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Note

Rapid and sensitive gas chromatographic method for the determination of alfentanil and sufentanil in biological samples

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Alfentanil (AF), or N-{1-[2-(4-ethyl-4,5-dihydro-5-oxo-1H-tetrazol-1-yl)-ethyl]-4-(methoxymethyl)-4-piperidinyl}-N-phenylpropanamide, and sufentanil (SF), or N-{4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl}-N-phenyl-propanamide, are two novel narcotic analgesics with structures similar to that of fentanyl. Both compounds are currently under clinical investigation in anaesthesiology [1, 2].

The purpose of the present paper is to describe a rapid, sensitive and specific gas chromatographic (GC) procedure for the determination of AF and SF in plasma and other biological samples. The method will be used for pharmacokinetic studies in animals and humans.

EXPERIMENTAL

Standards and reagents

Alfentanil hydrochloride (R 39 209), sufentanil citrate (R 33 800) and the internal standard (R 38 527; IS) or N-{1-[3-(4-ethyl-4,5-dihydro-5-oxo-1H-tetrazol-1-yl)propyl]-4-(methoxymethyl)-4-piperidinyl}-N-phenylpropanamide hydrochloride hemihydrate were synthesized in our research laboratories and were of analytical grade. Chemical structures are shown in Fig. 1.

Spectrophotometric grade *n*-heptane and methanol were used; the isoamyl alcohol was of analytical grade. The Clin Elut™ tubes were analytically pure (CE 1003; Analytichem International, Lawndale, CA, U.S.A.).

Using the salt forms of AF, SF and IS, stock solutions were prepared in methanol corresponding to 1 mg/ml as the free base. Standard solutions were obtained by diluting the AF and SF stock solutions with methanol to a concentration range of 0.01—10 μg/ml. Internal standard solutions were prepared at a concentration of 1 μg/ml for IS, the internal standard for the determina-

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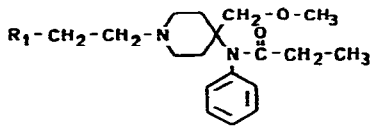


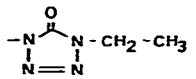
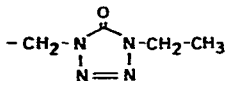
Compound	R_1
AF	
SF	
IS	

Fig. 1. Chemical structures of alfentanil (AF), sufentanil (SF) and the internal standard (IS).

tion of AF as well as for AF itself, which in turn was used as the internal standard for SF.

Apparatus

All the analyses were performed on a Varian Model 3700 gas chromatograph equipped with a thermionic specific detector, containing an electrically heated ceramic-alkali bead. The glass column (1 m × 3 mm I.D.) was packed with 3% OV-17 on 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The column, injector and detector temperatures were 290, 310 and 340°C, respectively. Nitrogen was used as a carrier gas at a flow-rate of 35 ml/min.

The detector was operated at a bias voltage of —4 V, the bead heating current was adjusted at 3 A and the hydrogen and air flow-rates were maintained at 4.5 and 175 ml/min, respectively, to ensure optimal detectability of the compounds. A Spectra-Physics Model 4000 data system was used for the integrations, the calculations and the plotting of the chromatograms.

Procedure

Extraction of plasma samples. To 1-ml aliquots of plasma, contained in 15-ml glass centrifuge tubes, were added 0.1 μg of the internal standard, 1 ml of 1 *M* sodium hydroxide and 4 ml of *n*-heptane—isoamyl alcohol (98.5: 1.5, v/v). The tubes were closed tightly with polyethylene stoppers, rotated for 10 min (25 rpm, Cenco rotary mixer) and centrifuged (5 min, 1000 *g*). The organic phase was carefully transferred to a second centrifuge tube by means of a disposable pasteur pipet and the plasma was extracted again with a 4-ml volume of the extraction solvent. The combined organic layers were back-extracted with 3 ml of 0.05 *M* sulphuric acid and removed after centrifugation. The acidic phase was made alkaline by the addition of 0.1 ml of concentrated ammonia and re-extracted twice with 2 ml of the heptane—

isoamyl alcohol mixture. The organic layers were combined into 5-ml glass test tubes and evaporated to dryness under nitrogen in a water bath of 60°C. The residues were reconstituted with 50 µl of methanol and 5 µl of the solutions were injected into the gas chromatograph.

Alfentanil standard curves were prepared by spiking blank human plasma with AF at concentrations ranging from 0.001 to 1 µg/ml, and with IS at a fixed concentration of 0.1 µg/ml. In the same way, sufentanil standard curves were prepared using 0.1 µg AF per ml as the internal standard. These samples were extracted and chromatographed as described above and the peak area ratios of AF and SF, relative to their corresponding internal standard, were plotted against the concentrations of AF and SF, respectively.

Preparation of tissue samples. Animal tissues were ground by means of a Waring commercial blender and homogenized (1:4, w/v) in distilled water using an Ultra-Turrax TP 18/10 homogenizer. To 2-ml volumes of these homogenates were added 0.1 µg of the internal standard (corresponding to 0.25 µg/g of tissue) and 2 ml of 1 *M* sodium hydroxide. The mixtures were extracted twice with 4 ml of the heptane—isoamyl alcohol mixture and re-extracted with 3 ml of 0.05 *M* sulphuric acid. The acidic phase was made basic with concentrated ammonia (pH 10) and added to the Clin Elut™ columns. Re-extraction into the heptane—isoamyl alcohol mixture was then achieved by adding two times 3 ml of extraction solvent to the extraction columns and allowing the organic phase to drip into 5-ml test tubes. The combined eluents were then evaporated to dryness, reconstituted with 50 µl of methanol and 5-µl aliquots were injected into the gas chromatograph.

Standard curves in tissues were prepared by spiking 2-ml aliquots of blank animal tissue homogenates with AF, SF and their corresponding internal standard as described for the plasma standard curves. The homogenates were then taken through the tissue extraction procedure described above to prepare the standard curves.

Urine samples. These were processed in the same way as the plasma samples.

RESULTS AND DISCUSSION

Typical chromatograms of a plasma extract from a rat treated with SF and of a heart tissue extract from a rat treated with AF are shown in Fig. 2. The retention times were 1.6, 2.7 and 3.6 min for SF, AF and IS, respectively.

Plasma extracts were pure and no interferences were noted. Extreme care must be taken not to withdraw any trace of the aqueous phase by aspirating the organic mixture in the last extraction step, since this results in interfering peaks and a severe loss of detector sensitivity.

In comparison to plasma, tissue extracts were more prone to the formation of emulsions, which obstructed the adequate removal of the organic phase. This problem was solved by using the Clin Elut™ disposable extraction columns, which resulted in clean extracts without noticeable loss of recovery.

The calibration curves for AF and SF, extracted from plasma and tissue were all linear in the range of the concentrations studied (Table I). The mini-

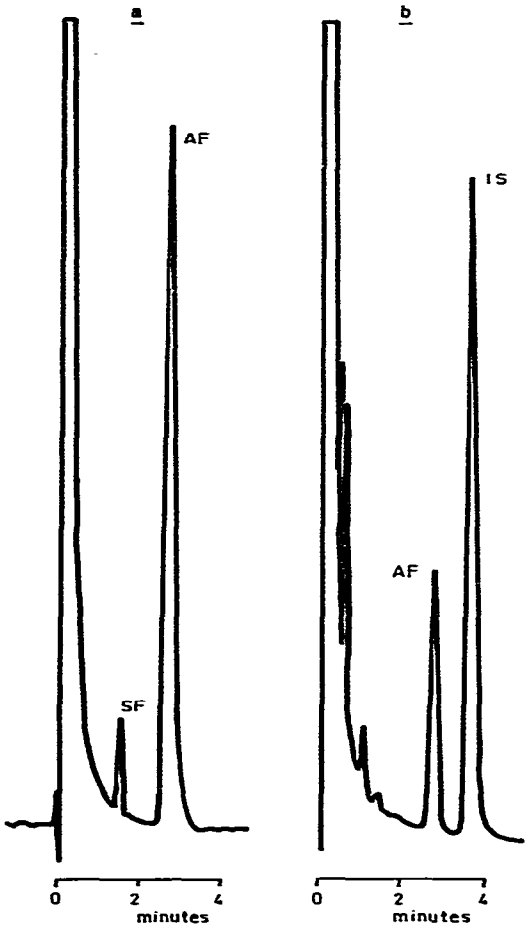


Fig. 2. Gas chromatograms of extracts from (a) rat plasma, 2 min after an intravenous dose of sufentanil (SF), spiked with AF as the internal standard, and (b) rat heart tissue, 8 min after an intravenous dose of alfentanil (AF), spiked with IS as the internal standard. GC conditions were as indicated in the text.

TABLE I

STANDARD CURVES FOR ALFENTANIL (AF) AND SUFENTANIL (SF) IN BIOLOGICAL SAMPLES

Compound	Sample	Internal standard (ng/sample)	Range (ng/sample)	Regression equation $y = ax + b$*		Correlation coefficient	
				a	b	r	n
AF	Plasma (1 ml)	100	1 —1000	1.046	—0.012	0.9998	11
	Tissue (1 g)	250	2.5— 500	1.071	—0.008	0.9998	9
SF	Plasma (1 ml)	100	1 — 100	0.628	+0.005	0.9994	5
	Tissue (1 g)	250	2.5— 250	0.650	—0.003	0.9996	8

*y = peak area ratios (AF/IS and SF/AF, respectively); x = concentration ratios (AF/IS and SF/AF, respectively).

mum detectable amount of AF and SF was 1 ng/ml of plasma and 2 ng/g of tissue. The reproducibility was checked by analyzing samples of different animal tissues (liver, kidney, pancreas, fat) spiked with several concentrations of alfentanil. The results are shown in Table II. The recovery over the

TABLE II

REPRODUCIBILITY DATA FOR THE DETERMINATION OF ALFENTANIL (AF) IN ANIMAL TISSUES

Added (ng/g)	Found, mean ± S.E.M.* (ng/g)	S.E.M. (%)
12.5	14.6 ± 1.4	9.6
25	25.5 ± 1.7	6.7
50	48.2 ± 1.4	2.9
125	118 ± 2.2	1.9
250	248 ± 10	4.0
500	503 ± 24	4.8

*S.E.M. = standard error of the mean (n = 4).

concentration range studied was 89± 4% (mean ± S.D., n = 6) and the precision was 3.0%.

The suitability of the method for AF was demonstrated by the analysis of plasma samples from a patient after an intravenous dose of 0.125 mg al-

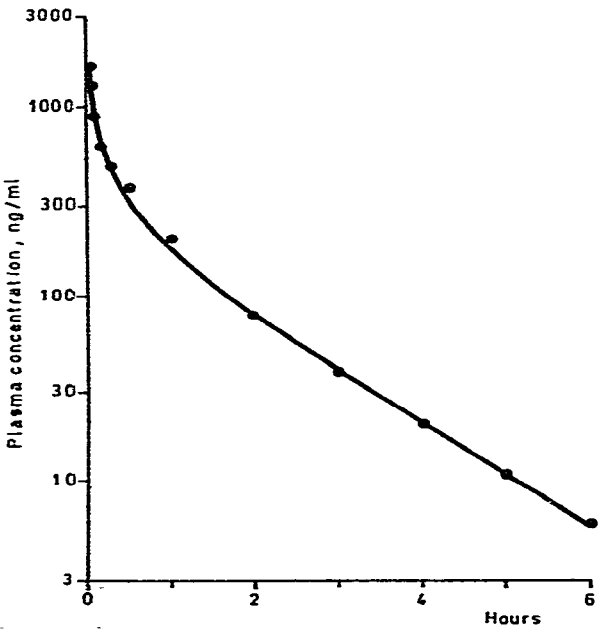


Fig. 3. Plasma levels of alfentanil (AF) in a patient after an intravenous dose of 0.125 mg/kg body weight.

fentanil per kg body weight. Plotting the AF plasma levels on a semilogarithmic scale (Fig. 3) permits the estimation of the biological half-life of the drug. It appears to be about 65 min.

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