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Research Article

A VALIDATED RP-UPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF LANSOPRAZOLE AND NAPROXEN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: A simple and precise RP-UPLC method development and validation for simultaneous estimation of lansoprazole (LAN) and naproxen (NAP) in bulk and tablet Dosage Form.
Method: Simple, accurate and cost efficient RP-UPLC method has been developed and validated for the simultaneous estimation of lansoprazole and naproxen in bulk and tablet dosage form. The optimum conditions for the analysis of the drug were established.
Results: The retention time (RT) was found to be 3.905 and 2.650 min for lansoprazole (LAN) and naproxen (NAP), for analysis the maximum wavelength (λ_{max}) was found 284nm for simultaneous estimation of LAN and NAP. The method was linear and found to be range between 5-30 µg/ml (r^2= 0.998) and 10-35 µg/ml (r^2= 0.999) for LAN and NAP respectively. The value of limit of detection and limit of quantification was 0.8397 and 2.979 µg/ml for LAN and 0.4678 and 1.5593 µg/ml for NAP, the recovery was found between 80,100 and 120% and RSD less than 2 for LAN and NAP. The method was satisfactorily validated as per the ICH guideline.
Conclusion: The proposed methods were simple, sensitive, precise, accurate, reproducible, quick and useful for routine quality control. This study shows that the proposed RP-UPLC method is useful for the routine determination of LAN and NAP in its bulk and tablet dosage form.

Keywords: Lansoprazole, Naproxen, RP-UPLC, Method validation and Tablets

INTRODUCTION

Lansoprazole {2-[[[3- methyl-4-(2,2,2- trifluoroethoxy)-2- pyridinyl] methyl] sulfinyl]1 Hbenzimidazole} as shown in (**Figure 1**) is a substituted benzimidazole with anti-secretory and anti ulceractivities . It is effective in treating various peptic diseases, especially those resistant to treatment with histamine H2 receptor antagonists; therefore it is successfully used for the treatment of duodenal ulcer, gastric ulcer, reflux Oesophagitis and Zollinger–Ellison syndrome [1-5]. Naproxen [6] is chemically 2-Naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-Methoxy-α-methyl-2-naphthaleneacetic acid as shown in (**Figure 1**). 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, Naproxen is capable of producing disturbances in the gastrointestinal tract. The combination of both is used in the treatment of rheumatoid arthiritis and oesteoarthiritis [7]. Several chromatographic methods have been reported for determination of NS in raw material, tablets, plasma, urine, intestinal perfusion samples and pharmaceutical [8-23]. So an attempt was taken to develop and validate an economic, rapid RP-UPLC method for the determination of naproxen and lansoprazole in combined tablet dosage form.

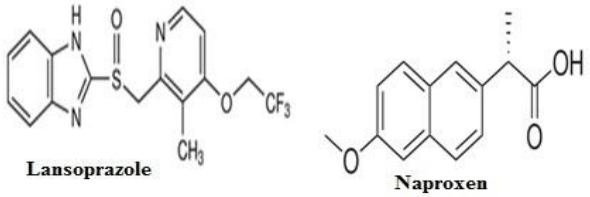

Figure 1- Chemical structure of LAN & NAP

MATERIALS AND METHODS

Drugs and chemicals

Lansoprazole was procured from Lupin pharmaceuticals limited, India. Naproxen was procured from Glenmark Pharmaceuticalc Limited, India. Methanol was of analytical grade. All other chemicals used were of analytical grade. The pharmaceutical dosage form used in this study was junior lanzol-30 labelled to contain 30mg LAN and naprosyn labelled to contain 250mg of NAP.

Apparatus

Shimadju LC ETAP UPLC system

The LC system consists of pump (Shimadzu LC-260 AD) with universal loop injector (Helminton syringe) of injection capacity 20 µL. Detector consists of photodiode array detector (PDA) for separation column used was phenomenex luna C18 (5 µm x 25 cm x 4.6 mm). The equipment was controlled by a PC work station equipped with software LC Solution software. The volume capacity of the reservoir was greater than 500 ml. The mobile phase velocity was within 1-2 ml/min.

Preparation of mobile phase

The mobile phase was prepared by mixing 80ml methanol with 20ml water. Further the method was optimized by changing the concentration of mobile phase and the results are reported. From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasonable run time and other parameters by use of 80:20 (v/v) ratio mixture of methanol:water mobile phase. The flow rate was determined by testing the effect of different flow rate on the peak area and resolution, flow rate of 1 ml/min found optimum. The mobile phase was sonicated for 15 min and then it was filtered through a 0.45 membrane filter paper.

Preparation of standard stock solutions

The stock solutions 1000µg/ml of LAN was prepared by accurately weighing 50mg drug separately in methanol in 50ml volumetric flask. Pipette out 10 ml of the solution from 1000 µg/ml solution and transfer to 50 ml volumetric flask and volume was make up with methanol and final dilution 100 µg/ml solution of LAN was prepared. The stock solution of NAP was prepared similarly.

Preparation of standard solutions for linearity study

From the standard stock solutions of 1000 µg/ml different dilutions were prepared for each drug having concentration. Then 20 µL of these solutions were injected into the LC system with the help of Hamilton syringe. Then the chromatograms were recorded at 284 nm, from the chromatogram it was cleared that LAN retention time 3.905 min and NAP at 2.650 min from which their area was noted and calibration curve was plotted between the peak area against their respective concentrations. From the calibration curve it was cleared that LAN 5-30 µg/ml and NAP has linearity range between 10-35 µg/ml respectively (**Figure 2,3,4 & 5**).

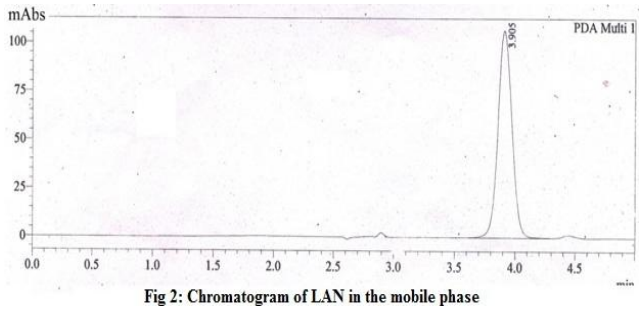

Fig 2: Chromatogram of LAN in the mobile phase

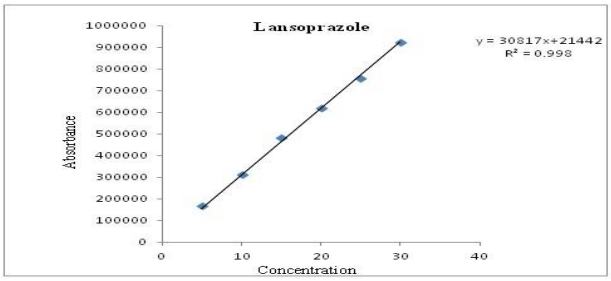

Fig 3: Calibration curve of LAN in the mobile phase

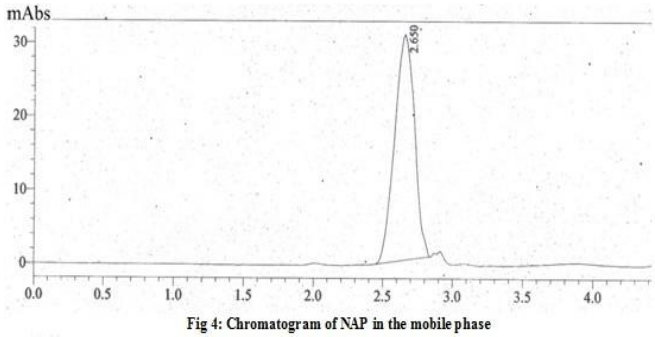

Fig 4: Chromatogram of NAP in the mobile phase

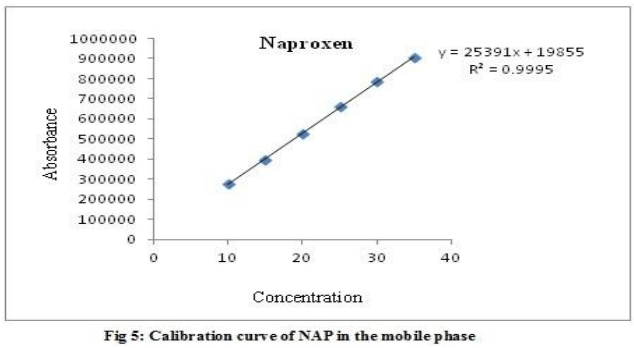

Fig 5: Calibration curve of NAP in the mobile phase

Analysis of mixed standard

From the standard stock solutions of 1000 µg/ml of the drugs different mixed standard solutions of known concentration were prepared and their 20µL solutions were injected into the LC system with the help of hamilton syringe and their chromatograms were recorded after that the concentration of individual drugs were calculated by extrapolating the value of area from their calibration curves respectively.

Analysis of Commercial formulation

20 tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of LAN (respective quantity of NAP) was taken in 50ml volumetric flask and dissolved in 30ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 100ml of volumetric flask through a 0.4 µm membrane filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml solvent. Six replicate of sample solutions were prepared of required concentrations of the three drugs. Then 20uL of each replicate were injected into the system and their chromatograms were recorded. From the chromatograms it was observed that LAN and NAP were eluted at 3.905 and 2.650 min respectively (**Figure 6**).

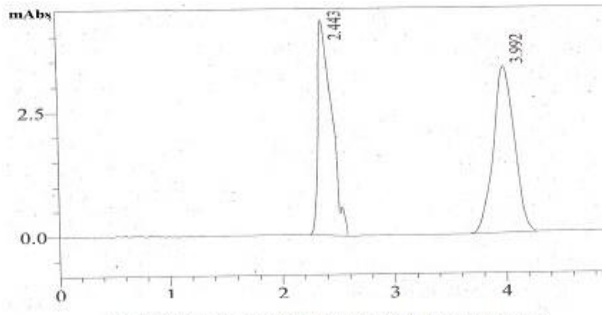

Fig 6: Chromatogram of LAN and NAP in Sample Solution

METHOD VALIDATION

Accuracy

Accuracy was confirmed by doing recovery study as per ICH norms, where to a preanalysed sample solution standard solutions of drugs were added equivalent to 80,100 and 120% of its drug content. The percentage of drug recoveries for LAN in 80,100 and 120% were 96, 97 and 99 and for NAP were 82, 88 and 88 respectively. As all the statistical results were within the range of acceptance i.e. % COV< 2.0 and S.D. < 1.0, hence the method was accurate for simultaneous quantitative estimation of these drugs.

Linearity and range

The six point calibration curve that were constructed were linear over the concentration range between 5-30 µg/ ml for LAN and 10-35 µg/ ml for NAP respectively.

PRECISION

The intra and inter-day precision was calculated by assay of the sample solution ones the same day and on different days at different time intervals respectively (n=3). Results obtained are given in table and for LAN and NAP respectively.

Limit of detection (LOD) and Limit of quantization (LOQ)

The LOD and LOQ of Lansoprazole and Naproxen were calculated by mathematical equation.

DL = SD x 3/slope……..(1)
QL = SD x 10/slope……..(2)

The LOD of LAN and NAP were found to be 0.8937µg/ml and 0.4678µg/ml and the LOQ of LAN and NAP were found to be 2.979µg /ml and 1.559µg/ml (Table.)

RESULTS AND DISCUSSION

The developed RP-UPLC method for estimation of Lansoprazole and Naproxen in tablet dosage form utilizing C_{18} column and water and methanol in the ratio of 20:80 as mobile phase. Detection of eluent was carried out using UV detector at 284nm. The method was developed. The method was analyzed marketed combination shown in **Table 1**. The run time per sample is just 10 min and linearity was performed successfully **Table 2**. Mean retention time of Lansoprazole and Naproxen was found to be 3.905 min and 2.650.

The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown in **Table 3**. Since none of the methods is reported for estimation of Lansoprazole and Naproxen in tablet dosage form, this developed method can be used for routine analysis of two components in formulation.

Table 1: Analysis of commercial preparation

Formulation	Drug	Label claim(mg)	%Label claim (Mean±SD)
Tablet	NAP	250	98%±0.005603
	LAN	30	102.23%±0.010460

Table 2: Linearity study for Lansoprazole and Naproxen

S.NO.	Parameter (units)	Lansoprazole	Naproxen
i.	Linearity range (µg/ml)	5-30 µg/ml	10-35µg/ml
ii.	Correlation coefficient (r^2)	0.998	0.999
iii.	Slope	9181.008	25391
iv.	Intercept		19855

Table 3: Validation of Lansoprazole and Naproxen

S.NO.	Parameter (units)	LAN	NAP
1.	Accuracy		
	(80%)	82±0.0101	96±0.0119
	(100%)	82±0.0051	97±0.0053
	(120%)	88±0.0001	99±0.0027
2.	Interday precision		
	(1st day)	84.69±0.0031	86.55±0.0021
	(2nd day)	83.10±0.0003	89.87±0.0086
	(3rd day)	83.55±0.0035	87.37±0.0575
3.	Intraday precision		
	1st hrs	86.34±0.0069	87.96±0.0046
	2nd hrs	83.72±0.0015	87.52±0.0016
	3rd hrs	84.01±0.0069	86.65±0.0005
4.	LOD	0.8937	0.4678
5.	LOQ	2.979	1.5593

CONCLUSION

Thus it can be concluded that Lansoprazole and Naproxen can be quantified simultaneously by the proposed RP-UPLC method using an isocratic mobile phase consisting of water:methanol (20: 80) using a UV detector at 284 nm. The proposed method is simple, sensitive, rapid, accurate and precise. It can be applied successfully for the estimation of Lansoprazole and Naproxen in bulk and its pharmaceutical formulations.

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