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Letter to the Editor

Simultaneous determination of fentanyl and its major metabolites and fentanyl analogues using gas chromatography and nitrogen-selective detection

Sir,

Fentanyl, N-phenyl-N-1(1-(2-phenylethyl)-4-piperidinyl)propanamide, is the prototype of the 4-anilinopiperidine series of narcotic analgesics. It has become widely used as a surgical anaesthesia in conjunction with nitrous oxide or droperidol and has a potency of 50 to 100 times that of morphine. Chemical analogues of fentanyl, including alfentanil, sufentanil, lofentanil and carfentanil, have recently been developed.

A previous study [1] of the metabolism of fentanyl identified two metabolites in the plasma of patients, namely 1-(2-phenethyl)-4-N-anilinopiperidine (metabolite 1) and 4-N-(N-propionylanilino)piperidine (metabolite 2).

To detect the extremely small concentration of the drug present in plasma (therapeutic concentration ca. 2–100 ng/ml) radioimmunoassays are commonly used [2], with all the disadvantages caused by the use of ^{3}H-labelled fentanyl in consenting patients. Phipps et al. [3] have demonstrated that it is possible to assay picogram amounts of fentanyl using gas chromatography (GC) with nitrogen detection. This can also be done for fentanyl and alfentanil by high-performance liquid chromatography [4].

In this paper, we present a rapid and simple GC method for the detection of picogram amounts of fentanyl metabolites or fentanyl analogues.

To a 15-ml screw-top tube were added 2 ml of plasma, 1 ml of phosphate buffer (50% K_2HPO_4, adjusted to pH 9.2), 20 μl of 1 μg/ml β-diethylaminoethyldiphenylpropyl acetate (SKF 525A) as an internal standard (structure close to methadone or propoxyphene) and 5 ml of chloroform–*n*-heptane–2-propanol (50:33:17, v/v). After vortexing and centrifugation for 10 min at 300 *g*, the solvent was evaporated to dryness. The residue was reconstituted in 15 μl of methanol, and 1 μl was injected into a 1.8 m $\times$ 2 mm I.D. glass column with 3% OV-17 on 100–120 mesh Chromosorb Q. The GC system consisted of a Perkin-Elmer (8500) chromatograph with a nitrogen–phosphorus detector adjusted for maximum sensitivity. The operating conditions were as follows: column, injection port and detector temperature, 280, 300 and 300°C, respectively; nitrogen carrier gas pressure, 250 kPa.

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TABLE I

DATA FOR FENTANYL ANALOGUES

The extraction recovery and precision ($n=9$) were studied for 1 ng/ml of each component.

Compound	Retention time (min)	Recovery (%)	Within-run precision (%)	Day-to-day precision over two weeks (%)	Limit of detection (ng/ml)
Fentanyl	4.83	84.7	7.8	9.4	0.08
Sufentanil	5.93	81.6	6.4	8 7	0.09
Carfentanil	7.44	78.3	5.0	6.1	0.19
Lofentanil	8.60	80.2	6.0	6.9	0.14
Alfentanil	9.77	83.1	4.9	6.2	0.32
Metabolite 1	2.48	82.4	4.9	7.1	0.10
Metabolite 2	0.91	79.5	6.3	7.0	0.07

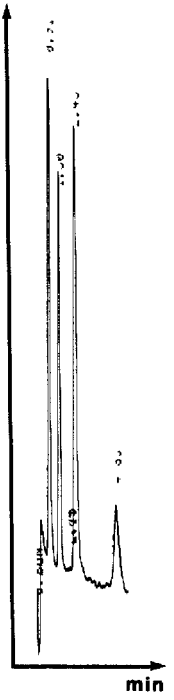

Fig. 1. Typical chromatogram obtained from a subject under fentanyl treatment: 0.91 min, metabolite 2 (8.4 ng/ml); 1.50 min, internal standard (SKF 525A); 2.48 min, metabolite 1 (6.7 ng/ml); 4.83 min, fentanyl (2.4 ng/ml).

Each drug was quantified by plotting peak-area ratios (drug to internal standard) against the concentration of standards to produce a standard curve, and by comparing the results for the samples with this curve.

The curve was linear over the range 0.4–100 ng/ml. The retention times, extraction efficiencies, precisions and limits of detection for the fentanyl analogues and metabolites are summarized in Table I.

A typical chromatogram obtained from a subject 15 min after an intravenous injection of 0.5 mg of fentanyl is shown in Fig. 1.

Fentanyl appears in the plasma of clinical patients in very small amounts, requiring a sensitive and selective detection method. Since the fentanyl molecule contains two nitrogen atoms, the nitrogen-selective detection offers the necessary sensitivity.

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