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DEVELOPMENT AND VALIDATION OF RP-HPLC FOR SIMULTANEOUS ESTIMATION OF RIZATRIPTAN AND NAPROXEN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
Objectives:
An accurate and precise RP-HPLC method was developed for the simultaneous determination of Rizatriptan and Naproxen in tablet dosage form.
Method:
Separation of the drug was achieved on an Agilent TC 1120 RP 18 column using a mobile phase consisting of acetonitrile and water in the ratio of 60:40v/v. The flow rate was 1.5 mL/min and the detection wavelength was 235 nm.
Results and conclusion:
The linearity was observed in the range of 60-140 ppm for Rizatriptan and Naproxen with a correlation coefficient of 0.998 and 0.997 respectively. The mean percentage recoveries for 80%, 100% and 120% accuracy were found to be 100%±0.853, 100%±1.909 and 100%± 0.436 respectively for RIZ. The mean percentage recoveries for 80%, 100% and 120% accuracy were found to be 100%±1.643, 100%±0.119 and 100%±1.493 respectively for NAP. The method showed good reproducibility and recovery with percent relative standard deviation less than 2. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Rizatriptan and Naproxen tablets.

Keywords : *Rizatriptan, HPLC, Naproxen, Tablet, Validation*

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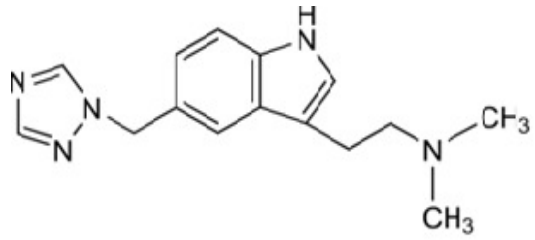
INTRODUCTION

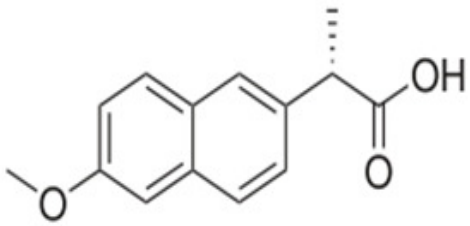
Rizatriptan (RIZ) is an anti-migraine drug used to treat migraines and is chemically known as *N*, *N*-Dimethyl–5-(1*H*- 1, 2, 4– triazol–1–yl methyl)-1*H*-indole–3–ethanamine. RIZ belongs to the class of drugs known as serotonin 5-Hydroxytryptamine ($5HT_1$) receptor agonist. RIZ acts as an agonist at specific $5HT_1$ receptor sites in intracranial vessels, causing vasoconstriction. RIZ also act on sensory trigeminal nerves reducing transmission along pain pathways. The molecular formula is $C_{15}H_{19}N_5$ and the structural formula is in figure 1. RIZ is a white crystalline solid, slightly soluble in water (42 g/L at 25°) as the free base[1]. It has a molecular weight of 269.4gm[2]. The literature survey revealed that various analytical methods such as UV-Visible (Vis) spectrophotometry[3-7] and High performance thin layer chromatography[8-10] are available for the estimation of RIZ alone or with some other drugs in formulations. Naproxen (NAP) is chemically as(*S*)-6-Methoxy-*α*-methyl–2–naphthalene acetic acid. It is a non-steroidal anti-inflammatory (NSAID) drug, commonly used as an analgesic and antipyretic drug used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both COX-1 and COX-2 enzymes. The molecular formula is $C_{14}H_{14}O_3$ and the structural formula is in figure 1. NAP is a White crystalline substance, sparingly soluble in water; soluble 1 in 25 of ethanol, 1 in 15 of chloroform, and 1 in 40 of ether[1]. It has a molecular weight of 230.3gm[11]. The literature survey revealed that various analytical methods such as UV-Visible (Vis) spectrophotometry[12-14], High performance liquid chromatography[15-17] methods have been reported for estimation of NAP from its formulations and biological fluids. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters which should be followed in the development and validation. A survey of literature reveals that simultaneous analytical methods are not available for the drug combination RIZ and NAP, even though very few methods of individual estimation of the above drugs are available. Hence it is proposed to develop new methods for the assay of RIZ and NAP in pharmaceutical dosage forms adapting method using RP-HPLC. The objective of the proposed method was to develop simple and accurate methods for the determination of RIZ and NAP simultaneously by RP-HPLC in pharmaceutical dosage forms.

MATERIALS AND METHODS:

RIZ and NAP standards and commercial sample of RIZ (5mg) and NAP (200mg) tablets were procured from local market and used within their shelf-life period. HPLC grade water and Acetonitrile were obtained from E. Merck (India) LTD., Mumbai. Quantitative HPLC was performed on Agilent TC (model no: 1120), with UV-Visible detector. Symmetry Shield RP 18 (250 x 4.6 mm, packed with 5 microns) column used for the chromatographic separation.

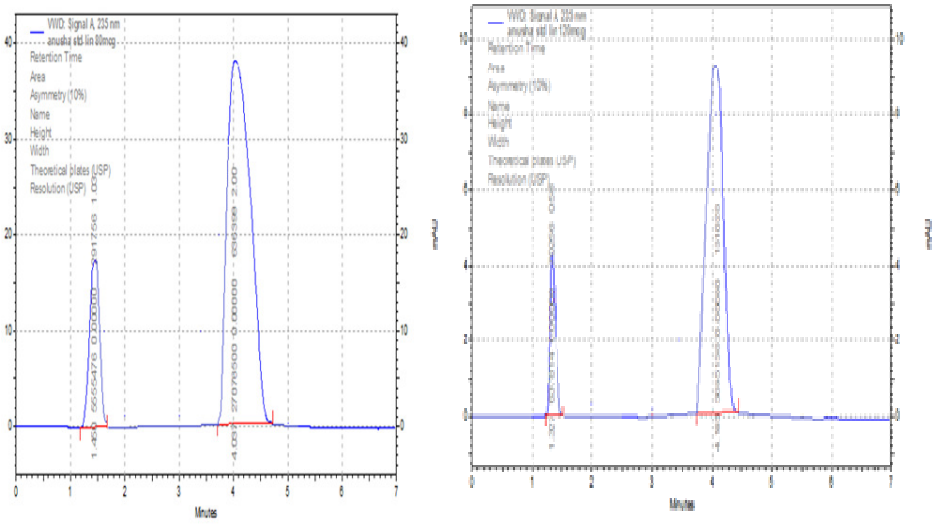
Manual injections (20 µl) were used. The column was maintained at ambient temperature. The detector wavelength was set at 235nm. To develop a suitable and robust HPLC method for the determination of RIZ and NAP, different mobile phases Acetonitrile: buffer, were used in different compositions of mobile phases at different flow rates. Finally, the mobile phase Acetonitrile: water at the ratio of 60:40v/v at a flow rate of 1.5 ml/ min gave peaks good resolution for RIZ and NAP. RIZ and NAP were eluted at retention times around 1.56 min and 3.94 min, respectively with symmetric peak shape. The mobile phase was degassed and filtered through 0.25 µm membrane filter before pumping into the HPLC system. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The run time was set at 10 minutes. The data were collected and analyzed with Ez chrome software in a computer system. A typical chromatogram showing the separation of the drug is given in Fig 2.





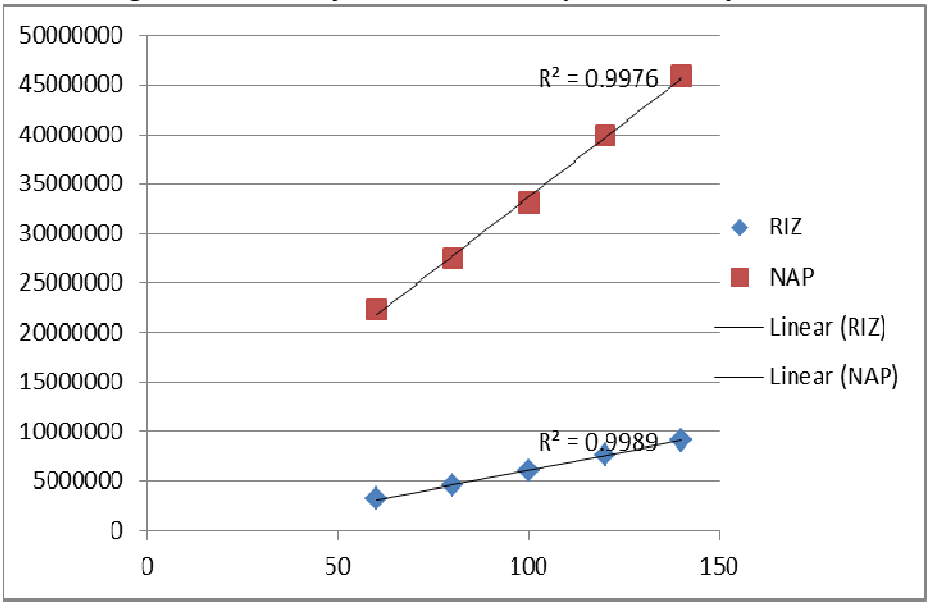
Rizatriptan **Naproxen**

Figure 2: Chromatogram for Rizatriptan and Naproxen



Standard mixture sample mixture

Figure 3: Linearity curve for Rizatriptan and Naproxen



Preparations:

Diluent preparation: 100ml volumetric flask was taken and 60 ml of acetonitrile and 40ml of water, which are of HPLC Grade were added.

Preparation of standard stock solution:

10 mg of RIZ and NAP working standards was weighed accurately and added to 10 ml volumetric flasks individually. 7.5ml of diluent to each volumetric flask was added and sonicated for about 15 minutes. Then the solution was made up to volume with diluent.

Standard Preparation:

1ml of each of the stock solutions prepared ie., 1ml of RIZ and NAP stock solution was taken and added to a 10ml volumetric flask, then made up to volume with diluent producing 100µg/ml of RIZ and NAP. Then final solutions were filtered using micro filtration unit of 0.25µm and injected.

Sample preparation:

Twenty tablets of RIZ and NAP individually were weighed and finely powdered and calculate the average weight. An accurately weighed sample of powdered tablet equivalent to 10mg of NAP and 10mg of RIZ were extracted with 7.5ml diluent in 10ml volumetric flask using Sonicator. This solution was filtered through 0.25 µm filter paper. The solution obtained was diluted with the diluent so as to obtain a concentration. 1ml of stock solution of RIZ and NAP each were added into 10 ml volumetric flask, then it was made up to the 10ml mark with diluent. All determinations were carried out in five to six injections.

Validation:

The described method has been validated for the assay of RIZ and NAP using following parameters. System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatography system. Peak area (A), Number of theoretical plates (N), Retention time (Rt) and resolution were evaluated for five replicate injections of the drug at a concentration of 100µg/ml. The linearity range was found to be 60- 140% concentrations of both drugs. To obtain proportionality, the slope of the regression line was calculated statistically by the method of average peak area Vs concentrations for RIZ and NAP and Correlation Coefficient was calculated. Precision was studied to find out variations in the test methods of RIZ and NAP of 100mcg/ml concentration when analysis carried out on the same day and on different day (Ruggedness) by using different analyst. The standard solution was injected for five times and measured the area for all five injections in HPLC. For ruggedness and precision, the %RSD and %content results were calculated. The accuracy of the method was shown by analyzing model mixtures which were obtained by spiking known amounts of RIZ and NAP to the placebo. The model mixtures contained 80%, 100%, and 120% of RIZ and NAP compared to the labeled drug amount. After injection, individual recovery values were calculated. Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. The equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate. Robustness of the method were determined by varying the chromatographic conditions such as the changing flow rate (± 0.1ml/min), mobile phase ratio (± 5 ratio) and the wavelength of detection (±5nm). Refrigerator solution stability was performed on 1st day.

RESULTS AND DISCUSSION:

A reverse phase isocratic procedure was proposed as a suitable method for the analysis of RIZ and NAP in tablets. Acetonitrile: water at the ratio of 60:40 v/v at a flow rate of 1.5 ml/ min was found to be an appropriate mobile phase allowing adequate and rapid separation of analyte. The retention time was found to be 1.56min and 3.94min for RIZ and NAP respectively. System suitability for the RIZ and NAP reported that theoretical Plates obtained from the standard injections was not less than 2500. System suitability studies for the RIZ and NAP reported that the %Relative standard deviation values of five replicate injections for RIZ and NAP were found to be 0.95 and 0.73. The theoretical plates for RIZ and NAP were found to be 5762 and

7985 respectively. The resolution was found to be 2.84. As shown in the Figure 2, the substances were eluted forming well shaped, symmetrical single peaks, well removed from the solvent front. The precision of the HPLC system was determined using the %RSD of the peak areas for five injections of the standard solution of RIZ and NAP. %RSD for RIZ and NAP were found to be 1.37 and 1.90 respectively. The %RSD of both the drug under this method was less than 2. Precision data were presented in table 1. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures of RIZ and NAP. The recovery of RIZ and NAP was evaluated from 80%, 100% and 120%. The mean percentage recoveries for 80% and 120% accuracy were found to be 104.5%, and 105.2 %for RIZ. The mean percentage recoveries for 80% and 120% accuracy were found to be 105.8%and 105.9% for NAP. The results of percentage recovery data were within the limit. Accuracy data were presented in table 2. For quantitative application a linear calibration curve was obtained over the concentration range from 60 to 140% for RIZ and NAP. The correlation coefficient for being 0.998 and 0.997 respectively. The regression equation of RIZ was found to be y=74662.015x-1374823.3 with coefficient of correlation 0.9987 where x is concentration, y is absorbance, 74662.015 is slope and -1374823.3 is intercept. The regression equation of NAP was found to be y=298566.85x+3886579 with coefficient of correlation 0.9985 where x is concentration, y is absorbance, 298566.85 is slope and 3886579 is intercept. Percentage curve fitting of RIZ and NAP was found to be 99.87% and 99.85% respectively. The calibration curve of both drugs was present in figure 3 and related linearity data in table 3. Ruggedness for RIZ and NAP were determined by varying analysts carrying out the procedure. Totally 2 analysts carried out the procedure and the results were within the limits. Limit of detection of RIZ and NAP were found to be 1.1001 and 0.3302mcg/mL respectively. Limit of quantitation of RIZ and NAP were found to be 3.3337 and 1.0006mcg/mL respectively. The results of robustness indicate that the variation in chromatographic conditions the method was not affected. Variation in parameters for robustness studies was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust. Stability studies (Refrigerator stability and Bench top stability) have expressed the percentage deviation from the true value within the limit for the both drugs. The results of validation parameters of proposed method were presented in table 4.

Table 1: Precision data for Rizatriptan and Naproxen

Samples	Peak area		Percentage content	
Concentration (mcg/ml)	Rizatriptan	Naproxen	Rizatriptan	Naproxen
100	5768721	3123163	98.14335237	98.0645092
100	5821345	3197432	99.03864541	100.396489
100	5899240	3265431	100.3638744	102.531596
100	5942138	3125463	101.0936989	98.1367271
100	5957816	3212534	101.3604289	100.870678
Mean	5877852	3184804.6	100	100
SD	80757.34308	60724.99398	1.373926106	1.90671019
%RSD	1.373926106	1.906710194	1.373926106	1.90671019

Table 2: Accuracy data for Rizatriptan and Naproxen

Number of injection = 6	Peak area		Percentage Content		Percentage Recovery	
	Rizatriptan	Naproxen	Rizatriptan	Naproxen	Rizatriptan	Naproxen
Accuracy 1(80%)						
Mean	4968927	26325984	83.64446	84.64372	104.56	105.8047
SD	116944.9	267970.8	1.968592	0.861584	2.46074	1.07698
%RSD	2.353524	1.017895	2.353524	1.017895	2.353524	1.017895
Accuracy 2(100%)						
Mean	5940533	31102110	100	100	100	100
SD	39863.85	33330.19	0.671048	0.107164	0.671048	0.107164
%RSD	0.671048	0.107164	0.671048	0.107164	0.671048	0.107164
Accuracy 3(120%)						
Mean	7506354	39554656	126.3582	127.1768	105.2985	105.9806
SD	39899.91	561052.5	0.671655	1.803905	0.559713	1.503254
%RSD	0.531549	1.418423	0.531549	1.418423	0.531549	1.418423

Table 3: Linearity data for mixture of Rizatriptan and Naproxen

	Rizatriptan		Naproxen	
Concentration (mcg/ml)	mean	SD	Mean	SD
60	3198153	14849.24	22310496	2121.32
80	4572234	8485.281	27522000	64346.72
100	5901845	113844.2	32975678	212132
120	7668375	32526.91	39959930	12091.53
140	9116284	14849.24	45948216	70639.97
Slope	74662.015	120.208145	298566.85	423.91055
Intercept	-1374823.3	24890.1597	3886579	29875.253
Correlation	0.998796175	0.08653107	0.998543955	0.159741492

Table 5: Results of validation parameters

Validation	**Parameters**	**Rizatriptan**	**Naproxen**
System suitability	%RSD	0.95	0.73
	Theoretical plates	5762	7985
	Resolution	2.84	
Linearity	Correlation coefficient	0.9987	0.998
	Slope	74662.015	298566.85
	Intercept	-1374823.3	3886579
LOD		1.100mcg/mL	0.330 mcg/mL
LOQ		3.333mcg/mL	1.0006 mcg/mL

Precision	%RSD	1.37	1.90
Ruggedness	%RSD for Analyst 1 variation	0.94	1.90
	%RSD for Analyst 2 variation	0.69	0.10
Accuracy	Mean % recovery for 80%, 100, 120% respectively	104.56,100 & 105.29	105.80,100 & 105.98
Refrigerator stability	%RSD	0.30	0.33
Specificity		No interference	No interference
Assay	% content	100	100
Robustness	%RSD for Flow rate 1.6 ml/min	1.45	1.31
	%RSD for Flow rate 1.4 ml/min	1.32	1.80
	%RSD for Mobile phase 65:35	1.32	1.82
	%RSD for Mobile phase 55:45	0.69	0.44
	%RSD for wavelength 240 nm	0.24	0.35
	%RSD for wavelength 240 nm	0.11	1.72

CONCLUSION:

The presented method is precise, sensitive and accurate. The advantages of the proposed method are its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of the proposed method for the routine analysis of mixtures of Rizatriptan and Naproxen in pharmaceuticals.

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