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Development and Validation of Analytical Methods for Simultaneous Estimation of Nimesulide and Pantoprazole in Synthetic Mixture

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ABSTRACT:A simple, rapid, and selective HPLC-UV method was developed for Nimesulide and pantoprazole.The 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. For this combination the RP-HPLC and UV methods was developing simultaneous estimation for nimesulide and pantoprazole A reversed phase high performance liquid chromatography and ultraviolet spectroscopy methods are developed and validated for the determination of the both drugs. with the help of RP-HPLC and UV its gives us to good resolution and simple, sensitive and accurate, separation for the both drugs. The separation was conducted by using shim pack ODS C18 column(4.6 mm× 250 mm, 5 µm) with the mobile phase consisting ACN, Methanol, Water (25:25:50 % V/V/V). The mobile phase was delivered at the flow rate of 1 ml/min. the eluent was monitored at wavelength 270 nm and found a sharp and symmetrical peak of PAN and NIM were found to be 9.69 min and 11.71 min respectively. The methods were validated for linearity, accuracy, precision, system suitability and stability. The method was found to be linear over the concentration range for both drugs are 2-10 µg/ml for PAN and 10-50µg/ml for NIM with coefficient $R^2$for PAN 0.9978 and NIM 0.9979. for UV spectrophotometric methods is first order derivative spectrophotometric has been developed for estimation of PAN and NIM in synthetic mixture in concentration range mentioned above in RP-HPLC for PAN (λmax= 249.60nm) and NIM (λmax=297.20 nm) respectively in methanol.Therefore, proposed methods can be successfully used for routine analysis of Nimesulide and Pantoprazole in bulk as well as synthetic mixture.

[Keywords: Pantoprazole (PAN), Nimesulide (NIM), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), Ultraviolet Spectroscopy (UV).]

I. 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. The 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.

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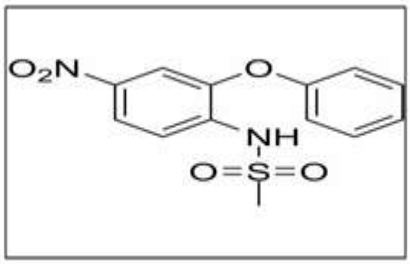
STRUCTURAL FORMULA:



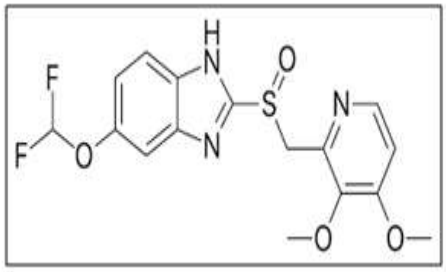


Figure 1. Structure formula of NIMFigure 2. Structure formula of PAN

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) and UV Visible Spectrophotometer (UV 1700 from Shimadzu Lab).

ANALYTICAL METHODS:
FOR RP-HPLC:
METHOD DEVELOPMENT OF RP-HPLC: Establishment of Optimum Condition for HPLC Method development. Various conditions used during the development of analytical methods should be optimizing for developing sensitive, accurate and reproducible method.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 proteolytic 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.

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.

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.

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.

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.

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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.

VALIDATION OF HPLC METHOD:

CALIBRATION CURVE: 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.

SYSTEM SUITABILITY TEST: System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard preparation (20µg/ml for NIM, 4µg/ml for PAN) and one injection of a check standard were made. The parameters measured were retention time, peak area, theoretical plates, and asymmetry of Pantoprazole (PAN) and Nimesulide (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 1.49. The repeatability of developed method was determined by analyzing 30µg/ml for NIM solution six times on the same day.The results of the intermediate precision (Intraday precision and Interday precision) experiments forPAN. 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) experimentsof 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 NIMrespectively.

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/mlof PAN and 10, 20, 30µg/mlof NIM in the preanalysed concentration4µg/mlPantoprazole (PAN)and 20 µg/mlNimesulide (NIM)by method of standard addition. The recoveries of PAN and NIMwere calculated by putting the peak area of the added concentration of PAN and NIMin 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.

LOD and LOQ: The Limit of detection (LOD) and limit of quantitation (LOQ). The detection limits for PAN and NIMwere found to be 0.11µg/ml and 0.26µg/ml respectively, while quantitation limits were found to be 0.33 µg/ml and 0.79µg/mlrespectively.

SPECIFICITY: 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 NIMwithout any interfering peak and the estimation of both the drugs were found to be satisfactory.

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.

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 4.51 and 6.42 minute for Pantoprazole (PAN) and Nimesulide (NIM), respectively, indicating that there is no interference of the excipients present in synthetic mixture.The Synthetic mixtures were analyzed using proposed method which gave percentage recovery of more

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than 98.23 forPantoprazole (PAN) and 99.17 for Nimesulide (NIM).

FOR UV SPECTROSCOPY:

METHOD DEVELOPMENT AND VALIDATION OF UV: Establishment of optimum condition for UV method development. condition used during the developing of analytical methods should be optimizing for developing sensitive, accurate and reproducible method.

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.

SELECTION OF WAVELENGTH: An ideal wavelength is the one that gives Maximum response for the drugs that was to be detected. From appropriate dilutions of the working standard stock solution, 6 µg/ml of PAN and 30 µg/ml of NIM were separately prepared and scanned in the UV range 200–400 nm.The overlain zero-order absorption spectra of PAN and NIM were obtained. These absorption spectra were converted to first-order derivative spectra by using the instrument mode. After observing the overlain first-order derivative spectra with scaling factor = 2 and $\Delta\lambda$ = 2 for PAN and NIM, zero crossing points of drugs were selected for the analysis of other drugs. The first wavelength selected was 249.80 nm (zero crossing of PAN), where NIM showed considerable absorbance. The second wavelength selected was 297.20 nm (zero crossing of NIM), where PAN showed considerable absorbance.

VALIDATION OF UV SPECTROSCOPY METHOD:

CALIBRATION CURVE:preparing solutions of six different concentrations of 2, 4, 6, 8 and 10 µg/ml of PAN, 10, 20, 30, 40 and 50 µg/ml of NIM respectively. Each concentration was repeated six times. Linearity was assessed in terms of slope, intercept, and correlation coefficient of Pantoprazole (PAN) and Nimesulide (NIM). The calibration curves were developed by plotting absorbance versus concentrations (n = 6).

PRECISION: The precision was studied as Repeatability, Intra and Interday precision and Reproducibility.

REPEATABILITY: Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time. The repeatability studies were carried out by estimating response of 6µg/ml for PAN and 30µg/ml for NIM six times and results are reported in terms of RSD.

INTRA AND INTER DAY PRECISION: The intraday and inter day precision study was carried out at three different concentrations of Pantoprazole (PAN) and Nimesulide (NIM). Intraday precision was evaluated by estimating the corresponding responses three times on the same day and inter day precision was evaluated by estimating the corresponding responses three times on three different days (first, third, and fifth day) at different concentrations of Pantoprazole (PAN) and Nimesulide (NIM). Intraday precision of the developed UV method was determined by analyzing sample solutions of PAN (2, 6, 10 µg/ml) and NIM (10, 30, 60 µg/ml) at three levels covering low, medium and high concentrations of the calibration curve three times on the same day (n = 3). Interday precision was determined by analyzing sample solutions of PAN (2, 6, 10 µg/ml) and NIM (10, 30, 60 µg/ml) at three levels covering low, medium and high concentrations over a period of 3 days (n = 3). The absorbance obtained were used to calculate mean and RSD values.

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

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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 absorbance of the added concentration of PAN and NIM in the regression equation of calibration curve respectively. The recoveries found to be 98.02 % - 102.06 % for PAN and 98.55 %-102.05% for NIM, respectively. result of the method is indicating good accuracy for chromatographic method.

LOD and LOQ: The detection limits for PAN and NIM were found to be 0.22 µg/ml and 0.207 µg/ml respectively, while quantitation limits were found to be 0.68 µg/ml and 0.629 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.

ROBUSTNESS: The method was determined in triplicate at a concentration level of 6 µg/ml Pantoprazole (PAN) and 30 µg/ml Nimesulide (NIM). Robustness of proposed method was performed by changing UV analyst and keeping the remaining conditions (solvent, dilution, UV spectrophotometer) same and RSD of absorbance calculated.

ANALYSIS OF SYNTHETIC MIXTURE: The developed first derivative UV spectrophotometry method was successfully applied for the estimation of Pantoprazole (PAN) and Nimesulide (NIM) in synthetic mixture. The absorbance of sample measured at ZCP of both drugs, indicating that there is no interference of the excipients present in synthetic mixture. The content of Pantoprazole (PAN) and Nimesulide (NIM) was calculated by measure the absorbances of sample and put this value in the regression equation.The Synthetic mixtures were analyzed using proposed method which gave percentage recovery of more than 98.80% forPantoprazole (PAN) and 98.20%for Nimesulide (NIM).

II. CONCLUSION:

CONCLUSION OF UV SPECTROSCOPY: Simple and sensitive first order derivative UV spectrophotometry method were developed for simultaneous estimation of Pantoprazole (PAN) and Nimesulide (NIM) in their synthetic mixture. Based on the results and the statistical parameters obtained, it was concluded that the proposed method of analysis is simple, rapid, accurate, precise and economical. The method did not utilize any extraction step for recovering the drug from the formulation excipient matrixes and they're by decreased the degree of error, time in estimation of drugs and the overall cost of the analysis. The developed method can be employed for routine quality control analysis of Pantoprazole (PAN) and Nimesulide (NIM) in bulk and pharmaceutical formulations.

CONCLUSION OF RP-HPLC CHROMATOGHRAPHY: 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.

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