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Note

High-performance liquid chromatographic determination of a new anti-inflammatory agent, nabumetone, and its major metabolite in plasma using fluorimetric detection

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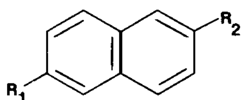
Nabumetone, 4-(6-methoxy-2-naphthyl)butan-2-one (I) (Fig. 1) is a new non-steroidal, anti-inflammatory agent that has excellent tolerance in fasting and non-fasting subjects [1]. The drug exhibits anti-inflammatory activity in acute and chronic animal models and has mild analgesic and anti-pyretic properties [2]. After oral administration of [^{14}C]nabumetone to three healthy male subjects, 80% of the radioactivity was recovered in urine and 9% in the feaces [3]. Intact nabumetone was not detected in plasma and the major labelled plasma component was 6-methoxy-2-naphthylacetic acid (II) (Fig. 1), a metabolite of nabumetone which has anti-inflammatory activity in animals [2].

This compound (II) is a more potent inhibitor of prostaglandin synthesis than nabumetone and although the mode of action of nabumetone is not known, the available evidence suggests that its activity resides in its metabolites [2].

Three other metabolites (Fig. 1), 4-(6-hydroxy-2-naphthyl)butan-2-one (III),



		R_1	R_2	RRT
4-(6-Methoxy-2-naphthyl)butan-2-one	(I)	OCH_3	$CH_2CH_2COCH_3$	1.10
4-(6-Hydroxy-2-naphthyl)butan-2-one	(III)	OH	$CH_2CH_2COCH_3$	0.57
4-(6-Hydroxy-2-naphthyl)butan-2-ol	(IV)	OH	$CH_2CH_2CHOHCH_3$	0.57
6-Methoxy-2-naphthylacetic acid	(II)	OCH_3	CH_2COOH	0.68
6-Hydroxy-2-naphthylacetic acid	(V)	OH	CH_2COOH	0.43
6-Chloro-2-naphthylacetic acid	(VI)	Cl	CH_2COOH	1.00
6-Methoxy-2-naphthoic acid		OCH_3	COOH	0.82

Fig. 1. Chemical structures and relative retention times (RRT) of nabumetone and its major metabolites. VI = internal standard.

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4-(6-hydroxy-2-naphthyl)butan-2-ol (IV) and 6-hydroxy-2-naphthylacetic acid (V) have been identified in human urine [3].

A gas chromatographic method for the determination of 6-methoxy-2-naphthylacetic acid in plasma lacks the sensitivity necessary for pharmacokinetic studies [1]. The present paper describes a high-performance liquid chromatographic assay (HPLC) with fluorescence detection, developed for the determination of nabumetone and its major metabolite (II) in plasma. This method has adequate sensitivity and specificity for single-dose pharmacokinetic studies.

MATERIALS AND METHODS

Chemicals and reagents

4-(6-Methoxy-2-naphthyl)butan-2-one (nabumetone, I, BRL 14777), 6-methoxy-2-naphthylacetic acid (II, BRL 10720), 6-chloro-2-naphthylacetic acid (VI, BRL 24333, internal standard) and other metabolites of nabumetone were obtained from Beecham Pharmaceuticals (U.K.). Stock solutions of nabumetone (10 mg per 5 ml), II (10 mg per 5 ml) and internal standard (4 mg per 10 ml) were prepared daily by dissolving the compounds in acetone.

Acetone (Hopkin and Williams, M.F.C.), hexane (Unichrom), ethyl acetate (Merck, GR), methanol (Unichrom), hydrochloric acid (Univar) and sodium acetate (Univar) were obtained from Ajax Chemicals (Sydney, Australia) and were used without further purification.

Extraction procedure

Plasma (0.5 ml) was placed in a 10-ml glass, screw-capped tube containing internal standard (1 μg for standard range 0.1—1 μg/ml and 50 μg for standard range 5—50 μg/ml). After thorough mixing on a whirlmixer, 6.0 ml of *n*-hexane—ethyl acetate (50:50) mixture and 0.7 ml of 1.5 mol/l hydrochloric acid were added. The closed tube was mechanically shaken for 30 min and then centrifuged for 10 min at 1500 *g*. The organic layer was transferred to a conical glass tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The sample residue was dissolved in mobile phase, centrifuged and 20-μl aliquots were injected into the chromatograph.

Apparatus and chromatographic conditions

Assays were carried out on a Varian 5000 liquid chromatograph (Varian, Sydney, Australia). Samples were injected with a Varian 8055 autosampler injector with a pneumatic-actuated Rheodyne 7126 injection valve (Reodyne, Berkeley, CA, U.S.A.) fitted with a 20-μl sample loop. A Schoeffel FS 970 LC fluorometer (Schoeffel, Westwood, NJ, U.S.A.) was used as the detector with the excitation monochromator set at 284 nm and the fluorescence emission at 320 nm (cut-off filter). Detector output was quantified using a 3390A integrator (Hewlett-Packard, Sydney, Australia). Calibration curves were constructed by calculating the ratio of the peak height of each compound (nabumetone or II) to that of the internal standard (VI).

Separations were performed on a 5-μm Ultrasphere ODS reversed-phase column (25 cm × 4.6 mm I.D.; Altex, Berkeley, CA, U.S.A.) with a Lichrosorb RP-8 guard column (Brownlee Labs., Santa Clara, CA, U.S.A.). The compounds

were eluted with a mobile phase of methanol in 0.05 mol/l sodium acetate buffer (pH 3.0), 70:30, v/v. The column temperature was 40°C and the flow-rate was 1 ml/min.

RESULTS AND DISCUSSION

Reversed-phase HPLC with fluorescence detection was an effective method of quantifying nabumetone and its major metabolite II in human plasma. Typical chromatograms obtained from blank plasma and plasma containing nabumetone, II and VI (internal standard) are shown in Fig. 2. Interference by endogenous substances did not occur from drug-free plasma (Fig. 2A). The three known metabolites of nabumetone (Fig. 1) were chromatographed as pure substances and did not interfere with nabumetone, II or the internal standard. Under the assay conditions described, naproxen, paracetamol, acetylsalicylic acid and salicylic acid had elution times of 7.0, 3.1, 3.1 and 3.1 min, respectively, and did not interfere with the nabumetone, II or internal standard peaks. However, acetylsalicylic acid and salicylic acid, at concentrations above 100 μg/ml, may interfere with the II peak due to the intense fluorescence of these compounds.

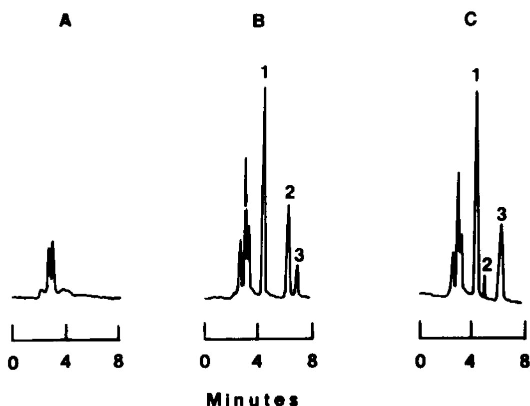

Fig. 2. Chromatograms of plasma extracts. (A) Blank human plasma; (B) human plasma containing 1 μg/ml II (1), 2 μg/ml internal standard VI (2) and 1 μg/ml nabumetone (3); (C) plasma collected 5 h after 500 mg nabumetone were administered orally to a male subject; II (1), 6-methoxy-2-naphthoic acid (2) and internal standard VI (3).

The standard curves for nabumetone and II in plasma were linear over the concentration range 0.25—50 μg/ml and 0.1—50 μg/ml, respectively. The correlation coefficient for the standard curves over this concentration range was 0.999 (n=4). The coefficient of variation was $<$ 6% for all concentrations measured (Table I). The sensitivity of the method was approx. 0.1 μg/ml of plasma for II and 0.25 μg/ml of plasma for I. The recovery for nabumetone was 92 ± 4% (mean ± S.D.), for II, 93 ± 4% and for VI (internal standard) 90 ± 4% (Table I).

The concentration of II, was measured in plasma after a single 500-mg oral dose of nabumetone (Fig. 3). Intact drug (nabumetone) was not detected in plasma for 72 h after administration of the compound, which is in agreement with a previous study performed using [^{14}C]nabumetone [2]. After the oral

TABLE I

REPRODUCIBILITY AND RECOVERY OF STANDARDS EXTRACTED FROM HUMAN PLASMA (n=4)

Concentration (μg/ml)	Coefficient of variation (%)		Recovery (%)	
	Nabumetone	II	Nabumetone	II
0.10	N.D.*	3.8	N.D.	93
0.25	5.9	2.0	99	94
0.50	4.0	1.8	90	99
0.77	4.7	1.3	94	93
1	1.6	1.2	92	94
5	4.8	3.7	91	94
10	4.6	3.6	89	87
25	4.6	3.5	90	90
33	3.3	1.1	88	89
50	1.0	1.9	97	96

*N.D. = not detected.

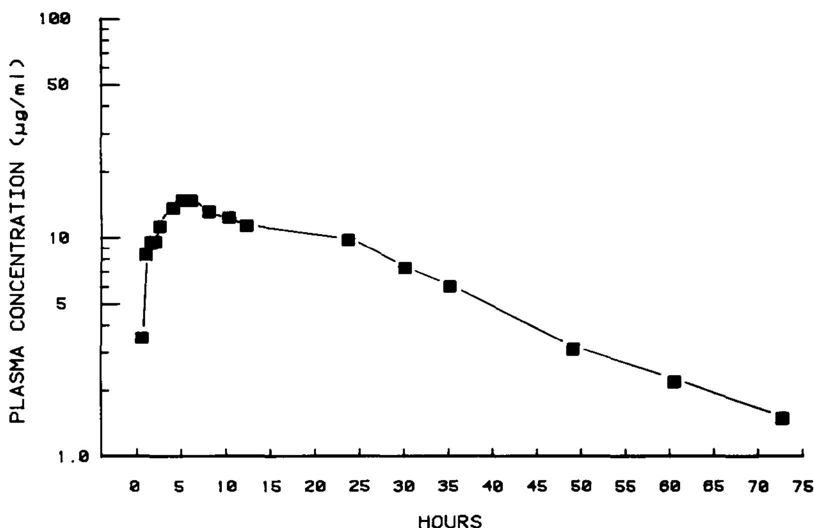

Fig. 3. Plasma concentrations of II after a single 500-mg oral dose of nabumetone.

administration of nabumetone, a peak that had not been detected in a previous study [1] appeared in plasma with a retention time of 5.2 min (Fig. 2C). This peak was identified as 6-methoxy-2-naphthoic acid, a known metabolite of nabumetone (Fig. 1), by collecting the HPLC fraction containing the unknown peak and subjecting this fraction to gas chromatographic—mass spectrometric analysis. This metabolite has little anti-inflammatory activity [4] and does not reach significant concentrations in plasma after a single oral dose.

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