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Note

Quantitative gas chromatographic determination of nefopam in plasma

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Nefopam (Aupan®) is a potent analgesic[1,2] with a unique heterocyclic structure (Fig. 1). This paper gives a method for the determination of nefopam in human plasma at the low nanograms per millilitre level using gas chromatography with flame-ionization detection.

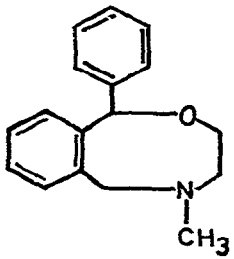

Fig. 1. Structure of nefopam.

EXPERIMENTAL

Apparatus

The gas chromatographic analysis was carried out with a Varian 1400 instrument equipped with a flame-ionization detector. The temperatures were: column 170°, injector 230° and detector 240°. The flow-rates were carrier gas (nitrogen) 25, oxygen 200 and hydrogen 25 ml/min.

The glass column (90 cm × 2 mm I.D.) was acid washed and silanized with 5% dimethyldichlorosilane in toluene and packed with 3% OV-225 on Gas-Chrom Q, 100–120 mesh. The column was flushed with carrier gas (nitrogen, flow-rate 25 ml/min) at 50° for 30 min and conditioned at 275° with no flow for 3 h then with a flow (25 ml/min) for 16 h.

The mass spectrometric analysis was performed on an LKB 9000 instrument equipped with an accelerating voltage alternator (ionizing energy 70 eV). The gas chromatographic column (150 cm × 2 mm I.D.) was packed with 5% OV-17 on Gas-Chrom Q, 80–100 mesh, and conditioned as described above.

The photometric determinations were performed with a Zeiss PMQ III Spek-

tralphotometer with 10-mm cells. The pH measurements were made with an Orion Research Model 701 digital pH meter equipped with an Ingold Type 401 combined electrode.

Chemicals

All solvents were of analytical grade. The aqueous and organic phases were carefully equilibrated before use in partition experiments. Phosphate buffers with an ionic strength of 0.1 were used. Nefopam and N-isopropyldemethylnefopam (hydrochlorides) were kindly supplied by Riker Labs., Northridge, Calif., U.S.A.

Determination of acid dissociation constants

The dissociation constant of the amino group was determined by potentiometric titration of $4 \cdot 10^{-3}$–10^{-2} *M* solutions of nefopam hydrochloride with 0.1 *M* sodium hydroxide solution at 25.0 ± 0.1°.

Determination of partition coefficients

The partition coefficients of nefopam were determined in batch experiments at 25.0 ± 0.1° using an equilibration time of 30 min. The concentration of nefopam was determined photometrically at 267 nm in both phases, in the aqueous phase by direct measurement and in the organic phase after re-extraction with 0.1 *M* orthophosphoric acid.

General procedure for the determination of nefopam in plasma

Plasma (5.00 ml) was mixed with an aqueous solution (0.200 ml) of the internal standard, N-isopropyldemethylnefopam hydrochloride (1.31 μg/ml), and 0.1 ml of 1 *M* sodium hydroxide solution. Toluene (5.00 ml) was added and the mixture was shaken for 30 min. The organic phase was separated, extracted with 5 ml of phosphate buffer (pH 7.3) for 10 min (aqueous phase discarded) and with 0.1 ml of 0.1 *M* sulphuric acid for 10 min. The aqueous phase was treated with nitrogen to remove trace amounts of toluene, mixed with 0.025 ml of 1 *M* sodium hydroxide solution and 0.025 ml of methylene dichloride and extracted for 5 min.

The organic phase (2 μl) was injected into the gas chromatograph. All quantitations were based on peak-height measurements.

RESULTS AND DISCUSSION

The following symbols are used:

$[\ ]_{org}$, $[\ ]$ = concentrations of ions and molecules in organic and aqueous phase, respectively;

C_{org}, C_{aq} = total concentrations in organic and aqueous phase, respectively;

$k'_{HA} = \dfrac{a_{H+}\,[A]}{[HA^+]}$ = apparent acid dissociation constant of HA^+;

$k_d = \dfrac{[A]_{org}}{[A]}$ = partition coefficient of A;

$$D_A = \frac{C_{A\ org}}{C_{A\ aq}} = \text{partition ratio of A.}$$

Determination of acid dissociation constant

The dissociation constant of the amino group was determined by potentiometric titration. A plot of pH *versus* log ([A]/[HA$^+$]) for $0.3 \leqslant [A]/[HA^+] < 3$ gave a straight line with $pk'_{HA} = 8.18$ as intercept and a slope of 1.00.

Determination of partition coefficients

The partition properties of nefopam were studied using toluene and methylene dichloride as the organic phase and phosphate buffers as the aqueous phase. Constants were evaluated graphically from eqn. 1 by means of a linear plot of D_A^{-1} *versus* a_{H^+}.

$$\frac{1}{D_A} = \frac{1}{k_d} + \frac{a_{H^+}}{k_d \cdot k'_{HA}} \qquad (1)$$

The products $k_d \cdot k'_{HA}$ calculated from the slopes are presented in Table I. The intercepts were too small for evaluation of k_d with sufficient accuracy. The partition coefficients included in Table I were calculated from $k_d \cdot k'_{HA}$ and the acid dissociation constant, determined as described above.

TABLE I

PARTITION COEFFICIENTS

Aqueous phase: phosphate buffer ($\mu = 0.1$).

Organic solvent	$C_{HA\ aq} \cdot 10^3$	$C_{A\ org} \cdot 10^3$	*pH*	$\log k_d \cdot k'_{HA}$	$\log k_d$
Toluene	0.89–2.23	1.24–2.59	4.99–5.72	−5.25	2.93
Methylene dichloride	0.48–1.11	0.97–1.60	4.36–4.95	−4.40	3.78

Recovery and precision

Fig. 2 shows a chromatogram from a plasma sample containing 9 ng/ml of nefopam. A standard graph prepared in the range 5–50 ng/ml is depicted in Fig. 3. No interfering peaks were observed when analyzing blank plasma. The recovery at the 10 and 100 ng/ml levels, obtained by using aliquots throughout the method, was 90% (calculated: 99%). The relative standard deviation was $\pm$ 2.2% at 10 ng/ml ($n = 5$) and $\pm$ 2.7% at 50 ng/ml ($n = 5$).

Plasma levels after oral dosing

Table II gives the concentration of nefopam in plasma after an oral dose of 60 mg. Analysis of the same plasma samples by gas chromatography–mass spectrometry (focusing at *m/e* 179) gave the same results, indicating that there were no interferences from metabolites of nefopam.

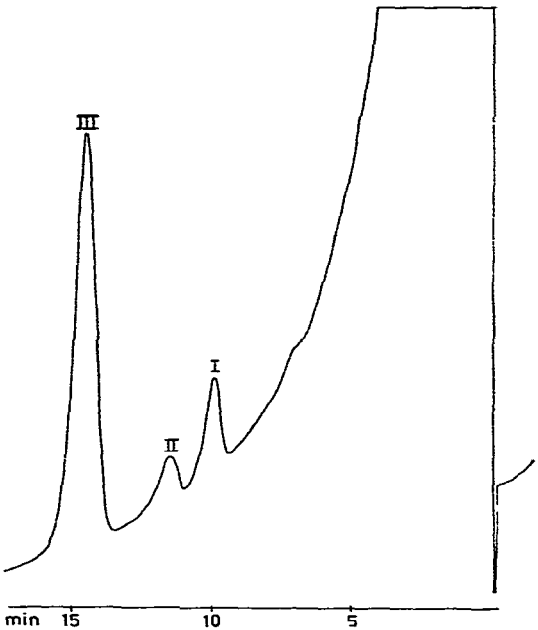

Fig. 2. Chromatogram of a plasma sample containing nefopam. Concentration of nefopam: 9.1 ng/ml (as base). Peaks: I = nefopam; II = unknown endogenous component; III = N-isopropyldemethylnefopam (internal standard).

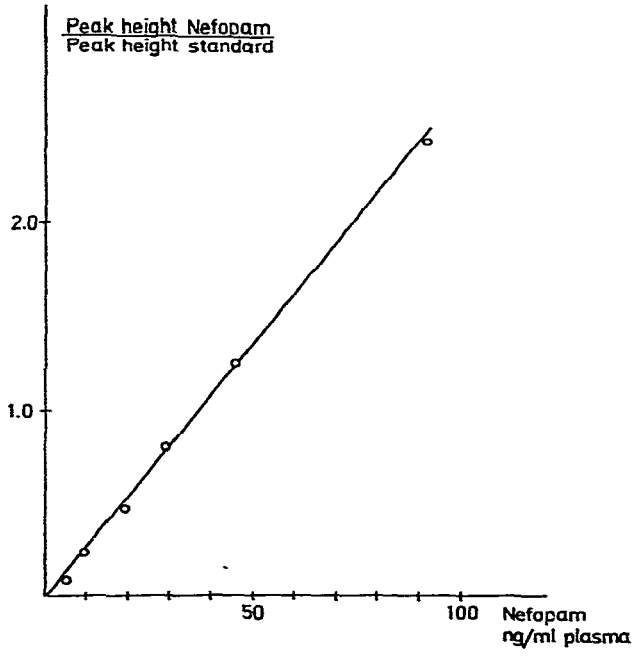

Fig. 3. Standard graph for the determination of nefopam in plasma.

TABLE II

CONCENTRATION OF NEFOPAM IN PLASMA AFTER AN ORAL DOSE OF 60 mg NEFOPAM HYDROCHLORIDE

The drug was given to one healthy volunteer. Weight: 70 kg.

Time after dose (h)	*Concentration (ng/ml)*
1	8.5
2	22.0
4	17.5
6	10.0

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