Journal of Analytical Chemistry, Vol. 58, No. 8, 2003, pp. 778–780. From Zhurnal Analiticheskoi Khimii, Vol. 58, No. 8, 2003, pp. 873–875.

ARTICLES

High-Performance Liquid Chromatographic Method for the Determination of Nimesulide in Pharmaceutical Preparations[1]

B. S. Nagaralli, J. Seetharamappa, B. G. Gowda, and M. B. Melwanki

Department of Chemistry, Karnatak University, Dharwad–580003, India

Received July 12, 2002

Abstract—A simple, rapid, and precise high-performance liquid chromatographic method for the determination of nimesulide in pharmaceutical preparations was proposed using Ibuprofen as an internal standard. The separation was performed on a CLC C_{18} (5 μm, 25 cm × 4.6 mm i.d.) column with a mobile phase consisting of an acetonitrile–0.05 M KH_2PO_4 buffer mixture of pH 7.00 (55 : 45, v/v). The detection was carried out at 230 nm and the linearity range was found to be 0.5–100 μg/mL. The method has been applied successfully to the determination of nimesulide in pharmaceutical formulations. The recovery values were found to be in the range of 99.23–100.13% with RSD values of less than 0.97%.

Nimesulide (NIM) is a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, antipyretic, and analgesic properties. NIM seems to cause less severe gastrointestinal side effects than do other NSAIDs and aspirin [1]. In view of its importance, several HPLC methods have been reported for the determination of NIM in biological fluids [2–8]. However, a critical literature survey indicated that no attempt has been made so far to assay NIM in a single-dosage form by HPLC using UV detection. Hence, it was considered worthwhile to develop a simple and rapid reversed-phase HPLC method for the determination of NIM in pharmaceutical formulations.

EXPERIMENTAL

Reagents. All chemicals used were of analytical or pharmaceutical grade. Acetonitrile (HPLC grade) and dihydrogen orthophosphate (AR grade) supplied by S.D. Fine-Chem, Ltd., India, and Merck (India) Ltd., respectively, were used. NIM and ibuprofen (IBF) were received as gift samples from Dr. Reddy's laboratory, India.

Preparation of solutions. (1) Standard solution: A stock solution of NIM was prepared by dissolving 100 mg of NIM in acetonitrile in a 100-mL calibrated flask.

(2) Internal standard solution: A stock solution of IBF was prepared by dissolving 150 mg of IBF in acetonitrile in a 100-mL calibrated flask. The stock solution of NIM was diluted with acetonitrile as and when required.

Instruments and chromatographic conditions. All HPLC measurements were made on a Shimadzu Corporation system (Analytical Instruments Division, Kyoto, Japan). The system consists of an LC10AT solvent pump, an SPD10AVP detector, and a data station with Win-chrome software, version 3.1. The separation was performed on a CLC C_{18} column (5 μm, 25 cm × 4.6 mm i.d.). A CLC ODS (4 cm × 4.6 mm, i.d.) was used as a guard column to protect the analytical column. A mixture of acetonitrile and 0.05 M KH_2PO_4 buffer of pH 7.00 [55 : 45, v/v] was used as a mobile phase at a flow rate of 1 mL/min with an operating pressure of 131–133 kg/cm^2. A Hamilton 702 μLR injector with a 25 μL loop was used for the injection of the samples. Detection was performed at 230 nm with a sensitivity of 0.22 AUFS. The mobile phase was filtered through a 0.45 μm Millipore membrane filter and degassed. The separation was carried out at room temperature.

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 the internal standard (IBF), diluted to volume with acetonitrile, and mixed well. Then, 20 μL of the solution was injected and a chromatogram was noted. A typical chromatogram is shown in Fig 1. A calibration graph was plotted.

Analysis of tablets. Twenty tablets of NIM were weighed and finely powdered and an amount equivalent to 100 mg of NIM was dissolved in acetonitrile and filtered. After keeping for 5 min in an ultrasonic, the solu-

[1] This article was submitted by authors in English.

1061-9348/03/5808-0778$25.00 © 2003 MAIK "Nauka / Interperiodica"

tion was diluted to volume, filtered through 0.45 μm Millipore membrane filter, and degassed. A 20 μL solution was then injected into chromatographic system.

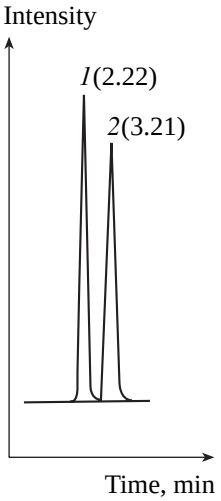
RESULTS AMD DISCUSSION

Method development. The mobile phase was chosen after several trials with methanol, acetonitrile, water, and buffer in various proportions and at different pH values. A mobile phase consisting of acetonitrile and 0.05 M 0.05 KH_2PO_4 (55 : 45, v/v) of pH 7.00 was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 2.0 mL/min were tried. It was observed that a flow rate of 1.0 mL/min gave an optimal signal-to-noise ratio with a reasonable separation time. IBF was selected as the internal standard after several trials due to its ideal retention time and reasonable separation.

Using a reverse-phase C_{18} column, the retention time for NIM and IBF was observed to be 2.22 and 3.21 min, respectively. A total time of 4 min was necessary for the analysis. NIM exhibited absorption maxima at 230 nm and 404 nm. However, the 230-nm wavelength was selected for the analysis in order to achieve an ideal retention time and reasonable separation. At 404 nm, IBF was not eluted. Under optimum conditions, the chromatograms of a series of NIM standard solutions were recorded. A computer controlled data station with Win-chrome software was used to plot the peak area versus the concentration in μg/mL.

Analytical features. To set up a linearity range, a series of NIM solutions of different concentrations were prepared in acetonitrile in the range of 0.5–100 μg/mL. The reproducibility of the detector response at each concentration level was examined by carrying out the experiment in triplicate. A typical chromatogram is shown in figure. A calibration curve was obtained by plotting the peak area ratio of the standard to the internal standard as a function of the NIM concentration. Further, NIM showed linearity in the concentration range of 0.5–100 μg/mL. The minimum detectable concentration of NIM under the optimized conditions was found to be 0.25 μg/mL. Precision of the method was studied with 5 replicates of the standard solution. The results are shown in Table 1. The low values of the relative standard deviation indicate the high precision of the method.

Recovery studies. To study the accuracy and reproducibility of the proposed method, recovery experiments were carried out. The recovery of the added standard was studied at five different levels. Each level was repeated five times. To an aliquot of the analyzed preparations, a known concentration of the standard solution was added. The NIM content was once again determined by the proposed method. From the amount of the drug present, the percent recovery was calculated using



The chromatogram of Nimesulide (*1*) and Ibuprofen (*2*) in pure form.

Table 1. Analysis of the formulations containing NIM

Drug	Amount mg/tablet		RSD, %
	Labeled	Found*	
Tablet			
NISE[1]	100	100.17	1.006
NIMUTAB[2]	100	100.31	0.814
N-LIDOT[3]	100	100.21	0.789
NIMFAST[4]	100	99.97	1.01
NIMBID[5]	100	99.92	0.960
NOVOGESIC[6]	100	100.27	1.03
NIMODOL[7]	100	99.94	0.913

Notes: Marketed by [1] Reddy's, [2] Centaur, [3] Orchid, [4] Indan, [5] Astra IDL, [6] Glen mark, and [7] Aristo.

*Average of six determinations.

Table 2. Results of the recovery analysis

Drug name	Amount label claims mg/tab	Amount of standard added mg/tab	Total amount recovered mg/tab	% Recovery
NIM	100	0.0	100.04	99.23
	100	10.0	109.26	100.08
	100	20.0	119.65	99.78
	100	30.0	130.59	99.51
	100	40.0	139.83	100.13

Mean Recovery 99.75%

the following formula:

$$\% \text{ Recovery} = \frac{N(\Sigma xy) - (\Sigma y)(\Sigma x)}{N(\Sigma x^2)(\Sigma x)^2},$$

where, x is the amount of standard drug added, y is the amount of drug found by proposed method, and N is the number of observations. The results obtained are shown in Table 2.

The proposed method gives a good resolution between NIM and internal standard. The total time of analysis was only 4 min. The method is simple, rapid, and does not involves complicated sample preparation. High percent recovery values show that the method is free from interference by the excipients used in the preparations. Hence, the present method could be used for routine quality control.

REFERENCES

1. Bernareggi, A., *Drugs*, 1993, suppl. 46, p. 64.
2. Chang, S.F., Miller, A.M., and Ober, R.E., *J. Pharm. Sci.*, 1977, vol. 66, no. 12, p. 1700.
3. Castoldi, D., Monzani, V., and Tofanetti, O., *J. Chromatogr., B*, 1988, vol. 69, no. 2, p. 413.
4. Jowarowicz, D.J., Jr., Filopowski, M.T., and Boje, K.M.K., *J. Chromatogr., B*, 1999, vol. 723, p. 293.
5. Khaska, G. and Udupa, N., *J. Chromatogr., B*, 1999, vol. 727, p. 241.
6. Carrasco-Portugal, M.C., Granasos-Soto, V., Camacho Vieyra, G.A., Perez-Vrizar, J., and Flores-Morrieto, F.J., *J. Liq. Chromatogr.*, 2000, vol. 23, p. 2237.
7. Ptacek, P., Macek, J., and Klima, J., *J. Chromatogr., B*, 2001, vol. 758, p. 183.
8. Nonzioli, A., Luque, G., and Fernandez, C., *J. High Resolut. Chromatogr.*, 1989, vol. 12, no. 6, p. 413.