eISSN: 2231-0541 CAS CODEN: PHARN8 An ELSEVIER Covered Journal

PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 4 | Issue 6 | November-December 2013 | Pages 1314-1321

Original Research Article

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR NAPROXEN AND RIZATRIPTAN IN BULK AND TABLET DOSAGE FORM USING ABSORPTION RATIO METHOD

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Received: 17-09-2013 Revised: 03-10-2013

Accepted: 19-10-2013 Available online: 01-11-2013

ABSTRACT

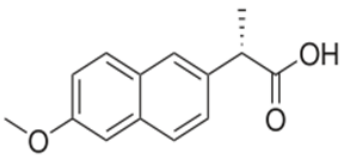
A simple, economic and accurate absorption ratio method was developed for the simultaneous estimation of Naproxen (NAP) and Rizatriptan (RIZ) in bulk and tablet dosage form.Phosphate Buffer pH 7.4 was used as a diluent. The absorptions were observed at 216.34nm and 230.19nm which were selected based on overlap spectra of NAP and RIZ. The Linearity range was found to be 2-4.5 µg/ml (r^2 =0.9905) at 216.34nm and (r^2 = 0.9998) at 230.19nm. The proposed method was validated. The reports was expressed that the proposed method was found to be simple, precise, accurate and rapid for the simultaneous estimation of NAP and RIZ in bulk and tablet dosage form using absorption ratio method.

Key words: Absorption ratio, Naproxen, Phosphate Buffer pH 7.4, Rizatriptan

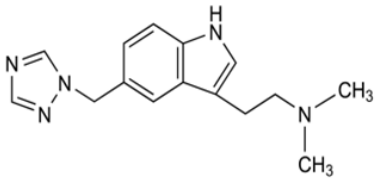
INTRODUCTION

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 analgesic and antipyretic drug used for 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, practically insoluble 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[2]. Literature survey revealed that various analytical methods such as UV-Visible (Vis) spectrophotometry[3-6], High performance liquid chromatography[7]and High performance thin layer chromatography[8] methods have been reported for estimation of NAP from its formulations and biological fluids. Rizatriptan (RIZ) is an antimigraine drug used to treat migraines and is chemically known as *N*,*N*-Dimethyl–5-(1*H*-1,2,4–triazol–1–ylmethyl)-1*H*-indole–3–ethanamine. RIZ belongs to the class of drugs known as serotonin 5-Hydroxytryptamine ($5HT_1$) receptor agonist. RIZ acts as 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].Literature survey revealed that various analytical methods such as UV-Visible (Vis) spectrophotometry[9,10], HPLC and High performance thin layer chromatography[11] are available for the estimation of RIZ alone or with some other drugs in formulations.

. Structures of Drugs:



Naproxen



Rizatriptan

Fig.1.Structures of Naproxen and Rizatriptan

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 NAP and

RIZ, even though very few methods of individual estimation of above drugs are available. Hence it is proposed to develop new methods for the assay of NAP and RIZ in pharmaceutical dosage forms adapting UV visible spectrophotometry. The objective of the proposed method was to develop simple and accurate methods for the determination of NAP and RIZ simultaneously using absorption ratio method by UV-Spectrophotometry in pharmaceutical dosage forms.

MATERIALS AND METHODS

NAP and RIZ obtained from Aurabindo Laboratories. A commercial sample NAP and RIZ tablets were procured from local market and used within their shelf-life period. Potassium Dihydrogen phosphate from S.D. Fine Chemical Limited, India was of Pharmaceutical or Analytical grade, Sodium hydroxide from Qualigens Fine Chemicals, India was of pharmaceutical or analytical grade. Quantitative estimation was performed on Labindia UV 3000+ and Elico SL 210 double beam UV visible spectrophotometers with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UV Win software was used along with quartz cuvette for the λmax prediction. To develop a suitable and robust absorption ratio method for the determination of NAP and RIZ, different diluents like methanol, 0.1M HCl etc., were tried based on the solubility and functional group present in the compound. Finally Phosphate buffer pH 7.4 was selected due its reproducible results. Absorbance were measured at selected λmax (216.34nm and 230.19nm) based on the overlap spectrum of both drugs. The data were collected and analyzed with software in a computer system.

Preparations

Stock solution of NAP (200µg/ml) was prepared by dissolving 10 mg of NAP in 50 ml of volumetric flask containing 20ml of Phosphate buffer pH 7.4. The solution was sonicated for about 15 minutes and then made up to volume with mobile phase. From the stock solution, 0.15ml was pipetted out and transferred into the 10ml volumetric flask to get 3µg/ml concentration. Same procedure followed for RIZ standard. The final solutions of both standard drugs solutions were scanned and spectra obtained are overlapped. From the overlap spectrum, two wavelengths were selected. Among the two, 230.19nm is a λmax of NAP and 216.34 nm is an isobestic point. Then the absorbance was measured at 216.34nm and 230.19nm and calculated the absorptivity.

Preparation of standard mixture:

From 200µg/ml of NAP and RIZ standard stock solutions, 0.15ml was pipette out individually and mixed in 10ml volumetric flask then it was made up to the mark with phosphate buffer pH 7.4 Absorbance were measured at selected λmax (216.34nm and 230.19nm)

Preparation of tablet mixture:

20 tablets were weighed and powdered. The amount of powder equivalent to 10mg of NAP and 10mg of RIZ were weighed and transferred into the 50ml of volumetric flask containing 10 ml of phosphate buffer pH 7.4. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase. The solution was filtered using 0.25 µ filter paper and vaccum associated filtration unit. From the filtrate, 0.15ml was pipetted out and transferred into the 10ml volumetric flask then made up to the mark with phosphate buffer pH 7.4. The amount of drug present in pharmaceutical formulation was calculated (Beckett & Stenlakke, 2007) through the following formula

$$Cy=(A1/ax1)-Cx$$
$$Cx=((Qm-Qy)/Qx-Qy))(A1/ax1)$$

Where, Cy is a concentration of RIZ in Mixture; Cx is a Concentration of NAP in Mixture; Qx (absorption ratio of drug 1) = ax2/ax1; Qy (absorption ratio of drug 2) = ay2-ay1; Qm (absorption ratio of mixture) = A2/A1; A1 is Absorption at 216.34nm in mixture; A2 is Absorption at 230.19nm in mixture and a is an absorptivity. A typical overlap spectrogram of standard NAP and RIZ and their mixture was shown in figure 2.

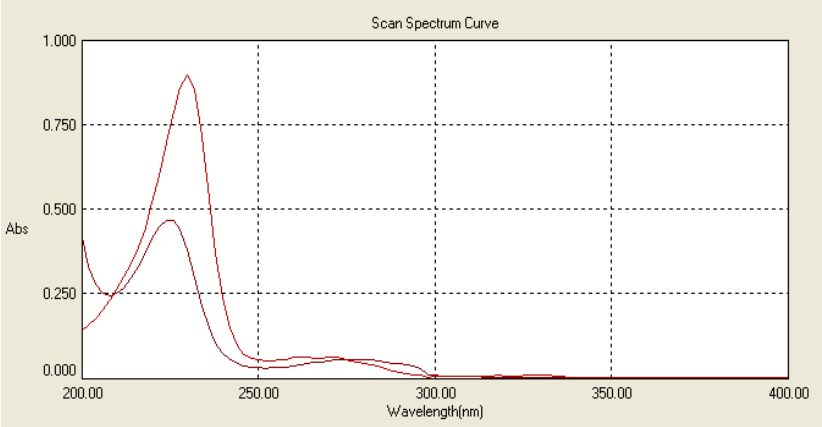


Fig.2.Overlap spectrogram of NAP and RIZ and their mixture

Validation:

The described method has been validated for the assay of NAP and RIZ using following parameters (ICH, 1995). Linearity was studied to find out the relationship of concentration with absorbance. The different concentrations of NAP and RIZ mixtures (2- 4.5 µg/ml of each drug in the mixture) were taken for linearity. The All Solutions were undergone for scanning and measured the absorbance at 216.34nm and 230.19nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL). Alternatively, the corresponding

regression equation was derived. Precision was studied to find out variations in the test methods of mixtures of NAP and RIZ (3µg/ml) on the same day and on different day by using different Instrument (Elico SL210, Lab India UV 3000+) (Ruggedness). The precision of each method was ascertained separately from the absorbance obtained by actual determination of six replicates of a fixed amount of drug (3µg/ml). Precision and ruggedness were done on the same day and the different day respectively and the %RSD was calculated for each. the accuracy of the method was shown by analyzing the model mixtures containing 2.4, 3, 3.6µg/ml of sample solution of mixture of NAP drug 2.4, 3 and 3.6µg/ml of sample solution of mixture of RIZ and along with 1µg/ml of bulk standard solutions of NAP and RIZ. After the measurement, the amount found, amount added for NAP and RIZ and individual recoveries were calculated. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the calibration curve method.

RESULTS AND DISCUSSION

An absorption ratio method procedure was proposed as a suitable method for the analysis of drugs NAP and RIZ in dosage forms. The λmax was found to be 216.34nm and 230.19nm. The regression equation for the method at 216.34nm was found to be y=0.185x-0.0725 (r^2=0.9905), where 0.185 is a slope; -0.0725is an Intercept; r^2 is correlation coefficient (0.9905) and found to be linear over Beer's Range 2- 4.5µg/ml respectively. The regression equation for the method at 230.19nm was found to be y=0.5925x-0.363 (r^2=0.9998), where 0.5925 is a slope; -0.363 is an Intercept; r^2 is correlation coefficient (0.9998) and found to be linear over beer's range 2-4.5µg/ml respectively. The Linearity graph of NAP and RIZ mixtures was shown in figure 3.

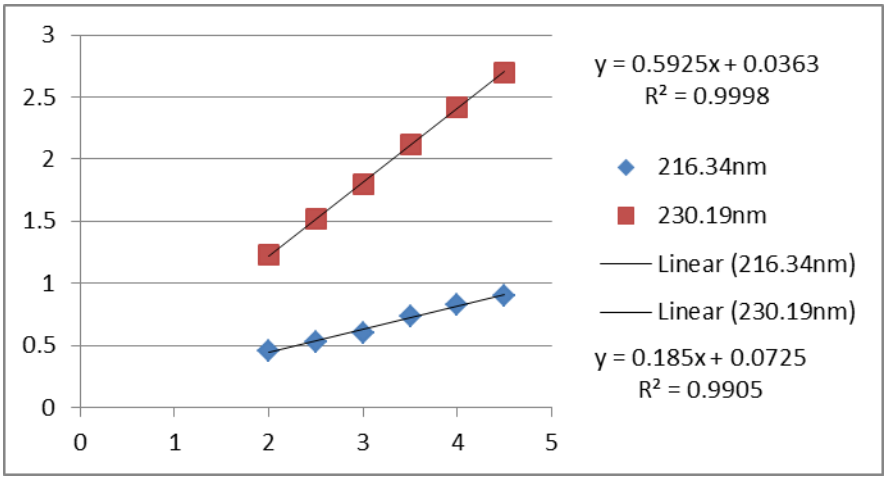


Figure 3: Linearity graph for absorption Ratio method for estimation of NAP and RIZ

The percentage of purity of NAP and RIZ in tablet dosage form was 102.5349% and 101.0021% respectively. The precision of the spectrophotometer system was determined using the %RSD of the absorbance for six replicate injections of the drug. The %RSD was less than 2. Precision data were present in table 1.

Table.1.Data for Precision

	Absorbance at 216.34nm	**Absorbance at 230.19nm**	**Absorption ratio**	**Concentration of drug 1**	**Concentration of drug 2**
Drug 1 standard	0.311	0.664	2.135048	-	-
Drug 2 standard	0.375	0.387	1.032	-	
mixture(m1)	0.628	0.995	1.584395	0.303372	0.302416
mixture(m2)	0.638	1.013	1.587774	0.310088	0.305346
mixture(m3)	0.635	1.008	1.587402	0.308423	0.304117
mixture(m4)	0.631	1.004	1.591125	0.308535	0.300146
mixture(m5)	0.636	1.012	1.591195	0.311019	0.302486
Mean	0.633	1.005	1.587674	0.307605	0.303006
SD	0.004397	0.007616	0.002753	0.002922	0.002253
%RSD	0.694624	0.757788	0.17343	0.950016	0.743693

In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 80%, 100% and 120% of sample solution of NAP and RIZ and along with 10µg/ml of bulk standard solution within the linearity ranges. The mean percentage recoveries were found to be 97.31, 91.15 and 90.27%w/w for 80%, 100% and 120% respectively. Accuracy data were present in Table 2.

Table.2.Data for Accuracy

Accuracy	**Absorbance at 216.34 nm**	**Absorbance at 230.19 nm**	**Absorption ratio**	**Concentration of drug 1**	**Concentration of drug 2**	**% Recovery for drug 1**	**% Recovery for drug 2**
1. (80%)	0.686	1.085	1.581	0.329733	0.332003	96.98	97.64
2. (100%)	0.756	1.195	1.580	0.362755	0.366505	90.68	91.62
3. (120%)	0.8605	1.361	1.581	0.413613	0.416451	90.02	90.53

The percent recoveries values indicate less interference from excipients used in formulation. LOD for NAP and RIZ was found to be 0.595437µg and 0.63478µg

respectively. LOQ for NAP and RIZ was found to be 1.804355µg and 1.923577µg respectively.

CONCLUSION

The presented method was precise, sensitive and accurate. the advantages of proposed method were its simple procedure for sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of naproxen and rizatriptan in pharmaceuticals.

ACKNOWLEDGEMENTS

The authors wish to express their deep sense of gratitude to the Management of Aditya Institute of Pharmaceutical Sciences and Research, Surampalem for carrying out the work and providing necessary facilities.

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