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Ravshankhon Makhmudov
Drug Development Department,
Nika Pharm Co., Tashkent,
Republic of Uzbekistan

Furkat Yunusov
Tashkent State Technical
University, State Unitary
Enterprise Science end Progress,
Tashkent, Uzbekistan

Simple and rapid RP-HPLC determination of nimesulide in tablets

Ravshankhon Makhmudov and Furkat Yunusov

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Abstract
The present investigation describes a fast and simplified RP-HPLC method for the evaluation of nimesulide in tablets. The method is specific, accurate, precise, and ensures good reproducibility. The sample was analyzed using Acetonitrile (ACN): Ethanol: Water in the ratio of 40:40:20 as a mobile phase at a flow rate of 1.0 ml/min and detection at 276 nm. The retention time for nimesulide was found to be about 3.4, and a recovery from tablet was 99%. The method can be used for the fast and simple quantitative estimation of nimesulide in tablets.

Keywords: Chromatography, HPLC, Nimesulide, Dysmenorrhea

1. Introduction
Nimesulide is a non-selective non-steroidal anti-inflammatory drug (NSAID). It is used to treat acute (short-term) pain, painful osteoarthritis (swelling in the joints), primary dysmenorrhea (period pains). Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis, and primary dysmenorrhea in adolescents and adults above 12 years old [1-3]. The IUPAC name of nimesulide is N-(4-nitro-2-phenoxyphenyl)-methanesulphonamide (Fig. 1).
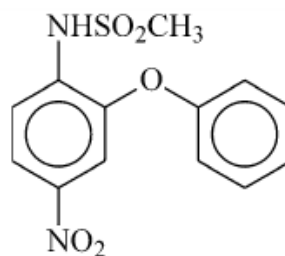

Fig 1: Chemical structure of nimesulide

There have been published several articles to estimate the quantity of nimesulide using HPLC [4-6], spectrophotometric [7, 8] and IR methods [9], as well as dissolution profile, validation of analytical methods for capsules having nimesulide [10].
Our goal of present investigation was to find a proper and environmentally safe eluent as well as to decrease the retention time of nimesulide peak in HPLC.

2. Instrumentation
The modular Shimadzu Nexera2 HPLC system is comprised of the following parts, each of those is produced by Shimadzu USA Manufacturing, Inc.: an SIL-30AC autosampler, an LC-30AD liquid chromatograph, a CTO-30A column oven; a binary pump; a SPD-20AV prominence UV–Vis detector; a DGU-20A5R degassing unit and a CMB-20A communications bus module. The chromatographic parameters (peak areas, retention times, theoretical plates etc.) were recorded and calculated via Shimadzu LabSolutions v.5.73 software running under MS Windows 7. The HPLC experiments were performed on a stainless-steel column 150 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles (Inert Sustain C18, 5µ, 250 mm x 4.6 mm, Japan). The chromatographic conditions are listed in Table 1 below.

Corresponding Author:
Ravshankhon Makhmudov
Drug Development Department,
Nika Pharm Co., Tashkent,
Republic of Uzbekistan

Table 1: Chromatographic conditions

Parameters	Chromatographic conditions
Chromatograph	Shimadzu Nexera2 HPLC
Column	InertSustain C18, 5µ, 250 mm x 4.6 mm, Japan
Mobile phase	ACN/Ethanol/Water
Flow rate	1.0 ml/min

3. Experimental
3.1 Chemicals and Materials
A pure sample of nimesulide was purchased from Aarti Drugs Limited (India). The HPLC grade orthophosphoric acid, acetonitrile and ethanol purchased from VWR Chemical (France). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

3.2 Preparation of standard stock solution
The standard stock solution of 2 mg/ml of nimesulide was prepared by dissolving 100 mg of the working standard of nimesulide in 35 ml of 96% ethanol, treated by an ultrasonic for 15 min, then the solution was adjusted to the volume of 50 ml with 96% ethanol.
The top of this paragraph illustrates a sub-subheading.

3.3 Preparation of test solution
378 mg of powder prepared of 20 tablets containing 100 mg of nimesulide was accurately weighed and placed into a 50 ml volumetric flask, then 35 ml of 96% ethanol was added, treated by ultrasonic for 15 min, the solution volume was adjusted to 50 ml with 96% ethanol, to get a concentration of 2 mg/ml of nimesulide.
Prior to injection all solutions were filtered through 0.45 µ membrane filters (Whatman). 3 µl of the solution was injected into HPLC system to obtain the chromatograms for the standard drug solution (five replicates) and the sample solution (five replicates). The concentrations of nimesulide in the formulation were calculated by comparing the AUC of the sample with that of the standard.

3.4 HPLC Procedure
All solutions of samples and standard (3µl) were injected by means of an autosampler and detected at 276 nm. ACN/Ethanol/Water (40/40/20) was used as the mobile phase while the flow rate was set at 1.0 ml/min at room temperature. Under the above-mentioned conditions, the analysis cycle was completed in 5 min.

4. Results
4.1 Validation of the HPLC Method
The fast and precise estimation of nimesulide in tablets by HPLC method was validated for linearity, precision (repeatability and reproducibility), and accuracy.

4.2 Linearity
The linearity and range of the method was determined by analyzing five different concentrations of standard solution ranging from 1.6 – 2.4 mg/ml. The calibration curve was plotted using the peak area versus the concentration of standard solution and correlation coefficient and regression line equation was determined. The linear plot of nimesulide is given in Fig. 2.
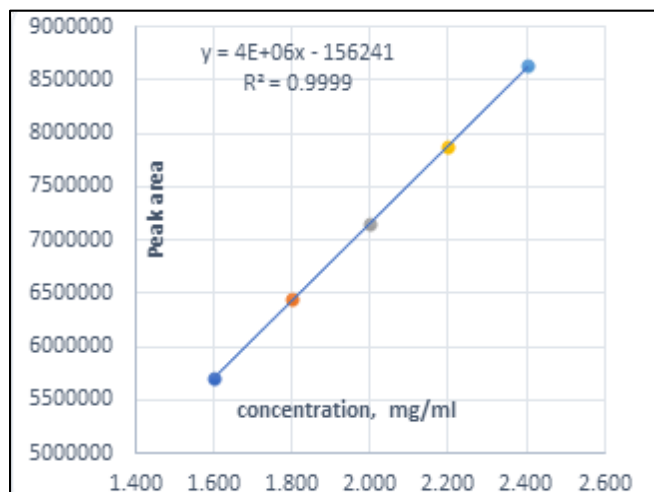

$$y = 4E+06x - 156241$$
$$R^2 = 0.9999$$

Fig 2: Calibration curve of nimesulide at 276 nm

4.3 Accuracy
Accuracy values shows that the estimated values are close to the reference ones prepared in laboratory, since in the range of 80% - 120%, the accuracy of the HPLC method for the quantitative determination of nimesulide was confirmed by appropriate tests of model solutions, based on the calculated results of the recovery rate. The results obtained are within the acceptable range of 98.0% - 102.0%. The average value of the degree of recovery was 100.16% of nimesulide (Table 2). Therefore, it is possible to conclude that these is acceptable for quality control of nimesulide in pharmaceutical formulation.

Table 2: The average value of the degree of recovery was 100.16% of nimesulide

Active compound	№	Quantity entered [mg] and [% of theoretical stated]		Detected amount, mg	Recovery rate, %
Nimesulide	1	80	80%	79.76	99.70
	2	80	80%	80.17	100.21
	3	80	80%	80.03	100.04
	4	100	100%	100.24	100.24
	5	100	100%	100.53	100.53
	6	100	100%	100.40	100.40
	7	120	120%	120.34	100.28
	8	120	120%	119.83	99.86
	9	120	120%	120.23	100.19
Average recovery rate [%]					100.16
95% confidence interval					100,16 ±0,16
Standard deviation					0.26
RSD [%]					0.26

4.4 Convergence

The convergence of the method was studied twice in two days by using six determinations at 100% test concentration, i.e. mixture of 2 mg/ml of nimesulide. The results are given in Table 3.

Table 3: The results of convergence study

Active compound	S. №	The quantitative content of the model drug from 100% of theoretical one	
		1st day	2nd day
Nimesulide	1	100.66	100.88
	2	100.74	100.32
	3	100.44	101.46
	4	99.74	101.18
	5	99.96	101.67
	6	99.83	101.38
Average		100.23	101.15
Standard deviation		0.44	0.49
RSD [%]		0.438	0.481
95% confidence interval		100,23 ±0,357	101,15 ±0,39

The obtained test results of nimesulide, performed by six determinations on different days, reveals that the relative standard deviations of the RSD are 0.438% and 0.481%, which indicates the acceptable degree of convergence of the quantitative content of model preparations (Fig 3).
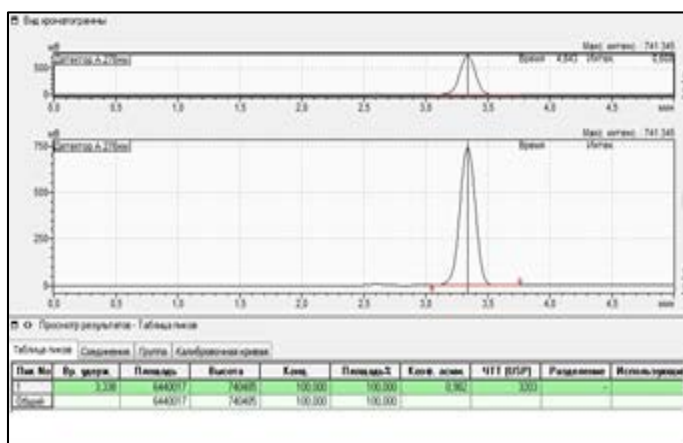

Fig 3: Chromatogram of nimesulide

5. Conclusions

The following validation characteristics have been confirmed for the validation of the quantitative determination method for nimesulide:

1. Specificity
2. Accuracy
3. Linearity
4. Convergence

Their compliance with the established acceptance criteria was experimentally proved.

These analytical methods should be considered validated, they correspond to the intended purpose: to determine the authenticity and quantitative determination of nimesulide. They can be used for quality control of this medicinal product.

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