Orthophosphoric acid (pH 3.8 adjusted with triethylamine) 550:450:1(v/v) and Acetonitrile: 10mM Ammonium acetate buffer (pH 3.8 adjusted with acetic acid) 550:450(v/v). The suitability of the mobile phase was decided on the basis of the sensitivity of the method, suitability for stability studies, time required for the analysis, ease of preparation and use of readily available solvents. Mobile phase were premixed and filtered through a 0.45µm nylon filter and degassed. Optimization and finalization of mobile phase was also done based on observations for various parameters such as retention time, theoretical plates and resolution. Acetonitrile–Water 50:50(v/v) was used as the diluent.

Procedure: Five tablets (each tablet containing 500 mg Naproxen) of Naproxen were weighed accurately. The tablets were finely powdered and powder equivalent to 250 mg of Naproxen was taken in a 500 ml volumetric flask and then dissolved in 250 ml of diluent. The powder mixture was dissolved in the diluent with aid of sonication for 15 min with occasional swirling and then diluted up to the mark with diluent. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady base line, the tablet sample solution was filtered through 0.45µm Nylon filter after discarding first 2 ml. The solution was injected into the chromatographic system and chromatogram was recorded.

Method Validation [15-16]

Linearity

Standard stock drug solution of Naproxen with concentration of 125µg/ml was prepared in diluent. For preparation of calibration curve of drug 1, 2.5, 5, 10, 12 ml of standard stock solution of Naproxen were transferred to series of 500 ml volumetric flasks and volume was made up to the mark with the diluent. Each solution was injected after filtration through 0.45µm membrane filter and chromatograms were recorded at 254 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

Determination of Response Factor

The response factor for all impurities with respect to Naproxen was evaluated at 3 concentration levels. This was done by spiking Naproxen and all Impurities on placebo. Data from the duplicate determinations was collected at 3 concentration levels from 50% to 150% of the impurity concentration. i.e. 0.5%. The ratio of the slope of individual impurity to the slope of the drug substance is the response factor for the particular impurity with respect to Naproxen in presence of excipients. Then this response factor was used in denomination of calculation formula of % Impurity.

Preparation of Concentration Levels: A solution containing Naproxen and each of the identified impurity at concentration of 125µg/ml was prepared. Placebo equivalent to 250 mg of Naproxen was weighed and transferred in separate 500 ml volumetric flask for each level and dissolved in 250 ml of diluent. To this, Naproxen and all impurities stock solution (125µg/ml) was spiked and then made up to the mark by diluent.

Accuracy as Recovery

Accuracy was determined by the standard addition method. Samples of Naproxen (500µg/ml) were spiked with 10, 25, 50, 100 and 120% impurities standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample absorbance was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For inter-day variation, six homogenous test solutions of Naproxen were analysed. The intermediate precision of the method was checked on a different instrument, analysis being performed in different laboratory.

Specificity

The specificity of the method was determined by analysing the standard solutions of Naproxen and impurities and the spiked samples and exposing a solution (500µg/ml) of the sample to acidic (conc. Hydrochloric acid), basic (5N Sodium Hydroxide) and oxidising (30% Hydrogen peroxide) stress conditions. The resulting solutions were then analyzed and the analyte peak was evaluated both for peak purity and for resolution from the eluting peaks of all impurities.

Detection (LOD) and Quantification (LOQ) Limits

LOD and LOQ were determined by the S/N Method. Samples spiked with impurities were injected and the peak areas were calculated.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of Related Substances of Naproxen. Robustness was determined by changing column manufacturer (two other brands of same specifications), column of different lot but of same manufacturer, changing buffer pH in mobile phase, change in column oven temperature to 20°C and 30°C, changing the mobile phase flow rate to 0.7 and 0.9 ml/min and the ratio of acetonitrile in the mobile phase to 520 and 605 ml. The stability studies and filter studies also come under robustness.

Stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the reference and spiked sample solutions for 24 hours at autosampler temperature (25 ± 1°C).

Filter Study

The 100% spiked test solutions were filtered through different makes of filters or centrifuged and analysed for their suitability for the proposed method.

RESULTS AND DISCUSSION

Method Development

The HPLC procedure was optimized with a view to developing a method for stability-indicating assay. No

internal standard was used because no extraction or separation step was involved. Of several solvents and solvent mixtures investigated, the mobile phase Acetonitrile: 10mM Ammonium acetate buffer in ratio 550:450 v/v (pH 3.8 adjusted with acetic acid) was found to furnish sharp, well-defined peaks with very good symmetry (1.1) and low t_R (about 5.98 min) at the flow rate 0.8 ml/min. The other mobile phases tried did not resulted in chromatographic system as good as proposed method.

Study of retention time: A standard dilution of pure drug containing 500µg/ml of Naproxen was prepared in diluent. 5 ml of Reference stock solution was diluted to 100 ml with Diluent. Further 5 ml of this solution was diluted to 50 ml with diluent and loaded in injection port of instrument fitted with 20µl fixed volume loop. The solution was injected and chromatogram was recorded. The mean retention time for Naproxen was found to be about 5.98 min. The representative chromatogram of Naproxen is reported in Fig. 2.

Observations of Naproxen Tablet Analysis

The results of analysis for tablet formulation are reported in Table 1.

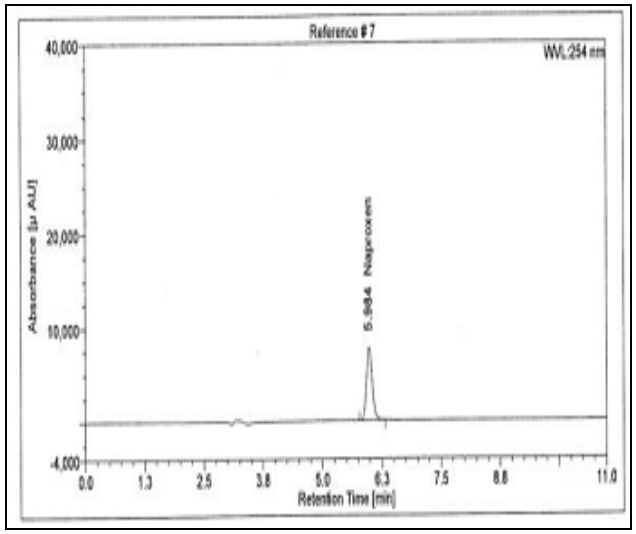

Fig. 2: Chromatogram of Naproxen

Table 1: % Content of Related Substances in Naproxen

S. No	% Content			
	Impurity I [6-methoxy-2-naphthoic acid]	Impurity II [2-acetyl-6-methoxy naphthalene]	Impurity III [Methyl(2S)-2-(6-methoxynaphthalene-2-yl)propionate]	Total Impurities
1.	N.D.	BLOQ	N.D.	BLOQ
2.	N.D.	BLOQ	N.D.	BLOQ
3.	N.D.	BLOQ	N.D.	BLOQ
4.	N.D.	BLOQ	N.D.	BLOQ
5.	N.D.	BLOQ	N.D.	BLOQ
6.	N.D.	BLOQ	N.D.	BLOQ
Mean	-	BLOQ	-	BLOQ
% RSD	-	-	-	-

BLOQ: Below quantification limit. (0.05%); ND: Not Detected

Method Validation

Linearity

The calibration curve plotted between concentration of drug and AUC of a peak of Naproxen is reported in Fig. 3. Linearity was established in concentration range of 0.25-3µg/ml for Naproxen.

The linear regression data for the calibration plot were indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance. The intercept of the ordinate showed the calibration plot did not deviate from linearity.

Table 2: Linearity Data for Naproxen Related Substances method with Acetonitrile: 10mM Ammonium acetate buffer in ratio 550:450v/v (pH 3.8) as mobile phase

Set No.	Concentration Level in (%)	Theoretical Concentration of Naproxen (ppm) (x – value)	Response (Area) (y – value)
Set I	LOQ (0.05%)-10%	0.2490	107.31368
	25%	0.6225	274.56520
	50%	1.2450	574.11203
	100%	2.4899	1088.57419
	120%	2.9879	1320.48796
Set II	LOQ (0.05%)-10%	0.2490	114.39526
	25%	0.6225	282.60135
	50%	1.2450	551.68503
	100%	2.4899	1103.21751
	120%	2.9879	1319.57599
Set III	LOQ (0.05%)-10%	0.2490	114.73356
	25%	0.6225	277.14292
	50%	1.2450	547.40852
	100%	2.4899	1101.43640
	120%	2.9879	1320.26826

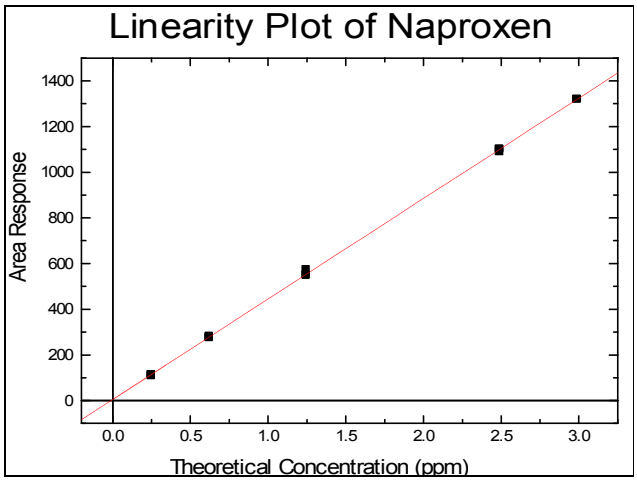

Fig. 3: Linearity Plot of Naproxen

Statistical Results

$R^2 = 1.000$

Table 3: Linear Regression Data for Naproxen Related Substances method

Parameters	Values
Detection wavelength	254 nm
Linearity range	0.25-3µg/ml
Slope	440.05
Intercept	1%
Correlation coefficient	1.000
Retention time for Naproxen peak	Appox. 5.98 min

Determination of Response Factor

Table 4: Linearity Response for Naproxen

Set No	Concentration Levels (%)	Theoretical Concentration of Naproxen (ppm) (X-value)	Area Response of Naproxen (Y-value)
Set-1	50	1.24	533.33
	100	2.48	1041.85
	150	3.72	1557.12
Set-2	50	1.24	522.67
	100	2.48	1040.93
	150	3.72	1554.02

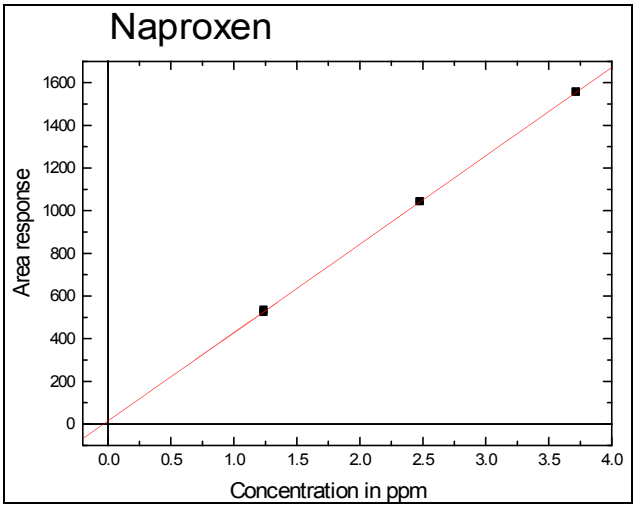

Fig. 4: Linearity Response for Naproxen: A graph of Concentration (x-value) against area response obtained (y-value) was plotted

Table 5: Linearity Response for All Impurities

Set No	Concentration Levels (%)	Theoretical Concentration of Impurities (ppm)			Area Response of Impurities		
		Imp I	Imp II	Imp III	Imp I	Imp II	Imp III
Set -1	50	1.23	1.25	1.22	3540.62	4244.95	509.92
	100	2.46	2.50	2.45	7022.32	8449.09	1025.57
	150	3.70	3.75	3.67	10516.03	12665.23	1540.87
Set -2	50	1.23	1.25	1.22	3508.42	4199.37	507.03
	100	2.46	2.50	2.45	7002.08	8450.44	1021.97
	150	3.70	3.75	3.67	10501.67	12658.16	1548.01

Accuracy, as Recovery

The mean recovery of the method, determined by spiking a known amount of impurity standards to test solution was 88% to 101% and the individual recovery was found to be 80.0% to 106.0%.

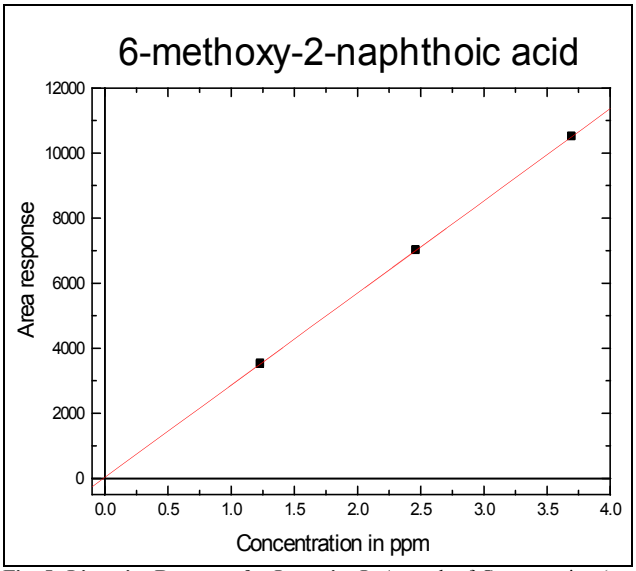

Fig. 5: Linearity Response for Impurity I: A graph of Concentration (x-value) against area response obtained (y- value) is plotted.

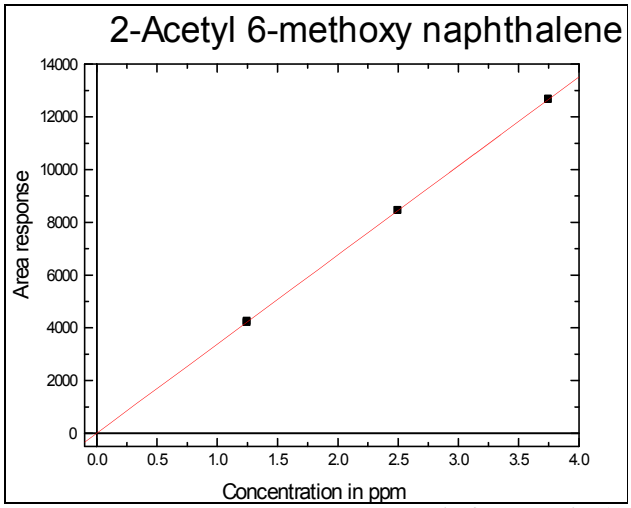

Fig. 6: Linearity Response for Impurity II: A graph of Concentration (x-value) against area response obtained (y- value) is plotted.

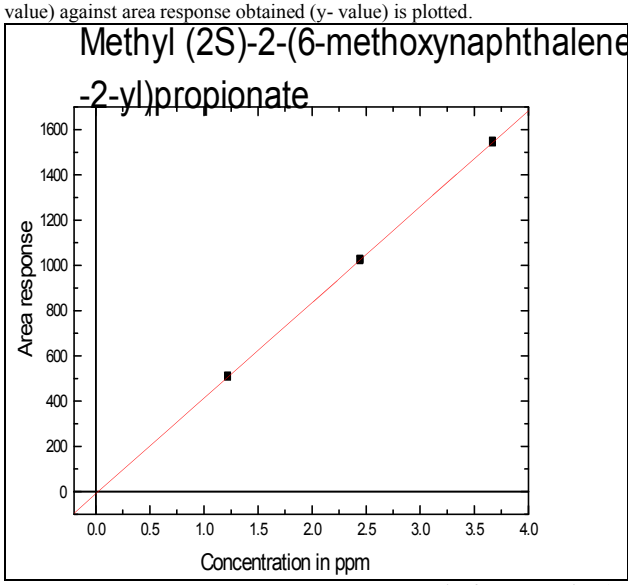

Fig. 7: Linearity Response for Impurity III: A graph of Concentration (x-value) against area response obtained (y- value) is plotted.

Table 6: Response Factor of Impurities

Parameter	Naproxen	Impurity I	Impurity II	Impurity III
Number for determinations	6	6	6	6
Correlation Coefficient(R)	1.000	1.000	1.000	1.000
% Y-axis Intercept	1	0	0	-1
Slope	414.41086	2834.77988	3377.57167	423.23908
Response Factor for Division = Slope of Impurity/Slope of Naproxen	1.00*	6.84	8.15	1.02

*It will be used for quantification of unknown impurities.

Precision

Analysis Repeatability

It was performed by carrying out the analysis of the six homogenous solutions of same test sample and content of impurities was calculated. The determinations were carried out one after the other under conditions as similar as possible. The relative standard deviation was calculated from the results of the obtained observations.

The % content of Impurity II and total impurities was found to be below quantification level for all the six homogenous test solutions, hence the method is precise.

Intermediate Precision

The intermediate precision of the method was checked by determining precision on a different instrument, analysis being performed in different laboratory on a different day. The % content of impurity II and total impurities was found to be below quantification level even when it is performed on a different instrument. The method is said to be precise with respect to the criteria of the intermediate precision.

Table 7: Accuracy of Naproxen in Related Substances Method

Concentration Levels in %	Volume of combined impurities stock solution 125ppm added (ml)	%Recovery 6-methoxy-2-naphthoic acid	2-acetyl-6-methoxy naphthalene	Methyl(2S)-2-(6-methoxynaphthalene-2-yl)propionate
		102	93	94
10%	0.2	102	90	80
		106	94	105
		99	89	90
25%	0.5	101	88	92
		102	90	95
		99	87	84
50%	1.0	98	88	88
		101	90	84
		98	88	85
100%	2.0	100	89	83
		101	90	86
		100	89	87
120%	3.0	99	89	83
		100	90	87
Mean Recovery rate		101	89	88
% Relative Standard deviation		2	2	7
Minimum		98	87	80
Maximum		106	94	105

Table 8: Analysis Repeatability (% Content of Impurities)

S. No	% Content			
	Impurity I	Impurity II	Impurity III	Total Impurities
1.	N.D.	BLOQ	N.D.	BLOQ
2.	N.D.	BLOQ	N.D.	BLOQ
3.	N.D.	BLOQ	N.D.	BLOQ
4.	N.D.	BLOQ	N.D.	BLOQ
5.	N.D.	BLOQ	N.D.	BLOQ
6.	N.D.	BLOQ	N.D.	BLOQ
Mean	-	BLOQ	-	BLOQ
% RSD	-	-	-	-

Table 9: Intermediate Precision of the method

Samples	% Content							
	Impurity I		Impurity II		Impurity III		Total Impurities	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1.	N.D.	N.D.	BLOQ	BLOQ	N.D.	N.D.	BLOQ	BLOQ
2.	N.D.	N.D.	BLOQ	BLOQ	N.D.	N.D.	BLOQ	BLOQ
3.	N.D.	N.D.	BLOQ	BLOQ	N.D.	N.D.	BLOQ	BLOQ
4.	N.D.	N.D.	BLOQ	BLOQ	N.D.	N.D.	BLOQ	BLOQ
5.	N.D.	N.D.	BLOQ	BLOQ	N.D.	N.D.	BLOQ	BLOQ
6.	N.D.	N.D.	BLOQ	BLOQ	N.D.	N.D.	BLOQ	BLOQ
Mean	-	-	BLOQ	BLOQ	-	-	BLOQ	BLOQ
% RSD	-	-	-	-	-	-	-	-

Specificity

The specificity of the method was determined by analysing the standard solutions of Naproxen and impurities and the spiked samples and exposing a solution (500µg/ml) of the sample to stress conditions i.e. chemical conditions like conc. Hydrochloric acid, 5N Sodium hydroxide, and 30% Hydrogen peroxide and physical conditions like treatment with heat, light and heat/humidity conditions. There was no degradation of Naproxen and no significant change in peak area and retention time of Naproxen in the presence of 5N Sodium hydroxide and in heat/humidity conditions. In the presence of conc. Hydrochloric acid, 30% Hydrogen peroxide and in thermal and photo stability conditions, it was found there was a substantial change in the peak area of Naproxen, but not in the retention time. The results from stress testing, including quantification of Naproxen after exposure to stress conditions show the method is stability-indicating.

Detection (LOD) and Quantification (LOQ) Limits

The LOD and LOQ of the method were 0.13 and 0.25µg/ml respectively, which indicated the method, can be used for detection and quantification of trace amounts of Naproxen impurities over a wide range of concentrations.

Robustness

There were no significant changes in the retention time of Naproxen when the composition, column oven temperature

Table 10: Degradation Conditions of Naproxen API

Stress conditions	% Impurity /Peak purity	Name of the Peak in Degraded Naproxen Sample				Assay of Untreated Naproxen API	Assay of Degraded Naproxen API	Total Imp. for Degraded Naproxen API	Mass Balance
		Naproxen	Imp. I	Imp. II	Imp. III				
Conc. HCl	% Impurity	–	ND	BLOQ	ND	99.0	96.6	1.0	98.6
	Peak purity	979	–	–	–				
30% H_2O_2	% Impurity	–	ND	BLOQ	ND	99.0	94.0	4.0	99.0
	Peak purity	987	–	–	–				
5N NaOH	% Impurity	–	ND	BLOQ	ND	99.1	99.8	0.0	100.7
	Peak purity	979	–	–	–				
Thermal Treatment 105°C	% Impurity	–	BLOQ	BLOQ	ND	99.2	98.8	0.0	99.6
	Peak purity	978	–	–	–				
Photolytic Exposure	% Impurity	–	BLOQ	0.16	ND	99.2	97.0	0.33	98.1
	Peak purity	980	–	1000	–				
Heat/ Humidity Conditions 40°C/75% RH	% Impurity	–	BLOQ	ND	ND	99.2	98.6	0.0	99.4
	Peak purity	979	–	–	–				

Note: The analysis of Untreated Naproxen API was carried out simultaneously with each forced degraded samples of Naproxen API and the % Impurity observed in Untreated Naproxen API was below Quantification limit (BLOQ).

Table 11: Degradation Conditions of Naproxen in Sample

Stress conditions	% Impurity/Peak purity	Name of the Peak in Degraded Naproxen Sample				Assay of Naproxen in Untreated Sample	Assay of Naproxen in Degraded Sample	Total Imp. for Degraded Naproxen API	Mass Balance
		Naproxen	Impurities I	II	III				
Conc. HCl	% Impurity	–	ND	0.1	ND	96.9	96.1	1.8	101.0
	Peak purity	979	–	1000	–				
30% H_2O_2	% Impurity	–	BLOQ	BLOQ	ND	96.9	90.1	5.4	98.5
	Peak purity	990	–	–	–				
5N NaOH	% Impurity	–	ND	BLOQ	ND	97.7	97.5	0.0	99.8
	Peak purity	979	–	–	–				
Thermal Treatment 105°C	% Impurity	–	BLOQ	BLOQ	ND	98.1	97.4	0.0	99.3
	Peak purity	979	–	–	–				
Photolytic Exposure	% Impurity	–	BLOQ	0.24	ND	98.1	96.1	0.43	98.4
	Peak purity	980	–	1000	–				
Heat/ Humidity Conditions 40°C/75% RH	% Impurity	–	ND	ND	ND	98.1	97.0	0.0	98.9
	Peak purity	979	–	–	–				

Note: The analysis of Untreated Naproxen Sample was carried out simultaneously with each forced degraded Naproxen samples and the % Impurity observed in Untreated Naproxen sample was below Quantification limit (BLOQ).

Table 12: LOQ Injection Repeatability

S. No.	Area			
	Naproxen	Impurity I	Impurity II	Impurity III
1.	127.91879	802.58208	829.62524	101.82284
2.	127.63196	854.84321	812.96645	98.26023
3.	126.99307	849.71601	788.87614	102.73160
4.	118.82421	854.50205	832.43516	93.30275
5.	139.19925	844.60157	820.57923	108.49061
6.	132.87135	826.96478	840.74767	93.59609
Mean	**128.90644**	**838.86828**	**820.87165**	**99.70069**
% RSD	**5.26**	**2.45**	**2.24**	**5.87**
S/N Ratio of LOQ	**15**	**33**	**24**	**10**
S/N Ratio of LOD	**10**	**16**	**12**	**4**

Table 13: Results from testing of the Robustness of the method by small changes in chromatographic conditions

Condition	Retention time for Naproxen peak (t_R)	Theoretical plates for Naproxen peak	Tailing factor for Naproxen peak	Resolution between Naproxen and Impurity-I	Resolution between Naproxen and Impurity-II	Resolution between Naproxen and Impurity-III
Normal (D-192)	6.155	5912	1.2	2.5	6.0	13.3
Different lot (LC-223)	5.993	5810	1.2	2.8	6.7	13.9
Different lot (D-193)	7.728	21910	1.1	6.9	14.9	33.1
Different column Cosmosil (D-150)	7.777	19872	1.3	6.9	13.7	31.2
Different column Kromasil (A-310)	7.845	19799	1.1	7.9	15.6	33.1
Mobile Phase composition - 520:480 v/v	6.516	5853	1.2	3.3	7.2	15.5
Mobile Phase composition - 605:395 v/v	5.306	5985	1.2	1.9	5.3	10.9
Buffer pH 4	7.029	6261	1.2	3.5	7.2	16.1
Buffer pH 3.7	6.169	5927	1.2	2.4	5.8	12.9
Flow rate - 0.9 ml/min	6.801	21661	1.0	6.8	14.7	32.5
Flow rate - 0.7 ml/min	8.724	22399	1.0	6.9	14.8	32.1
Column oven -20°C	7.855	21222	1.0	6.7	14.7	28.9
Column oven -30°C	7.441	22903	1.1	7.0	14.9	32.6

(The concentration of the spiked test solution analysed was of 500µg/ml)

Table 14: Stability of the Reference Solution

Time in hrs	Area of Naproxen	% Content w. r. t. 0 hr	% Change w. r. t. 0 hr	Reported % change w. r. t. 0 hr
0.0	1092.9	100.0	0.0	0
5	1109.2	101.5	-1.5	-1
8.7	1087.8	99.5	0.5	0
12:5	1082.5	99.1	0.9	1
14.0	1093.6	100.1	-0.1	0
19.0	1088.8	99.6	0.4	0
23.0	1085.2	99.3	0.7	1
27.0	1101.8	100.8	-0.8	-1
28.5	1094.1	100.1	-0.1	0

and flow rate of the mobile phase were changed. Changes were observed in the retention time of Naproxen when there is a change in column and buffer of the mobile phase. For all changed parameters, system suitability parameters of the proposed method were fulfilled. The theoretical plates and tailing factor of Naproxen and resolution of impurities, shown in Table indicated the robustness of the method.

Stability

The stability of the drug in solution during analysis was determined by repeated analysis of the reference and spiked sample solutions during the course of experimentation on the same day and also after their storage at autosampler temperature (25 ± 1°C).

The reference solution stability was established up to 28 hrs and the spiked sample stability was established up to 23 hrs.

Filter Suitability Test

The filter study was carried out by analyzing following solutions

- 100% spiked Test solution unfiltered but centrifuged.
- 100% spiked Test solution filtered with different makes of the filters.

No significant change was observed in the filtered spiked test solutions when compared with unfiltered spiked test solutions. For filtration of test solution, different makes of nylon filters can be used. Therefore, MDI make SY25NN 0.45µm and Valuprep make 0.45µm Nylon filters are suitable filters for the filtration of test solution.

Table 15: Stability of the Spiked Sample Solution: Impurity I

Time (hrs)	% Impurity I	% Impurity I change w. r. t. 0 hr	Reported % Impurity I change w. r. t. 0 hr
0.0	0.20	0.00	0
3.6	0.20	0.00	0
7.5	0.20	0.00	0
9.1	0.20	0.00	0
10.9	0.20	0.00	0
15.1	0.20	0.00	0
19.4	0.20	0.00	0
23.5	0.20	0.00	0

Table 16: Stability of the Spiked Sample Solution: Impurity II

Time (hrs)	% Impurity II	% Impurity-II change w. r. t. 0 hr	Reported % Impurity II change w. r. t. 0 hr
0.0	0.16	0.00	0
3.6	0.16	0.00	0
7.5	0.16	0.00	0
9.1	0.16	0.00	0
10.9	0.16	0.00	0
15.1	0.16	0.00	0
19.4	0.16	0.00	0
23.5	0.16	0.00	0

Table 17: Stability of the Spiked Sample Solution: Impurity III

Time (hrs)	% Impurity III	% Impurity-III change w. r. t. 0 hr	Reported % Impurity III change w. r. t. 0 hr
0.0	0.17	0.00	0
3.6	0.16	0.01	0
7.5	0.16	0.01	0
9.1	0.15	0.01	0
10.9	0.16	0.01	0
15.1	0.16	0.01	0
19.4	0.16	0.01	0
23.5	0.15	0.02	0

Table 18: Solution Stability of Spiked Test Sample: Total Impurities

Time (hrs)	% Total Impurities	% Total Impurities change w. r. t. 0 hr	Reported % Total Impurities change w. r. t. 0 hr
0.0	0.52	0	0
3.6	0.52	0	0
7.5	0.51	1.9	2
9.1	0.51	1.9	2
10.9	0.51	1.9	2
15.1	0.51	1.9	2
19.4	0.51	1.9	2
23.5	0.50	3.8	4

Table 19: Observations of Filter Suitability test

Filter used	% Impurity I	% Impurity II	% Impurity III	Total % Impurity content
Centrifuged & unfiltered	12.41	11.19	10.20	33.8
Millipore 0.45µ, nylon filter	12.45	11.07	10.31	33.8
Valuprep 0.45µ, nylon filter	12.39	11.12	10.70	34.2
MDI SY25NN 0.45µ, nylon filter	12.42	11.10	10.41	33.9

This HPLC method is sensitive, accurate, precise, reproducible, specific, and stability-indicating. The proposed method was found to be simple and linear in the concentration range 0.25-3.0µg/ml for Naproxen. This method was found to be accurate and precise as indicated by the recovery studies and relative standard deviation. The Relative Response factor for impurities determined from validation can be used for quantification of impurities in Naproxen in routine testing, by which there is no need to use impurities' standard. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, simplified extraction procedure, low retention time (t_R) and no internal standard. All these factors make this method suitable for quantification of impurities in Naproxen in pharmaceutical dosage forms without interference and can be successfully used for routine analysis. It can therefore be concluded that it can be used in small laboratories with very high accuracy and a wide linear range.

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