Short Communication

HPTLC determination of nimesulide from pharmaceutical dosage forms

Vandana B. Patravale *, Susan D'Souza, Yogeeta Narkar

Department of Chemical Technology, Pharmaceutical Division, University of Mumbai, Nathalal Parikh Marg, Matunga, Mumbai 400 019 India

Abstract

A simple, rapid, reproducible and stability indicating high performance thin-layer chromatographic method for the analysis of Nimesulide both the bulk drug and from pharmaceutical formulations is reported. The mobile phase selected consists of Cyclohexane–Ethylacetate [60:40, v/v]. It gave compact spots both for Nimesulide and its degraded products at Rf values 0.44 and 0.712, respectively. Densitometric analysis of nimesulide was carried out at 295 nm. The calibration curve of Nimesulide in methanol was linear in the range of 100–900 ng. The mean value of correlation coefficient, slope and intercept were 0.9989 ± 0.0011, 504.655 ± 0.0013 and 85331.56 ± 0.0253, respectively. The limits of detection and quantitation were 60 and 100 ng, respectively. The drug content was within the limits ($\pm 5\%$ of the labelled content of the formulations). The recovery of Nimesulide was about 99.5%. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Nimesulide; Nimesulide analysis in pharmaceuticals; High performance thin layer chromatography

1. Introduction

Nimesulide [1–3] [4′-Nitro-2′-phenoxymethane sulphonanilide, Fig. 1] is one of the most potent NSAIDs advocated for use in a variety of inflammatory conditions. It is official only in Martindale [4], the extra pharmacopoeia, 29th edition. Methods of analysis reported for Nimesulide include,

Ultraviolet spectroscopy [5], High speed liquid chromatography [6], Polarography [7] and High Performance Liquid Chromatography [8]. Nimesulide in plasma is reported to be quantified by High Performance Liquid Chromatography [9] and High Performance Thin Layer Chromatography [10]. The present investigation was aimed at developing a stability indicating HPTLC method of analysis for Nimesulide, which could also be used for routine analysis from dosage forms.

High performance Thin Layer Chromatography [HPTLC] is a simple microanalytical separa-

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* Corresponding author. Tel.: + 91-22-4145616; fax: + 91-22-4145614.

E-mail address: vbp@pharma.udct.ernet.in (V.B. Patravale).

tion technique where a large number of samples can be handled at a time. The entire chromatogram is visible for easy inspection. The technique is economical, as the consumption of solvent is low and there is virtually no solvent disposal problem. The uniform particle size [7 μm] of precoated HPTLC plates enables achievement of a greater resolution and an easy reproducible separation. The method of detection does not place any restriction on the choice of the mobile phase.

This paper describes a simple, economic, rapid and stability indicating HPTLC method for the estimation of Nimesulide from bulk as well from pharmaceutical dosage forms.

2. Experimental

2.1. Chemicals and reagents

Nimesulide provided by Nivedita Chemicals Ltd. [Mumbai, India] was used for the analysis. Analytical grade reagents and solvents were purchased from Ranbaxy [New Delhi, India].

2.2. Preparation of the standard solution

A stock solution of Nimesulide [1 mg/ml] was prepared in methanol. A standard solution of 100 μg/ml was used.

2.3. Instrumentation

The samples were spotted on HPTLC aluminium plates[10 × 10 cm] precoated with silica gel 60F$_{254}$ (layer thickness 0.2 mm) using Camag
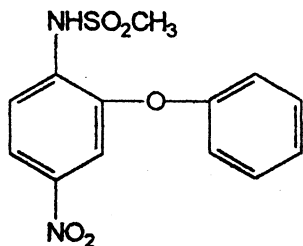

Fig. 1. Structure of Nimesulide.

Linomat IV model. The samples were streaked in the form of narrow bands of length 4 mm, 10 mm from the bottom edge, 5 mm from the margins, 4 mm apart at a constant rate of 10 s/μl using a nitrogen aspiration. Linear ascending development of chromatogram was carried out in a Camag twin trough chamber saturated with the mobile phase. The chromatogram run was 8 cm. The time of run was 15 min. Densitometric analysis was carried out utilizing Camag TLC scanner II in the absorbance mode at 295 nm. The source of radiation was Deuterium lamp. The slit dimensions were 5 × 0.3 mm. Scanning speed was kept at 1 mm/s. Integration was performed by using Camag TLC Scanner/ Integrator system LCI-100.

2.4. Degradation of Nimesulide

Nimesulide was forcibly degraded by refluxing in 5 N HCl and 5 N NaOH in a water bath for 3 h. The resulting solution along with the residue was allowed to cool, neutralized and extracted with chloroform.

2.5. Selection of mobile phase

The following solvent systems were tried in different proportion:
1. Toluene–chloroform–methanol
2. Toluene–chloroform
3. Toluene–ethyl acetate
4. Cyclohexane–chloroform
5. Cyclohexane–ethyl acetate

2.6. Techniques of HPTLC for analysis of Nimesulide

A number of precautions were exercised in addition to the classic rules described by Stahl [11] and Touchstone [12] (constant temperature, saturated chambers, freshly prepared mobile phases and plates without drafts) in order to avoid variations during the HPTLC analysis. The samples were freshly prepared and the analysis was carried out as rapidly as possible. After

spotting, the plates were developed immediately and scanned immediately after development.

2.7. Preparation of Calibration curve

Calibration curve of Nimesulide was prepared using the stock solution [100 μg/ml] of Nimesulide in the concentration range of 100–900 ng. The data of peak area Vs drug concentration was treated by linear least square regression analysis.

2.8. Extraction of drug from the developed and marketed formulation and drug content analysis

Marketed and developed formulations, for both oral and transdermal use were analyzed using this technique.

Ten tablets of the conventional marketed formulation [A: label claim 100 mg per tablet] and 10 capsules of the developed controlled release thermocaps [B: label claim 130 mg per capsule] were crushed separately to a fine powder. Powder equivalent to 100 mg of Nimesulide for the marketed formulation and 130 mg of Nimesulide for the developed formulation was weighed accurately and dissolved in 100 ml of methanol. One milliliter of this solution was diluted to 10 ml with methanol. For the marketed transdermal gel [C: label claim 1% w/w] and the developed microemulsion based gel [D: label claim 1% w/w], formulations equivalent to 10 mg of Nimesulide were accurately weighed and dissolved in 100 ml of methanol. Four microliter of each of these solutions were streaked on plates and analyzed for drug content.

2.9. Recovery studies

As an additional check on the accuracy and precision of the method, recovery experiments were carried out. Recovery experiments were performed by adding three different amounts of standard drug i.e. 25, 50 and 75% of the amount of drug analyzed from the marketed formulations, were added to the marketed formulations and the resultant was reanalyzed.

3. Results and discussion

3.1. Selection of mobile phase

Since no literature report of an HPTLC method of analysis for Nimesulide for routine analysis and for stability studies were available, the selection of the mobile phase was carried out on the basis of polarity. A solvent system that would give dense and compact spots with appropriate and significantly different Rf values for Nimesulide and its degraded product was desired.

Both the pure drug and the acidic and basic degraded products were spotted on TLC plates and run in different solvent systems. Among these, the solvent system Cyclohexane-Ethyl acetate [60:40, v/v] gave compact spots both for Nimesulide and its degraded products with Rf values of 0.44 and 0.712, respectively. Hence for further studies, a combination of Cyclohexane and Ethyl acetate [60:40, v/v] was used as the mobile phase.

The degraded product obtained after acidic and basic degradation had the same Rf values which was higher than that of Nimesulide. Therefore, it was concluded that there was only one degraded product of Nimesulide under acidic and basic conditions.

3.2. Calibration curve of Nimesulide

All the calibration curves prepared [$n = 6$] were linear over the concentration range 100–900 ng.

The reproducible values for slope on repetition, indicated the validity of the technique.

The limits of detection for Nimesulide was 60 ng. The limit of quantitation was 100 ng with coefficient of variation 1.02% [$n = 6$].

3.3. Drug content analysis and recovery studies

The results of drug content of the developed and marketed formulations for oral and transdermal use, by HPTLC, are depicted in Table 1 and that for recovery studies are depicted in Table 2.

The Nimesulide content of the developed and the marketed formulations was found to be well within the limits [$\pm 5\%$ of the theoretical value].

Table 1
Drug content

Formulation	Amount of drug spotted (ng)	Mean concentration of drug found (ng) ± SD	Coefficient of Variation (%)
A	400	401.52 ± 6.05	1.50
B	520	521.01 ± 9.60	1.84
C	400	413.03 ± 9.90	2.39
D	400	402.49 ± 5.32	1.32

Table 2
Recovery studies

Formulation	Amount of drug analyzed (ng)	Amount of drug added (ng)	Theoretical concentration (ng)	Total amount of drug analyzed	% Recovery ± S.D.
A	400	100	500	501.98	100.39 ± 0.83
	400	200	600	601.52	100.25 ± 0.63
	400	300	700	700.82	100.11 ± 0.41
B	400	100	500	514.45	102.89 ± 0.67
	400	200	600	612.12	102.02 ± 0.49
	400	300	700	708.89	101.27 ± 0.60

The higher drug content found for the marketed gel may be because of the high alcohol content [66% w/w] of the formulation. On evaporation of alcohol, the product becomes more concentrated. The recovery of nimesulide was greater than 99.5%. There was no interference from the common excipients present in tablets, capsules and gels.

4. Conclusion

The proposed method is simple, rapid, sensitive, economic and stability indicating for the estimation of Nimesulide from bulk as well as from pharmaceutical dosage forms.

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