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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE IMPURITIES OF FORMULATED NIMESULIDE GRANULES BY RP-HPLC METHOD

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ABSTRACT

The object of the analytical work is to develop an analytical method for the quantification of related substances of formulated Nimesulide Granules which is used as Non Steroidal Anti Inflammatory drug. In this newly developed method, two processes related impurities, Impurity-C and impurity-D were well separated. This method utilized silica based RP-C_8 column i.e. X Terra RP8 (100 x 4.6 mm, 3.5µ) and better resolution between the impurity peaks is achieved at temperature of 30°C with the flow rate of 1.0 ml/min. Acetonitrile & Buffer of Ammonium dihydrogen phosphate which is adjusted to pH 7.0 in ratio of (35:65) and wavelength of HPLC UV-Detector was fixed to 230nm.This method is validated according to ICH guidelines.

INTRODUCTION

Nimesulide is N-(4-Nitro-2-phenoxyphenyl) methane sulfonamide and its molecular formula is $C_{13}H_{12}N_2O_5S$ with molar mass of 308.311 g/mol. Structure of Nimesulide was given in Fig 1 with related impurities. Nimesulide is a relatively COX-2 selective, Non Steroidal Anti-Inflammatory drug (NSAID) with analgesic and antipyretic properties[1].It is administered to treat musculosk eletal disorder, dental pain, thrombophlebitis an d inflammation[2].Only few studies in the literature have been devoted to the Investigation and quantification of Nimesulide and its related impurities in then finished formulation[3]. In the current study, attempts were made to develop an RP-HPLC method which separate impurities of Nimesulide. The method which is developed is validated according to ICH guidelines[4].

Fig.1: Nimesulide and its related impurities

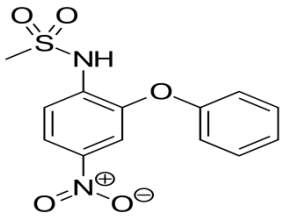

Fig 1(a): Structure of Nimesulide

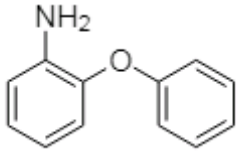

Fig 1(b): Structure of impurity-C

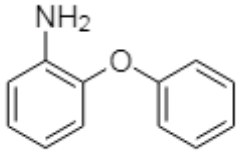

Fig 1(c): Structure of impurity-D

MATERIALS AND METHODS

Regent and Chemicals

Samples of formulated Nimesulide granules and working reference standards of Impurities were acquired from Dr.Reddy's laboratories limited, Hyderabad, Telangana. Acetonitrile (HPLC grade) purchased from Standard REAGENTS Pvt. Ltd. Hyderabad.Ammonium dihydrogen phosphate (AR grade) used in the preparation of buffer and aqueous ammonia (ammonia solution 30%) preparation of mobile phase purchased from Rankem. Milli Pore water was obtained from Thermo Scientific system.

Instrumentation

This method was developed using Waters HPLC e2695 equipped with UV-Visible detector. The data integration was done using the software Empower 3. With the help of Ultrasonicator of model Power Sonic 420, the samples were Sonicated and degassed and pH meter (Thermo Scientific) was used for pH adjustment of the buffer. Digital balance (Sartorius) aided in weighing of the drugs for preparation of stock solutions.

Chromatographic conditions

Method - Isocratic elution, Column used - Reverse phase chromatographic column, X Terra RP8, (100 x 4.6 mm, 3.5µ), Mobile Phase - Acetonitrile: Buffer of Ammonium dihydrogen phosphate (35:65) of pH 7.0, Flow rate - 1.0mL/min. Injection volume - 20µL,Temperature - 30˚ C, Diluent – Water: Acetonitrile (50:50 v/v),Wavelength – 230nm.

Preparation of Standard Solution

About 20mg of Nimesulide Reference standard and 70mL of acetonitrile were added into 100mL volumetric flask and dissolved using Sonicator for 5 mins. It was allowed to come to room temperature. Then the volume made up with acetonitrile and mixed well. From this, 5 mL was transferred into 100mL volumetric flask and made up to the volume using diluent. Again, 5 mL was taken and transferred to 50 mL volumetric flask, then made up the volume with diluent and mixed well.

Resolution solution

About 2.5 mg of each, Impurity-C and Impurity-D working standard was weighed and 70 mL of acetonitrile were added into 100mL volumetric flask and dissolved using Sonicator for 5 mins. Cooled down to room temperature. Then the volume made up with acetonitrile and mixed well. From this, 3 mL pipetted out in to 50 mL volumetric flask, then made up the volume with diluent and mixed well.

Sample Solution

About 1000 mg of Sample (equivalent to 50 mg Nimesulide) weighed accurately and 70 mL of acetonitrile was added in 50 mL volumetric flask and dissolved using sonicator with intermittent shaking for 15 mins. Then the sample solution centrifuged at 4000 rpm for 10 mins. About 5 mL of the supernatant sample solution pipetted out into 10 mL volumetric flask and made up the volume with purified water. With the help of 0.45µ PVDF membrane filter, the solution was finally filtered.

Method Development:

In the current development, column X Terra RP8 and an isocratic elution of mobile phase is used in which better separation and resolution was achieved between Nimesulide and its related impurities given in figure 2(a), 2(b) and 2 (c) and their retention times were given in table 1.

Specificity: Specificity is checked for the interference of any blank peaks with the impurity peaks. The Chromatogram of placebo not showing inference at the retention time of impurity peaks /analyte peaks given in figure 3.

System Suitability: System suitability was determined by 5 injections of Impurity–C and impurity-D and it was found that system is suitable as resolution >5, Tailing factor is <1.5 and theoretical plate count is > 8000.

Linearity:

Linearity was developed by the preparation of standard concentration over the range of 30%, 50%, 75%, 100%, 150% and 200% specification levels. Slope, intercept and correlation coefficient were calculated. For suitable linearity of a method, the value of correlation coefficient factor should be above 0.99.The correlation coefficient for Nimesulide and its impurities were found to be more than 0.99. The HPLC method for the determination of Nimesulide related substances is therefore linear. The results showing the slopes, correlation coefficient, y-intercept for assay and related substances is given in Table 2 and figure 4.

Precision Studies:

Method was observed for reproducibility under the developed chromatographic conditions with an excellent mean % recovery at 100 % test drug concentration. The system is sufficiently precise with the %RSD is not greater than 10% which implies the method is rugged. The percent recovery and percent RSD of precision studies is given in Table 3.

Accuracy/ Recovery Studies:

Accuracy of the developed method has been determined by spiking the impurities at 200% level to the drug substance. It was inferenced that the developed method was successful with the mean recovery within the range of 85 – 115 %. The percent recovery studies are given in Table 4.

Limit of Detection and Limit of Quantification (LOD & LOQ):

S/N Ratio method was used for the determination of LOD and LOQ of Nimesulide and its impurities. LOD and LOQ were the smallest analyte concentration which gives a response that can be detected and quantifies respectively. LOD and LOQ were calculated and stated in Table 5.

Robustness:

Robustness studies are carried out by altering the conditions like flow rate, pH, column temperature. Study conducted by changing the flow rate to 0.8mL/min, and 1.2 mL/min. Mobile pH is changed to 6.5 and 7.5 and column temperature to 25˚C and 35˚C. This method is found to be robust with change in the flow rate, temperature and pH and there is no change in the system suitability parameters without any change in tailing factor and resolution.

Stress Degradation Studies:

According to ICH guidelines the stress degradation studies were performed Q1A (R2). Using a validated analytical method the stability studies of Nimesulide product were carried out.

Acid degradation studies:

The API and placebo was subjected to Reflux with 5N HCl solution for about 14 hrs. Following the 10µl of sample injected.

Basic Degradation Studies / Alkali Degradation Studies:

The API and placebo was subjected to Reflux with 0.5N NaOH solution for about 10 min. Following the 10µl of sample injected.

Oxidation Degradation Studies: The API and placebo was subjected to Reflux with 15% Hydrogen peroxide for about 15 min. Following the 10µl of sample injected.

Thermal Degradation Studies: The API and placebo was subjected to dry heated at 105 °C for about 48 hrs. Following the 10µl of sample injected. In the Forced degradation studies it was found to be peak purity was found to be meeting the acceptance criteria of mass balance. As it being a rock stable molecule no significant degradation was obtained. No purity flag was detected, hence peak purity was found to be passed and Purity angle was obtained less than the purity threshold. Results were given in Table 6.

Table 1: Nimesulide and its impurities retention times

S.No	Name	Retention Time (min)	Peak Area
1	Nimesulide	4.665	98502
2	Impurity C	11.494	144982
3	Impurity D	16.041	99423

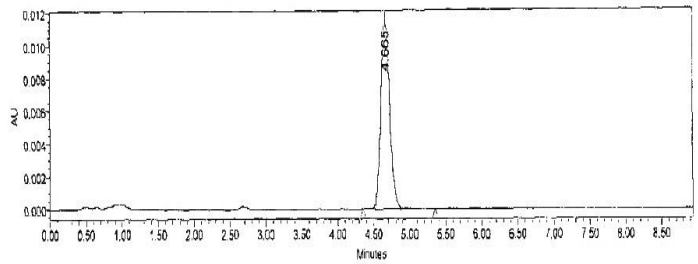

Fig 2 (a): Chromatogram of Nimesulide diluted standard in X Terra RP8.

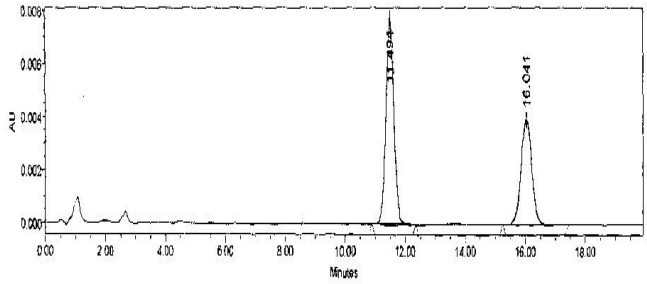

Fig 2 (b): Chromatogram of Nimesulide related compounds in X Terra RP8.

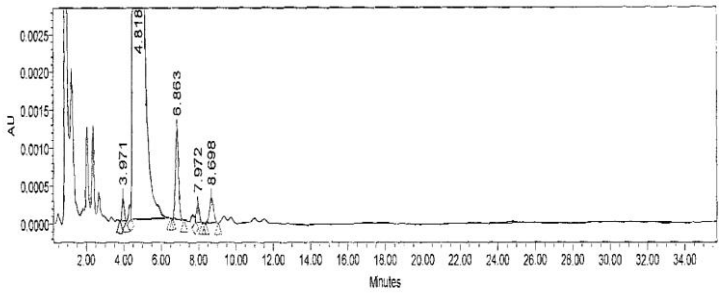

Fig 2 (c) Chromatogram of sample solution

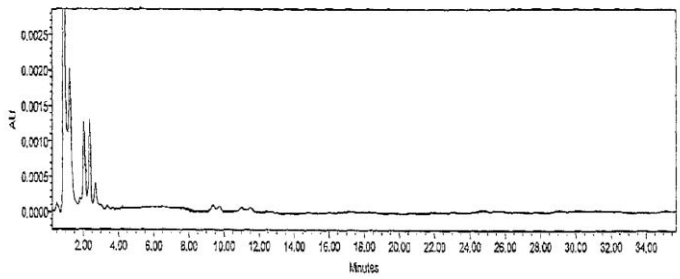

Fig. 3: Placebo chromatogram

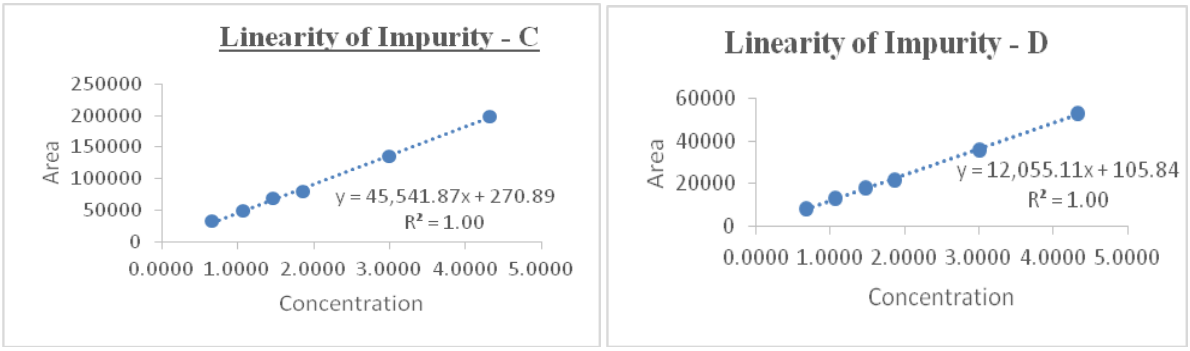

Fig 4: Linearity of Nimesulide impurity-C and impurity-D

Table: 2.Linearity data for Nimesulide related impurities

Component	Correlation Coefficient	Slope	y-Intercept	Bias
Impurity - C	0.999	45542	270.9	0.3
Impurity - D	1	12055	105.8	0.5

Table 3: Precision studies of Nimesulide related compounds

S.No	Impurity Name	% Recovery	% RSD from 6 injections
1	Impurity - C	100	1.1
2	Impurity - D	87	2.0

Table 4: % Recovery studies at 200%

S.No	Impurity Name	% Recovery
1	Impurity - C	101
2	Impurity - D	87

Table 5: Limit of Detection and Limit of Quantification

S.No	Peak Name	Conc. (%w/w)		LOD S/N Ratio	LOQ S/N Ratio
		LOD	LOQ		
1	Impurity - C	0.012	0.04	3	10
2	Impurity - D	0.015	0.05	3	10

Table 6: Table for forced degradation studies

Stress Condition	Nimesulide			
	% Assay	% Impurity	Mass Balance (NLT 95.0)	Purity Flag
As such sample	94.75	NA	NA	NO
Refluxed with 5N HCl solution for about 14 hrs	93.38	1.73	100.4	NO
Refluxed with 0.5N NaOH solution for about 10 min	93.75	1.00	100.0	NO
Refluxed with 15% Hydrogen peroxide for about 15 min.	94.02	0.8	100.1	NO
Photolytic Degradation	94.02	0.8	100.1	NO

CONCLUSION

The method which is developed has been validated for its accuracy, precision, linearity, detection limit, quantitation limit, robustness and stress degradation studies for the process related impurities Impurity-C and impurity-D of Nimesulide and according to ICH guidelines. From the above results, as the sample contains no impurities and even if present they can be quantified with the developed method. It was concluded that the RP-HPLC method for Nimesulide and its related Substance was precise, accurate, rapid, specific and economical.

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