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Comparative biotransformation and disposition studies of nabumetone in humans and minipigs using high-performance liquid chromatography with ultraviolet, fluorescence and mass spectrometric detection

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Abstract

The disposition of the non-steroidal anti-inflammatory drug (NSAID) nabumetone after a single oral dose administration of nabumetone tablets to humans and minipigs was investigated. Nabumetone is a prodrug, which is metabolized in the organism to the principal pharmacodynamically active metabolite — 6-methoxy-2-naphthylacetic acid (6-MNA), and some other minor metabolites (carbonyl group reduction products, O-desmethylation products and their conjugates with glucuronic and sulphuric acids). Standards of the above-mentioned metabolites were prepared using simple synthetic procedures and their structures were confirmed by NMR and mass spectrometry. A simple HPLC method for the simultaneous determination of nabumetone, 6-MNA and the other metabolites was developed, validated and used for xenobiochemical and pharmacokinetic studies in humans and minipigs and for distribution studies in minipigs. Naproxen was chosen as the internal standard (I.S.), both UV (for higher concentrations) and fluorescence detection (for very low concentrations) were used. The identity of the nabumetone metabolites in biological samples was confirmed using HPLC-MS experiments. Pharmacokinetics of nabumetone, 6-MNA and 6-HNA (6-hydroxy-2-naphthylacetic acid) in human and minipig plasma was evaluated and compared. The concentration levels of nabumetone metabolites in urine, bile and synovial fluid were also evaluated.

Keywords: Nabumetone; 6-Methoxy-2-naphthylacetic acid; Metabolites; NSAIDs; Biotransformation; Pharmacokinetics; HPLC with ultraviolet, fluorescence, APCI-MS and ESI-MS detection; Complete NMR analysis

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1. Introduction

Nabumetone (4-(6-methoxy-2-naphthyl)-butan-2-one, see Fig. 1, compound **1**) was synthesized in the laboratories of Beecham Pharmaceuticals in 1978 [1], and introduced into therapeutical use as a non-acidic non-steroidal anti-inflammatory drug (NSAID) for the symptomatic treatment of rheumatic and inflammatory conditions [2,3].

Being administered orally, nabumetone is well (about 80% of an administered dose) absorbed from gastrointestinal tract, primarily from the duodenum [3–5]. However, its plasma concentrations are too small to be measured, as it undergoes rapid and extensive first-pass biotransformation in the liver to form the principal active compound, 6-methoxy-2-naphthylacetic acid (6-MNA, see Fig. 1, compound **2**), and some other pharmacologically inactive metabolites — see Fig. 1 [4,6,7]. The peak plasma concentration of 6-MNA is reached approximately 8–12 h after nabumetone administration [5,8,9], but the inter-subject variability of t_{max} is extremely high: 3–24 h according to Kendall et al. [8] and even 1.5–48 h according to de Jager et al. [10]. De Jager et al. [10] withdrew blood samples more often than other researchers [8,9], and found two peaks on the 6-MNA plasma concentration versus time curve — the first one approximately 10 h, and the second one approximately 24 h after nabumetone administration.

The mean absolute bioavailability of 6-MNA is about 35% [11]. It is dose independent in the nabumetone dose range 250–1000 mg, and not affected by co-administration of aluminium hy-

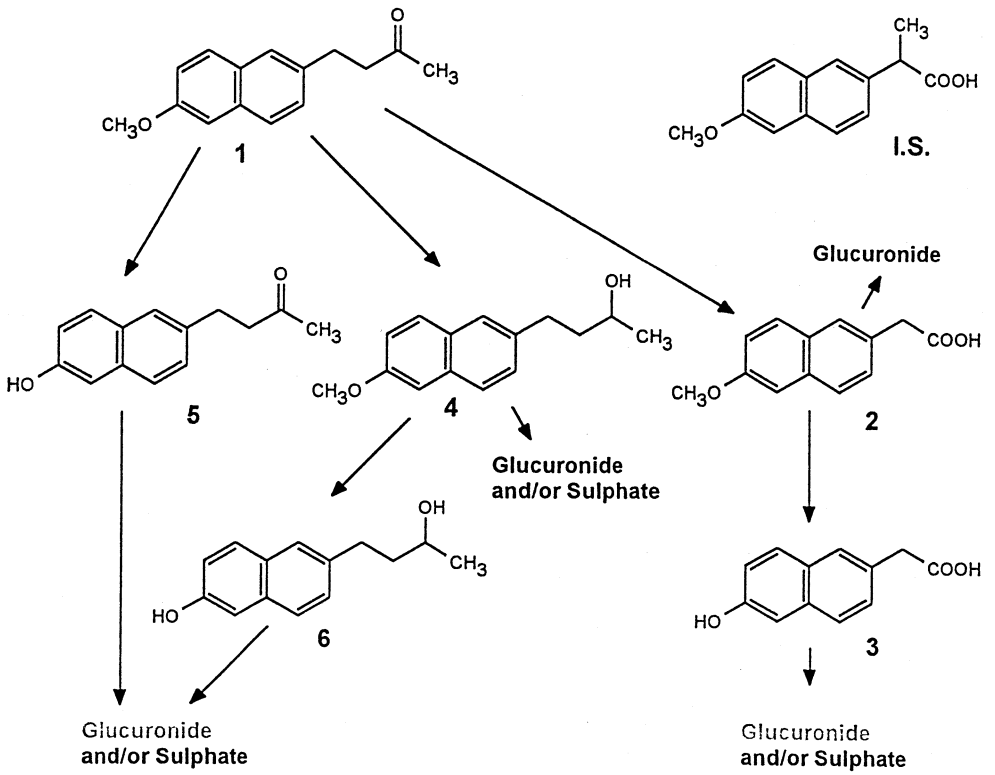

Fig. 1. Chemical structures of nabumetone (1), naproxen (I.S.), 6-methoxy-2-naphthylacetic acid (2) and the other metabolites (3–6) formed during the biotransformation of nabumetone.

droxide, aspirin and paracetamol [12]. On the other hand, it increases in the presence of milk or food [12].

6-MNA is more than 99% bound to plasma proteins [3,5,7], with the volume of distribution being small-7.5 l after intravenous administration [3]. 6-MNA penetrates well into the synovial fluid [5,7], and does not undergo enterohepatic circulation [5].

The biotransformation of nabumetone to 6-MNA occurs in the liver via oxidative cleavage of the side chain. 6-MNA undergoes further metabolism by O-desmethylation to yield 6-hydroxy-2-naphthylacetic acid [3,5,9]. Three other metabolites of nabumetone, 4-(6-hydroxy-2-naphthyl)butan-2-one, 4-(6-hydroxy-2-naphthyl)butan-2-ol and 4-(6-methoxy-2-naphthyl)butan-2-ol, have been identified in human urine [5,9]. Both 6-MNA, and four other metabolites are partially conjugated to glucuronides and/or sulphates [3–5].

About 80% of a nabumetone dose is excreted in the urine as inactive or conjugated metabolites and less than 1% as unchanged 6-MNA [4,5,7].

The average terminal phase elimination half-life of 6-MNA is approximately 21–27 h, but its inter-individual variability is extremely high, in the range 13–33 h [8–10].

The mode of action of 6-MNA consists in a potent and relatively selective inhibition of cyclo-oxygenase-2 (COX 2). The anti-inflammatory effects of 6-MNA are thought to be related to its ability to inhibit prostaglandin synthesis in synovial tissue and fluid and in other inflammatory exudates. Because nabumetone is absorbed as a non-acidic unionized prodrug, it does not appear to exert a significant direct toxic effect on the gastric mucosa during absorption. Nabumetone causes a lower incidence of gastrointestinal erosions or microbleeding than aspirin, naproxen, piroxicam and ibuprofen [3].

Interspecies differences in nabumetone disposition were studied after oral administration to rats, mice, rabbits, dogs, rhesus monkeys and healthy human subjects using radiotracer methodology, mass-spectrometry and independent syntheses of metabolite standards [4]. In all species, the major circulating metabolite was 6-MNA; the parent compound was not practically detected. The other four metabolites (see Fig. 1) were excreted mainly in urine. The metabolic products of the oxidation of nabumetone side-chain, O-desmethylation and carbonyl reduction occurred in all species, but the ratios of the final metabolites tended to be species-dependent.

High-performance liquid chromatographic methods prevail among the analytical techniques used for the determination of nabumetone and/or its metabolites in biological materials [10,13–19]. In the sample-handling step preceding the instrumental analysis, a liquid–liquid extraction (LLE) [13–17] or solid-phase extraction (SPE) [19] procedure was usually employed. Methods describing the extractionless (protein precipitation) procedure [10] or direct injection HPLC analysis [18] were also published. 6-Chloro-2-naphthylacetic acid [13], β-naphtol [15] or naproxen [10,17,20] were used as internal standards (I.S.) for the determination in biological samples. Nabumetone and/or its metabolites were determined using ultraviolet [10,15–19] or fluorescence [13,19] detection.

The above-mentioned bioanalytical methods were used for pharmacokinetic, metabolic and biodistribution studies. The determination of 6-MNA in plasma or serum [10,13–18], urine [19], synovial fluid and adjacent tissue [20] prevails among the described bioanalyses. The study of the disposition and excretion of 6-MNA in horses was also reported [21].

The goal of this study was to make a comparison of nabumetone disposition in humans and minipigs. Pharmacokinetics and metabolism of nabumetone in minipigs have not been described yet, although one recent paper [22] indicates the similarity in the enzymatic equipment of both above-mentioned biological species. For these purposes, the standards of five metabolites of nabumetone were synthesized and a bioanalytical HPLC method based on the fluorescence, ultraviolet and mass spectrometry detection was developed, validated and used for the identification and determination of nabumetone metabolites in some biomatrices (blood plasma, synovial fluid, bile and urine).

2. Experimental

2.1. Chemicals, preparations and materials

Nabumetone, 4-(6-methoxy-2-naphthyl)-butan-2-one ($C_{15}H_{16}O_2$, FW = 228.3 g/mol, Fig. 1, compound **1**) was obtained from PRO.MED.CS Praha a.s., Czech Republic, naproxen, (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid (Sigma Aldrich, Prague, Czech Republic) was used as the I.S. (in Fig. 1) for HPLC determination. 6-Methoxy-2-naphthylacetic acid ($C_{13}H_{12}O_3$, 216.24 g/mol, 6-MNA; Fig. 1, compound **2**), 6-hydroxy-2-naphthylacetic acid ($C_{12}H_{10}O_3$, 202.21 g/mol, 6-HNA; Fig. 1, compound **3**), 4-(6-methoxy-2-naphthyl)-butan-2-ol ($C_{15}H_{18}O_2$, 230.31 g/mol, 6-MeOnphBuOH; Fig. 1, compound **4**), 4-(6-hydroxy-2-naphthyl)-butan-2-one ($C_{14}H_{14}O_2$, 214.26 g/mol, 6-HOnphBu=O; Fig. 1, compound **5**), 4-(6-hydroxy-2-naphthyl)-butan-2-ol ($C_{14}H_{16}O_2$, 216.28 g/mol, 6-HOnphBu-OH; Fig. 1, compound **6**) were synthesized in the laboratories of our institute and used as the standards of nabumetone metabolites. 6′-Methoxy-2′-acetonaphthone (98%), morpholine (99+%) and sulphur (all from Sigma-Aldrich) were used for the synthesis of 6-methoxy-2-naphthylacetic acid. Boron tribromide (1 M solution in dichloromethane, Sigma-Aldrich) was employed in O-desmethylation reactions (for the preparation of metabolites 3, 5 and 6 in Fig. 1). Lithium aluminium hydride ($LiAlH_4$, powder, Lachema Brno, Czech Republic) and tetrahydrofuran (anhydrous, 99.9%, Sigma-Aldrich) were used for carbonyl reduction (for the preparation of metabolites 4 and 6 in Fig. 1). Acetonitrile, methanol (both HPLC grade, Merck, Darmstadt, Germany), diethyl ether (analytical grade, Merck), hydrochloric acid (35% water solution of HCl, analytical grade), sodium hydrogenphosphate dodecahydrate, potassium dihydrogenphosphate and sodium hydroxide (all analytical grade, Lachema Brno, Czech Republic), acetic acid (99%, analytical grade, HiChem s r.o., Prague, Czech Republic), ultra-high-quality (UHQ) water (prepared using Elgastat UHQ PS apparatus, Elga Ltd., Bucks, UK) were used for the LLE of biomatrices and chromatography of nabumetone derivatives. A Visiprep Solid Phase Extraction Vacuum Manifold (12-port, Supelco) with SPE columns (Supelclean LC-18, 1 ml tubes, Supelco, Bellefonte, USA) was used for the SPE.

A phosphate buffer (pH 7.4) was prepared by mixing 800 ml of 0.067 M sodium hydrogenphosphate dodecahydrate (23.976 g in 1000 ml of UHQ water) and 200 ml of 0.067 M potassium dihydrogenphosphate (2.2695 g in 250 ml of UHQ water).

A nabumetone formulation (coated tablets containing 500 mg of the parent compound) was tested in this study.

2.2. NMR analyses

A Varian Mercury-Vx BB 300 NMR spectrometer was used for the NMR analyses of the synthetic standards of nabumetone metabolites. The NMR spectra were recorded at 300 MHz for ^1H, and 75 MHz for ^{13}C. Chemical shifts are given as δ values in ppm, the coupling constants are given in Hz. Analytical sample (15–20 mg) was dissolved in CD_3OD (0.7 ml) and the solution was transferred via pipette into an NMR tube (203 mm length, 5 mm diameter).

2.3. HPLC/MS analyses

High-performance liquid chromatography-mass spectrometry (HPLC/MS) analyses were performed on an ion trap analyzer Esquire3000 (Bruker Daltonics, Bremen, Germany) and a liquid chromatograph HP 1090 (Hewlett–Packard, Palo Alto, CA, USA). Mass spectra were recorded in the range *m/z* 15–400 using positive-ion atmospheric pressure chemical ionization (APCI) and averaged over 10 scans. The pressure of the nebulizing gas was 70 psi and the flow rate of the drying gas was 5 l/min. The temperatures of the drying gas and APCI heater were 350 and 400 °C, respectively. The mass spectrometer was tuned to give a maximum response for *m/z* 230. The positive-ion and negative-ion electrospray ionization (ESI) mass spectra of all compounds under study were measured using the direct infusion technique under the following conditions: ion source temperature 300 °C, the flow rate and pressure of nitrogen 4 l/min and 10 psi, respec-

tively. For direct infusion ESI experiments, the compounds were dissolved in pure acetonitrile. For HPLC/MS experiments, plasma samples or synthetic compounds were dissolved in a mobile phase (acetonitrile–UHQ water–acetic acid, 45:55:0.1, v/v/v), and 25 μl of the solution was injected into HPLC/MS system.

2.4. Syntheses of the standards of nabumetone metabolites

2.4.1. Synthesis of 6-methoxy-2-naphthylacetic acid

A mixture of 6′-methoxy-2′-acetonaphthone (5 g, 0.025 mol), morpholine (4.4 g, 0.05 mol) and sulphur powder (1.6 g, 0.05 mol) was heated under reflux for 6 h. The hot reaction mixture was then poured into warm ethanol (40 ml), and the crystallization of the thiomorpholide was accelerated using a glass rod. The reaction mixture was allowed to stand overnight. The crude crystalline product was filtered off, washed with water and cold ethanol. 6-Methoxy-2-naphthylthioacetomorpholide (7.52 g, 0.025 mol) was hydrolyzed in a mixture of 50% potassium hydroxide (40 ml) and 35 ml of ethanol (reflux for 6 h). Ethanol was then removed, and the solution was diluted with water, filtered and acidified with concentrated hydrochloric acid to furnish 6-methoxy-2-naphthylacetic acid, which was crystallized from methanol–water ($C_{13}H_{12}O_3$, 216.24 g/mol, 4.7 g, 87% yield). Melting point 176–177 °C (175 °C according to [23]).

^{1}H NMR (300 MHz, CD_3COCD_3) δ: d 7.75 (bd, $J = 8.5$ Hz, 2H, H4, H8); 7.72 (bs, 1H, H1); 7.42 (dd, $J_{3,4} = 8.5$ Hz, $J_{3,1} = 1.7$ Hz, 1H, H3); 7.26 (d, $J_{5,7} = 2.4$ Hz, 1H, H5); 7.13 (dd, $J_{7,8} = 8.5$ Hz, $J_{7,5} = 2.4$ Hz, 1H, H7); 3,89 (s, 3H, $-OCH_3$); 3,75 (s, 2H, $-CH_2-$). ^{13}C NMR (300 MHz, CD_3COCD_3) δ: 128.331(C1); 130.786(C2); 128.813-(C3); 127.424(C4); 134.352(C4a); 106.265(C5); 158.277(C6); 119.434(C7); 129.625(C8); 129.610-(C8a); 55.478 ($-OCH_3$); 41.175($-CH_2-$); 172.683(–COOH). Positive-ion APCI-MS: *m/z* 232, relative abundance 26%, $[M+16]^+$; *m/z* 217, 17%, $[M+H]^+$; *m/z* 216, 28%, $M^{+\bullet}$; *m/z* 187, 69%, $[M-29]^+$; *m/z* 171, 100%, $[M-H-CO_2]^+$. Positive-ion ESI-MS: *m/z* 255, 4%, $[M+K]^+$; *m/z* 239, 100%, $[M+Na]^+$; *m/z* 217, 3%, $[M+H]^+$. Negative-ion ESI-MS: *m/z* 215, 4%, $[M-H]^-$; *m/z* 187, 66%, $[M-29]^-$; *m/z* 171, 100%, $[M-H-CO_2]^-$.

2.4.2. O-Desmethylation of 6-methoxynaphthalene derivatives (preparation of compounds **3** and **5** in Fig. 1)

Nabumetone (2 g, 0.00876 mol) or 6-methoxy-2-naphthylacetic acid (1 g, 0.0046 mol) was dissolved in dichloromethane (30 ml for nabumetone or 25 ml for 6-MNA) and cooled to −50 °C (a bath of solid carbon dioxide in methanol). A 1 M solution of boron tribromide in dichloromethane [30 ml (0.03 mol) for nabumetone or 10 ml (0.01 mol) for 6-MNA] was subsequently added in several portions, and the reaction mixture was stirred for 50 min. The mixture was then allowed to warm up to room temperature and poured onto ice. The value of pH was adjusted to 3, and the resultant substance was filtered off and washed with water. The crude product was then dissolved in dichloromethane, the solution filtered, dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure to yield the final product. Via this procedure, the following two standards were prepared: 4-(6-hydroxy-2-naphthtyl)-butan-2-one ($C_{14}H_{14}O_2$, 214.26 g/mol, 1.45 g, 77% yield), melting point 125–126 °C (122–124 °C according to [1], recrystallized from petroleum ether, 123–125 °C according to [4], recrystallized from ethanol–water).

^{1}H NMR (300 MHz, CD_3OD) δ 2.11 (3H, s), 2.75–2.88 (2H, m), 2.88–3.00 (2H, m), 7.02 (1H, dd overlapped, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz), 7.05 (1H, bd overlapped, $J = 2.5$ Hz), 7.22 (1H, dd, $J_1 = 1.9$ Hz, $J_2 = 8.5$ Hz), 7.49 (1H, bs), 7.54 (1H, d, $J = 8.5$ Hz), 7.61 (1H, d, $J = 8.8$ Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 30.0, 30.8, 45.8, 109.6, 119.1, 127.1, 127.3, 128.2, 129.8 (2 C), 134.8, 136.6, 155.8, 210.9. Positive-ion APCI-MS: *m/z* 230, relative abundance 16%, $[M+16]^+$; *m/z* 214, 100%, $M^{+\bullet}$; *m/z* 213, 39%, $[M-H]^+$; *m/z* 195, 5%, $[M-H-H_2O]^+$; *m/z* 171, 7%, $[M-CH_3CO]^+$; *m/z* 157, 17%, $[M-CH_3COCH_2]^+$. Positive-ion ESI-MS: *m/z* 237, 100%, $[M+Na]^+$; *m/z* 215, 13%, $[M+H]^+$.

6-Hydroxy-2-naphthylacetic acid ($C_{12}H_{10}O_3$, 202.21 g/mol, 0.6 g, 64% yield), melting point 207–209 °C (210 °C according to [23]).

^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (2H, s), 7.05 (1H, dd, $J_1 = 2.5$ Hz, $J_2 = 8.7$ Hz), 7.08 (1H, bd, $J = 2.5$ Hz), 7.30 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.5$ Hz), 7.58 (1H, d overlapped, $J = 8.5$ Hz), 7.60 (1H, bs overlapped), 7.65 (1H, d, $J = 8.7$ Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 41.9, 109.8, 119.5, 127.4, 128.8 (2C), 129.8, 130.2, 130.4, 135.3, 156.3, 176.0. Positive-ion APCI-MS: *m/z* 202, relative abundance 36%, $M^{+\bullet}$; *m/z* 173, 14%, $[M-29]^+$; *m/z* 157, 100%, $[M-H-CO_2]^+$. Positive-ion ESI-MS: *m/z* 225, 100%, $[M+Na]^+$; *m/z* 203, 78%, $[M+H]^+$; *m/z* 157, 73%, $[M+H-HCOOH]^+$. Negative-ion ESI-MS: *m/z* 201, 100%, $[M-H]^-$; *m/z* 187, 77%; $[M-15]^-$, *m/z* 157, 66%, $[M-H-CO_2]^-$.

2.4.3. Reduction of the carbonyl function to a secondary alcoholic group (preparation of metabolites 4 and 6 in Fig. 1)

Nabumetone (1 g, 0.00438 mol) or 4-(6-hydroxy-2-naphthtyl)-butan-2-one (1 g, 0.0047 mol, see Section 2.3.2) was dissolved in anhydrous tetrahydrofuran, the solution cooled to 0 °C and $LiAlH_4$ (2 g, 0.00876 mol) was added in several portions. After stirring for 2 h at 0 °C, the reaction mixture was warmed to ambient temperature and stirred for a further 1 h. Tetrahydrofuran was removed under reduced pressure, unreacted $LiAlH_4$ decomposed by water (10 ml), and the mixture extracted with diethyl ether. The organic layer containing the product was dried over anhydrous Na_2SO_4.

Two standards of nabumetone metabolites were prepared via this procedure: 4-(6-methoxy-2-naphthyl)-butan-2-ol ($C_{15}H_{18}O_2$, 230.31 g/mol, 0.71 g, 70% yield), melting point 99–100 °C (94–95 °C according to [1]).

^{1}H NMR (300 MHz, CD_3OD) δ 1.19 (3H, d, $J = 6.0$ Hz), 1.65–1.90 (2H, m), 2.65–2.95 (2H, m), 3.65–3.82 (1H, m), 3.85 (3H, s), 7.06 (1H, dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz), 7.14 (1H, bd, $J = 2.5$ Hz), 7.28 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.5$ Hz), 7.53 (1H, bs), 7.63 (1H, d overlapped, $J = 8.8$ Hz), 7.65 (1H, d overlapped, $J = 8.5$ Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 23.6, 33.1, 42.1, 55.6, 67.9, 106.5, 119.4, 127.0, 127.7, 128.6, 129.7, 130.5, 134.4, 138.5, 158.4.

Positive-ion APCI-MS: *m/z* 247, relative abundance 31%, $[M+15]^+$; *m/z* 230, 44%, $M^{+\bullet}$; *m/z* 229, 24%, $[M-H]^-$; *m/z* 213, 28%, $[M-17]^+$; *m/z* 187, 25%, $[M-43]^+$; *m/z* 171, 100%, $[M+H-CH_3COOH]^+$. Positive-ion ESI-MS: *m/z* 269, 29%, $[M+K]^+$; *m/z* 253, 27%, $[M+Na]^+$; *m/z* 231, 100%, $[M+H]^+$.

4-(6-Hydroxy-2-naphthyl)-butan-2-ol ($C_{14}H_{16}O_2$, 216.28 g/mol, 0.65 g, 65% yield), melting point 116–118 °C (117–119 °C according to ref. [4]).

^{1}H NMR (300 MHz, CD_3OD) δ 1.19 (3H, d, $J = 6.3$ Hz), 1.65–1.90 (2H, m), 2.65–2.90 (2H, m), 3.65–3.80 (1H, m), 7.02 (1H, dd, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz), 7.06 (1H, bd, $J = 2.5$ Hz), 7.23 (1H, dd, $J_1 = 1.7$ Hz, $J_2 = 8.5$ Hz), 7.49 (1H, bs), 7.53 (1H, d, $J = 8.5$ Hz), 7.60 (1H, d, $J = 8.8$ Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 23.6, 33.0, 42.0, 67.9, 109.8, 119.1, 127.1, 127.3, 128.6, 129.9, 130.0, 134.8, 138.0, 155.7. Positive-ion APCI-MS: *m/z* 232, relative abundance 34%, $[M+16]^+$; *m/z* 216, 100%, $M^{+\bullet}$; *m/z* 215, 31%, $[M-H]^+$; *m/z* 198, 61%, $[M-H_2O]^{+\bullet}$; *m/z* 183, 15%, $[M-33]^+$; *m/z* 173, 20%, $[M-CH_3CO]^+$; *m/z* 158, 88%, $[M-CH_3COCH_3]^{+\bullet}$; *m/z* 157, 81%, $[M-CH_3CHOHCH_2]^+$.

In addition, the APCI-MS and ESI-MS spectra of nabumetone and naproxen (I.S.) were measured for the sake of comparison: nabumetone, molecular weight 228. Positive-ion APCI-MS: *m/z* 244, relative abundance 43%, $[M+16]^+$; *m/z* 228, 67%, $M^{+\bullet}$; *m/z* 227, 100%, $[M-H]^+$; *m/z* 211, 53% $[M-17]^+$; *m/z* 171, 47%, $[M-CH_3COCH_2]^+$. Positive-ion ESI-MS: *m/z* 251, 25%, $[M+Na]^+$; *m/z* 228, 100%, $M^{+\bullet}$; *m/z* 171, 13%, $[M-CH_3COCH_2]^+$.

Naproxen (I.S.), molecular weight 230. Positive-ion APCI-MS: *m/z* 246, relative abundance 28%, $[M+16]^+$; *m/z* 231, 22%, $[M+H]^+$; *m/z* 230, 42%, $M^{+\bullet}$; *m/z* 201, 43%, $[M-29]^+$; *m/z* 185, 100%, $[M-H-CO_2]^+$, *m/z* 171, 7%, $[M-59]^+$. Positive-ion ESI-MS: *m/z* 253, relative abundance 100%, $[M+Na]^+$; *m/z* 231, 1%, $[M+H]^+$. Negative-ion ESI-MS: *m/z* 265, 80%, $[M+Cl]^-$; *m/z* 229, 6%, $[M-H]^-$; *m/z* 185, 100%, $[M-H-CO_2]^-$; *m/z* 170, 91%, $[M-H-CO_2-CH_3]^{\bullet -}$.

2.5. Volunteers, minipigs and biological material

Two healthy human male volunteers (H1: 46 years, 193 cm, 96 kg; H2: 42 years, 178 cm, 72 kg) were treated with one 500 mg nabumetone coated tablet.

Blood samples of 5 ml volume were taken from the cubital vein before and in 0.5, 1.5, 2.5, 3.5, 4.5, 6.5, 8.5, 10.5, 12.5, 24, 36, 48, 72, 96 and 120 h (and also 168 h in the case of the H2 volunteer) after the tablet administration. Blood in the heparinized Monovette® syringes (Sarstedt, Germany) was centrifuged immediately at 3000 rpm for 10 min, the plasma was separated and stored in polypropylene tubes at −70 °C until the analysis. According to stability studies, nabumetone and its metabolites in the plasma did not decompose significantly for at least 65 days.

Human urine samples were also taken during the first 2 days after the administration of nabumetone, and were stored under the same conditions as the plasma samples.

Two white castrated male minipigs (P1: 32 kg and P2: 35 kg of body weight) were used in pharmacokinetic and biodistribution experiments (approved by the regional ethics committee). The blood samples of 5 ml volume were withdrawn from the cannulated vena cava cranialis via vena jugularis externa using a permanent central catheter in the following time intervals: 0 (predose), 0.5, 1, 2, 4, 6, 8, 24, 32 and 48 h after the oral administration of one 500 mg nabumetone coated tablet and processed in an analogous way as the human blood.

After a 2-week wash-out period, a dose of 1000 mg of nabumetone (two 500 mg tablets) was administered orally to the above-mentioned minipigs. Five hours after the administration (corresponding approximately to t_{max} determined in the preceding pharmacokinetic experiments), the animals were sacrificed and fluid samples taken immediately: urine from urinary bladder, bile from gall bladder, synovial fluid from knee joint. Tissue samples from liver, kidney, heart, lung, duodenum, jejunum, illeum, thigh muscle, subcutaneous fatty tissue and skin were also taken (the results will be published later). They were stored at −70 °C until the analysis.

2.6. Sample preparation, liquid–liquid extraction (LLE) and solid phase extraction (SPE)

The frozen plasma samples were thawed out and centrifuged (2000 × *g* for 10 min at 20 °C). To 0.5 ml of plasma in a 9-ml glass tube equipped with a ground-glass stopper, 250 μl of 10^{-4} M naproxen (I.S.) and 25 μl of 36% aqueous HCl were added and the plasma was shortly vigorously shaken. Diethyl ether (3 ml) was added and the content of the tube was vortexed-mixed for 1 min. After centrifugation (2000 × *g*, 12 min), the tubes were stored in a deep freezer (−34 °C for 60 min) until the water layer froze to ice. The organic layer containing the analytes was decanted into another clean 3-ml tube and the solvent was evaporated (water bath 45 °C, stream of nitrogen). The dry extract in the glass tube was reconstituted in 600 μl of the mobile phase and transferred into the vial of the autosampler. 100 μl of the sample were injected into the chromatographic column. Minipig blood plasma was treated in an analogous way (75 μl of 10^{-4} M of I.S. was used in this case).

SPE was also tested. A sample of plasma (0.5 ml) was diluted with 0.5 ml of the phosphate buffer (pH 7.4), spiked with 250 μl of 10^{-4} M naproxen and the solution was shortly shaken. The sample was then acidified with 25 μl of 36% aqueous HCl and again shaken. SPE columns (Supelclean LC-18) were activated on the SPE vacuum manifold with 2 ml of methanol followed by 2 ml of the phosphate buffer (pH 7.4). The diluted and acidified plasma sample spiked with the I.S. was passed through an SPE column. The washing step was performed with the same buffer (pH 7.4). Finally, the captured analytes (nabumetone, its metabolites and naproxen) were eluted from the column with 2 ml of methanol. The methanolic extract was evaporated (45 °C, stream of nitrogen) to dryness. Further procedures were the same as in the case of LLE.

Urine and synovial fluid samples were processed in an analogous way as the plasma samples. No incubations of urine with a mixture of β-glucuronidase and aryl sulphatase (for the enzymatic hydrolysis of conjugates) were performed at this stage.

Bile samples (100 μl) were spiked with I.S., acidified with 5 μl of HCl and diluted with the mobile phase (900 μl), and 100 μl of the supernatant after the centrifugation (2000 × *g*, 12 min) were injected on the chromatographic column.

2.7. HPLC with ultraviolet and fluorescence detection

Routine chromatographic analyses were performed using a Thermo Separation Products (formerly Spectra Physics) chromatograph. The chromatographic system was composed of an SCM400 solvent degasser, P4000 quaternary gradient pump, AS 3500 autosampler with a 100-μl sample loop, FL3000 fluorescence detector, SpectraFOCUS high-speed scanning UV–VIS detector, SN4000 system controller and a data station (Intel-Pentium 166 MMX, RAM 64 MB, HDD 2GB) with the analytical software CHROMQUEST 2.1 (ThermoQuest, Inc., San Jose, CA, USA) working under the operating system WINDOWS NT WORKSTATION 4.0 (Microsoft Corporation). A LiChroCART® 125-4 mm packed with LiChrospher®100 RP-C18, 5μm, precolumn LiChroCART® 4-4 with the same stationary phase (Merck, Darmstadt, Germany) were used for the analyses. The mobile phase composition was acetonitrile–UHQ water–acetic acid (45:55:1, v/v/v). The flow rate was 1 ml/min. UV detection could be performed at 225, 265 and 325 nm (see Fig. 2). For the collection of the UV spectra, a high-speed scanning mode (in the range 195–365 nm with a 1 nm distance) was used. Fluorescence detection (Exc. 230 nm/Emiss. 350 nm) was used for lower concentrations.

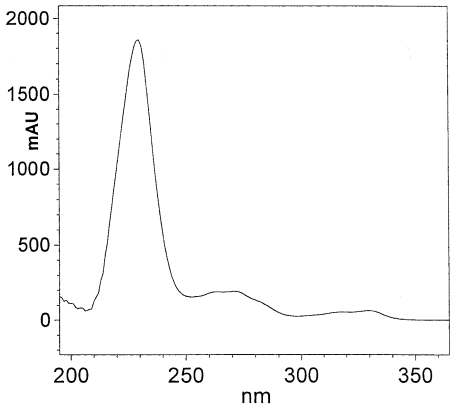

Fig. 2. UV spectrum of nabumetone derivatives obtained on a SpectraFOCUS detector during the HPLC analysis.

2.8. Calibration

Standard 10^{-3} M stock solutions (22.83 mg of nabumetone, 21.62 mg of 6-MNA, 20.22 mg of 6-HNA, 23.03 mg of 4-(6-methoxy-2-naphthyl)-butan-2-ol, 21.43 mg of 4-(6-hydroxy-2-naphthtyl)-butan-2-one, 21.63 mg of 4-(6-hydroxy-2-naphthyl)-butan-2-ol and 23.03 mg of naproxen (each in 100 ml of the respective solution) were prepared. Lower concentrations (10^{-4}, 10^{-5} M) of each compound were obtained by dilution with UHQ water. A calibration series of nabumetone+its metabolites/naproxen (I.S.) mixtures with the concentrations 0.2, 1, 2, 19, 36, 53, 70, 87, 104 and 120 nmol/ml of each analyte, and with the same naproxen concentration (50 nmol/ml) was made. Six individual samples were prepared at each calibration level. The same concentrations were used to make a calibration curve with a drug-free human plasma spiked with nabumetone, its metabolites and naproxen (using appropriate concentrations in order to keep the volumes at a minimum). This calibration series was measured using the UV detector under the condition mentioned in Section 2.7. An analogous ten-level calibration series with the concentration 100-times lower than that in the previous one was prepared and measured using the fluorescence detector. The extraction procedures were the same as described in Section 2.6.

2.9. Testing and statistical evaluation of the analytical procedure

As mentioned in Section 2.8, ten-level calibration series with six analyses at each concentration level was measured. On-line statistical processing of the calibration analyses by the least-squares method was performed automatically using the CHROMQUEST software. The linearity of the calibration curve from the aqueous solutions of nabumetone, its metabolites and naproxen (I.S.)

and from extracts of a drug-free human plasma spiked with the above-mentioned analytes was tested and evaluated [$y = k \cdot x + q$, where x is the concentration ratio of nabumetone (or one of its metabolites, respectively) to naproxen (I.S.) and y is the corresponding peak-area ratio nabumetone (one of its metabolites)/naproxen (I.S.)] and the correlation coefficient (r) was expressed. The accuracy was determined as a relative error (%) found on the standard curve and was calculated from the following equation: Accuracy (%) = $100(C_{real} - C_{determined})/C_{real}$. The precision of the method, expressed as the relative standard deviation (percentage of coefficients of variation; R.S.D. = 100 · S.D./mean), was also assessed. Both statistical parameters were calculated for each concentration level. The range of the applicability of HPLC method was enclosed within the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ). The LLOQ was determined as the lowest concentration on the standard calibration curve which was measured with a precision of 20% and accuracy of 80 or 120% [24,25]. ULOQ was equal to the highest concentration in ten-level human plasma calibration, which was chosen as the 1.2-multiple of c_{max} in average human pharmacokinetics of nabumetone. The recovery for nabumetone and its four metabolites was also calculated [24,25].

The determination of nabumetone derivatives in urine was validated in the analogous way using a six-level calibration.

3. Results and discussion

3.1. Syntheses of the standards and their NMR spectra

To obtain the standards of nabumetone metabolites, simple synthetic and degradation methods starting from commercially accessible reactants were employed.

The principal pharmacodynamically active metabolite of nabumetone, 6-methoxy-2-naphthylacetic acid, was prepared using the Willgerodt–Kindler reaction [26], see Section 2.4.1.

Nabumetone and 6-methoxy-2-naphthylacetic acid served as the starting materials for the preparation of the other metabolite standards (compounds **3**–**6** in Fig. 1). These standards were obtained by the reduction of the carbonyl group (Section 2.4.3), O-desmethylation (according to [27], see Section 2.4.2) or using both reactions in a sequence.

Both ^{1}H and ^{13}C NMR spectra were recorded, in the case of the principal metabolite—6-methoxy-2-naphthylacetic acid—the structure was fully assigned by 2D NMR spectroscopy using gHSQC and gHMBC experiments.

3.2. Mass spectra of the nabumetone derivatives

In mass spectrometric experiments, the ionization and the fragmentation of all synthetic compounds with ESI and APCI were studied in order to confirm their structures. In the negative-ion ESI mode, the signal was obtained only for compounds containing a carboxylic group. The spectra of all carboxylic acids (i.e. compounds **2**, **3** and the I.S.) showed the deprotonated molecules $[M-H]^-$ and the loss of carbon dioxide $[M-H-CO_2]^-$ which is typical for carboxylic acids. The positive-ion ESI mass spectra yielded $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions for all compounds with two exceptions. Compound **6** gave no signal in the positive-ion ESI $M^{+\bullet}$. The sodiated adducts appeared as the base peaks in most spectra, but the abundances of these adducts depend strongly on the concentration of sodium ions in the initial samples. The positive-ion APCI mass spectra yielded the most valuable information for the structure confirmation and the best sensitivity. Since this was universal for all studied, this ionization technique was chosen for HPLC/MS experiments. The radical cation $M^{+\bullet}$ was an abundant ion in all spectra, and it was the base peak for compounds **5**, **6** and the I.S. The radical cations in the spectra of soft ionization technique are not usually observed. The $M^{+\bullet}$ ions in the APCI and ESI spectra are formed by charge exchange mechanism and their presence can be probably explained by low ionization energy of studied aromatic compounds. In addition to the mentioned ions, the even-electron ions were observed in the spectra, such as [M+

$H]^+$, $[M-H]^+$ and some fragment ions with varying abundances (see Section 2.3). A characteristic fragment ion was formed by the cleavage of the side alkyl chain between the first and second carbon atom attached to the aromatic ring. This abundant ion corresponds to *m/z* 171 in the case of the 6-methoxy derivatives (i.e. compounds **1**, **2**, **4** and the I.S.) or *m/z* 157 for the 6-hydroxy derivatives (compounds **3**, **5** and **6**). In summary, the molecular weights of all compounds were unambiguously confirmed and some other structural features were evident from the mass spectra.

3.3. Sample preparation procedure

Even though a very simple extractionless procedure [10] has been described, in our experience, a sample preparation step involving a LLE or a SPE is very suitable for the removal of nabumetone and its Phase I metabolic products from biomatrices. Fewer amounts of ballast compounds were contained in the extracts and the corresponding chromatograms were clearer. With a view to the fact that polar metabolites having short retention times should have been in our method determined by our method as well, the presence of every interferring ballast compound was very undesirable.

Both LLE and SPE were tested. In the LLE, 0.5 ml of plasma (urine, synovial fluid) was spiked with the I.S. As the presence of acidic metabolites (compounds **2** and **3**) together with the neutral ones (compounds **4**, **5**, **6**) was expected in biomatrices, the samples were acidified in order to suppress the dissociability of the carboxylic group of compounds **2**, **3** and the I.S., and to increase the extractability of the analytes into the organic solvent. Three different extraction media were tested (dichloromethane, diethyl ether and ethyl acetate). The best results (according to the validation parameters) were achieved with diethyl ether. The separation of the aqueous and organic phases by freezing the aqueous phase is an elegant and effective method, because the upper organic phase is easily removable by simple decantation.

During the SPE, the plasma was diluted by the phosphate buffer and after the addition of the I.S. and acidification of the mixture, passed through an SPE column. The acidification is probably a critical moment of the SPE. When insufficient amount of HCl is added, the retention of acid metabolites (compounds **2**, **3** in Fig. 1) and of naproxen (I.S.) on octadecylsilyl silica gel of the SPE column is poor, and during the washing step of the SPE, a part of these acidic analytes is washed out together with the ballast compounds.

Both above-mentioned extraction methods were found to be comparable (according to the validation parameters—see Table 1).

Only in the case of bile samples, better interpretable chromatograms were obtained from 10-fold diluted and acidified bile.

3.4. Chromatography

Because the principal metabolites of nabumetone in the plasma and urine were 6-methoxy- and 6-hydroxy-2-naphthylacetic acid (6-MNA, 6-HNA), naproxen (also a NSAID, a higher homologue of 6-MNA) was chosen as an I.S. Naproxen was commercially available, possesses the same chromophore (fluorophore) as nabumetone and all its metabolites, and exhibited a very similar behavior in the course of the extractions (LLE, SPE) and HPLC analyses.

All analytes under study had very similar ultraviolet and fluorescence spectra. The UV spectrum of nabumetone and its derivatives is shown in Fig. 2. From the three maxima (225, 265 and 325 nm) found in the spectrum, the best results (according to the validation parameters) were achieved at the wavelength of 265 nm. The fluorescence excitation and emission spectra are shown in Fig. 3. The excitation maximum at 230 nm was common for all analyzed compounds. The emission spectra were slightly different for 6-methoxy derivatives (350 nm for compounds **1**, **2**, **4** and I.S.) and for 6-hydroxy derivatives (358 nm for compounds **3**, **5** and **6**).

The chromatographic analysis of the extract from the blank human plasma spiked with nabumetone, its metabolites and naproxen (I.S.) under the conditions mentioned in Section 2.7 lasted 12 min and is shown in Fig. 4A. For comparison, a chromatogram of a drug-free plasma is shown in the same picture. The mutual separation of all

Table 1
Validation results of the bioanalytical HPLC method for nabumetone and its metabolites using SPE with a UV-detection (A), LLE with a UV-detection (B), and LLE with a fluorescence detection (C)

Compound (number in Fig. 1)	Regression equation	r	Precision (%)	Accuracy (%)	LLOQ (nmol/ml)	ULOQ (nmol/ml)	Recovery (%)
(*A*)							
6-HNA (**3**)	$y = 0.9375x - 0.0020$	0.998	0.92–7.43	97.60–102.60	9	120	82
6HOnphBuOH (**6**)	$y = 0.722x + 0.0009$	0.995	0.82–11.53	97.93–107.60	9	120	87
6-MNA (**2**)	$y = 0.9857x - 0.0016$	0.999	0.29–11.75	86.52–100.93	0.2	120	78
6MeOnphBuOH (**4**)	$y = 1.0034x - 0.0071$	0.998	1.36–10.06	89.85–102.07	1	120	83
Nabumetone (**1**)	$y = 0.9397x + 0.045$	0.998	1.22–6.93	95.26–109.69	1	120	80
(*B*)							
6-HNA (**3**)	$y = 1.0004x - 0.0235$	0.999	1.14–3.99	82.95–102.89	9	120	81
6HOnphBuOH (**6**)	$y = 0.7958x - 0.017$	0.999	1.29–6.56	85.41–104.34	2	120	85
6-MNA (**2**)	$y = 1.0188x - 0.0063$	0.999	0.50–2.67	98.55–109.81	1	120	78
6MeOnphBuOH (**4**)	$y = 1.0126x - 0.006$	0.999	0.61–7.93	99.14–101.13	1	120	82
Nabumetone (**1**)	$y = 0.9918x - 0.0037$	0.999	0.48–12.71	83.38–101.47	1	120	81
(*C*)							
6-HNA (**3**)	$y = 0.4459x - 0.0069$	0.994	1.21–9.05	95.27–107	0.02	1.200	81
6-MNA (**2**)	$y = 1.1104x + 0.0281$	0.999	0.30–3.46	88.3–110.4	0.01	1.200	79
6MeOnphBuOH (**4**)	$y = 1.4531x + 0.021$	0.999	0.50–8.10	86.6–107.04	0.01	1.200	84
Nabumetone (**1**)	$y = 0.4969x + 0.0027$	0.999	1.02–6.31	95.61–101.51	0.01	1.200	80

nabumetone derivatives in the chromatogram was found to be sufficient. Only the metabolite 4-(6-hydroxy-2-naphthyl)-butan-2-one (6-HOnphBu=O, $t_R \cong 2.9$–3 min) interfered with some ballasts from the plasma and in higher concentrations, also with the principal metabolite, 6-MNA ($t_R = 3.46$ min). Because 6-HOnphBu=O was found in no real plasmatic samples, it was excluded from the validation experiments in the plasma.

The HPLC method with ultraviolet and fluorescence detection was transferred to the HPLC/MS system. The mass spectrometric detection was applied to the confirmation of the expected metabolites. The concentration of acetic acid in the mobile phase was reduced from 1 to 0.1% for the HPLC/MS analysis, all other parameters were identical as described before for HPLC/UV and fluorescence detection. This change led to only a small decrease of the retention, and the resultant time shift did not complicate the peak assignment. The positive-ion APCI was applied to the HPLC/MS identification of the metabolites on the basis of the previous study with ESI and APCI, as discussed above. The presence of two principal metabolites (6-MNA, 6-HNA) in the biomatrices was unambiguously confirmed (see Sections 2.4 and 3.2).

For pharmacokinetic and biodistribution studies of nabumetone and its metabolites in different biomatrices, routine bioanalytical HPLC methods involving either a LLE or a SPE, and based on the ultraviolet or fluorescence detection were validated. The validation parameters summarized in Table 1 show that the UV detection is suitable for 6-MNA in the range 0.2–120 nmol/ml. In our study, the value of LLOQ (0.2 nmol/ml) is comparable with that published by de Jager [10] (70 ng/ml $\cong$ 0.35 nmol/ml). The fluorescence detection covers lower concentrations (0.01–1.2 nmol/ml). The use of both liquid–liquid and SPE in the sample preparation step furnished similar results. The recovery for different metabolites ranged between 78 and 87%.

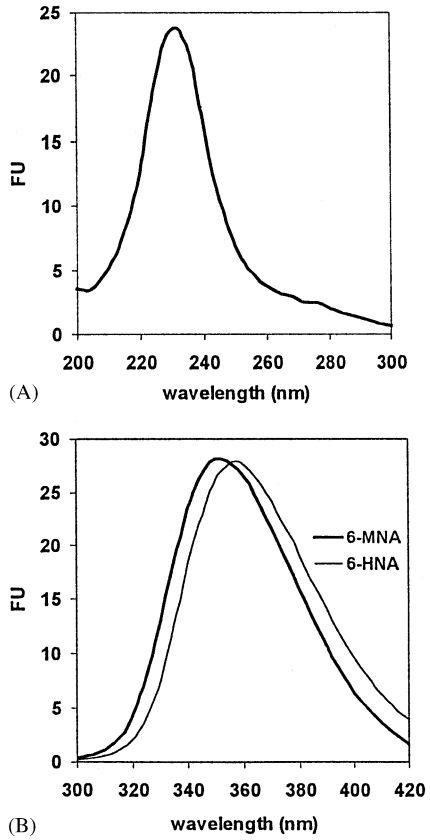

Fig. 3. Excitation spectrum (A, at the emission wavelength of 350 nm) and emission spectra (B, at the excitation wavelength of 230 nm) of nabumetone derivatives. The spectra were recorded during the HPLC analysis using an FL3000 fluorescence detector.

3.5. Pharmacokinetics and distribution of nabumetone metabolites

The HPLC method (involving the LLE and UV detection) was used for interspecies comparison of the biotransformation of nabumetone, and body fluid disposition of its metabolites. Nabumetone and its metabolites were determined in the blood plasma and urine of humans, and in the plasma, urine, bile and synovial fluid of minipigs, after the oral administration of nabumetone coated tablets.

The principal nabumetone metabolite found both in human and minipig blood plasma was 6-methoxy-2-naphthylacetic acid (6-MNA; Fig. 4B and C). Another detected metabolite in the plasma of both species was 6-hydroxy-2-naphthylacetic acid (6-HNA). However, its concentrations were approximately ten-times lower in humans as compared with minipigs. Parent compound (nabumetone) was not detected in the plasma.

Similar situation, as regards 6-HNA and 6-MNA, was found out in the synovial fluid of minipigs. But, in contrast to the plasma, nabumetone and its metabolite, 4-(6-methoxy-2-naphthyl)-butan-2-ol, were detected in synovial fluid samples.

While the plasma and synovial fluid appeared as simple biomatrices with a minimum of interfering ballast compounds in their extracts (see Fig. 4, Fig. 5D), the analyses of urine extracts and diluted bile samples were complicated by the presence of polar ballast compounds, which interfered with the most polar metabolites (see Fig. 5E and F). From all five metabolites and trace amounts of nabumetone found in these biomatrices, 6-hydroxy-2-naphthylacetic acid (6-HNA) prevailed.

These body fluid analyses based on HPLC with the UV and mass spectrometric detection suggest that metabolic pathways of nabumetone involve O-desmethylation, reduction of the carbonyl group and oxidation of the butanone side-chain to substituted 2-naphthylacetic acid in both species under study (human and minipig).

The utility of the developed HPLC method was also demonstrated by the quantitative determination of the plasma concentrations of nabumetone metabolites after the oral administration of 500 mg of nabumetone to two humans (Fig. 6), and two minipigs (Fig. 7). Table 2 summarizes the pharmacokinetic parameters of 6-MNA calculated from the concentrations determined using non-compartmental methods.

The results show that 6-MNA reached considerably higher levels than 6-HNA both in humans and in minipigs. However, while 6-HNA reached reasonable concentrations to be measured in minipigs (the c_{max} ratios of 6-HNA and 6-MNA are 0.15 (P1) and 0.11 (P2)), the compound was below the LLOQ in human plasma.

The human pharmacokinetic parameters are comparable with the previously published data [8–10] but the minipig data have not been published yet. The time to reach the maximum

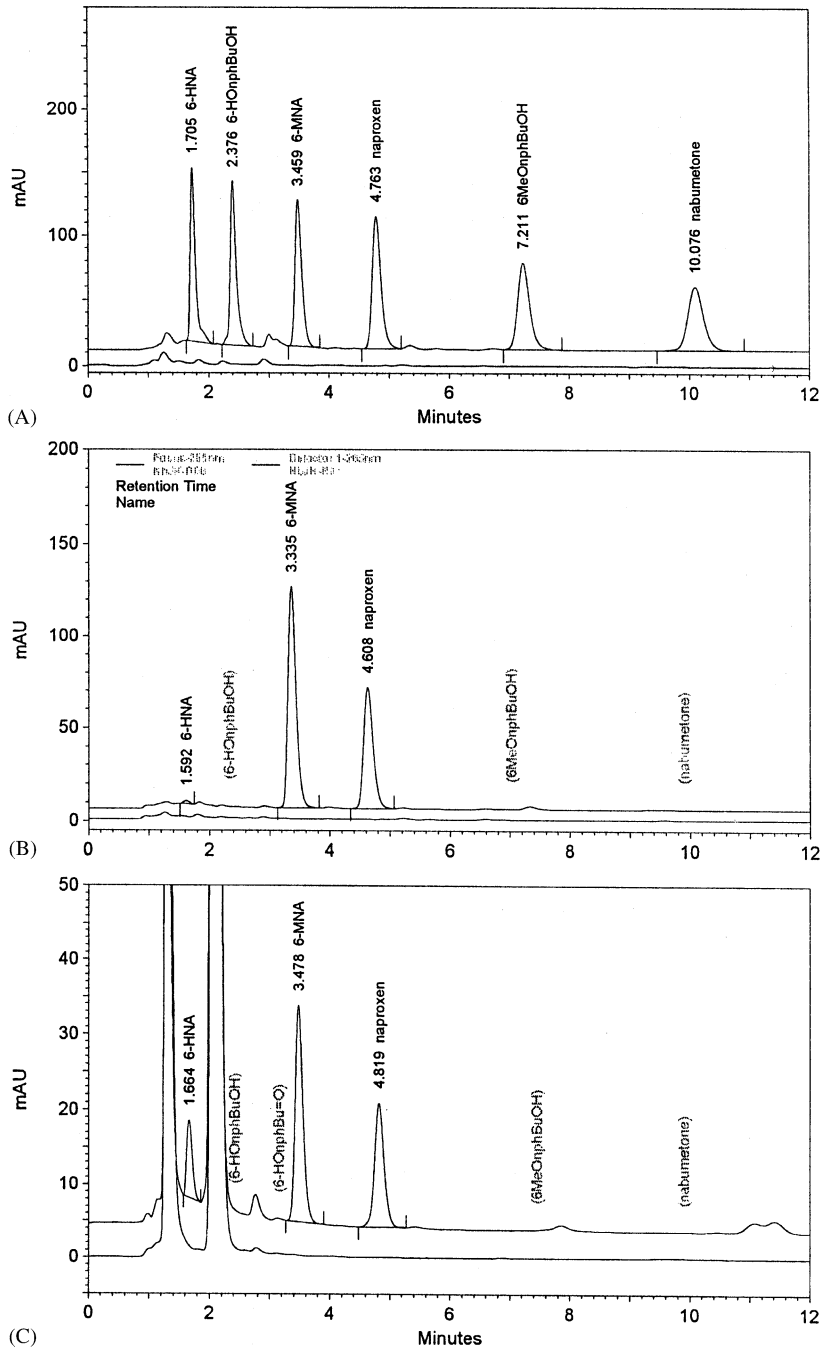

Fig. 4. Typical chromatograms of the extracts from human and minipig plasma samples: a blank human plasma spiked with the same amounts of nabumetone, its four metabolites and naproxen (upper chromatogram in A with 4167 pmol of each compound) in comparison with a drug-free human plasma (lower chromatogram in A). Analyses from human pharmacokinetics before (lower chromatogram in B) and 10.5 h after the administration of nabumetone (upper chromatogram in B). Analyses from minipig pharmacokinetics before (lower chromatogram in C) and 6 h after the administration of nabumetone (upper chromatogram in C).

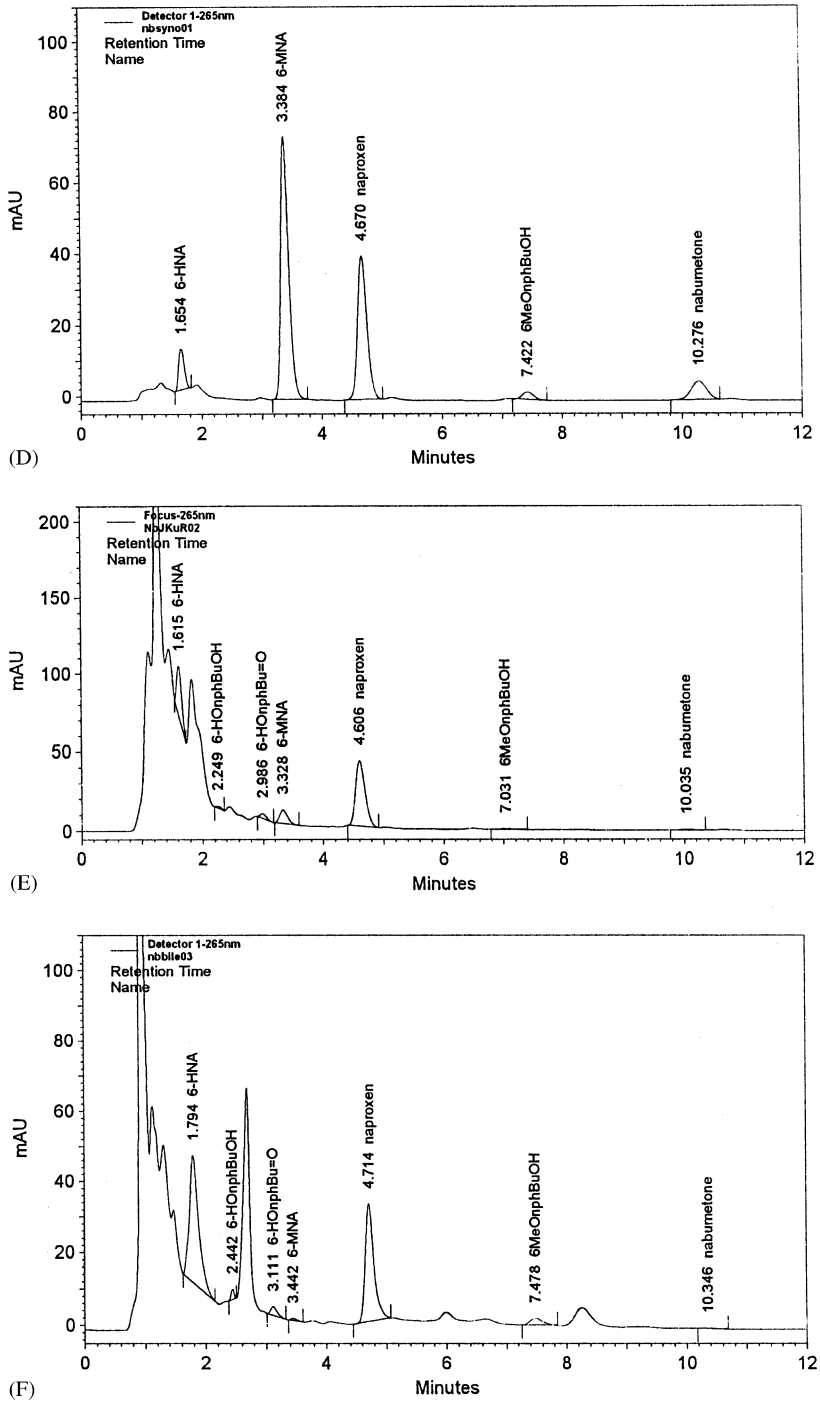

Fig. 5. Typical chromatograms of the extracts from a minipig synovial fluid (D), human urine (E) and from a diluted minipig bile (F) after the oral administration of nabumetone.

plasma concentrations of 6-MNA in minipigs is similar to that in humans. Taking the body weight into account, the same applies to the c_{max} parameter. However, the other three parameters (AUC0 $\rightarrow \infty$, $t_{1/2}$, k_e) are different for both compared species.

The time courses of the plasma 6-MNA concentrations show that the technique described is sensitive enough to allow the determination of 6-MNA longer than five half-lives both in humans (>150 h) and in minipigs (>35 h) following the oral administration of a nabumetone dose of 500 mg.

4. Conclusions

Bioanalytical methods involving either the extractions (LLE, SPE) of the plasma, synovial fluid, urine or just the dilution of the bile (for the direct injection), and subsequent high-performance liquid chromatographic determination of nabumetone and its metabolites based on the ultraviolet, fluorescence and mass spectrometric detection were developed and validated. Simple methods for the preparation of the standards of five nabumetone metabolites (Phase I metabolites) were designed. The identity of the standards was confirmed using NMR, APCI-MS and ESI-MS analyses. HPLC-MS analyses were used for the identification of the nabumetone metabolites separated from the biomatrices analyzed. The validated bioanalytical method (LLE $\rightarrow$ HPLC $\rightarrow$ UV detection) was applied to pharmacokinetic and biodistribution studies, where the disposition of nabumetone and its five metabolites (including the pharmacodynamically active 6-methoxy-2-naphthyl-acetic acid, 6-MNA) was followed in two biological species, in humans and minipigs.

The HPLC-UV (FL, MS) methods presented here were also applied to the biodistrubution studies of nabumetone and its metabolites in the tissues of minipigs (as mentioned in Section 2.5).

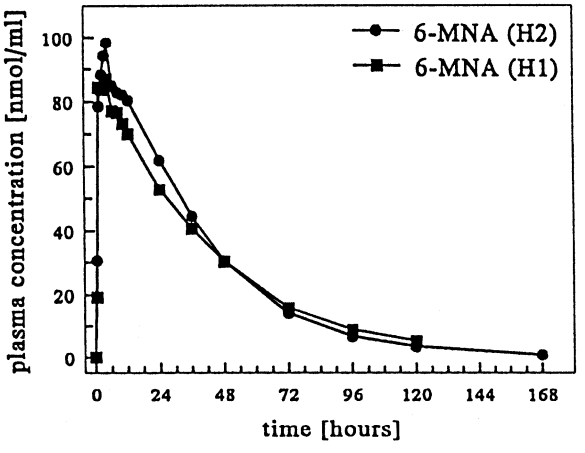

Fig. 6. The time course of plasma concentrations of 6-MNA following a single oral dose of 500 mg of nabumetone administered to two healthy male volunteers (H1 and H2).

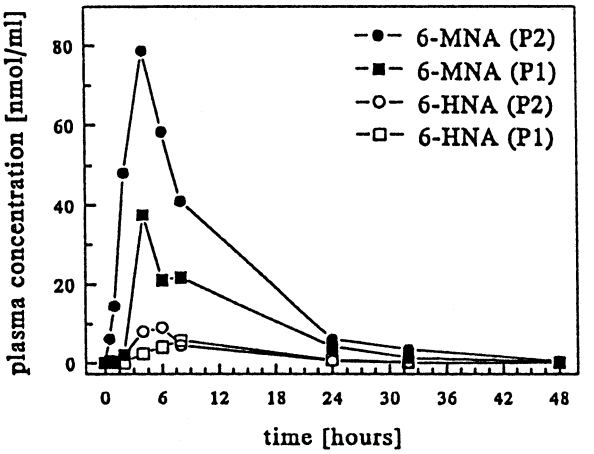

Fig. 7. The time course of plasma concentrations of 6-MNA and 6-HNA following a single oral dose of 500 mg of nabumetone administered to two minipigs (P1 and P2).

Table 2
Pharmacokinetic parameters of 6-MNA in two healthy human male volunteers and two castrated male minipigs following a single oral administration of 500 mg of nabumetone

Parameter	Human volunteers		Minipigs	
	H1	H2	P1	P2
$AUC_{0\rightarrow\infty}$ (nmol h/ml)	3813	3865	343	758
c_{max} (nmol/ml)	86.9	98.3	37.6	78.6
t_{max} (h)	4.5	4.5	4.0	4.0
$t_{1/2}$ (h)	30.0	23.1	6.5	6.7
k_e (per h)	0.0231	0.0300	0.1065	0.1027

Results from these experiments will be presented in another contribution.

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