IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF RP-HPLC METHODS FOR SIMULTANEOUS ESTIMATION OF NIMESULIDE AND PANTOPRAZOLE IN SYNTHETIC MIXTURE

Het Patel [1], Prof. Priyanka Malani [2],

Dr. Umesh Upadhyay[3]

Student[1], Professor [2], Principal[3]

Sigma Institute of Pharmacy (Sigma University), Bakrol, Vadodara, Gujarat,390019, India.

ABSTRACT: It is simple, sensitive and accurate RP-HPLC method was developed for simultaneous estimation for Nimesulide and Pantoprazole. A reversed–phase high-performance liquid chromatography method is developed and validated for the determination of both drugs. With the help of RP-HPLC it gives us to good resolution and better separation for the both drugs. The separation was conducted by using Shimpack C18 column (250 mm×4.6 mm×5 µm) with mobile phase consisting ACN: Methanol: Water in ratio of (25:25:50%v/v/v). The mobile phase was delivered at the flow rate of 1.0 ml/min. The eluent was monitored at wavelength 270nm and found shorter retention time of Drug and peak shape was proper, resolution good of Pantoprazole and Nimesulide were found to be 9.69 min and 11.71 min respectively. The method was validated for linearity, accuracy, precision, system suitability, and stability. The method was found to be linear over the concentration range for PAN was 2-10 µg/ml and for NIM was 10-50 µg/ml with coefficient R^2 for PAN 0.9978 and NIM 0.9979. Therefore, proposed method can be successfully used for routine analysis of Nimesulide and Pantoprazole in bulk as well as synthetic mixture.

[KEYWORDS: Nimesulide (NIM), Pantoprazole (PAN), Reversed-Phase-High performance Liquid Chromatography (RP-HPLC).]

INTRODUCTION: Nimesulide is a nonsteroidal anti-inflammatory medicine (NSAID) with capabilities for treating pain and lowering temperature. Its approved uses include the relief of severe pain, the symptomatic management of osteoarthritis, and the treatment of primary dysmenorrhea in adolescents and adults over the age of 12. A nonsteroidal anti-inflammatory medication (NSAID), nimesulide primarily functions as a relatively selective cyclooxygenase-2 inhibitor. Pantoprazole was first studied in 1985, and in 1994 it was approved for medicinal usage in Germany. It is available as a generic drug. It's sold under the brand name Protonix, among others, is a proton pump inhibitor used for the treatment of stomach ulcer short-term treatment of erosive esophagitis due to gastroesophageal reflux diseases (GERD), maintenance of healing of erosive esophagitis, and pathological hypersecretory conditions including Zollinger Ellison syndrome. he investigation medicinal drug was once designed to deal with OA pain alongside with dyspepsia, frequent adverse effect of stand-alone osteoarticular pain (musculoskeletal) pain drugs. The study outcomes suggest that pantoprazole may also have really useful effects on gastrointestinal function. The need of validation of the analytical method development and validation emerged due to international competition, new development, maintaining the standard of products in high commercial & market value and ethical reasons. High Performance Liquid Chromatography is the most common analytical separation tool, components by distributing between mobile phase and stationary phase.

STRUCTURAL FORMULA:

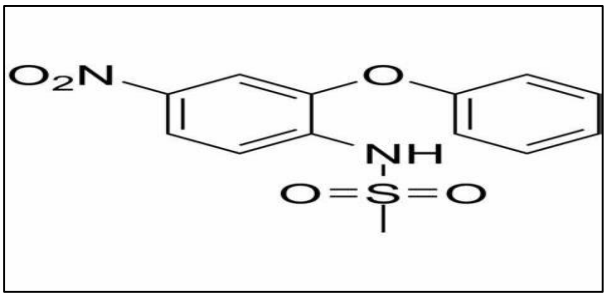

Figure 1. Structure formula of NIM

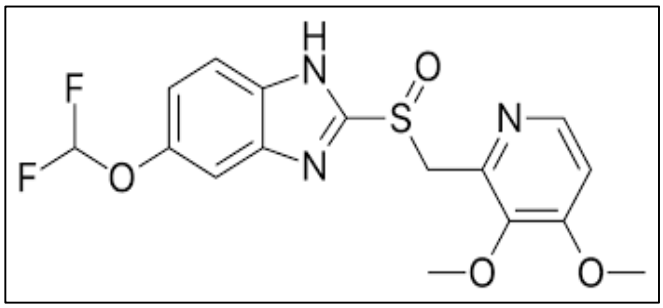

Figure 2. Structure formula of PAN

MATERIALS AND METHODS: Sample of Nimesulide and Pantoprazole procured from Zota Healthcare Pvt Ltd, Surat, Gujarat.

INSTRUMENT: Instrument use for the development and validation for NIM and PAN are RP-HPLC (LC-20 AD from Shimadzu Lab).

SELECTION OF DILUENT: Based on solubility, Nimesulide (NIM) and Pantoprazole (PAN) was soluble in methanol. Hence, methanol was selected as diluent.

PREPARATION OF STOCK SOLUTION: Accurately weighed and transferred about 50 mg of Nimesulide (NIM) and 10mg of Pantoprazole (PAN) in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with diluent. Concentration of Nimesulide (NIM) is 500 µg/ml and Pantoprazole (PAN) 100 µg/ml. Further diluted 5 ml of above solution to 50 ml volumetric flask and volume was make up to the mark with diluent. Concentration of Nimesulide (NIM) is 50 µg/ml and Pantoprazole (PAN) 10 µg/ml. The optimum wavelength was selected for the estimation was 270 nm where gives good absorbance.

Preparation of Pantoprazole (PAN) solution: Pipette out 0.2, 0.4, 0.6, 0.8 and 0.10 ml of Pantoprazole (PAN) 100 µg/ml in 10 ml of volumetric flask. Further diluted above solution to 10 ml volumetric flask and volume was make up to the mark with diluent to get 2, 4, 6, 8 and 10 µg/ml concentration of Pantoprazole (PAN).

Preparation of Nimesulide (NIM) solution: Pipette out 1, 2, 3, 4, 5 and 6 ml of Nimesulide (NIM) 500 µg/ml in 10 ml of volumetric flask. Further diluted above solution to 10 ml volumetric flask and volume was make up to the mark with diluent to get 10, 20, 30, 40, 50 and 60 µg/ml concentration of Nimesulide (NIM).

SELECTION OF MOBILE PHASE: The water, buffer, pH of the buffer, organic solvent, and buffer-to-solvent ratio were all factors in the mobile phase selection process. The HPLC technique selection is influenced by the sample's nature, physicochemical properties, molecular weight, and solubility. pH management necessitates the use of a buffer. The pH of the acidic component is kept low, while the pH of the base is kept high. Separation, peak purity, tailing factor, theoretical plate, and other parameters were used to optimize the mobile phase for HPLC system. Various mobile phases in various compositions and pH levels were tried to achieve a sharp peak of Nimesulide (NIM) and Pantoprazole (PAN).

SELECTION OF WAVELENGTH: An ideal wavelength is the one that gives Maximum response for the drugs that was to be detected. For High Performance Liquid Chromatography system with PDA detector give 270 nm wavelength where both Nimesulide (NIM) and Pantoprazole (PAN) show good absorbance.

PREPARATION OF MOBILE PHASE: Acetonitrile, Methanol, and water were filled in different mobile phase reservoir after filter and sonicate to degas the mixture. Mobile phase Acetonitrile: Methanol: water in the volume ratio 25:25:50 v/v/v were used.

SELECTION OF COLUMN: Nimesulide (NIM) and Pantoprazole (PAN) are polar in nature. So, C18 analytical column were selected for HPLC method. The column was used Shimpack ODS C18 column (250 mm × 4.6 mm, 5 µm) was used for the development of the method.

RESULT AND DISCUSSION:

OPTIMIZATION OF RP-HPLC CHROMATOGRAPHIC CONDITION:

The method development, top priority was given for the complete separation of drugs by optimization of mobile phase. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic solvent and buffer used in the mobile phase and composition of the mobile phase on trial error basis by varying one parameter and keeping all other conditions constant. Before beginning the method development, we need to review what is known about the sample; also, the goal of the analysis should be defined at this point and considerations must be given regarding how many samples will be analyzed and what HPLC equipment are available.The nature of the sample (e.g., whether it is hydrophilic or hydrophobic, whether it contains protolytic functions etc.) determines the best approach to HPLC method development. The objective is to develop HPLC method for determination of Pantoprazole (PAN) and Nimesulide (NIM) in bulk and synthetic mixture.

METHOD VALIDATION:

LINEARITY AND RANGE: The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity and range of the method was determined by plotting a calibration curve over the concentration range of 2 - 10 µg/ ml for PAN and 10- 50 µg / ml for NIM, respectively. The calibration curve was constructed by plotting peak areas versus concentrations of 2 - 10 µg/ ml for PAN and 10- 50 µg / ml for NIM, respectively shown in figure 3 and figure 4. Linearity Data shown Table 01. The regression equation was found to be y = 24108x - 9344.3 and correlation coefficient was found to be 0.9978 for PAN. The regression equation was found to be y = 6721.8x + 977.5 and correlation coefficient was found to be 0.9979 for NIM. Each response was the average of three determinations. The Statistical analysis data of calibration curve intercept, slope, and regression equation are shown in Table 02. The linearity of chromatogram over the concentration range of 2 - 10 µg/ ml for PAN and 10- 50 µg / ml for NIM, respectively shown in chromatogram 6.22

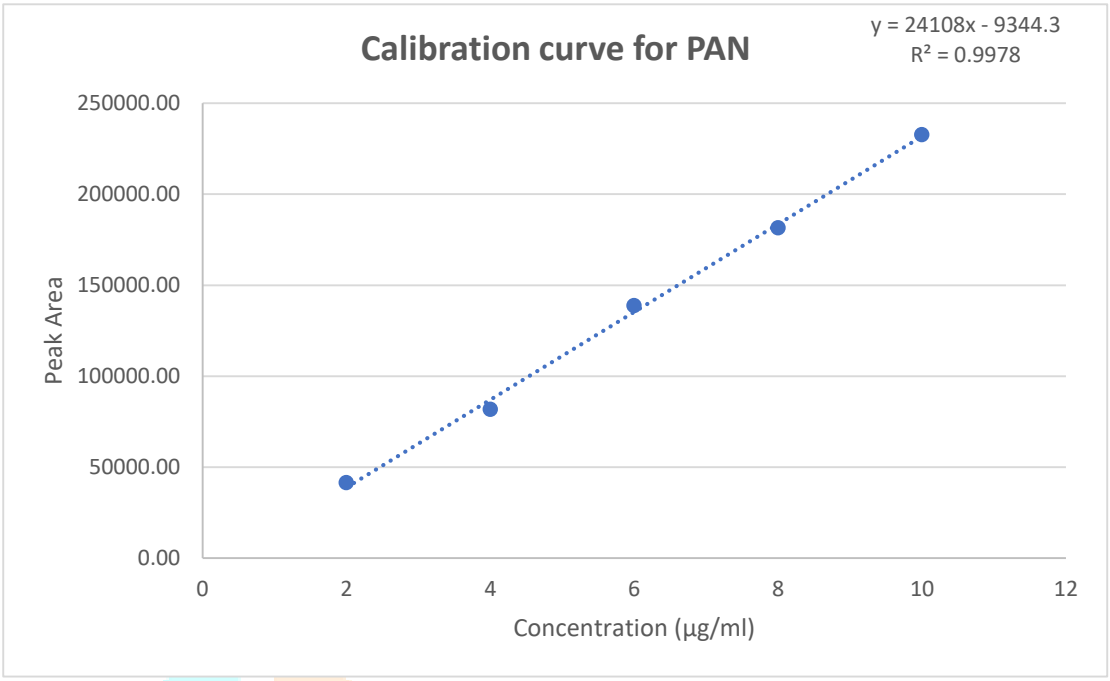

Figure 3. Calibration curve of PAN standard

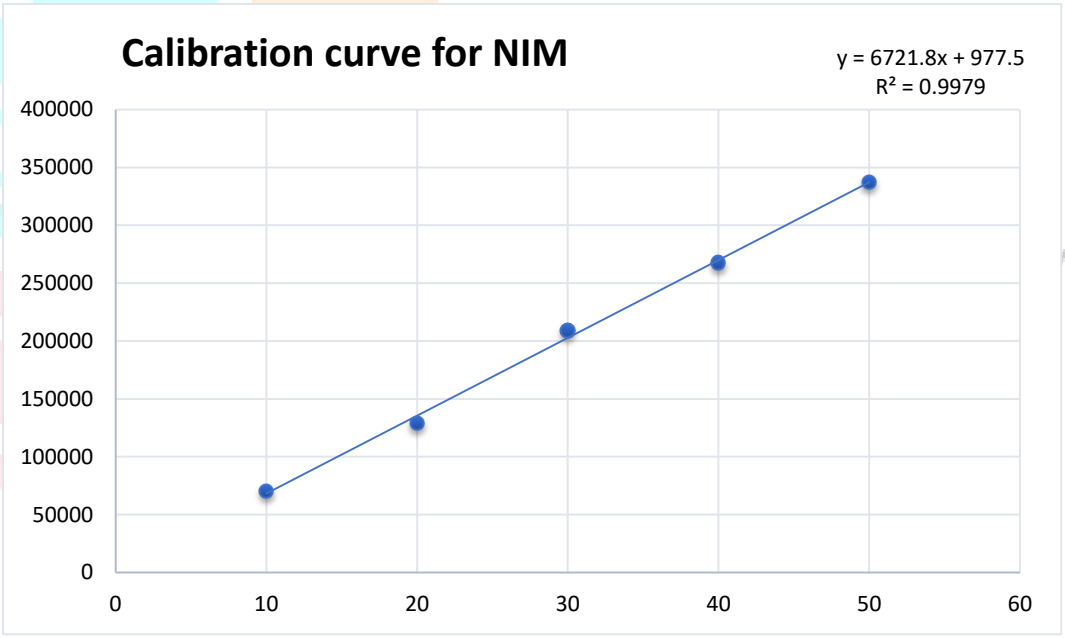

Figure 4. Calibration curve of NIM standard

Table 01. Linearity Data

Drug	Conc.	Peak Area	% RSD	Drug	Conc.	Peak Area	% RSD
PAN	2	41515.67 ± 725.96	1.75	NIM	10	70338 ± 567.07	0.81
	4	81899.17 ± 826.82	1.01		20	129055 ± 953.05	0.74
	6	138766 ± 878.34	0.63		30	209118 ± 3128.75	1.43
	8	181573 ± 1519.31	0.84		40	267388 ± 2631.72	0.99
	10	232756 ± 1780.36	0.76		50	337263 ± 5273.82	1.57

Table 02. Statistical analysis data of calibration curve

Parameters	PAN	NIM
Linear Range	2 – 10 µg/ml	10 – 50 µg/ml
Regression Equation	y = 24108x - 9344.3	y = 6721.8x + 977.5
Regression Coefficient (r^2)	0.9978	0.9979
Standard deviation of slope	143.01	562.51
Standard deviation of intercept	809.68	2654.33
LOD (µg/ml)	0.11	0.26
LOQ (µg/ml)	0.33	0.79

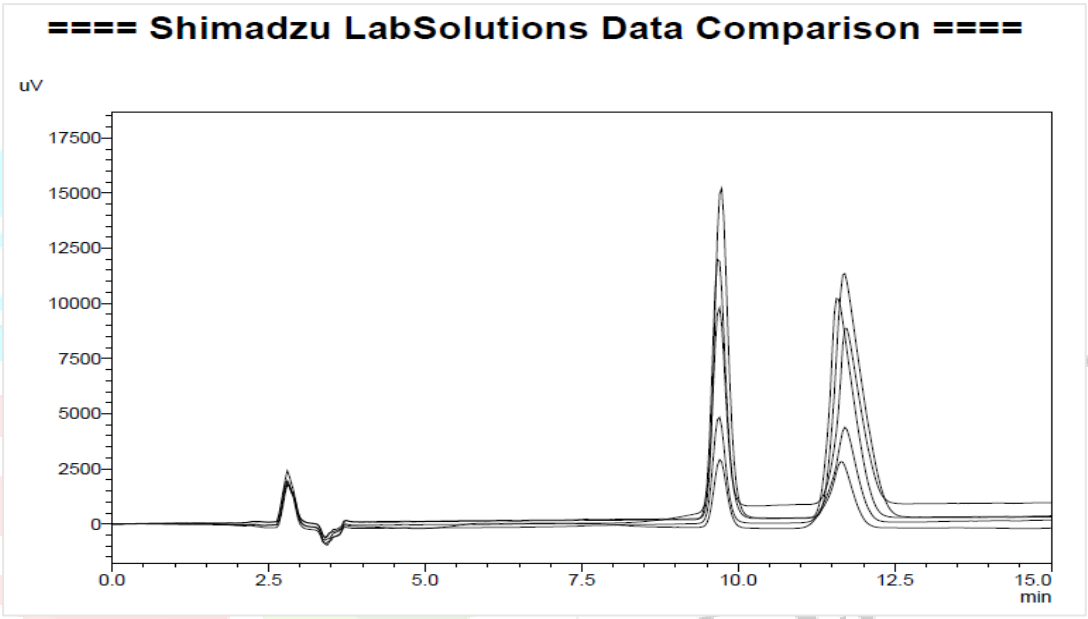

Figure 5. The HPLC chromatogram over the concentration range of 2 - 10 µg/ ml for PAN and 10- 50 µg / ml for NIM.

PRECISION: The repeatability of developed method was determined by analyzing 6µg/ml for PAN solution six times on the same day. The percentage RSD was found to be 0.87. The repeatability of developed method was determined by analyzing 30µg/ml for NIM solution six times on the same day. The percentage RSD was found to be 1.39. The results of repeatability data are shown in Table 3.

Table 3. Repeatability study

Concentration	PAN 6 (µg/ml)	NIM 30 (µg/ml)
Peak Area	139162	216118
	138068	218545
	139145	216525
	141125	222115
	138545	221351

	137651	214510
Mean	138949	218194
SD	1220.07	3037.47
% RSD	0.87	1.39

SD: Standard Deviation

RSD: Relative Standard Deviation

The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in Table 40 for PAN. Replicate analyses of three different concentrations PAN (2, 6, 10 µg/ml) solutions showed good reproducibility. The percentages RSD of intraday and interday studies were found to be 0.30–0.93% and 0.57-1.80% respectively for PAN. The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in Table 41 for NIM. Replicate analyses of three different concentrations NIM (10, 30, 60 µg/ml) solutions showed good reproducibility. The percentages RSD of intraday and interday studies was found to be 0.41 – 1.37 % and 0.46–1.91% respectively for NIM. The developed method was found to be precise and repeatable on the basis of the mean CV values for the repeatability and intermediate precision studies which were < 2 for PAN and NIM respectively. The separations of the drug and various degradation products in a mixture of stressed samples were found to be similar when the analyses were performed with an LC system on different days.

Table 4. Intraday and Interday Precision study for PAN

Intraday Precision		
Conc. (µg/ml)	**(Area ± S.D) (n=3)**	**% RSD**
2	42007.33 ± 389.08	0.93
6	138758.33 ± 599.28	0.43
10	231334.33 ± 692.08	0.30
Inter day Precision		
2	41190.67 ± 740.80	1.80
6	138773.67 ± 1252.75	0.90
10	233077.00 ± 1326.96	0.57

n=Three determination

Table 5. Intraday and Interday Precision study for NIM

Intraday Precision		
Conc. (µg/ml)	**(Area ± S.D) (n=3)**	**%RSD**
10	70186.67 ± 285.82	0.41

30	217062.67 ± 1759.66	0.81
50	335416.67 ± 4605.01	1.37
Interday Precision		
10	70566.33 ± 783.66	1.11
30	219325.33 ± 4187.66	1.91
50	333153.00 ± 1523.39	0.46

n=Three determination

Accuracy: The recovery of the method was carried out by the standard addition to the preanalysed test sample at three different concentration levels 50%, 100% and 150%. Triplicate determinations were made at each concentration level. The accuracy of the method was determined by calculating recoveries of 2, 4, 6 µg/ml of PAN and 10, 20, 30µg/ml of NIM in the preanalysed concentration 4 µg/ml Pantoprazole (PAN) and 20 µg/ml Nimesulide (NIM) by method of standard addition. The recoveries of PAN and NIM were calculated by putting the peak area of the added concentration of PAN and NIM in the regression equation of calibration curve respectively. The recoveries found to be 97.97 % - 102.74% for PAN and 96.49 %-102.30% for NIM, respectively. The result of the method is indicating good accuracy for chromatographic method. The accuracy result shown in Table 6.

Table 6. Accuracy study

Level	**Drug added (µg/ml)**	**Drug Recovered (µg/ml) [a]**	**% Drug Recovered ± SD**
PAN			
0	4	3.87	96.74 ± 0.68
50	6	6.16	102.63 ± 0.71
100	8	7.89	98.67 ± 0.26
150	10	10.08	100.75 ± 0.75
NIM			
0	20	19.30	96.49 ± 0.69
50	30	30.69	102.30 ± 1.42
100	40	39.55	98.87 ± 1.14
150	50	50.15	100.30 ± 1.59

a=Average of Three determination

LIMIT OF DETECTION AND LIMIT OF QUANTITATION: According to ICH, the approach based on the standard deviation of the response and mean of slope was used for determining the Limit of detection (LOD) and limit of quantitation (LOQ). The detection limits for PAN and NIM were found to be 0.11 µg/ml and 0.26 µg/ml respectively, while quantitation limits were found to be 0.33 µg/ml and 0.79 µg/ml respectively. The above data shows that a microgram quantity of PAN and NIM the drugs can be accurately and precisely determined. The values of LOD and LOQ of PAN and NIM respectively indicate the sensitivity of proposed method. Data shown in table no 7.

Table 7. LOD and LOQ Data

	NIM	PAN
LOD	0.26	0.11
LOQ	0.79	0.33

ROBUSTNESS: Robustness is the measure of the capacity of a method to remain unaffected by small variations in the method parameters. Robustness of the method was determined in triplicate at a concentration level of 6µg/ml for PAN and 30µg/ml for NIM. After small changes in this parameter effect peak areas were determined and mean and RSD of peak areas calculated. Deliberate changes in the following parameters which affects % assay of PAN and NIM system suitability parameters were studied.

a) Change in % organic phase of mobile phase by ± 5.0 %

b) Change in the flow rate of the mobile phase by ± 10 % of the original flow rate.

c) Change in detection wavelength by ± 5.0 nm

The method was found to be robust, as small but deliberate changes in method parameters have no detrimental effect on the method performance as shown in table 8. The low value of percentage relative standard deviation indicates that the method is robust.

Table 8. Robustness study

Parameters	**Change in condition**	**PAN**		**NIM**	
		Peak Area	**%RSD**	**Peak Area**	**%RSD**
Flow rate Changed (1 ml/min)	0.9	143528.33	0.58	218727.00	1.36
	1.1	138046	0.73	219061.67	0.84
Mobile Proportion Changed Acetonitrile: Methanol: Water (25:25:50 % V/V/V)	Acetonitrile: Methanol: Water (30:25:45 %V/V/V)	137146.00	0.74	219561.67	1.41
	Acetonitrile: Methanol: Water (25:30:45 %V/V/V)	137612.67	1.08	219257.85	1.17
Detection wavelength (270nm)	265 nm	136830.00	0.88	219159.76	1.17
	275nm	138075.33	1.44	218365.00	1.56

SPECIFICITY: Selectivity of a method refers to the extent to which it can determine particular analytes under given conditions in mixtures or matrices, simple or complex, without interferences from other components. The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The HPLC chromatogram showed peaks of the drugs PAN and NIM without any interfering peak and the estimation of both the drugs were found to be satisfactory. Test solution is prepared by mixing of PAN and NIM with the tablet powder excipients. Specificity is proven by comparing the chromatogram of Diluent, standard solution and test preparation solution and show that there was no any interference of excipients with the peak of PAN and NIM as shown in figure 6.

ANALYSIS OF SYNTHETIC MIXTURE: The developed RP-HPLC method was successfully applied for the estimation of Pantoprazole (PAN) and Nimesulide (NIM) in synthetic mixture. The chromatogram of sample showed only drug peaks at retention time (Rt) value of 9.69 and 11.71 minute for Pantoprazole (PAN) and Nimesulide (NIM), respectively, indicating that there is no interference of the excipients present in synthetic mixture. The content of Pantoprazole (PAN) and Nimesulide (NIM) was calculated by comparing peak areas of sample with that of the standard. The Synthetic mixtures were analyzed using proposed method which gave percentage recovery of more than 97.08 ± 1.06 for Pantoprazole (PAN) and 98.07 ± 1.39 for Nimesulide (NIM) (Table 9). No interference from the excipients present in the marketed tablet formulation was observed which is shown in figure 6.

Table 9. Results of synthetic mixture

Formula^n	**Drug**	**Amount Taken (µg/ml)**	**Amount Found^n (µg/ml)**	**%PAN ± SD**	**%NIM ± SD**
Synthetic mixture	Pantoprazole (PAN)	4	3.88	97.08 ± 1.06	98.07 ± 1.39
	Nimesulide (NIM)	20	19.61		

n= Average of Three determination

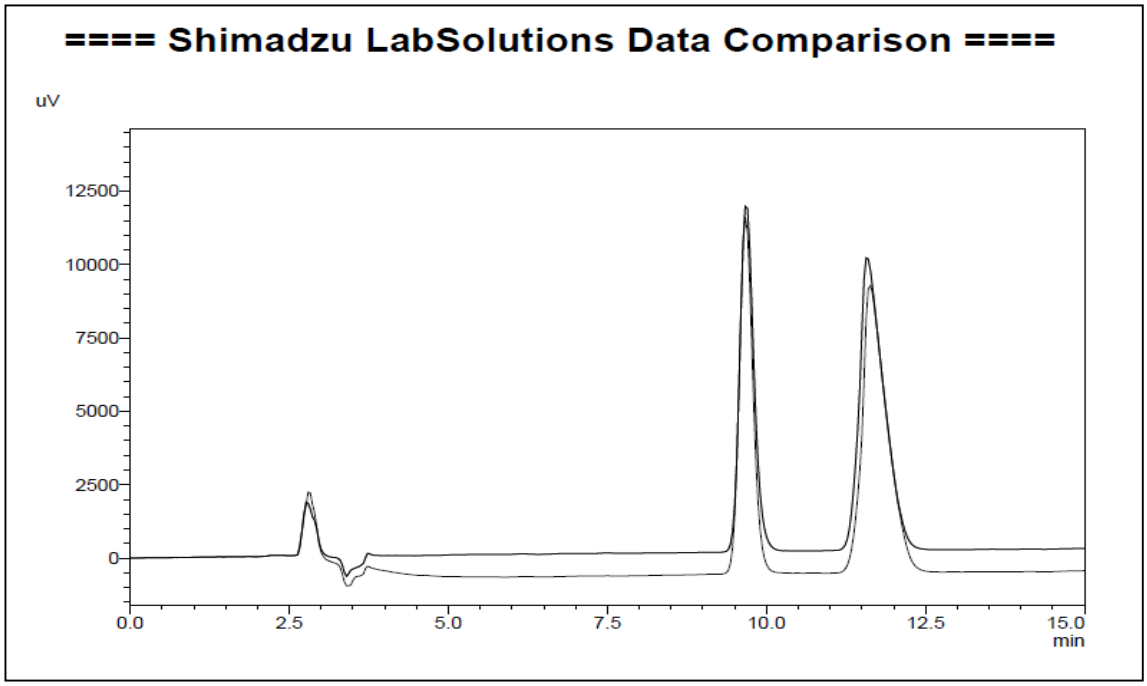

Figure 6. overlay chromatogram of API and synthetic mixture of NIM and PAN

CONCLUSION: Simple and sensitive RP-HPLC were developed for simultaneous estimation of Pantoprazole (PAN) and Nimesulide (NIM) in their synthetic mixture. RP-HPLC method was developed using Cyber Lib C18 (250 x 4.6mm, 5µm) column as a stationary phase and Acetonitrile: Methanol: Water (25:25:50 % V/V/V) as mobile phase. The flow rate was maintained at 1 ml/ min and detection was carried out at 270 nm where Pantoprazole (PAN) and Nimesulide (NIM) have significant absorbance. The retention times of Pantoprazole (PAN) and Nimesulide (NIM) was found to be 9.69 minute and 11.70 minute respectively. RP-HPLC method is linear in the concentration range of 2- 10 µg / ml for PAN and 10- 50 µg/ ml for NIM, with correlation coefficient found to be 0.9978 for PAN and 0.9979 for NIM. The recovery was in the range of be 96.74 % - 102.63% for PAN and 96.49 %- 102.30% for NIM, respectively. The detection limits for Pantoprazole (PAN) and Nimesulide (NIM) were found to be to be 0.11 µg/ml and 0.26 µg/ml respectively, while quantitation limits were found to be 0.33 µg/ml and 0.79 µg/ml respectively. The method was found to be accurate, precise, specific, selective, repeatable, robust and reproducible.

REFERENCES:

1. Molfetta L, Saviola G et al, "Osteoarticular Pain: Therapeutic Approach by Paradigms." *Eur. Rev. Med. Pharma*.**2022**, *26*, 4054-4068.
2. Zhang Y, Glynn RJ, Felson DT,"Musculoskeletal disease research: should we analyze the joint or the person?" *J Rheumatol*, PMID: 8823682,**1996**, Jul,*23(7),*1130-4.
3. Carton, James (Ed), "Osteoarticular Pathology", In James Carton (Ed.), Oxford Handbook of Clinical Pathology 2e,2 Edn, Oxford Medical Handbook (Oxford,2017; Online Edn, Oxford Academic,1july 2017.
4. M De Vos, L Van Praet, D Elewaut, "Osteoarticular Manifestation: Specific Treatments And/Or Treating Intestinal Disease?", Departments of A Gastroenterology and B Rheumatology, Ghent University Hospital, Ghent University, Ghent, Belgium,**2013**, *31,*239-243.

5. Scheinberg M, H Pott Júnior et al, "Efficacy and Safety of a Fixed-Dose Combination of Nimesulide/Pantoprazole Compared to Naproxen/ Esomeprazole for Pain Relief in Patients with Osteoarticular Diseases and Dyspeptic Symptom." *Drug Ded Devel Ther. (Dovepress*). **2018**,12,2775-2783.
6. Pathuri R, Muthukumaran M et al, "A Review Analytical Method Development and Validation of Pharmaceutical Technology." *Curr Pharm Res*.**2013**, *3*, 855-70.
7. Michael ES, Ira SK, Introduction of Analytical Method Development; 3rd Edition; *John Wiley & Sons.* London, **2004**, 25-46.
8. Chatwal GR, And Sham KA, Instrumental Methods of Chemical Analysis; 5th Edition; *Himalaya Publishing House.* Mumbai, **2011**, 2.615-2.626.
9. ICH Q2 (R1); "Validation of Analytical Procedures: Text and Methodology."

Geneva, Switzerland, November 2005.

10. Sawale V, Dr.D. Umamaheshwari, "A Review on Novel Analytical Techniques Used in Method Development and Validation of Pharmaceuticals."*J. Pharm. Sci.***2020**, *12(2),* 321-328.
11. Donovan J W, "Ultraviolet Difference Spectroscopy- New Techniques and Application."Methods in Enzymology Book, *J. Sci. Pharma.* **1973**, *27*, 497-525.
12. Verma G, and Dr. Mishra M, "Development and Optimization of UV-Vis Spectroscopy - A Review." *W. J. Pharm. Res.* **2018**, *11(7),* 1170-1180.
13. Atole D, Rajput H, "Ultraviolet Spectroscopy and Its Pharmaceutical Applications- A Brief Review" *Asian J Pharm Clin Res.***2018**,*11(2),*59-66.
14. Kutty N, Dr. Parag S, "UV-Visible Spectroscopy" Summer Research Fellowship Programme of India's Science Academies, *Indian sci. J.***2016,**1.
15. Upstone S L, "Ultraviolet/Visible Light Absorption Spectrophotometry in Clinical Chemistry" *The Encyclo. of Analy. Chem.,* **2013**, 2.
16. Royal Society of Chemistry, "Ultraviolet /Visible Spectroscopy", Unilever,**2015**. Https://Edu.Rsc.Org/Resources/Ultraviolet/Visible-Spectroscopy/1300.Article.
17. Snyder L.r., Kirkland J.L., And Glajch J.l, "Practical HPLC Method Development"2nd Edn, John Wiley & Sons Inc. New York, **1997**, *21,*613,653.
18. Ahuja S, Scypinski S, "Handbook of Modern Pharmaceutical Analysis",1st Edn; Harcourt Science and Technology Company Limited, San Diego, **2001**, 445.
19. Giri D, "High Performance Liquid Chromatography Principle, Types, Instrumentation and Applications"December ,**2021**.
20. Sethi PD, "Introduction-High Performance Liquid Chromatography; 1st Edn; *CBS Publishers.* New Delhi, **2001**, 1-28.
21. Yadav V, Bharkatiya M, "A Review on HPLC Method Development and Validation" *Res. J. Pharm. Boil. Chem. Sci.* **2017**, *2(6),*166.
22. Ms P Patil, "HPLC Method Development - A Review"*J. Pharm. Res. Int.* **2017**, *1(2)*, 243-260.

23. R. Snyder; J. J. Kirkland and J. L. Glajch: Practical HPLC Method Development. Second Edition, XXVI, And 765 Pages, Numerous Figures and Tables. *John Wiley & Sons Inc.,* New York, Chichester, Weinheim, Brisbane, Singapore, Toronto 1997.
24. Sahu PK, Ramisetti NR, Cecchi T et at, "An Overview of Experimental Designs in HPLC Method Development and Validation."*J Pharm Biomed Anal*.**2018** Jan 5,*147,* 590-611.
25. Drug profile of Nimesulide.
https://go.drugbank.com/drugs/DB04743.
26. Drug profile of Pantoprazole.
https://go.drugbank.com/drugs/DB00213.
27. The European Pharmacopeia (Ph. Eur), Published by Council of Europe, **2016**,*10*, 3368-3369.
28. Patil Sudhir V, "Electroanalytical And UV- Spectroscopic Study for Analysis of Nimesulide in Pharmaceutical Samples" *Int. J. Chem. Stud*.**2022**, *10(1),* 07-12.
29. D Shrivastava, S Dwivedi, "Estimation of Nimesulide Using Mixed Solvency Approach*" Int. J. Pharm. Life. Sci.***2020**, *11(5),* 6629-6634.
30. Sajeev Chandran, Sachin Saggar et al, "New Ultraviolet Spectrophotometric Method for The Estimation of Nimesulide" *Drug Develop. and Indu. Pharm*. **2000**, *26(2),* 229–234.
31. S J Shakkor, "Spectrophotometric Determination of Reduced Nimesulide Using 8- Hydroxyquinolinol Reagent in Pharmaceutical Preparations" *Kirkuk Uni. J. /Sci. Studies (KUJSS)*. March **2015**,*10(1),*143-157.
32. Renjitha JR, Jose Kurien, "Method Development and Validation of Nimesulide and Camylofin Dihydrochloride in Combined Tablet Dosage Form by UV - Absorbance Ratio Method"*The J. Pharm. Innov.***2019**, *8(3),* 443-448.
33. S S Chitlange, Soni R, "Spectrophotometric Methods for Simultaneous Estimation of Nimesulide and Drotaverine" *Int J. Chem tech Res.***2009**, *1(2),* 135-138.
34. Mr J Patel, Dr R K Patel, Ms N S Mochi, "Q-Absorbance Ratio Spectrophotometric Method for Simultaneous Estimation of Nimesulide and Diclofenac Sodium in Combined Pharmaceutical Dosage Form"*Int. J. Res. Manag. Pharm.***2017**,*6(3),*1-7.
35. Prasad R K, Sharma R, "Spectrophotometric Quantitative Estimation and Validation of Nimesulide and Drotaverine Hydrochloride in Tablet Dosage Form" *Int. J. Pharm. Sci. Res.***2010**, *2(1),* 67-70.
36. R Kirtawade, Pallavi Salve et al, "Simultaneous UV Spectrophotometric Method for Estimation of Paracetamol and Indian Nimesulide in Tablet Dosage Form"*Int. J. Chemtech Res.* April-June **2010**, *2(2),* 818-821.
37. V A Patil, Sanjay B Bari, D A Patil, "Q-Absorbance Ratio Spectrophotometric Method for The Simultaneous Estimation of Nimesulide and Chlorzoxazone in Dosage Form"*Anal. Chem. J.***2013**,*13(3),* 96-101.
38. A Álvarez-Lueje, P Vásquez et al, "HPLC Determination of Nimesulide in Tablets by Electrochemical Detection" *J.Analy. Let*.**1998**,*31(7),* 1173-1184.

39. G H Ragab, Magda M, "Stability-Indicating HPLC Method For Determination of Nimesulide in Bulk Powder and In Tablet Dosages Form" *Asian J. Pharm. Clin. Res.***2014**,*2(4),*230-239.
40. Paraskevas D T, Demetrius G T, "Validated High-Throughput HPLC Assay for Nimesulide Using a Short Monolithic Column" *J. Pharm. Biomed. Anal*.**2007**,*43*,1483-1487.
41. S Altino, O O Dursun, "Determination of Nimesulide in Pharmaceutical Dosage Forms by Second Order Derivative UV Spectrophotometry" *J. Pharm. Biomed Analysis*.**2000**, *22*, 175–182.
42. S J More, S S Tandulwadkar et al, "Application of HPLC For the Simultaneous Determination of Paracetamol, Chlorzoxazone, And Nimesulide in Pharmaceutical Dosage Form" *ISRN Chromatography*. **2012**.
43. Aly et al, "Simultaneous Determination of Cetirizine, Phenyl Propanolamine and Nimesulide Using Third Derivative Spectrophotometry and High-Performance Liquid Chromatography in Pharmaceutical Preparations" *J. Chem*.**2017**,11-99.
44. Rafael et al, Y Vannuchi, "Quantification of Nimesulide in Human Plasma by High-Performance Liquid Chromatography/Tandem Mass Spectrometry. Application To Bioequivalence Studies" *J. Mass Spectrom*. **2001**,*36*,1281–1286.
45. I Singhvi et al, "Spectrophotometric and HPLC Method For Simultaneous Estimation of Nimesulide and Paracetamol from Tablets" *Philippine J. Sci.***2002**,*131(1),*59-64.
46. K E V Nagoji et al, "Simultaneous Reverse Phase HPLC Estimation of Nimesulide and Diclofenac Sodium" *Indian J. Pharm. Sci.* **2003**, *1*,407-409.
47. K Chouhan, Navin R et al, "Validated RP-HPL Method for Simultaneous Determination of Drotaverine and Nimesulide and Its Application in Drug Formulation" *Pharmacol. onli. 2*.**2011**, 1245-1252.
48. K R Singh et al, "Stability Indicating Method for Simultaneous RP HPLC Determination of Camylofin Dihydrochloride and Nimesulide in Pharmaceutical Preparations" *ISRN Analytical Chemistry*.**2012**, 1-7.
49. P Reddy et al, "Determination of Nimesulide in Pharmaceutical Formulations and In Human Serum by Reverse-Phase High performance Liquid Chromatography" *Int. J. Pharm tech Res.***2009**, *1(2),* 206-209.
50. Sami B, K M Badr et al, "Development and Validation of a Versatile UPLC-PDA Method For Simultaneous Determination of Paracetamol, Tizanidine, Aceclofenac, And Nimesulide in Their New Combinations" *J. Anal. Chem.***2018**,7.
51. V B Patravale et al, "HPTLC Determination of Nimesulide from Pharmaceutical Dosage Forms" *J. Pharm Biomed Anal.***2001**, *25*, 685–688.
52. S R Dhaneshwar et al, "Validated HPTLC Method For Simultaneous Estimation of Levocetirizine Hydrochloride and Nimesulide in Formulation" *Der Pharmacia Sinica.* **2011**, *2 (4),*117-124.
53. D Halder, Shubhasis Dan et al, "LC-MS/MS Determination of 4-Hydroxynimesulide, An Active Metabolite of Nimesulide and Application to Bioequivalence Study in Indian Subjects" *Eur. J. Mass Spectrom.***2019**,1–13.
54. The Indian Pharmacopeia-Volume, Published by Indian Pharmacopeial Commission for Ministry of Health and Family Welfare, **2018**, Vol (3), 2848-2849.
55. The United States Pharmacopeia, USP 43, NF 38, **2020.**

56. British Pharmacopeia, British Pharmacopeia Commission, London, **2022**, Vol (2), 532-534.
57. H Basnett et al, "A Novel Difference Spectrophotometric Method for The Determination of Pantoprazole in Tablet Dosage Form*" Res. J. Pharm. Biol. Chem. Sci.***2019**,*5(1),*725-734.
58. Madhukara B M et al, "Validated Spectrophotometric Estimation of Pantoprazole in Pure and Tablet Dosage Form" *Int. J. Pharm.tech Res.***2015**,*8(2)*, 176-179.
59. Madhukara B M et al, "Validated Derivative Spectrophotometric Estimation of Pantoprazole in Bulk and Tablet Dosage Form" *Int. J. Adv. Res. Publ.***2015**, *3(4),* 366-370.
60. R. Kumar et al, "Development of UV Spectrophotometric Method for Estimation of Pantoprazole in Pharmaceutical Dosage Forms" *J. Chem. Pharm.***2011**, *3(2),*113-117.
61. Vaishali S., "Development and Validation of UV Spectrophotometric Method for Estimation of Pantoprazole Sodium in Bulk and Tablet Dosage Form" *Cibtech J.Pharm. Sci.***2016,***5(4),* 22-26.
62. S S Patil et al, "Spectrophotometric Estimation of Pantoprazole in Tablet Dosage Form" *Int. J. Chem. Sci.* **2008**, *6(4),* 1984-1990.
63. A Z Mirza et al, "Quantitative Analysis of Pantoprazole Sodium Sesquihydrate in Bulk and Solid Dosage Form Via UV-Spectrophotometric Method" *Indian. J. Pharm. Edu. Res.***2020**,*5(4),* 448-455.
64. V Khanvilkar et al, "Simultaneous Determination of Pantoprazole Sodium and Itopride Hydrochloride in Pharmaceutical Dosage Form by First Order Derivative UV Spectrophotometry" *Asian J. Pharm. Clin. Res.***2010**,*3(3),* 221-223.
65. S Mujbaile et al, "Simultaneous Estimation of Ondansetron and Pantoprazole in Solid Dosage Form by First Derivative Spectroscopy Method" *IOSR J. Pharm. Bio. Sci.***2012**,*4(4),* 05-08.
66. D A Shah et al, "Simultaneous Estimation of Pantoprazole Sodium and Levosulpiride in Capsule Dosage Form by Simultaneous Equation Spectrophotometric Method" *Hindawi Publis. Corpo*.**2013**,4.
67. A. M. Mansour et al, "High Performance Liquid Chromatographic Determination of Pantoprazole in Tablet Dosage Form" *Chromato. Supple*.**2001**,53.
68. J. Emami et al, "Quantification of Pantoprazole by High Performance Liquid Chromatography (HPLC) Method: In Vitro, In Vivo Applications" *J.Liq. Chromato. Relat. Technol.***2013**,1-26.
69. F Dias Marques et al, "A Fast, Validated HPLC Method Applied to The Dissolution Test of Gastro-Resistant Capsules of Pantoprazole Pellets"*J. Liq. Chromato. Relat. Technol.***2007**,*30*, 1833–1843.
70. S Pandey et al, "A Validated Stability Indicating HPLC Method For the Determination of Process-Related Impurities in Pantoprazole Bulk Drug and Formulations" *Brazil. J. Pharm. Sci.***2013**,*49(1),*175-184.
71. Srinivasarao T et al, "Dissolution Test Method and HPLC Analysis for Pantoprazole Pellets Dosage Forms" *J. Emerg. Technol. Innov. Res.***2019,***6(4),*535-543.
72. Q B Cass et al, "Enantiomeric Determination of Pantoprazole in Human Plasma by Multidimensional High-Performance Liquid Chromatography" *J. Chromato*.**2003**, 766(1),153–160.
73. D Zhong et al, "Simultaneous Determination of Pantoprazole and Its Two Metabolites in Dog Plasma By HPLC" *J. Chromato. Sci.* **2005**,43.

74. Safwan A, Soulafa O, "A Modified High-Performance Liquid Chromatographic Method for The Analysis of Pantoprazole Sodium in Pharmaceutical Dosage Forms Using Lansoprazole as Internal Standard" *Arab. J. Chem*. **2011**,2.
75. R Ahmad et al, "Development and Validation of An HPLC Method For the Determination of Meloxicam and Pantoprazole in A Combined Formulation" *Analytica.***2022**, *3*, 161–177.
76. Patel G H et al, "Development and Validation of HPLC Method for Simultaneous Estimation of Cinitapride and Pantoprazole in Pharmaceutical Dosage Form" *Int. J. Pharm. Technol.***2012**,*4(2),* 4253-4260.
77. M Srinivas et al, "Method Development and Validation Of RP-HPLC Method For the Simultaneous Estimation of Pantoprazole and Ondansetron Hydrochloride in Bulk and In a Synthetic Mixture" *Int. J. Pharm. Res*.**2014**,*6(6),*1794-1802.
78. A B N Nagewara Rao et al., "RP-HPLC Method For Simultaneous Estimation of Domperidone and Pantoprazole in Capsule Dosage Form" *Sch. Resea. Libr*. **2012**, *4 (6),*1712-1720.
79. K Nagappan et al, "Stability Indicating RP-HPLC Method for The Simultaneous Estimation of Pantoprazole and Levosulpiride in Pharmaceutical Dosage Form" *Int. J. Pharm. Sci. Res.***2019**, *10(11),* 4949-4958.
80. Lakshmi Durga K et al, "Analytical Method Development & Validation for Simultaneous Estimation of Pantoprazole and Mosapride in Pharmaceutical Dosage Form By RP-HPLC Method" *J. Pharm. Chem.***2014**,*8(4),*14-20.
81. R T Addo, Kenneth Davis, R Ubale et al, "Development and Validation of a UPLC Method for Rapid and Simultaneous Analysis of Proton Pump Inhibitors" *AAPS Pharma. SciTech*.**2014**,1-5.
82. A Patil, S Mulla et al, "Development and Validation of HPTLC Method for The Simultaneous Estimation of Naproxen and Pantoprazole in Combined Dosage Form" *Int. J. Pharm. Sci.***2013**,5(3),222-225.
83. Roshdy E S et al, "High-Performance Thin-Layer Chromatography Method for The Simultaneous Determination of Itopride, Pantoprazole, And Mosapride in Their Formulations and Spiked Human Plasma" *J. Planar Chromat.***2017**,*30(4),* 299–306.
84. Y Chen, Song Wu, And Q Yang, "Development and Validation Of LC-MS/MS For Analyzing Potential Genotoxic Impurities in Pantoprazole Starting Materials" *J. Anal. Methods. Chem.***2020**,1-8.
85. Dr A Singh, Jain R.Therapeutic Composition Containing Nimesulide and Process for Their Preparation IP 51/Del/2001,2006.
86. V Kukkar, R Parveen, N Sharma, V Saini, S Bhatt, M Kumar. A Novel Composition and Method for Analysis of Nimesulide in Tablet Dosage Form by HPLC IP 202011018038,2022.
87. V Kukkar, R Parveen, N Sharma, S Sandhu, VSaini, S. Bhatt, M. Kumar, A. Mittal, M. Kapoor. A Novel HPLC Methods for Combined Dosage Form of Nimesulide, Phenylepherine Hydrochloride Cetrizine Hydroch Paracetamol and Caffeine in Combined Dosage Form and Composition Thereof IP 202011018199,2022.

88. Da He, Wei Chen, Jiegen Chu, Bin Zhou, Tianjiang Sun, Yubin Zhang, Xianbin Zhang, Bixi Yin. High Performance Liquid Chromatography (HPLC) Method for Analyzing and Separating Optical Isomer of Pantoprazole Sodium CN102141547,2011.
89. Zhou Zhidong, Zong Yeliang. HPLC (High Performance Liquid Chromatography) Method for Separating and Analyzing Pantoprazole Thioether Related Substances CN109917057,2019.
90. Sharma BK. In Instrumental Methods of Chemical Analysis; 24th Edn; GOEL Publishing House, Meerut,2005, pp S-193- S-333.