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## Development and Validation of a Gas Chromatographic Method for the Assay of Nimesulide

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A simple, rapid, precise and accurate gas liquid chromatographic method was proposed for the determination of nimesulide in pure and pharmaceutical preparations using flame ionization detection. Ibuprofen was used as an internal standard. The separation was performed on a 3 % OV-101 stainless steel column of 6 feet length with an internal diameter of 2 mm. Nitrogen was used as a carrier gas at a flow rate of 1.75 kg/cm<sup>2</sup>.min. The column was maintained at 270° while that of injection port and detector were maintained at 300°. The procedure gave a linear response in the concentration range of 1.5-16.0 mg/ml with sufficient reproducibility. The system suitability parameters were studied throughout the study. The method has been applied successfully for the assay of nimesulide in pharmaceutical formulations. The recovery values were found to be in the range of 99.31-100.13% with RSD values less than 0.53%.

Nimesulide (NIM) is a non-steroidal antiinflammatory drug (NSAID) with antiinflammatory, antipyretic and analgesic properties<sup>1</sup>. NIM seems to cause less severe gastrointestinal side effect compared with other NSAIDs and aspirin<sup>2</sup>. The literature survey revealed HPLC methods<sup>3-9</sup> and a thin layer chromatographic method<sup>10</sup> for the determination of NIM in biological fluids. Recently, a GC method<sup>1</sup> has been reported for the determination of NIM in pharmaceutical formulations. But, it is applicable at higher concentrations of the drug and requires more time for analysis. Hence, it was considered necessary to develop and validate a simple, specific, rapid and accurate GC method for the determination of NIM in pure form as well as from pharmaceutical formulations.

### MATERIAL AND METHODS

A nucon-5700 GC (AIMIL India, Ltd., New Delhi) with a dual flame ionization detector (FID) was used in the study. The stationary phase was 3% OV-101 (60-80 mesh) packed into a steel column of 6 feet in length and internal diameter of 2 mm. Nitrogen was used as a carrier gas at a flow rate

of 1.75 kg/cm<sup>2</sup>.min. The column was maintained at 270° while that of injection port and detector were maintained at 300°. All chemicals used were of analytical or pharmaceutical grade. Pure NIM and ibuprofen (IBF) were received as gift samples from Dr. Reddy's laboratory, Hyderabad. HPLC grade acetonitrile was used in the study.

Standard solutions of NIM and IBF were prepared separately by dissolving 1.5 g of NIM and 1.0 g of IBF in acetonitrile and diluting up to the mark in 50 ml volumetric flasks with the same solvent to give a concentration of 30 mg/ml of NIM and 20 mg/ml of IBF. These solutions were stored in amber colored bottles.

### Standard assay procedure:

Suitable amounts of aliquots of standard NIM were transferred into a series of 5 ml calibrated flasks. To these were added a fixed amount of IBF (5 mg/ml), made up to the volume with acetonitrile and mixed well. Then 2 µl of the solution was injected and a chromatogram was noted. A typical chromatogram was shown in fig. 1. The retention time of NIM and IBF are respectively 2.2 min and 0.38 min. The ratio of peak areas of NIM to that of the internal standard was calculated and plotted against the concentration of NIM

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to obtain a calibration graph. Through out the study, the suitability of the chromatographic system was monitored by calculating the capacity factor ( $k'$ ), the resolution ( $R$ ), the selectivity ( $\alpha$ ) and the peak asymmetry ( $T$ ).

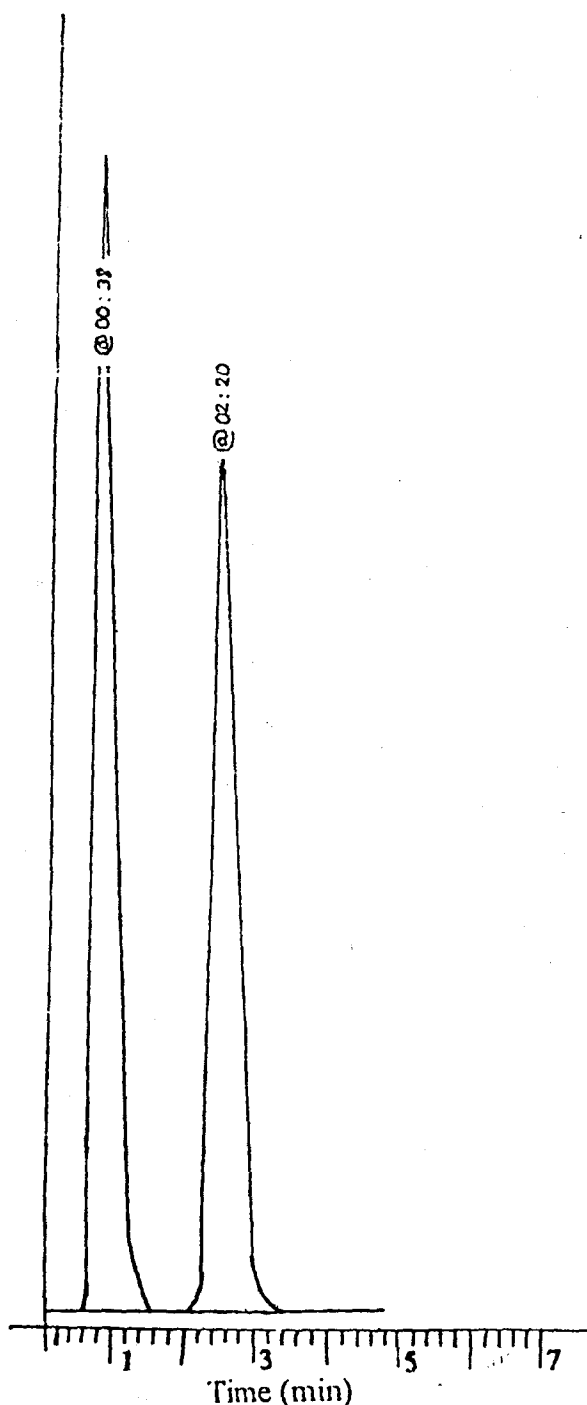


Fig. 1: Chromatogram of Nimesulide and Ibuprofen in pure form.

#### Assay procedure for tablets:

Twenty tablets of NIM were finely powdered. An amount of powder containing the equivalent of about 100 mg of NIM was weighed accurately and transferred into a 100 ml beaker. Using a mechanical stirrer the powder was completely disintegrated in acetonitrile. The solution was filtered and the filtrate was made up to 50 ml with the same solvent. An aliquot of the drug solution was analyzed as described earlier.

#### RESULTS AND DISCUSSION

Optimum conditions, which are necessary for the quantitative determination of the drug with maximum sensitivity, were established by a number of preliminary experiments. Optimum conditions were fixed by varying one parameter at a time fixing other parameters constant and observing its effect on the response factor and also on the peak resolution. Solvents like methanol, butanol, acetonitrile, hexane and n-heptane were tried as the extracting and injecting solvents. But satisfactory resolution and recoveries were observed only in acetonitrile. Moreover, the retention times of both NIM and IBF were increased in other solvents. Hence, acetonitrile was selected as the extracting and injecting solvent. The choice of IBF as an internal standard was based on the proper resolution, less time for analysis and chromatographic behavior (ideal retention time). After evaluating the various stationary phases such as 10 % OV-101, 5 % OV-101, 3 % OV-101 and 10 % OV-210, the stationary phase of 3 % OV-101 was selected as an ideal column for efficient separation of the component with good peak shapes. The effects of nitrogen flow rates were examined for recording chromatograms. The nitrogen flow rate of 1.75 kg/cm<sup>2</sup>.min was selected because of its ideal retention time, proper resolution and less time for analysis. The oven temperature was varied from 200-300° and finally it was fixed at 270° due to good resolution, shorter time of analysis, sharp peaks and ideal retention time. The injector and detector temperature were maintained at 300° throughout the analysis for sharp peaks and ideal chromatographic behavior. Under these optimum conditions, we have observed good resolution between standard and internal standard peak.

Linearity and range of the method were evaluated by analyzing 10 different concentrations ( $n=5$ ) of the mixed standard containing 1-17 mg/ml of NIM and 5 mg/ml of IBF under the chromatographic conditions mentioned above. The calibration curve was plotted and it was found to be linear over the concentration range of 1.5-16 mg/ml. The data were

analyzed by linear regression least-squares method. The calibration graph shows negligible intercept and is described by the calibration equation  $y=a+bx$ , where  $y$  is the peak area, ' $b$ ' is the slope, ' $a$ ' is the intercept and ' $x$ ' is the concentration of the analyte. 'Relative standard deviation' ( $\% \pm$  RSD) values of ' $b$ ' and ' $a$ ' are found to be 0.76 and 0.78, respectively. To calculate limit of detection (LOD) and limit of quantification (LOQ), signal to noise ratio of 3:1 and 10:1, respectively were used. Linear regression least squares fit data are given in Table 1.

Chromatographic parameters such as resolution, selectivity and peak asymmetry were satisfactory for the selected drugs (Table 2). The calculated resolution values between each peak-pair were not less than 9.0 and the selectivity were not less than 7.0. Capacity factors ( $k'$ ) were

TABLE 1: LINEAR REGRESSION LEAST SQUARES FIT DATA FOR THE ASSAY OF NIM

Parameters	NIM
Linear dynamic range (mg/ml)	1.5-16
Regression equation ( $Y^a$ )	
Slope ( $b$ )	0.198
Intercept ( $a$ )	0.019
Standard deviation of the slope ( $S_b$ )	0.0015
Standard deviation of the Intercept ( $S_a$ )	0.00016
$\% \pm$ RSD of $b$	0.76
$\% \pm$ RSD of $a$	0.78
Correlation coefficient	0.9991
LOQ (mg/ml)	0.423
LOD (mg/ml)	0.251

TABLE 2: SYSTEM PERFORMANCE PARAMETERS OF IBF AND NIM

Parameters	IBF	NIM
Retention time $t_r$	0.38	2.2
Capacity factor ( $k$ )	1.11	7.81
Peak asymmetry ( $A_s$ )	1.40	1.25
Selectivity factor ( $\alpha$ )	-	7.027
Resolution ( $R$ )	-	9.86

TABLE 3: ANALYSIS OF NIM IN PHARMACEUTICAL FORMULATIONS

NIM formulations, (mg/tablet)	NIM found* (mg/tablet)	Relative standard deviation (%)	% Recovery
Tablets			
Nise <sup>1</sup> (100)	101.07	0.53	99.31
NIMUTAB <sup>2</sup> (100)	99.27	0.36	99.89
N-LIDOT <sup>3</sup> (100)	100.89	0.44	100.13
NIMFAST <sup>4</sup> (100)	99.19	0.40	99.68

\*Average of five determinations. Marketed by 1, Dr. Reddy's Labs Ltd., 2, Centaur Lab. Pvt. Ltd., 3, Orchid Health Care, and 4, Indan Ltd.

found to be 1.11 and 7.81 for IBF and NIM, respectively. Analytical recovery of NIM from pharmaceutical formulations was carried out at different concentrations. The supernatant solution was injected and chromatogram was recorded. Peak height ratios of NIM to IBF were compared with the peak height ratio obtained by the injection of the drug. The results of analysis of pharmaceutical formulations presented in Table 3 are in good agreement with labeled values.

Analyzing five replicates of fixed amount of NIM checked precision and accuracy of the proposed method. The precision of the method was calculated in terms of the relative standard deviation. Low values of relative standard deviation (0.53%) and percentage errors at 95% confidence limits (0.79) indicated high precision and accuracy of the proposed method. In order to study the selectivity of the method, the interference of commonly associated excipients in the determination of NIM was carried out. It was observed that none of the excipients interfered in the determination as evident from the similar retention times of NIM and internal standard.

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