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Note

Detection of picogram concentrations of fentanyl in plasma by gas—liquid chromatography

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The popularity of fentanyl and related compounds as narcotic analgesics in clinical anaesthesia has resulted in a number of studies requiring measurement of plasma concentration. Normally, only relatively low doses of these drugs are administered and plasma concentration tends to fall rapidly to low levels — often in the pg/ml range. The detection of such low levels is important to a full understanding of the pharmacokinetics of these drugs. Radioimmunoassay [1], with a lower detection limit of 30 pg, has been the method of choice for measuring such small quantities as the gas chromatographic methods at present described are not sufficiently sensitive. Using gas—liquid chromatography (GLC) with a flame ionization detector, Van Rooy et al. [2] described a detection limit of fentanyl in plasma of 3.3 ng/ml. Gillespie et al. [3] using a more sensitive nitrogen—phosphorus detector (NPD) measured plasma fentanyl concentrations of 0.25 ng/ml. Using a gas chromatograph equipped with an NPD, a modification of the extraction method described by Van Rooy et al. [2] is presented which allows detection of picogram concentrations of fentanyl and similar compounds in plasma.

EXPERIMENTAL

Equipment

A Perkin-Elmer Sigma 1B gas chromatographic system [Perkin-Elmer (Canada) Ltd.] equipped with an NPD is used. The conditions for the analyzer in which the detector is housed are shown in Table I and are adjusted to give maximum component separation in minimum time. The voltage to the rubidium bead of the detector is adjusted for maximum sensitivity as described in the manufacturer's manual. Recorder range and attenuation are set for maxi-

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TABLE I

ANALYZER CONDITIONS FOR MEASUREMENT OF PLASMA FENTANYL CONCENTRATION BY GLC USING A NITROGEN–PHOSPHORUS DETECTOR

Column	3.05 m × 3.2 mm silanized glass
Packing	3% OV-17 on Gas-Chrom Q (80—100 mesh)
Injector temperature	310°C
Oven temperature	290°C
Detector temperature	310°C
Carrier gas flow-rate (helium)	35 ml/min
Detector gas flow-rates:	
hydrogen	3 ml/min
air	108 ml/min

mum sensitivity. The area and base sensitivities used in the analysis method are calculated using a computer programme supplied by Perkin-Elmer.

Materials and chemicals

For the NPD flame ultra high pure hydrogen and air, ultra zero gas (Matheson, Edmonton, Canada) are used. The carrier gas is ultra high pure helium (Matheson, Edmonton, Canada). Kimax 10-ml culture tubes with PTFE-lined caps are used. To prevent adsorption of drugs to the glass, the tubes are initially silanized with a 5% solution of dimethyldichlorosilane in toluene (Eastman Kodak, Rochester, NY, U.S.A.). The extraction solvent is 99% molecular pure benzene (Fisher Scientific, Fairlawn, NJ, U.S.A.).

Alfentanil is used as the internal standard. A solution of a known concentration of alfentanil in benzene is prepared using pure alfentanil hydrochloride powder.

Method

All glassware is rinsed with benzene prior to use to remove interfering substances. 1.0 ml of plasma sample, 0.1 ml of 4 *M* sodium hydroxide, 1 ml of internal standard solution and 5 ml of benzene are pipetted into clean culture tubes. These are sealed, mixed for 5 min and centrifuged for 10 min at 8000 *g*, following which the supernatants are transferred to clean tubes and evaporated to dryness at 40°C. The residues are reconstituted with 10 μl of distilled, deionized water of which 1 μl is injected onto the GLC column. Following extraction, a number of samples of known concentration of fentanyl in human plasma protein fraction are injected onto the column. Relative response factors for fentanyl as compared to the internal standard are then calculated by the Sigma 1B computer and an average factor is inserted into the analysis method to allow unknown fentanyl concentrations to be determined.

RESULTS

Using this method we have been able to measure plasma fentanyl concentration as low as 20 pg/ml. A chromatogram from a 20 pg/ml sample is shown in Fig. 1. The initial large deflection is caused by air and distilled water. The

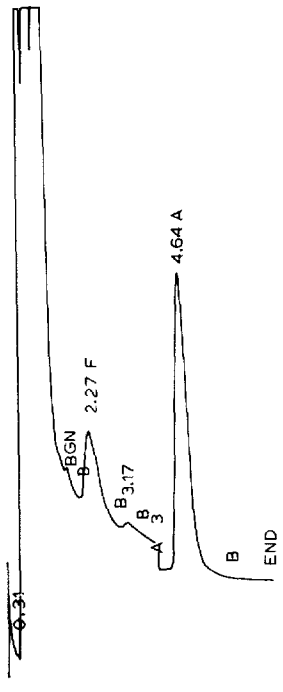

Fig. 1. Chromatogram from a 20 pg/ml sample of fentanyl. Peaks: F = fentanyl, A = alfentanil, BGN = data start time, B = computer recognition of baseline and A 3 = increase of signal attenuation. 0.31, 2.27, 3.17, 4.64 = elapsed time in min.

first peak shown (F) is from fentanyl and the second (A) is from alfentanil. As a relatively large concentration of alfentanil (2 μg/ml) is used, the signal attenuation from the detector to the computer is increased before the alfentanil is eluted otherwise maximum deflection of the pen would occur and the peak would not be displayed properly. Recovery of 20 pg/ml fentanyl was 69.4 ± 5.3%, determined by injecting known concentrations of fentanyl onto the column and comparing with extracted samples.

DISCUSSION

The major modification in the current method from that of Van Rooy et al. [2] was to use distilled water as the final solvent prior to injection of the sample onto the column. The use of an organic solvent such as benzene produces a very broad deflection over the first few minutes of the chromatogram. This completely masks the fentanyl peak from low plasma concentrations and from larger concentrations gives at best a peak superimposed on the trailing side of the solvent peak. In this situation the fentanyl peak is produced on an unstable baseline and corrections for this have to be inserted into the analysis method to enable the computer to distinguish the peak from the baseline. The current method eliminates the need for such correction factors as a stable baseline is produced.

The problem of an initial broad deflection was encountered with a wide

range of organic solvents but was eliminated with the use of distilled water. This produces a narrow initial peak, allowing the pen to return to baseline before fentanyl is eluted from the column. It seems that distilled water, being relatively inert as compared to the organic solvents, is retarded less by partitioning as it passes through the column. Also, as water is an inorganic substance, the NPD is insensitive to it. Baseline is recognised by the computer, which prints a letter B (see Fig. 1). The stable baseline enables the computer to distinguish very small peaks and thus measure accurately low concentrations of fentanyl. Furthermore, the Sigma 1B system calculates concentrations by measuring peak areas which is more accurate, particularly at low concentrations, than the measurement of peak heights described by earlier workers using less sophisticated equipment.

The internal standard is added prior to the extraction process to allow for variability in drug recovery from different samples. The computer is programmed to recognize the concentration of alfentanil as being the same in every sample and thus variability in recovery is taken into account.

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