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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SUMATRIPTAN AND NAPROXEN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple and precise RP-HPLC method was developed and validation for the determination of Sumatriptan and Naproxen in pharmaceutical dosage forms. Chromatography was carried out on a C_8 (4.6x150mm.3.5µm) using a mixture of Buffer: acetonitrile (50:50) as the mobile phase at 0.7 ml/min flow rate. The analytes were monitored using UV detector at 285nm. The retention times of the drugs are 5.87 and 2.24min for Sumatriptan and Naproxen respectively. The proposed method is found to be having linearity in the concentration range of 60-100 µg/ml with correlation coefficient of r =0.999. The developed method has been statistically validated and found simple and accurate. The accuracy studies of RP-HPLC method was performed at three different levels, i.e., 50%, 100%, 150% and recovery was found to be in range 99.0-101.0 %. Sumatriptan and Naproxen respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 3.36µg/ml and 3.206µg/ml for sumatriptan and 9.906µg/ml and 9.86µg/ml for naproxen. The % R.S.Dreported was found to be < 2 %. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Sumatriptan and Naproxen in bulk and dosage forms.

KEYWORDS

Sumatriptan, Naproxen, Acetonitrile, UV and RP-HPLC.

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INTRODUCTION

Sumatriptan1-{3-[2-(dimethylamino)ethyl]-1H-indol -5-yl}-N-Methyl methane sulfonamide Figure No.1 is avaso constrictor agent selective serotonin agonist, which inhibit the firing of serotonin neurons and a reduction in the synthesis and release of serotonin upon activation. After sumatriptan binds to these receptors, adenylate cyclase activity is inhibited via regulatory G proteins, increases intracellular calcium, and affects other intracellular

events. This results in vasoconstriction and inhibtion of sensory nociceptive (trigeminal) nerve firing and vasoactive neuropeptide release and is used in the treatment of migraine disorder. Naproxen 2-(6-methoxynaphthalen-2-yl) propanoic acid Figure No.2 is an anti-inflammatory agent with analgesic and antipyretic properties. This inhibits the cyclo-oxygenase enzyme action. Both the acid and its sodium salt are used in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrhoea and acute gout[1-2]. As per literature survey it is revealed that a few methods reported for the determination of sumatriptan and naproxen in biological fluids and HPLC methods in tablet formulation [3-8].In the present work, a simple reliable and reproducible RP-HPLC method was developed validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines[9-10].

MATERIALS AND METHOD

Samples

Chemicals and reagents

Sumatriptan and Naproxen was obtained as a gift samples from Vircho laboratories, Hyderabad, Acetonitrile (HPLC grade), Potassium dihydrogen Phosphate (AR grade), Sodium di hydrogen phosphate (AR grade), Orthophosphoric acid (AR grade), Trimethylamine (AR grade) was purchased from Merck.

Instrumentation

Analysis was performed using high performance liquid chromatography system (HPLC) Waters separation model 2695 equipped with a UV-Visible detector. The output signal was monitored and processed using acquisition software.

Chromatographic conditions

Mobile phase consists of buffer and acetonitrile in the ratio 50:50 delivered at a flow rate of 0.7 ml/min, whereas run time set was 8min. The column was maintained at 25 ^{0}C and the volume of each injection was 20 µL. The column effluent was monitored at 285 nm.

Preparation of mobile phase

Mix a mixture of above buffer 500 mL (50%) and 500 mL of Acetonitrile HPLC (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of standard solution (Stock solution)

Accurately weigh and transfer 10 mg of Sumatriptan and Naproxen working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 5ml of Sumatriptan and Naproxen the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluents. Further pipette 8ml of Sumatriptan and Naproxen the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution (Stock solution)

Accurately weigh and transfer equivalent to 10 mg of Sumatriptan and Naproxen sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 5ml of Sumatriptan and Naproxen of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan and Naproxen the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents. The optimized chromatographic condition was shown in Table No.1 and the standard chromatogram for Sumatriptin and Naproxen were shown in Figure No.3. Assay of Sumatriptan and Naproxen shown in Table No.1 and 2.

METHOD VALIDATION

The following parameters were considered for the analytical method validation of Sumatriptan and Naproxen in pharmaceutical dosage form.

System suitability

A Standard solution of Sumatriptan and Naproxen working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Accuracy

For accuracy determination three different concentrations were prepare separately i.e. 50%, 100% and 150% for the analytes and chromatograms are recorded for the same.

Precision

The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Robustness

As part of the Robustness, deliberate change in the mobile phase composition and flow rate variation was made to evaluate the impact on the method.

Linearity and range

Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 60 ppm to 100 ppm of test concentration, into the chromatograph, covering minimum 5 different concentrations. Inject each level into the chromatographic system and measure the peak area. A calibration curve was plotted for concentration v/s peak area and calculates the correlation coefficient.

Ruggedness

Establish the ruggedness of the analytical method by using the assay of 4 different sample preparations of same batch by a different analyst using a different HPLC System.

Limits of detection and limits of quantification

The LOD and LOQ were determined by using the slope and SD of response. The mean slope value and the SD of response were obtained from the calibration curve.

RESULTS AND DISCUSSION

The present study was aimed at developing a simple economical precise and accurate HPLC method for the analysis of sumatriptan and naproxen in bulk and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with buffer in different combinations were tested as mobile phase on a C_8 stationary phase. The optimized method was validated as per ICH guidelines.

System suitability

System suitability tests were carried out on freshly prepared standard solutions and the parameters are summarized in TableNo.2. A typical chromatogram of sumatriptan and naproxen is shown Figure No.2 and 3 respectively.

Accuracy

The results of recovery study (99.8 to 101.9% for sumatriptan and 99.5% to 101.7% for naproxen) suggest that the method is accurate. The results are shown in Table No.3 and 4.

Precision

The precision of proposed method was carried out and from the results it reveals that the method is precise. The results are shown in Table No.5.

Linearity

The calibration curve of sumatriptan and naproxen were constructed by plotting the peak areas of the drug to the concentration and correlation coefficient was found to be 0.999 for both drugs shows that the good correlation coefficient exists between the drug and response. The linearity results are shown in Table No.6 and 7.

The results of robustness in the present method show that method is robust. The results are shown in Table No.8 and 9. The method is rugged by using the assay of 4 different sample preparations of same batch by a different analyst using a different HPLC System. The results are in shown in Tale No.10.

LOD and LOQ

The LOD and LOQ values for sumatriptan and naproxen show that the method is sensitive. The values are reported in Table No.11. The results of analysis of specific study indicated that no interference due to commonly used Tablet Excipients.

Table No.1: Optimized Chromatographic Condition

S.No	Column	c_8 (4.6X 150mm,3.5µm,)
1	Flow rate	0.7mL / min
2	Wavelength	285nm
3	Column oven	Ambient
4	Injection volume	20µL
5	Run time	8min

Table No.2: System Suitability Parameters of Sumatriptan and Naproxen

S.No	Name	Retention Time (min)	Area (µv*sec)	Height (µv)	USP Plate count	USP Tailing
1	Naproxen	2.249	1531670	147652	2187.1	1.6
2	Sumatriptan	5.875	1573899	84946	2196.7	1.3

Table No.3: Accuracy Results of Sumatriptan

S.No	% Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	50%	984243	6.15	6.23	101.4%	101.0%
2	100%	1567396	10.0	9.98	99.8	
3	150%	2497228	15.5	15.79	101.9%	

Table No.4: Accuracy Results of Naproxen

S.No	% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	50%	958196	6.15	6.25	101.7%	101.0%
2	100%	1532695	10.0	9.96	99.5%	
3	150%	2425792	15.6	15.8	101.7%	

Table No.5: Precision results of Sumatriptan and Naproxen

S.No	Injection number (80 mcg/ml)	Retention Time of Naproxen	Retention Time of Sumatriptan	Area of Naproxen	Area of Sumatriptan
1	Injection-1	2.412	6.374	1549491	1579612
2	Injection-2	2.259	5.900	1530248	1574521
3	Injection-3	2.259	5.884	1530713	1566564
4	Injection-4	2.261	5.863	1527834	1570849
5	Injection-5	2.257	5.857	1537667	1567180
AVRG		---	---	1535191	1571745
STDEV		---	---	8792.8	5433.0
% RSD		---	---	0.57	0.35

Table No.6: Linearity Results of Naproxen

S.No	Conc.in µgml	Response
1	60	1219297
2	70	1531332
3	80	1856868
4	90	2252060
5	100	2560402

Table No.7: Linearity Results of Sumatriptan

S.No	Conc.in µgml	Response
1	60	1189032
2	70	1497165
3	80	1851004
4	90	2186380
5	100	2548658

Table No.8: Robustness of Sumatriptan

S.No	Proposed variations		USP Plate Count	USP Tailing
1	Variation in mobile phase composition	10% less	2396.9	1.8
		*Actual	2183.4	1.6
		10% more	2218.9	1.5
2	Variation in flow rate	0.6ml/min	2228.3	1.6
		0.8ml/min	2183.4	1.6
		1.0ml/min	2142.7	1.6

Table No.9: Robustness of Naproxen

S.No	Proposed Variations		USP Plate Count	USP Tailing
1	Variation in mobile phase composition	10% less	2963.1	1.2
		*Actual	2203.7	1.3
		10% more	2268.8	1.3
2	Variation in flow rate	0.6ml/min	2519.4	1.3
		0.8ml/min	2203.7	1.3
		1.0ml/min	2474.4	1.3

Table No.10: LOD and LOQ Results of Sumatriptan

S.No	Parameters	Sumatriptan	Naproxen
1	LOD	3.36µv	3.206µv
2	LOQ	9.906µv	9.866µv

Table No.11: Ruggedness of Sumatriptan and Naproxen

S.No	Naproxen		Sumatriptan	
1	Spl. Area	1525384	Spl. Area	1503.654
2	Std.Area	1532594	Std.Area	1457.193
3	Std. Wt	10mg	Std. Wt	10mg
4	Spl.Wt	117mg	Spl.Wt	19.8mg
5	LC	500mg	LC	85mg
6	Avg.Wt	997.5mg	Avg.Wt	997.5mg
7	Std.Purity	99.8	Std.Purity	99.6
8	Assay %	98.8	Assay %	98.6

Table No.12: Assay of Sumatriptan and Naproxen by RP-HPLC

S.No	Analysis	Retention Time of Naproxen	Retention Time of Sumatriptan	Area of Naproxen	Area of Sumatriptan
1	Standard(80mcg)	2.293	5.951	1976857	1990948
2	Analyst(1)(80mcg)	2.291	5.955	1971778	1993690
3	Analyst(2)(80mcg)	2.290	5.944	1970279	1995386
4	Analyst(3)(80mcg)	2.290	5.935	1979007	1992472
5	Analyst(4)(80mcg)	2.286	5.923	1970631	1993363
6	AVRG			1973711	1993172
7	STDEV			3966.9	1631.1
8	%RSD			0.20	0.08

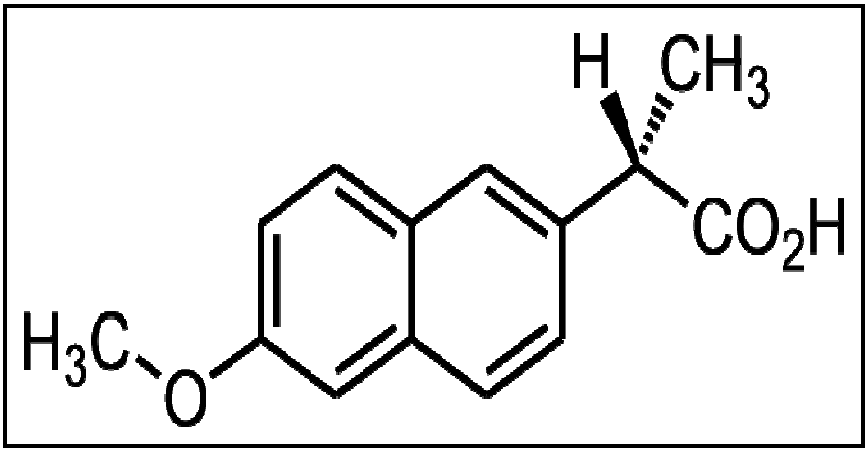

Figure No.1: Sumatriptan

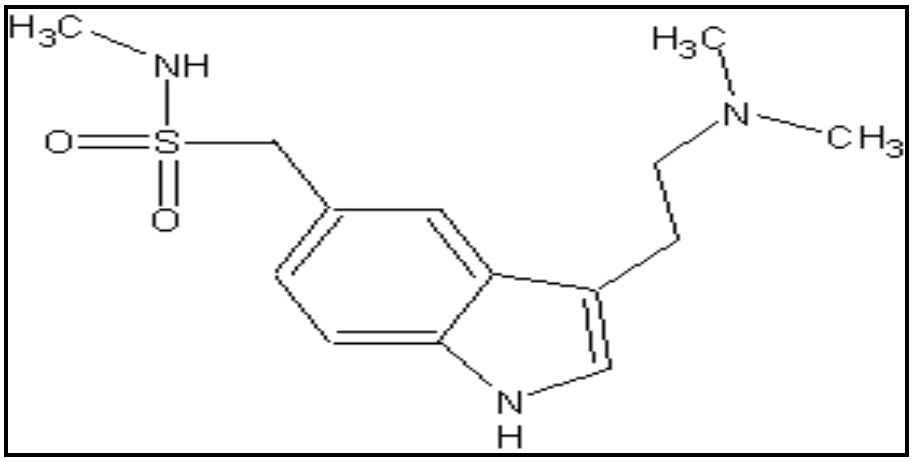

Figure No.2: Naproxen

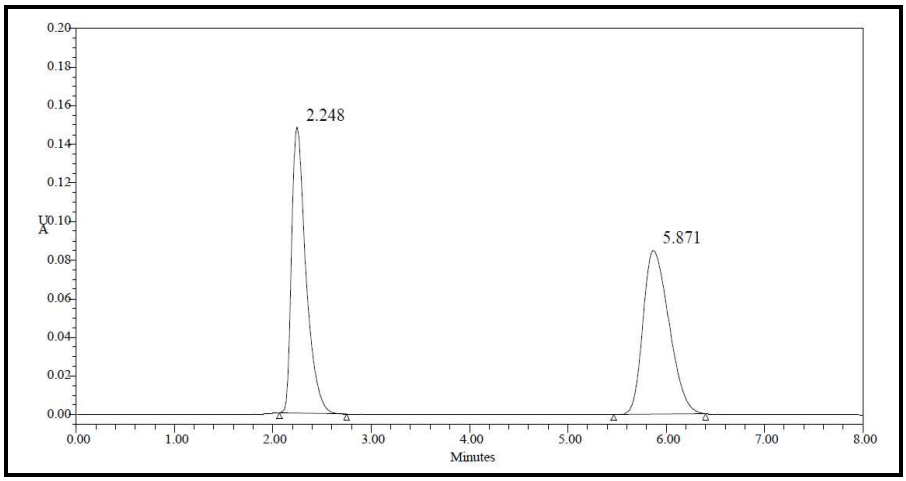

Figure No.3: Chromatogram of Standard

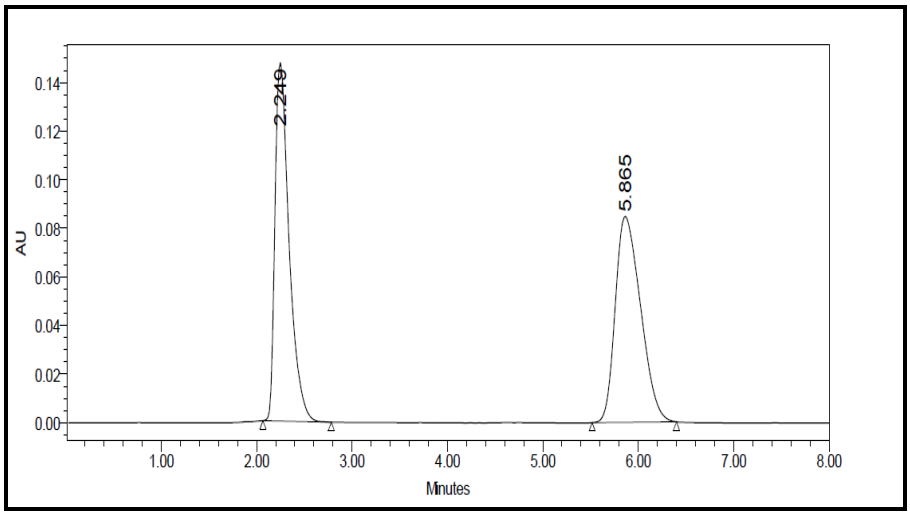

Figure No.4: Chromatogram of Sample

CONCLUSION

The proposed method is found to be simple, presice, accurate and rapid for the determination of sumatriptan and naproxen from pure and its pharmaceutical dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good with their respective label claims and they suggest the non-interference of formulation exciepients in the estimation. Hence, this method can be used for routine analysis of two drugs in their combined pharmaceutical dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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