Journal of Chromatography, 310 (1984) 193—198
Biomedical Applications
Elsevier Science Publishers B.V. Amsterdam — Printed in The Netherlands

CHROMBIO. 2182

Note

Determination of morphine in serum and cerebrospinal fluid by gas chromatography and selected ion monitoring after reversed-phase column extraction

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(First received January 19th, 1984; revised manuscript received April 13th, 1984)

A wide range of methods for the determination of nanogram levels of morphine in biological fluids have been reported. Assays based on scintillation counting of radioactively labelled morphine and radioimmunoassay are capable of detecting picogram amounts of this drug, but are relatively non-specific [1—5].

Gas chromatographic procedures require extraction and formation of a volatile morphine derivative by silylation or alkylation. Quantitation is achieved by electron capture detection when a fluoroalkyl derivative is prepared [6—10]. Assessment of morphine after extraction by liquid chromatography with ultraviolet as well as electrochemical detection has been described [11—14]. Gas chromatography combined with mass spectrometry offers a highly specific and sensitive method and is a most suitable assay to obtain an unequivocal identification of morphine [15—23].

0378-4347/84/$03.00

Extraction procedures of morphine from biological specimens prior to analysis are mainly performed by liquid—liquid extraction. However, this isolation technique is rather time consuming. Todd et al. [13] described an extraction procedure on a Clin-Elut CE 1001 silica gel column. Reversed-phase column extraction offers the advantage of smaller elution volumes compared to normal-phase silica gel column extraction. Sep-Pak C_{18} cartridges were used for extraction by Svensson et al. [14].

In this paper we describe a rapid and efficient extraction procedure for the isolation of morphine from biological fluids. Morphine is extracted onto a reversed-phase Bond-Elut C_{18} column and the derivatized extract is analysed by selected ion monitoring, under chemical ionization conditions. This technique is being applied in a combined pharmacokinetic and clinical study; preliminary results illustrate the practical use of the method.

MATERIALS AND METHODS

Reagents

Morphine hydrochloride (pharmacopee grade) was obtained from Onderlinge Pharmaceutische Groothandel (Utrecht, The Netherlands). Deuterated morphine was prepared by reaction of normorphine and deuterated methyl iodide ($C[^2H_3]I$) according to the procedure of Ebbinghausen et al. [16]. Methanol was high-performance liquid chromatography grade from Baker (Deventer, The Netherlands). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce (Oud Beijerland, The Netherlands) and β-D-glucuronidase was obtained from Sigma (Amsterdam, The Netherlands). All other chemicals were analytical grade. Baker octadecyl C_{18} columns were used for the extraction procedure.

Instrumentation

Gas chromatography was performed isothermally at 300°C on a 25 m × 0.32 mm I.D. fused-silica column with a chemically bonded stationary phase CP Sil 8 from Chrompack (Middelburg, The Netherlands). The carrier gas was helium, flow-regulated at 1.2 ml/min. A splitless type of injector was employed. Mass spectrometry was performed on a Finnigan 3200 F instrument in chemical ionization (CI) mode. Ammonia—methane (1:5) as reagent gas was directly introduced into the ion source. In selected ion monitoring at high sensitivity levels, a four-channel peak selection (PROMIM) was used. The temperature of the CI ion source was digitally controlled at 140°C ± 1°C. The temperature stated was measured at the elution time of morphine. The mass spectrometer was tuned for optimal sensitivity at the selected ions.

Sample preparation

Before use the columns were prewashed with 5.0 ml of methanol, 3.0 ml of distilled water and 1.0 ml of 0.05 mol/l borax buffer pH 9.0. Serum or liquor (100 μl to 1.0 ml) was mixed with 50 μl (= 50 ng) of internal standard and 1.0 ml of buffer pH 9.0, and transferred to the Bond-Elut C_{18} column. The column was washed twice with 500 μl of water and once with 100 μl of 80% methanol. Morphine was eluted with 0.5 ml of methanol and collected in a 1-ml reaction

vial. The solvent was evaporated under a stream of nitrogen and the residue was derivatized with 20 μl of BSTFA in pyridine (1:1) by heating at 85°C for 15 min; 2 μl of the mixture were injected into the gas chromatograph.

The total amount of morphine (i.e. free morphine base and morphine glucuronide) was estimated as follows: 0.5 ml of the serum sample was buffered at pH 5.0 and hydrolysed by 5 mg of β-D-glucuronidase at 37°C for 16 h. The mixture obtained was made alkaline with 4 *M* sodium hydroxide solution and adjusted to pH 9.0 with borax buffer. The precipitate was centrifuged and the supernatant was transferred to the extraction column and treated as described above.

RESULTS AND DISCUSSION

Previous reported procedures [17—23] for the determination of morphine in biological specimens using liquid—liquid extraction for the isolation of the drug have been examined. Compared to the present extraction procedure for serum and liquor these methods were less efficient and also time consuming.

To establish a standard curve we added known amounts of morphine and [N-C^2H_3]morphine to aliquots of morphine-free serum and liquor. The standard curve indicates that the measurement of morphine concentrations is linear over the range 5—200 ng/ml. The linear regression line of the data was calculated using the least-squares regression method and is expressed by the equation $y = 0.033 - 0.015x$ (y = peak ratio of morphine versus internal standard, x = morphine concentration in ng/ml) with a correlation coefficient of 0.996. Day-to-day precision of the morphine concentration of 50 ng/ml in serum ($n = 5$) was calculated, with a coefficient of variation of 5.4%.

The recovery of morphine at a concentration of 10 ng/ml from serum as well as from liquor was 90%.

Using the described procedure, morphine could not be detected when morphine glucuronide was added to serum at a concentration of 100 ng/ml. Svensson et al. [14] reported that morphine and its conjugate were eluted from a Sep-Pak C_{18} column. After hydrolysis with β-glucuronidase of the same concentration a 95% recovery of morphine was obtained.

Selected ion monitoring was applied to the analysis of morphine using a quadrupole mass spectrometer under CI conditions. Two masses were monitored at *m/e* 340 and *m/e* 343, respectively. They correspond to the prominent fragment ion $(M—89)^+$ [loss of $(CH_3)_3SiOH$] and the corresponding deuterated compound in the ammonia—methane CI spectrum of bis(trimethylsilyl)morphine and [N-C^2H_3] bis(trimethylsilyl)morphine (Fig. 1).

The procedure was applied to serum and liquor samples of two patients. One of them was undergoing surgery by standardized epidural anaesthesia, the other by intravenous administration of morphine. Morphine hydrochloride (1 mg/kg body weight) was injected intravenously and 0.1 mg/kg was injected through the epidural catheter. Eight liquor samples as well as serum samples were taken simultaneously within the time interval 0—240 min after injection.

In order to exclude any interference in the determination of morphine by drugs administered as premedication before surgery (e.g. methylatropine, diazepam and promethazine or/and their metabolites), a serum sample was

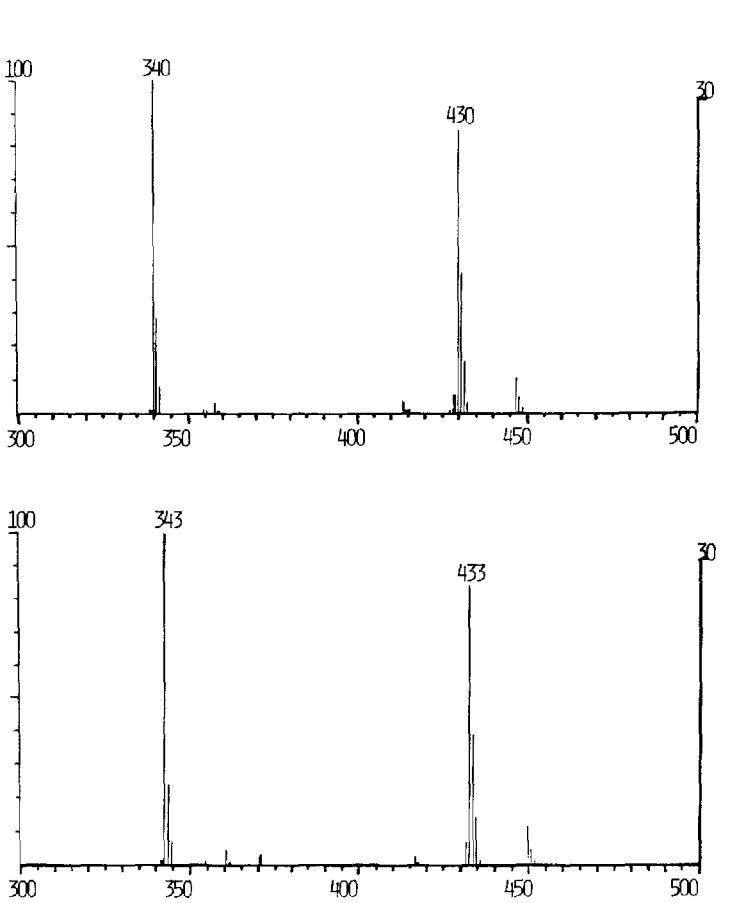

Fig. 1. Ammonia—methane chemical ionization spectra of bis(trimethylsilyl)morphine (A) and [N-$^2H_3$]bis(trimethylsilyl)morphine (B).

taken just before anaesthesia was initiated. No response was observed in these samples for the ion pair 340/343. These patients did not receive any other structurally related drugs like codeine, since morphine is a possible metabolite of codeine.

Fig. 2 shows the serum morphine concentration of the patient receiving the drug by intravenous route before and after hydrolysis. The major metabolite of morphine in man is the glucuronide conjugate. By examining the concentration of morphine before and after hydrolysis with β-glucuronidase, the decline is demonstrated between free and conjugated morphine in serum. The results indicate that the maximum ratio of morphine glucuronide to morphine in the serum of this patient was 10 to 1, which is in good agreement with the findings of Murphy and Hug [24]. From the free morphine concentrations, a terminal half-life of about 2 h was calculated, which is also in agreement with previous estimates [24].

Fig. 3 presents the morphine concentration in liquor and serum of a patient undergoing epidural anaesthesia, taken at regular time intervals. From this experiment it can be deducted that the decay of the morphine concentration in

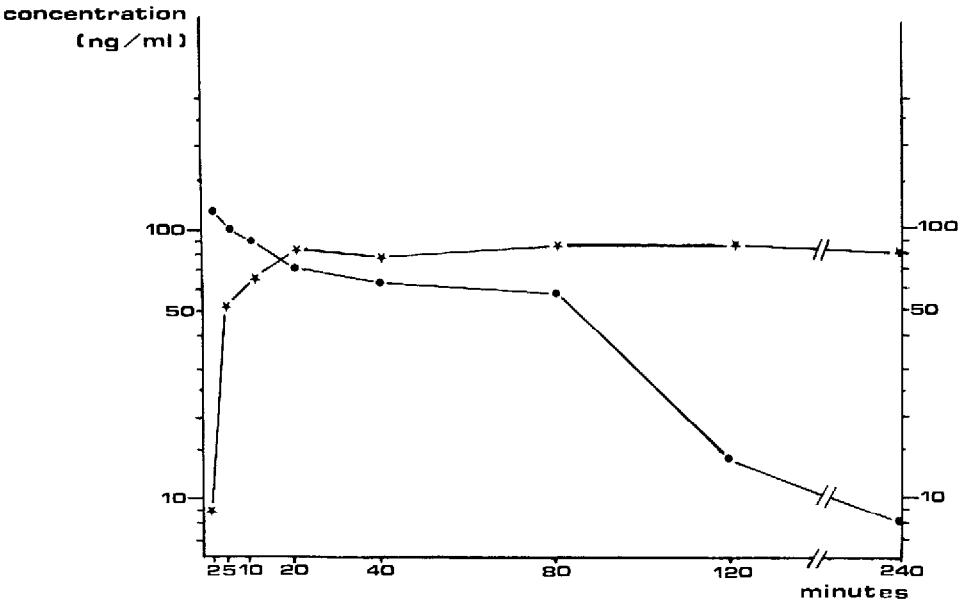

Fig. 2. Morphine concentration in serum before and after hydrolysis with β-glucuronidase. (•), free morphine (ng/ml); (⋆), morphine glucuronide (ng/ml).

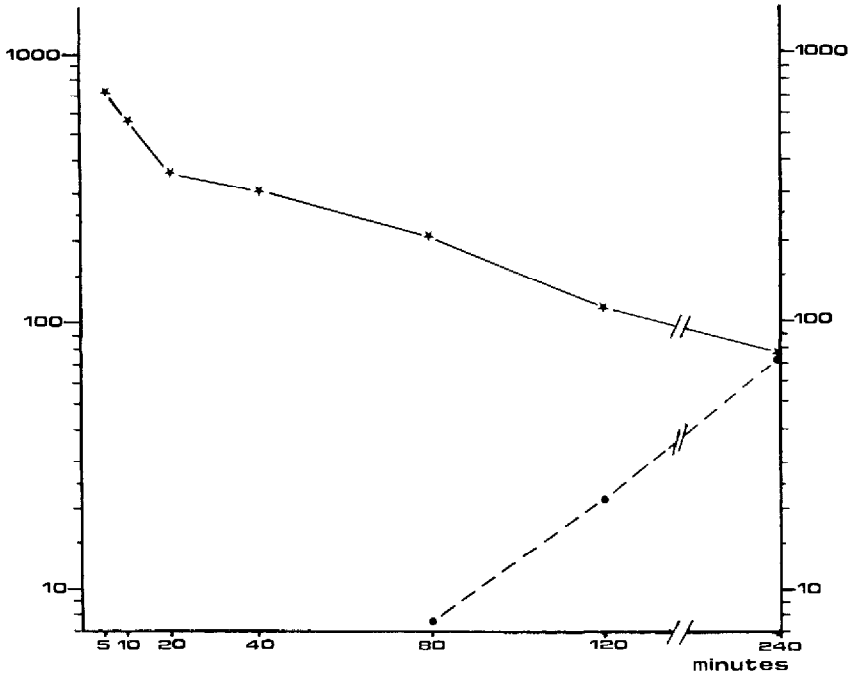

Fig. 3. Morphine concentrations (ng/ml) in liquor (⋆) and serum (•) after epidural administration of 0.1 mg of morphine per kg body weight.

liquor parallels the serum levels after intravenous administration. Furthermore, these data show that 1 h after epidural administration morphine can be detected in the serum.

In summary, it can be concluded that the described procedure offers a reliable analytical method for the determination of morphine in biological fluids, and had proved to be a suitable procedure for studying the pharmacokinetics and metabolism of the drug.

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