DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NAPROXEN AND RANITIDINEHYDROCHLORIDE

TASNUVA HAQUE[1*], MD. MESBAH UDDIN TALUKDER[2], SUSMITA LAILA[3] AND KANIJ FATEMA[1]

[1]*Department of Pharmacy, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217, Bangladesh*
[2]*Department of Pharmacy, The University of Asia Pacific, Dhaka-1209. Bangladesh*
[3]*Product Development Department, Eskayef Bangladesh Limited, Tongi, Gazipur, Bangladesh*

ABSTRACT

A simple, specific and accurate reverse phase liquid chromatographic method has been developed for the simultaneous determination of naproxen and ranitidine HCl. Both the drugs are official with British Pharmacopoeia 2007, but do not involve simultaneous determination of naproxen and ranitidine HCl. The separation was carried out using 4.6×250 mm Symmetry Shield ™ RP 18 with a particle diameter of 5 µm and mobile phase containing 0.1M orthophosphoric acid: methanol (35:65, pH 3.1) in isocratic mode. The flow rate was 1.00 ml/min and effluent was monitored at 240 nm. The retention times (average) of ranitidine HCl and naproxen were 2.36 min and 12.39 min, respectively. The linearity for naproxen and ranitidine HCl was in the range of 5-35 µg/ ml and 1.5-12 µg/ ml, respectively. The potencies of naproxen and ranitidine HCl were found 99.40 % and 99.48 %, respectively. The proposed method was validated and successfully applied to the estimation of naproxen and ranitidine HCl in newly formulated combined tablet and in plasma.

Keywords: Development and validation of a method, naproxen, ranitidine HCl, RP-HPLC.

INTRODUCTION

Naproxen (NAP) is chemically 2-Naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-Methoxy-α-methyl-2-naphthaleneacetic acid (United States Pharmacopoeia 2003). Ranitidine HCl (RAN) is chemically *N*-[2-[[[5-[(Dimethylamino)methyl]furan-2-yl]methyl] sulphanyl]-ethyl]-*N¢*-methyl-2-nitroethene-1,1-diamine hydrochloride (Indian Pharmacopoeia 1996). Naproxen is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both the COX-1 and COX-2 enzymes. Like other NSAIDs, NAP is capable of producing disturbances in the gastrointestinal tract (MIMS Bangladesh 2002). RAN is a H_2-receptor antagonist. It is a classification of drugs used to block the action of histamine on parietal cells in the stomach, decreasing acid production by these cells. These drugs are used in the treatment of dyspepsia (MIMS Bangladesh 2002).

NAP and RAN combination is widely prescribed by the physicians in order to avoid NSAID induced ulcers (Sharma and Ahuja, 2003). This paper describes a simple reverse phase high performance liquid chromatographic method (RP-HPLC) method for estimation of naproxen and ranitidine in a new combined formulation and in the plasma of patients.

**Corresponding author:* e-mail: shoume_du@yahoo.com

EXPERIMENTAL

Materials

Naproxen and ranitidine HCl were gift samples from Eskayef Bangladesh Ltd. HPLC grade methanol was obtained from Merck (Germany). Distilled deionized water was prepared by Aquatron deionizing water system. Hydrochloric acid (37%) and tribasic sodium phosphate were purchased from Merck (Germany) and orthophosphoric acid from Sigma-Aldrich (Switzerland). Tablets of naproxen and ranitidine were purchased from the local market. Ultrapure water system (Sartorius, Germany), vacuum pump (Alltech, Germany), filter tips-0.22 µm (Sartorius, Germany) were also used. Waters 1525 high performance liquid chromatographic system (Ireland) was used for this experiment. The HPLC system equipped with Waters 1525 binary HPLC pump, Waters 2487 dual λ absorbance detector, in-line degasser AF and manual injector, which was controlled by Breeze software (version 3.30).

Buffer preparation

In a 1 litre of volumetric flask, 750 ml of 0.1 N HCl was taken and 180 ml of previously prepared 0.3 M tribasic sodium phosphate was added and rest amount of water was added up to 1000 ml. The pH of the buffer solution was 7.4.

Preparation of mobile phase and chromatographic condition

To prepare 0.1 M orthophosphoric acid (OPA), 400 ml HPLC grade deionized water was taken in 500 ml of

volumetric flask. Then, 0.34 ml of orthophosphoric acid (pH =2.78) was added to it and the final volume was made up to 500 ml with water. The final pH of methanol and 0.1 M OPA was 3.03. If needed, pH (3±0.05) adjustment was made with diluted phosphoric acid or with 0.1 N NaOH. The total content was mixed, filtered with 0.22 µm nylon filter and degassed properly. The λ_{max} for UV detection was set at 240 nm. AUFS (absorbance unit full scale) was kept at 0.002. The injection volume was 20 µl for all standards and samples. Before every injection, every sample was filtered through 0.22 µm filter tips.

Preparation of stock and standard solutions of NAP and RAN

50 mg of naproxen and 17.12 mg of ranitidine hydrochloride (equivalent to 15 mg of ranitidine) were weighed accurately and transferred to a 100 ml volumetric flask, pH 7.4 phosphate buffer was added to dissolve the drug and the resultant solution was diluted up to the mark. A combined solution of 10, 15, 20, 25, 30, 35 µg/ ml concentrations of naproxen and 3, 4.5, 6, 7.5, 9, 11 µg/ ml concentrations of ranitidine HCl were prepared by diluting with a mixture of methanol and 0.1 M OPA (in 65: 35 ratio), which was used as diluent.

Sample preparation

Twenty tablets of marketed brands of NAP and RAN were weighed separately. Their average weights were determined. Powder of tablets equivalent to 25 mg of RAN and 35 mg of NAP were weighed and taken in a 50 ml volumetric flask, 30 ml of water and 10 ml of methanol were added to it and it was sonicated for 30 min to dissolve it. The solution was filtered through Whatmaan filter paper (0.45µm) and made 50 ml with water. Further dilution was carried out by using diluent.

Mobile phase selection

The mobile phase was chosen after several trials with methanol and 0.1 M OPA in various proportions like 15:85, 25:75, 30:70 and 35:65 at different pH values. A mobile phase consisting methanol and 0.1 M OPA buffer (65:35) was selected to achieve maximum separation and sensitivity.

Flow rate selection

Different flow rates in between 0.50 to 1.20 ml /min were studied. A flow rate of 1.0 ml /min gave an optimal signal to noise ratio with a reasonable separation time.

Wavelength selection

NAP and RAN were scanned separately in the UV region. Then, the spectrum of combined product either 1:1 or 1:1.5 of NAP: RAN was taken. While performing HPLC individual active and combined standard was measured at 228, 240, 278, 314, and 332 nm. Finally, all the analysis were done at 240 nm as at this wavelength, both drugs absorb light better and two peaks could be distinguished properly.

Peak identification

At first a blank sample or diluent was injected three times to observe the peak of impurities of the blank. A peak (at retention time of 3.12 min) along with a negative peak was found in the blank, shown in fig. 1. Then single NAP and RAN containing samples were injected. Single NAP at 12.50, 6.25 and 5.00 µg/ ml concentrations gave peak areas of 1984893, 1159918 and 875846 with retention times of 12.46, 11.72 and 12.62 min, respectively at width of 50, threshold of 500 and minimum area of 100000. Single RAN at concentrations of 12.50, 10 and 6.25 µg/ ml, gave peak area of 586139, 612688 and 354859 with retention times of 2.29, 2.20 and 2.21 min, respectively at the same width, threshold and minimum area. Then combined solution of NAP and RAN with concentration 6.25 µg/ ml (both for two) gave the peak area of 973171 (for NAP) and 328143 (for RAN) which produced two peaks separately with the same retention times 12.39 min (for NAP) and 2.36 min (for RAN) as found and identified by single compound of these two (fig. 2).

RESULTS AND DISCUSSION

Representative chromatograms are illustrated in figs. 1 and 2. From the peak identification results of blank sample (diluent), single NAP, single RAN and combined solution of NAP and RAN, it is shown that the developed analytical method was specific for the analysis of NAP and RAN from standard mixture as well as tablet formulation (fig. 2). Table 1 represents the relative standard deviation (RSD %) values of the slopes and R^2 and table 2 represents system suitability (Kothekar *et al*., 2007) of this method. Excellent linearity was obtained for NAP between 10-35 µg/ml with R^2 values of 0.997, 0.998, 0.998 and for RAN linearity was observed between 1.5-12 µg/ml with R^2 0.999, 0.999 and 0.996 (Razib *et al*., 2006). The precision (Kothekar *et al*., 2007) of the method {intraday and interday (5 d) variation of replicate determination} was checked by injecting one concentration of combined NAP (10 µg/ml) and RAN (3 µg/ml) for five times. The precision of the method, expressed as the RSD % of intraday and interday variation is given in table 1. The sensitivity (Sathe and Bari, 2007) of measurement of NAP and RAN was estimated in terms of the limit of quantification (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOQ and LOD were calculated by use of the equations: LOD = 3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs, taken as a measure of noise and B is the slope of the corresponding calibration plot. LOQ and LOD for NAP were 2.57 and 0.77 µg/ml respectively. For RAN

Table 1: Linearity, precision and sensitivity (values were shown mean ± SD) of this proposed method.

Parameters	NAP	RAN
Regression equation (*Y)		
R^2		
mean±SD	0.997±0.0005	0.998±0.0013
**RSD, %	0.05	0.13
Slope		
mean±SD	52625±200.92	51521.67±370.18
**RSD, %	1.61	0.88
Precision		
Intraday (n = 5)		
mean±SD	1724534±30844.36	144926±2599.21
**RSD, %	1.79	1.79
Interday (n =5)		
mean±SD	1742534.4±26476.82	144619.79±2537.71
**RSD, %	1.52	1.75
LOD	0.77	0.31
LOQ	2.57	1.04
Reproducibility (**RSD, n =3)	0.37-1.65	0.51-1.63

Y** = mX+C; where Y = peak area, m = slope, X = concentration (µg/ml) and C = intercept. ****RSD (%) = Relative standard deviation = (Standard deviation X 100)/mean**, R^2** = Correlation coefficient, **LOD** = Limit of detection and **LOQ** = Limit of quantification.

Table 2: System suitability of this proposed method.

Compound	*R	*k' (mean±SD)	*n (mean±SD)	*Tailing Factor (mean±SD)	*RT, min (mean±SD)	RT (%RSD)
NAP	31.91±1.87	12.82±0.15	11988.34±501.91	10.31±0.14	12.39±0.06	1.34
RAN	-	1.36±0.07	5481.80±1173.04	68.23±4.36	2.36±0.17	2.58

***** Data obtained by eight determinations. **R =** Resolution**, k/** = Capacity factor, **n** = Theoretical plates, **RT** =Retention time and **SD** =Standard deviation.

Table 3: Determination of drug content present in marketed brands of NAP and RAN by this proposed method.

Compound	Measured amount (mean±SD) (mg) in 50 ml	Added amount (mg) in 50 ml	*% potency
NAP	34.79±0.02	35	99.40
RAN	24.87±0.31	25	99.48

*% Potency = Measured amount × 100/ Claimed amount.

they were 1.04 and 0.31 µg/ml respectively. Results are shown in table 1.

A standard working solution containing combined NAP and RAN, producing final concentration of 10, 12.5 and 20 µg/ml of NAP and 3, 3.75 and 6 µg/ml of RAN were prepared. The prepared mixture of standard solution was injected three times as a test sample. From the respective area counts, the concentrations of NAP and RAN (reproducibility) were calculated using the detector response (table 1). The potency was determined for two different marketed brands of NAP and RAN tablets (table 3). The potencies were found 99.48 % for RAN and 99.40 % for NAP respectively.

Thus in the proposed study, RP-HPLC method has been developed for simultaneous determination of NAP and RAN. The method was validated and found to be simple, sensitive, accurate and precise. This study has acceptable correlation coefficient and RSD (%) which make it versatile and valuable. It was successfully applied for determination of drugs in their newly formulated pharmaceutical formulations and plasma drug concentration monitoring hence method can be used for routine quality control analysis of NAP and RAN.

CONCLUSION

The validation study shows that the developed method is accurate, rapid, precise, reproducible and inexpensive

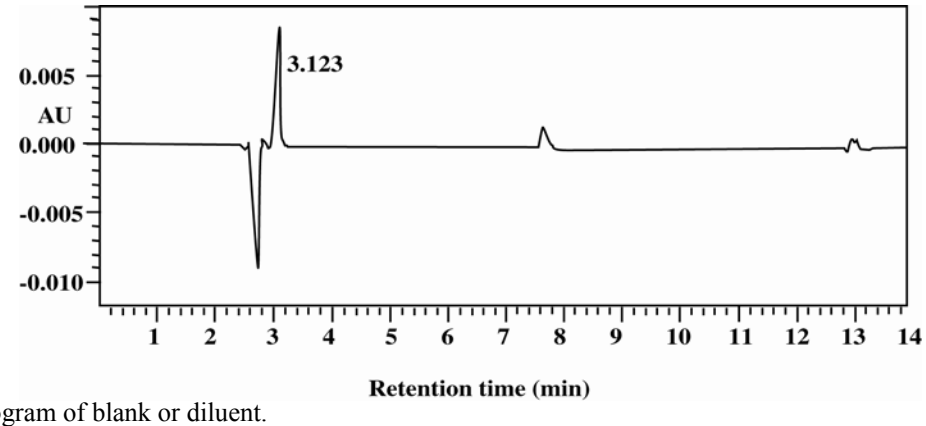

Fig. 1: Chromatogram of blank or diluent.

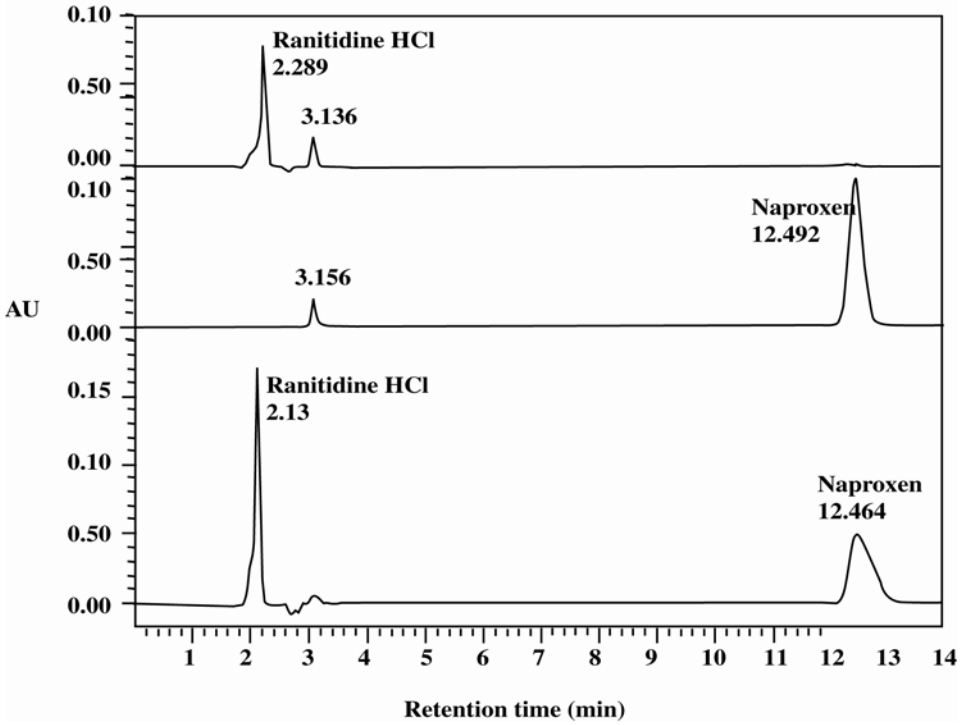

Fig. 2: Chromatogram of single and combined NAP and RAN.

with acceptable correlation co-efficient, RSD (%) and standard deviations which make it versatile and valuable for simultaneous determination of NAP and RAN in plasma and pharmaceutical dosage form; especially new formulated tablet. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed method is simple and do not involve laborious time-consuming sample preparation. So this RP-HPLC method can be used in the quality control department.

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