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# Analysis of nabumetone in human plasma by HPLC. Application to single dose pharmacokinetic studies

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**Abstract**

A simple and sensitive high performance liquid chromatography method for the determination of nabumetone in human plasma is described. The procedure involves liquid–liquid extraction with ethyl acetate and reversed-phase chromatography with fluorimetric detection (excitation 230 nm, emission 356 nm). The chromatographic conditions and the extraction procedure gave a clean chromatogram for the compound. The limit of quantitation was established as 0.313 ng/ml and the calibration curve was linear up to 20 ng/ml. The within-day and between-day relative standard deviations were less than 10% and the accuracy of the assay was in the range of 99–104%. The suitability of the method is shown for pharmacokinetic studies.


*Keywords:* Nabumetone; NSAID; HPLC; Human plasma; Fluorescence detection; Pharmacokinetics

## 1. Introduction

Nabumetone, 4-(6-methoxy-2-naphthyl)-butan-2-one, (EP 4, 2002, p. 1609), is a nonsteroidal anti-inflammatory drug (NSAID) of naphtylalkanone class. The drug has proved to be effective in the treatment of rheumatoid arthritis, osteoarthritis and acute soft tissue injuries [1,2]. Nabumetone is a prodrug which undergoes extensive first pass metabolism to 6-methoxy-2-naphthylacetic acid (6-MNA), the major circulating metabolite. 6-MNA is largely responsible for the therapeutic efficacy of nabumetone. It decreases prostaglandin synthesis via inhibition of cyclooxygenase, an enzyme involved in the arachidonic acid conversion pathway [3].

Although the clinical applications of nabumetone have expanded, little information is available on its pharmacokinetics because of the lack of a sensitive assay method in biological fluids. Preliminary studies to quantify this drug in biological fluids involved radioassay scintillation counting of isotopes and gas chromatography with flame ionisation detection and reverse-phase high performance liquid chromatography (HPLC) using fluorescence or ultraviolet detection [4–9]. However, these methods were not sensitive enough to measure the unchanged compound after drug

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administration. We have developed a simple and sensitive HPLC method to determine the concentration of nabumetone in human plasma and examined the pharmacokinetics of nabumetone in healthy volunteers.

## 2. Experimental

### 2.1. Chemicals and reagents

Nabumetone was purchased from Esteva Quimica S.A. (Barcelona, Spain) and cisapride from Dr Reddy's Holding Ltd (Hyderabad, India). HPLC-grade methanol and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ), phosphoric acid from BDH (Pool, England). Triethylamine was from Sigma-Aldrich (Steinheim, Germany). All other reagents were of analytical grade.

### 2.2. Apparatus

The HPLC system consisted of a Model LC-10ADvp pump coupled to a Model SIL-10ADvp autosampler, a Model RF-10$A_{XL}$ fluorescence detector operated at 230 nm for excitation and 356 nm for emission wavelength, a Model SCL-10Avp system controller (Shimadzu Europa, Duisburg, Germany). The apparatus was connected to a personal computer with a Class-Vp 5.03 Chromatography Data System software (Shimadzu).

### 2.3. Chromatographic conditions

The chromatographic separation was performed using a Nova-Pak C18 analytical column (150 × 3.9 mm I.D., 5 μm; Waters Corporation, Milford, MA), preceded by 4 × 3 mm I.D. C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile and 0.02% triethylamine adjusted with phosphoric acid (85%) to pH 7 (50:50 v/v) and was delivered at a flow-rate of 1.4 ml/min. The solution was filtered using 0.45 μm nylon membrane (Supelco) and ultrasonically degassed prior to use.

### 2.4. Plasma samples

Venous blood samples (8 ml) were withdrawn into the heparinized tubes. Blood samples were centrifuged at 1000 × $g$ for 10 min at 4 °C and the plasma obtained was stored at −70 °C until analysis. Stability was determined in samples comprising 0.625, 5.0 and 15 ng/ml of nabumetone in human blank plasma stored at −70 °C for 2 months.

### 2.5. Standards solutions

Stock solutions of nabumetone (1 mg/ml) and internal standard cisapride (1 mg/ml) were prepared in methanol and were stored at −20 °C in a freezer.

Standard solutions were made by serially diluting the nabumetone stock solution with drug-free plasma from healthy volunteers to provide concentrations of 0.313, 0.625, 1.25, 2.5, 5, 10, 15 and 20 ng/ml. Quality control (QC) samples were prepared in the same way at concentrations of 0.625, 5 and 15 ng/ml, divided into 1 ml portions and stored at −70 °C.

Internal standard was prepared from stock solution diluted with methanol to 100 μg/ml and 25 μl included in each plasma sample (corresponding to 2.5 μg).

### 2.6. Extraction procedures

To 0.5 ml plasma in a glass tube, 25 μl of an internal standard working solution and 0.2 ml 0.01 M phosphate buffer pH 10, were added. Then, the plasma was briefly mixed with a vortex mixer and 3 ml of ethyl acetate was added. The solution was shaken for 5 min (IKA Labortechnik, Staufen, Germany), and centrifuged at 1500 × $g$ for 5 min (MPW 370, MPW Med-Instruments, Warsaw, Poland). After freezing at −70 °C for 20 min, the organic layer was transferred to another glass tube and evaporated to dryness at 45 °C under a gentle stream of nitrogen (Turbo Vap®, Zymark, Hopkinton, MA). The sample residue was dissolved in 200-μl freshly prepared mobile phase, centrifuged (1500 × $g$, 2 min), transferred to auto-

Table 1
Stability of nabumetone and cisapride in plasma control samples

| | Storage condition | Nabumetone | | | Cisapride 5 μg/ml |
|---|---|---|---|---|---|
| | | 0.625 ng/ml | 5 ng/ml | 15 ng/ml | |
| Autosampler stability | 24 h, 21 ± 1 °C | | | | |
| % of initial ($n = 6$) | | 98.84 | 98.29 | 98.93 | 99.52 |
| Freeze–thaw ($n = 6$) | | | | | |
| 1st cycle ng/ml | | 0.624 | 5.02 | 15.12 | – |
| (% of initial) | | (99.8) | (100.4) | (100.8) | |
| 2nd cycle ng/ml | | 0.620 | 4.98 | 14.80 | – |
| (% of initial) | | (99.2) | (99.8) | (98.7) | |
| 3rd cycle ng/ml | | 0.610 | 4.88 | 14.50 | – |
| (% of initial) | | (97.6) | (97.6) | (96.7) | |
| Plasma sample storage (ng/ml) | 2 months, −70 ± 2 °C | 0.608 | 4.98 | 14.6 | – |
| % of initial | | 97.1 | 99.6 | 97.3 | |

sampler vials and 40-μl aliquot was injected onto the HPLC system for analysis.

### 2.7. Application

The method of analysis was applied to the plasma from 24 healthy volunteers (male), who participated in pharmacokinetic studies of nabumetone. The study was performed according to the ethical guidelines of the revised Declaration of Helsinki. All subjects gave written informed consent and the study protocol was approved by the Ethical Committee of the Rzeszów Hospital. The subjects received Relifex tabl. (SmithKline Beecham Pharmaceuticals, Brentford, England) 500 mg orally in 1000 mg dose (two tablets). Blood samples were taken from a forearm vein into heparinized tubes immediately before (time zero) and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48 and 80 h, after drug administration.

### 2.8. Analysis of data

The pharmacokinetic parameters for nabumetone were determined from the plasma concentration-time data with the aid of the Pharm/PCS program [10]. The maximum plasma concentration ($C_{max}$) and the time to peak ($t_{max}$) for nabumetone were taken directly from the experimental data. The elimination rate constant ($k_{el}$) was estimated by least square regression analysis from the data of the last 3–4 points of each plasma concentration–time curve. The terminal elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/k_{el}$. The area under the concentration versus time curve ($AUC_t$) was calculated using the trapezoidal rule and then extrapolated to infinity.

## 3. Results

### 3.1. Validation

#### 3.1.1. Stability

In the present study, the stability of processed samples in autosampler (24 h), plasma storage −70 °C (2 months) and after three freeze–thaw cycles was demonstrated (Table 1). No significant degradation of both compounds of interest was observed.

#### 3.1.2. Selectivity

The selectivity of the analytical method was investigated in order to assume that the method can be used to quantitate nabumetone and cisapride in the presence of other constituents in the sample. The selectivity was studied by analysing six individual blank plasma samples. The chromatogram of blank plasma (Fig. 1A) did not show any interfering compounds extracted from the sample. A typical chromatogram of a drug-free human plasma sample spiked with nabumetone (5

ng/ml) and internal standard (5 μg/ml) is shown in Fig. 1B. The chromatogram of the extract of plasma sample from a volunteer who received Relifex tabl. 500 mg orally in 1000-mg dose is shown in Fig. 1C. The retention times of nabumetone and I.S. were 5.4 and 6.8 min, respectively.

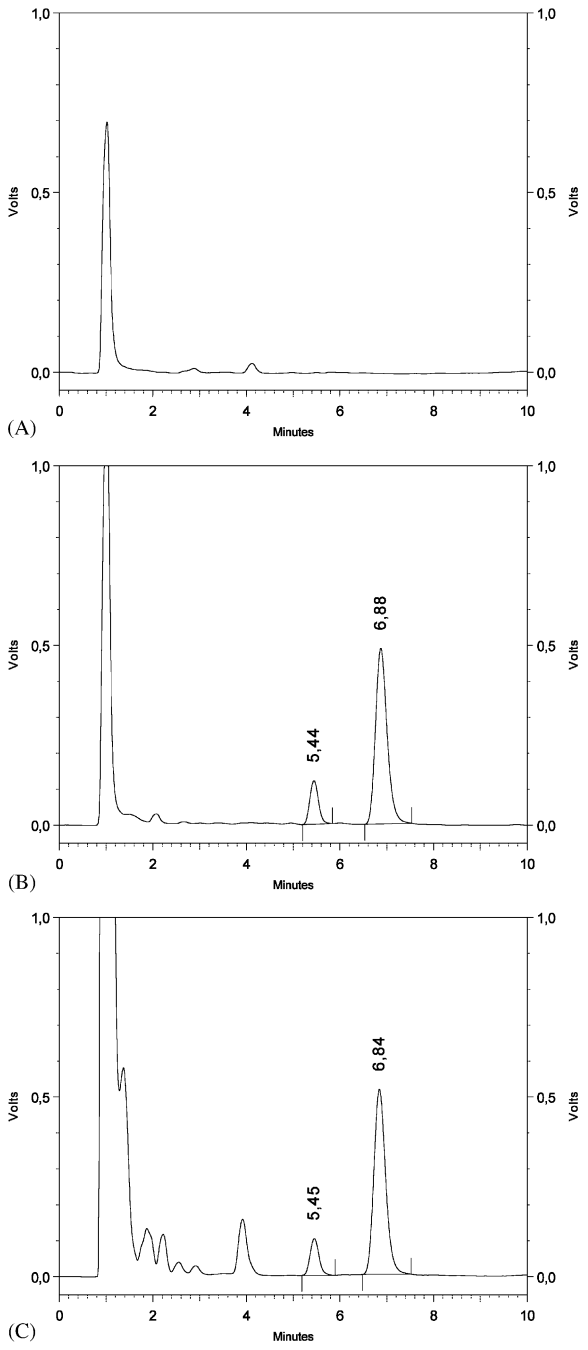


Fig. 1

### 3.1.3. Extraction efficiency

The absolute recovery of nabumetone from human plasma was calculated by comparing the peak area obtained from extracts of spiked plasma samples and the peak area obtained from the direct injection of known amounts of standard solutions of nabumetone. The overall extraction yields of nabumetone in plasma were $83.78 \pm 3.31$, $82.37 \pm 2.68$ and $81.26 \pm 1.33$ at 0.625, 5.0 and 15 ng/ml, respectively. The mean recovery for internal standard was $88.45 \pm 4.14\%$ ($n = 6$).

### 3.1.4. Linearity

Standard curves were constructed by plotting the peak area ratio of nabumetone to the I.S. against the concentration of nabumetone. Sample with an analyte concentration exceeding the standard curve were reassayed upon appropriate dilution with drug-free plasma. The standard curve for nabumetone was linear over the range 0.313–20 ng/ml. The standard curve was calculated by linear regression method: $y = ax + b$, where $y$ is the peak area ratio of drug to internal standard, $a$ and $b$ are constant, and $x$ is the nabumetone concentration (ng/ml). Typical values for the regression parameters $a$ (slope), $b$ (y-intercept) and $r$ (correlation coefficient) were calculated to be 0.036973, 0.002625 and 0.999, respectively ($n = 6$).

### 3.1.5. Limits of detection and quantitation

The limit of detection (LOD) was defined as the sample concentration of nabumetone resulting in peak height 3 times of the noise level. The limit of quantification (LOQ) was the lowest point on the calibration curve which could be detected with variation below 15%. The minimum detectable

Fig. 1. Chromatograms of (A) blank human plasma, (B) spiked human plasma with 5 ng/ml of nabumetone and 5 μg/ml of internal standard and (C) a plasma sample from a volunteer at 10 h after drug administration, containing 4.11 ng/ml of nabumetone. The retention times for nabumetone and internal standard are 5.4 and 6.8 min, respectively. See Section 2.3 for chromatographic conditions.

Table 2
Precision and accuracy of the method of nabumetone determination

| Nominal concentration added (ng/ml) | Concentration found (ng/ml) ± S.D. | RSD (%) | Accuracy (% mean ± S.D.) |
|---|---|---|---|
| *Intra-assay* ($n = 6$) | | | |
| 0.625 | 0.64 ± 0.04 | 6.25 | 102.82 ± 6.82 |
| 5.0 | 5.21 ± 0.28 | 5.38 | 104.20 ± 6.46 |
| 15.0 | 15.39 ± 0.40 | 2.59 | 102.59 ± 2.65 |
| *Inter-assay* ($n = 6$) | | | |
| 0.625 | 0.64 ± 0.05 | 7.81 | 102.40 ± 7.56 |
| 5.0 | 4.99 ± 0.28 | 5.61 | 99.87 ± 5.67 |
| 15.0 | 15.51 ± 0.38 | 2.45 | 103.40 ± 2.50 |

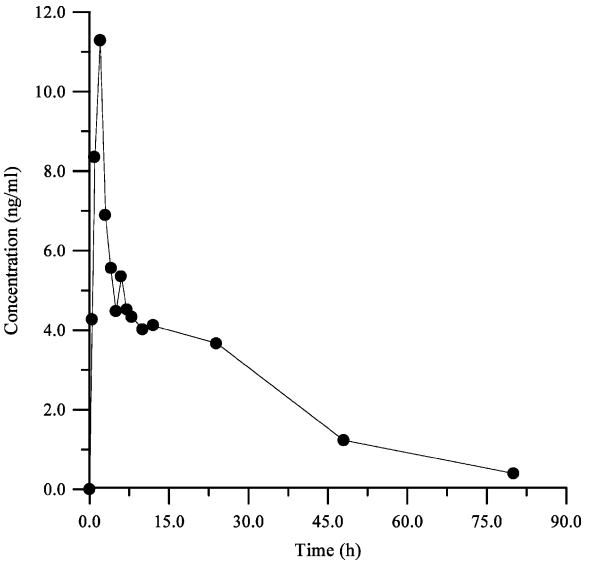


Fig. 2. Mean plasma levels of nabumetone in 24 healthy volunteers following a single oral dose of 1000-mg Relifex tabl.

concentration of nabumetone (LOD) was determined to be 0.05 ng, whereas the quantitative limit (LOQ) was 0.313 ng/ml and relative standard deviation (RSD) of replicate determinations was 12.45% ($n = 6$).

### 3.1.6. Precision and accuracy

The within-day assay variations were determined by analyzing six 0.5-ml aliquots of spiked plasma samples containing 0.625, 5 and 15 ng/ml of nabumetone. The between-day assay variations were determined by analyzing 0.5-ml aliquots of spiked plasma samples containing 0.625, 5 and 15 ng/ml of nabumetone in duplicates on three separate days. In both cases, the RSD was less than 10% at any concentrations studied (Table 2). Accuracy was within the range 99–104% in all concentrations investigated.

Table 3
Pharmacokinetic parameters of nabumetone following a single oral administration of 1000 mg Relifex tabl. to 24 healthy volunteers

| Pharmacokinetic parameter | Mean value ± S.D. ($n = 24$) |
|---|---|
| $k_{el}$ (/h) | 0.045 ± 0.01 |
| $t_{1/2}$ (h) | 16.82 ± 5.57 |
| $C_{max}$ (ng/ml) | 13.48 ± 16.25 |
| $t_{max}$ (h) | 3.48 ± 3.43 |
| $AUC_t$ (ng h/ml) | 186.38 ± 115.70 |
| $AUC_\infty$ (ng h/ml) | 204.98 ± 121.38 |

### 3.2. Pharmacokinetics

The applicability of the assay procedure is illustrated in Fig. 2, which shows an average plasma concentration–time curve of nabumetone after administration of Relifex tabl. to 24 volunteers. Resulting pharmacokinetic parameters are summarised in Table 3. Maximum concentrations of nabumetone were reached approximately 3 h ($t_{max}$) after application. The individual $t_{max}$ value varied over the range 0.5–12 h. Maximum concentrations ranged from 2.57 to 77.58 ng/ml with an average of 13.48 ng/ml. Terminal elimination half-life of the compound was about 16.8 h. AUC

for nabumetone showed an average of 204.98 ng h/ml (range 66.97–639.95).

## 4. Discussion

Most of the published studies on pharmacokinetics of nabumetone included data obtained for its metabolite-6-MNA [1,3–5,11–13]. The parent compound nabumetone was not generally detected intact in plasma. Using radioassay scintillation counting of isotopes, unchanged nabumetone ($<$ 0.5 μg/ml) was not detected in plasma for up to 72 h after administration [4]. However, other studies indicate that plasma nabumetone concentrations of about 0.03 to 0.3 μg/ml occasionally occur in some individuals [4]. Moreover, Miehlke et al. [6] reported that the steady state plasma concentrations of nabumetone were $21 \pm 20$ ng/ml.

In the present study, we have developed a simple and sensitive HPLC method to determine the concentration of nabumetone in human plasma and examined the pharmacokinetics of nabumetone after oral administration to healthy volunteers. Several published nabumetone assays did not have the sensitivity to yield a limit of quantitation low enough to allow for the full pharmacokinetic profiles of nabumetone in humans [4–9]. The proposed method has a limit of quantitation (0.313 ng/ml) which is enough to measure plasma nabumetone concentration for up to 80 h. The method is precise and accurate. The composition and pH of the mobile phase used in the present assay provide good separation of nabumetone and the internal standard with no interferences from endogenous components in plasma (Fig. 1). Moreover, because of the simple extraction procedure and the short time of the chromatographic analysis, this method allows the quantification of a large number of samples daily. In our study we showed that after oral administration of a 1000-mg dose of Relifex tabl. the parent drug concentrations were low (Fig. 2). The mean maximum concentration of nabumetone was 13.48 ng/ml (range 2.6–77.6 ng/ml) and demonstrated high interindividual variations. This variation in plasma concentration was expected because nabumetone is a prodrug and conversion to the active metabolite is dependent on the extent of hepatic biotransformation of each individual.

In summary, the described method for the determination of nabumetone in human plasma is sensitive, specific and reproducible. This method would allow pharmacokinetic studies of nabumetone after oral administration to be conducted.

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