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Determination of Nimesulide in Pharmaceutical formulations and in Human serum by Reverse-Phase High-Performance Liquid Chromatography

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Abstract: A simple, selective, accurate reversed-phase high performance liquid chromatographic (RP-HPLC) method was determination of Nimesulide. Chromatographic separation achieved isocratically on a C_{18} column [Use ACE 5 C_{18},4µm , 250 mm x 4.0 mm] utilizing a mobile phase of acetonitrile-triethylamine (TEA)-water (50:0.5:49.5, v/v/v), adjusted to pH 5.0 with formic acid, at a flow rate of 1.0 ml/min with UV detection at 230 nm. The retention time of nimesulide was 7.545 min. The method is accurate (99.15-101.85%), precise (intra-day variation 0.13-1.60% and inter-day variation 0.30-1.70%) and linear within range 0.1-30µg/ml (R^2=0.999) concentration and was successfully used in monitoring left over drug. The detection limit of nimesulide at a signal-to-noise ratio of 3 was 1.70ng/ml in human serum while quantification limit in human serum was 5.80 ng/ml. The proposed method is applicable to routine analysis of nimesulide in pharmaceutical formulations as well as in human serum samples.

Keywords: Nimesulide, RP-HPLC, Validation, Human blood samples, Pharmaceutical dosage forms.

Introduction

Nimesulide is an anti-inflammatory drug. Chemically, nimesulide is N-(4-nitro-2-phenoxyphenyl) methane sulphonamide.It is approved for used in treatment of musculoskeletal disorder, dyemenorrhoea, thrombophlebitis and dental pain, inflammation. Some HPLC[1, 2] and spectrophotometric[3, 4], methods have been reported in the literature for its estimation. This compound, a derivative of p-nitrophenylmethane-sulfonamide, is structurally a unique nonsteroidal anti-inflammatory drug. It belongs to selective COX-2 inhibitors, with a potent anti-inflammatory and analgesic activity, when administered orally, rectally, or topically[5]. Due to its analgesic and antipyretic properties, nimesulide is widely used for the treatment of various inflammatory processes[6]. Besides it is better tolerated and causes fewer adverse effects than other currently used non-steroidal anti-inflammatory drugs[7].

Experimental

Materials and Chemicals

Nimesulide working standard obtained from Local aboratories. For the estimation of Nimesulide in bulk and commercial formulations of nimesulide brand (Nile-100mg, Intra laboratories), 20 tablets were obtained from retail pharmacies. Each tablet was labeled contain 100 mg of nimesulide and had an expiry of not less than 365 days at the time of study. HPLC grade triethylamine (TEA), acetonitrile and formic acid (98-100%) of analytical purity were procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system.

Instrumentation

A High Performance Liquid Chromatograph system, with LC solutions data handling system (Shimadzu-LC2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The purity determination performed on a stainless steel column 250 mm long, 4.0mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 4µm diameter (ACE 5 C_{18}, 4µm, 250 mm x 4.0 mm). The optimized chromatographic conditions were listed in Table 1.

Standard solutions

Nimesulide was powdered finely. A quantity equivalent to 25 mg was transferred into a 25 ml volumetric flask and dissolved in 10 ml of methanol. The volume was then diluted with the mobile phase and filtered through Whatman No. 1 filter paper. One milliliter of the

resulting solution was then diluted to 10 ml with mobile phase. From this 1,2and 3 ml samples were taken and their volume was made up to 10 ml each. A chromatogram of these solutions was obtained by imjecting 20 µl of each sample into the chromatographic system. All solutions were freshly prepared before the analysis.

Method validation

The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, system suitability parameters were also calculated. To demonstrate specificity in the presence of excipients used in formulation, nimesulide was spiked (at approximately 25 µg/ml) in drug product, chromatogram was observed and compared with that of raw material. To evaluate the linearity, the LOD and LOQ of the method in reference drug and in serum, different serial dilutions (0.0980, 0.195, 0.80, 1.50, 3.10, 6.20, 12.50 and 25 µg/ml) were prepared from the standard stock solutions in 25 ml volumetric flasks and volume made up with diluent which is mobile phase. The samples were injected (10 µl) and signals from the samples were recorded at 7.01 minute which were compared with those of blank. LOD and LOQ values were calculated as signal-to-noise ratio of 3:1 and 10:1 respectively. To determine accuracy of the method, working standard of nimesulide was prepared in triplicate at three concentration levels (10, 20 and 25 µg/ml) and analyzed. Repeatability of the method was checked by analyzing six replicate samples of nimesulide (at the 100% concentration level) and calculating relative standard deviation (%RSD). To determine intermediate precision, standard solutions of nimesulide at eight concentration levels were analyzed three times within the same day (intra-day variation) and three other days (inter-day variation).

Assay in formulations

In case of marketed formulations, five accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10 mg of nimesulide was added into different 100 ml volumetric flasks and volume was made up with acetonitrile and methanol mixture. The samples were filtered through a 0.45-µm-membrane filter; different serial dilutions (3.10, 6.20, 12.40, 25µg/ml) were made from this solution in 25 ml volumetric flask and were injected for HPLC analysis.

Assay in serum

One volume of plasma was de-proteinated by nine volume of acetonitrile and filtered through 0.45 µm Millipore filter paper that was used to make serial dilution of nimesulide (0.0980 µg/ml to 25 µg/ml). Three replicates of each dilution were injected to HPLC system and linearity was evaluated. Repeatability of the method was checked by analyzing six replicate samples of nimesulide (at the 100% concentration level) and calculating relative standard deviation (%RSD).

Results and discussion

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996][8] and [USP 2002][9] have recommended the accomplishment of accuracy tests, precision, specificity, linearity of the method

System suitability

The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation.

The system suitability parameters including capacity factor >2, resolution>3 and asymmetric factor<2. All parameters were satisfactory with good specificity for the stability assessment of nimesulide. Theoretical plates of the column were >3000.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to true value. In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (i.e.,"to spike"). In our studies, the later technique was adopted and nimesulide was spiked in drug product. The result of accuracy given in (Table-2) revealed that the method was found accurate for all above purposes.

Precision

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The method passed the test for repeatability as determined by %RSD of the area of the peaks of six replicate injections at 100% test concentration. The results of intra-and inter-day variation are shown in (Table 3).

Range and Linearity

The linearity of an analytical method is its ability to elict test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed with in the

expected concentration range demonstrating its suitability for analysis. The correlation coefficient (r^2) was found to be 0.999 and value of intercept was less than 25 of the response of 100% of the test concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak

Table 1: Optimized Chromatographic conditions

Parameter	Optimized condition
Chromatograph	Shimadzu-HPLC
Column	ACE 5 C_{18}, 4µm, 250 mm x 4.0 mm
Mobile phase*	Acetonitrile-triethylamine (TEA)-Water (50:5:49.5, v/v/v), adjusted to pH 5.0 with formic acid,
Flow rate	1.0ml/min
Detection	UV at 230 nm
Injection volume	20µl
Temperature	Ambient
Reaction time-Nimesulide	7.545 min

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated

Table 2: Accuracy/recovery of Nimesulide

Parameters	Conc (µg/ml)	% Recovery	% RSD
Assay	10	96.02	1.70
(Spiking method)	20	101.54	4.80
	25	95.15	4.60
Assay	6.20	99.18	0.5
	12.40	100	1.5
	25	99.99	0.3
Assay (in serum)	12.40	100	0.5
	6.20	100	1.2
	3.10	100	0.7

Table 3: Intermediate precision of the method

Concentration (µg/ml)	Assay in formulation		Serum
	Intra-day variation (%RSD)	Inter-day variation (%RSD)	Intra-day variation (%RSD)
0.0980	0.13	0.94	4.15
0.195	0.35	0.33	0.08
0.80	0.40	1.70	2.70
1.50	0.85	1.08	3.20
3.10	0.26	0.20	1.15
12.5	1.65	0.70	0.40
25	0.25	1.08	3.30

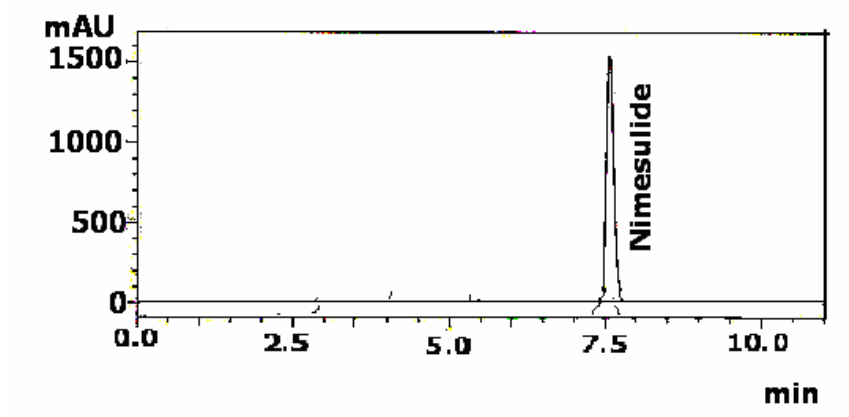

Figure 1. Chromatogram of Nimesulide

Limits of Detection and Quantitation

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1 (ICH Q2B guidelines, 1997, FDA, Guidance for Industry 2000)[10, 11]. The lower limit of detection for nimesulide is 2.40ng/ml in reference material and formulation and 1.70ng/ml serum. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOQ values were found to be 8.15ng/ml for raw material, formulations and 5.80ng/ml for serum.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Figure-1). The excipiants used in different formulation products did not interfere with the drug peak and thus, the method is specific for Nimesulide. To further confirm the specificity of the method, UV scans of spiked drug were taken in the range 200-400nm and no significant change was found by comparing the absorbance of pure drug and spiked drug at the analytical wavelength of drug.

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