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High-performance Thin-layer Chromatography for the Determination of Nimesulide in Human Plasma, and its Use in Pharmacokinetic Studies

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Abstract

A rapid and sensitive high-performance thin-layer chromatographic assay has been developed for the measurement of nimesulide in human plasma. Its use for pharmacokinetic studies has been evaluated.

The method includes a single-stage extraction procedure without the use of an internal standard. Analysis was performed on plasma containing known amounts of the drug, on drug-free plasma, and on plasma containing an unknown quantity of the drug. Known amounts of extract and nimesulide (100 and 200 ng, as external standard) were spotted on precoated silica-gel $60F_{254}$ plates by means of a Camag Linomat IV autosampler. Quantification was achieved using a Camag TLC scanner 3. The recovery of the method was $97{\cdot}10 \pm 2{\cdot}22\%$. The method was applied for the determination of plasma levels and pharmacokinetic parameters of nimesulide after oral administration of two formulations (100 mg) in healthy volunteers.

The method is a sensitive, economical, rapid and specific assay for nimesulide in human plasma, and is suitable for pharmacokinetic studies after therapeutic doses.

Nimesulide (4-nitro-2-phenoxymethanesulphonanilide, Fig. 1), is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties (Biscarini et al 1988; Ward & Brogden 1988). Nimesulide differs from conventional NSAIDs in both structure and pharmacological profile and has been shown to be efficacious and well tolerated in adult patients with a wide variety of inflammatory and painful conditions (osteoarthritis, soft tissue and oral cavity lesions, respiratory-tract inflammation, dysmenorrhoea) and fever (Ward & Brogden 1988; Perucca 1993).

Analytical methods reported for pharmacokinetic studies of nimesulide include high-speed liquid chromatography (HSLC; Chang et al 1977) and high-performance liquid chromatography (HPLC; Castoldi et al 1988). A few, mostly unpublished studies have also examined the pharmacokinetic properties of nimesulide after single and repeated doses in healthy volunteers. Because the pharmacologically effective dose for animals and the therapeutic doses projected for man are relatively low, a sensitive and specific method is required for studying the pharmacokinetics of the drug. The aim of this study was to detect nimesulide in human plasma samples by high-performance thin-layer chromatography (HPTLC).

Materials and Methods

Chemicals

Nimesulide was obtained from Dr Reddy's Laboratory, India. Dichloromethane (analytical grade) was used for extraction. Toluene and acetone (analytical grade) were used for chromatography.

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Standard solutions

A stock solution of nimesulide (1 mg mL^{-1}) was prepared in methanol. Working standard solutions were obtained by diluting the stock solution to concentrations ranging from 1 to 25 $\mu g\ mL^{-1}$.

Procedure

Nimesulide working standard (10 $\mu g\ mL^{-1}$; 0, 10, 20, 40, 50 or 100 μL) was added to drug-free plasma in 15-mL graduated centrifuge tubes to give calibration standards containing 0 (no nimesulide added), 100, 200, 400, 500 or 1000 ng mL^{-1}. All test samples were extracted with dichloromethane (2 × 3 mL) by shaking the mixture on a vortex mixer for 2 min and centrifuging for 10 min at 2500 rev min^{-1}. The combined dichloromethane extract was evaporated to dryness in a water-bath at 45°C. The residue was reconstituted in methanol (100 μL), vortex-mixed for 30 s, and samples (5–10 μL) were spotted on silica gel $60F_{254}$ TLC plates (E. Merck, Darmstadt, Germany) by means of a Camag Linomat IV sample applicator. Nimesulide reference standard (100 and 200 ng) was separately spotted on each TLC plate as external standard and

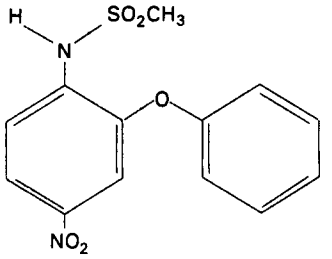

FIG. 1. The chemical structure of nimesulide.

the plates were developed in a Camag twin-trough chamber. Plasma samples from volunteers who had received a 100 mg nimesulide tablet were prepared in an identical manner except for the addition of nimesulide.

Chromatography

Mobile phases of different composition were evaluated to obtain optimum separation of nimesulide. When development was performed with toluene-acetone (100:10) the drug R_f value was 0·31 and the spot was well separated from those of other plasma components. The TLC chamber was saturated with the solvent system to ensure a concentrated zone of the compound and hence better resolution. After development, the TLC plates were dried completely using a hot-air drier. Quantitation of nimesulide was achieved by scanning with Camag TLC Scanner 3; the wavelength used for quantitation was 310 nm.

Analysis of commercial formulations

The nimesulide content of commercial nimesulide tablets was analysed by use of the same mobile phase. A nimesulide tablet (100 mg) was dissolved in methanol and the solution diluted to 10 and 20 $\mu g\ mL^{-1}$. Each solution (10 μL) was spotted on to the plate which was then developed and scanned as described above.

Bioavailability study

Six healthy young male volunteers entered the study, giving written informed consent, and having met the eligibility criteria (routine physical examination and laboratory tests). The study protocol was approved by the local ethical committee of Cadila Pharmaceuticals, R & D unit. Subjects were aged 30·4 ± 1·2 (mean ± s.e., range 22–38), with mean body weight 52·6 ± 1·8 kg (range 46–60). Each subject received a nimesulide tablet, 100 mg, according to a randomized 2-way cross-over design, with a 1-week wash-out period between two phases. One tablet of either product A (reference product; Nimulid, Panacea Biotec, India) or product B (test product; Nilide, Cadila Pharma, India) was administered with water (200 mL) after an overnight fast. A standard breakfast and lunch were provided after 2 h and 4 h, respectively. Venous blood samples were drawn into heparinized tubes before drug administration and 1, 2, 3, 4, 6, 8 and 12 h thereafter, all blood samples being taken via an indwelling catheter. The samples were immediately centrifuged for 10 min at 3000 rev min^{-1} and the plasma was separated and frozen at − 20°C until analysis.

Pharmacokinetic parameters were calculated using a model-independent method (Gibaldi & Perrier 1982). The peak plasma level (C_{max}) and time to reach peak level (T_{max}) were the observed data. The elimination rate constant (K_{el}) and terminal elimination half-life ($t_{\frac{1}{2}}$) were estimated by linear regression of the terminal part of the log concentration–time curve. The area under the plasma concentration–time curve (AUC) was determined by the linear trapezoidal rule, and extrapolated to infinity ($AUC_{0\rightarrow\infty}$) by dividing the last measurable concentration by the elimination rate-constant. The mean residence time (MRT) was calculated according to the equation of Yamaoka et al (1978):

$$MRT = AUMC_{0\rightarrow\infty}/AUC_{0\rightarrow\infty}$$

Where $AUMC_{0\rightarrow\infty}$ is the area under the first moment curve from time zero to infinity. $AUMC_{0\rightarrow\infty}$ is defined by the equation (Benet & Galeazzi 1979):

$$AUMC_{0\rightarrow\infty} = AUMC_{0\rightarrow t} + (t.Ct)/K_{el} + C_t/K_{el}$$

Where C_t is the last plasma concentration of the drug at time t and K_{el} is the apparent elimination rate constant in the terminal phase. $AUMC_{0\rightarrow t}$ is calculated by the trapezoidal rule and from the equation:

$$AUMC_{0\rightarrow t} = \{[(C(n+1) + C(n)]/2\} \times T(n+1) - (Tn) \times \{[T(n+1) + T(n)]/2\}$$

Results and Discussion

Quantitation

Quantitation was performed by using the ratio of the peak area of the unknown to that of a standard. A representative standard curve of nimesulide was obtained by plotting the nimesulide peak area against concentration over the range 10–250 ng. Such curves were constructed on 10 different days to determine the variability of slopes and intercepts. The results showed little day-to-day variability and a good linear relationship (r = 0·999) over the concentration range studied. The R_f value of nimesulide was found to be 0·31 ± 0·05.

Sensitivity

The minimum quantifiable concentration of nimesulide in human plasma samples was 100 ng mL^{-1}. This value is based on the lowest amount of compound detected by the scanner in the amount spotted (5–10 μL), i.e. the actual sensitivity of the instrument back-calculated to give a result mL^{-1} plasma. Below this level instrument sensitivity was variable.

Precision

The intra-day precision (random analytical variation) was evaluated by triplicate analysis of drug-free plasma samples to which nimesulide had been added at concentrations of 100, 200 or 500 ng mL^{-1}. All specimens used to study precision and bias were interspersed with clinical samples during analysis. The inter-day precision was determined by analysing 200-, 400- and 1000 ng standards, simultaneously with subjects, plasma, daily for five days (Table 1). The linearity of the detector response was tested by spotting standards in triplicate for each concentration over the range 10–250 ng.

Table 1. Precision data for HPTLC assay of nimesulide.

Amount (ng)	Peak area[a] (mean ± s.d.)	Coefficient of variation (%)
Intra-day[b]		
100	2495·60 ± 28·13	1·13
200	4991·01 ± 49·06	0·98
500	11629·07 ± 130·46	1·12
Inter-day[b]		
200	4931·99 ± 58·90	1·19
400	9750·89 ± 107·44	1·10
1000	24483·17 ± 243·56	0·99

[a]Calculated for total concentration (integrated value). [b]Plasma (1 mL) contained added drug at the indicated concentrations.

Table 2. Accuracy and precision of HPTLC method for determination of nimesulide in plasma.

Added (ng mL^{-1})	Detected (mean ± s.d., n = 5) (ng mL^{-1})	Coefficient of variation (%)	Accuracy[a] (%)
100	98·84 ± 1·11	1·13	101·79
200	197·68 ± 1·94	0·98	101·79
400	386·20 ± 4·26	1·10	96·55
500	464·98 ± 4·78	1·03	95·77
1000	982·71 ± 10·07	1·02	101·21

[a]After correction for recovery.

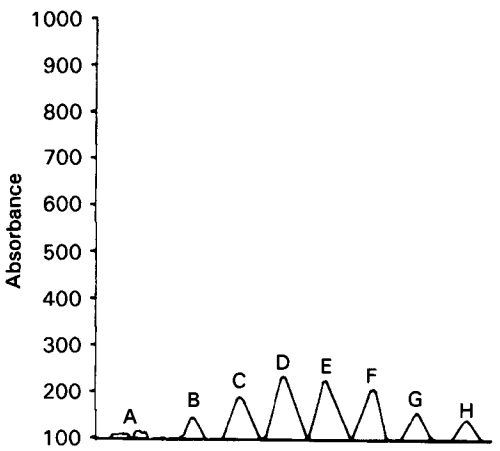

FIG. 2. HPTLC-densitometric scans of nimesulide in patient samples before and after oral administration of 100 mg tablets. A, 0 h; B, 1 h; C, 2 h; D, 3 h; E, 4 h; F, 6 h; G, 8 h; H, 12 h. Wavelength: 310 nm.

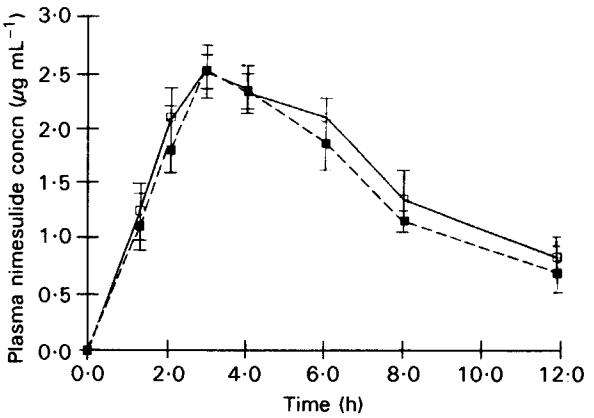

FIG. 3. Plasma concentration after oral administration of nimesulide (100 mg) as two commercial products: □ A and ■ B. Each point represents the mean ± s.e. (n = 6, cross-over design).

Recovery

The recovery of nimesulide from plasma was determined by comparing peak areas obtained from plasma to which nimesulide had been added at 100, 200, 400, 500 or 1000 ng mL^{-1} with the peak areas obtained from standards (Table 2). The recovery of nimesulide by application of the extraction procedure to 1 mL plasma was found to be 97·10 ± 2·22%.

Analysis of commercial formulations

When commercial products were analysed for nimesulide content R_f values were found to be the same for tablets and for nimesulide standard and there was no interference from excipients. Nimesulide content was found to be 99·16 ± 2·12 and 99·66 ± 1·81 for products A and B, respectively.

Bioavailability

Fig. 2 (a composite made up of the peaks from a number of individual chromatograms) shows the levels of nimesulide in the plasma of an individual volunteer at different times. The mean plasma concentration–time curve for the two brands of nimesulide are shown in Fig. 3, and the pharmacokinetic parameters are summarized in Table 3. Nimesulide C_{max} was 2·615 μg mL^{-1} at 3·33 h for product A and 2·659 μg mL^{-1} at 3·17 h for product B. Statistical analysis showed that dif-

Table 3. Pharmacokinetic parameters calculated from the plasma-time data obtained after administration of nimesulide (100 mg; products A and B) to healthy volunteers.

	Product	Subject						Mean ± s.e.
		1	2	3	4	5	6	
Peak plasma level (μg mL^{-1})	A	2·16	2·38	3·53	3·46	2·38	1·78	2·615 ± 0·267
	B	1·905	2·571	3·100	3·430	2·660	2·290	2·659 ± 0·22
Time to reach peak level (h)	A	4	3	3	4	3	3	3·33 ± 0·19
	B	4	2	3	4	3	3	3·17 ± 0·28
Area under the plasma concentration–time curve extrapolated to infinity (μg mL^{-1} h)	A	13·66	21·98	22·93	28·46	21·32	18·21	21·09 ± 1·84
	B	12·85	28·44	25·52	28·89	23·51	14·93	22·36 ± 2·56
Elimination rate constant (h^{-1})	A	0·302	0·145	0·226	0·268	0·176	0·120	0·206 ± 0·026
	B	0·435	0·151	0·243	0·214	0·170	0·202	0·236 ± 0·04
Terminal elimination half-life (h)	A	2·29	4·78	3·07	2·58	3·94	5·78	3·74 ± 0·505
	B	1·59	4·59	2·85	3·24	4·08	3·43	3·30 ± 0·39
Mean residence time (h)	A	3·17	5·17	3·51	3·36	4·76	5·93	4·30 ± 0·42
	B	2·90	4·96	3·56	4·11	4·67	3·48	3·96 ± 0·30
Relative bioavailability of product B compared with product A (%)		94·07	129·39	111·29	101·51	110·27	81·99	104·75 ± 6·06

ferences between the C_{max} and T_{max} values for the two products were not significant. $AUC_{0\rightarrow\infty}$ values were $21{\cdot}09 \pm 1{\cdot}84$ and $22{\cdot}36 \pm 2{\cdot}56$ for products A and B, respectively. MRT was not significantly different for the two products. These results indicate that the pharmacokinetic profiles of the two products are identical and bioavailability is not significantly different ($P < 0{\cdot}05$) as analysed by two-way analysis of variance, indicating the bioequivalence of two products.

Conclusions

The proposed HPTLC method constitutes a sensitive and specific assay for nimesulide in human plasma and is suitable for pharmacokinetic studies after administration of therapeutic doses. It is economical and faster than previously published methods; solvent consumption is less and after a single-stage extraction process at least 10–12 samples can be analysed in 4–5 h on a single plate. Unlike earlier methods, an internal standard is not required – quantification can be performed using an external standard.

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