Journal of Chromatography, 274 (1983) 372—375
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1620

Note

Determination of therapeutic concentrations of codeine by high-performance liquid chromatography

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(First received October 11th, 1982; revised manuscript received December 28th, 1982)

Various analytical methods have been reported for the determination of codeine, a narcotic analgesic and antitussive drug. Gas—liquid chromatographic methods were often used to determine codeine in various media [1—3], sometimes in combination with thin-layer chromatography [4] or mass spectrometry [5]. Several high-performance liquid chromatographic (HPLC) methods have been described using reversed-phase or ion-pair systems [6—9]. In addition, a radioimmunoassay procedure has been reported to study the disposition of codeine in man [10]. However, not all these methods are always available, and some of them are not sensitive enough for measuring therapeutic plasma levels (10—200 ng/ml) after a single dose of codeine phosphate (60 mg).

In order to study the biopharmaceutics of codeine after oral and rectal administration in man, we developed a rapid and accurate determination of codeine by straight-phase HPLC with a detection limit of 5 ng/ml. Methadone hydrochloride was used as an internal standard.

EXPERIMENTAL

Chromatographic system

The analyses were performed on a Waters liquid chromatograph consisting of a 6000A pump, a U6K injector and Model 440 UV detector set at 254 nm and operating at 0.005 AUFS (Waters Assoc., Milford MA, U.S.A.). A μPorasil (10 μm) straight-phase column (30 cm × 3.9 mm I.D.) was used (Waters Assoc.) guarded with a pre-column of Vydac 101 SC, 10 cm × 2.1 mm I.D. (Chrompack, Middelburg, The Netherlands). The eluent was dichloromethane—

0378-4347/83/$03.00

methanol—ammonia solution (33%) (90:10:0.1) with a flow-rate of 1.5 ml/min resulting in a back-pressure of 6.9 MPa (1000 p.s.i.).

Materials

Reagent-grade dichloromethane, methanol and ammonia solution (33%) were obtained from Merck (Darmstadt, G.F.R.); codeine phosphate (Ph.Eur.) and methadone hydrochloride (Ph.Eur.) were from Brocacef (Maarssen, The Netherlands). Standard solutions of 2 μg/ml codeine phosphate in dichloromethane and 80 ng/ml methadone hydrochloride in dichloromethane were made.

Methods

To 1.0 ml plasma in a 20-ml glass-stoppered tube, 1.0 ml of ammonia solution (10%) and 4.0 ml of the methadone hydrochloride solution were added. After mixing for 1 min on a Vortex mixer and centrifugation for 5 min at 2500 *g*, most of the upper layer was removed. The tube was held in liquified nitrogen to freeze the rest of the upper layer. The dichloromethane layer was poured into a smaller tube and the volume was reduced to 200—300 μl by evaporation. The whole residue was injected into the liquid chromatograph.

The retention times were: codeine 6.3 min and methadone 7.2 min. Peak height ratios were used to calculate codeine concentrations, based on standard curves prepared from spiked plasma samples (Table I).

With 1.0-ml plasma samples this method is accurate to concentrations as low as 5 ng/ml codeine.

RESULTS AND DISCUSSION

A typical chromatogram from a plasma extract is shown in Fig. 1. For a good separation the amount of ammonia in the eluent turned out to be very critical. During storage and the analysis ammonia is gradually lost from the mobile phase mixture. Therefore fresh solvent should be used daily in order to keep retention times constant and to maintain effective separation.

The standard curve was linear over the range 10—160 ng/ml ($y = 0.0061x - 0.0028$; $r = 0.9993$). From Table I it can be calculated that the mean recovery of extraction from plasma is 99.8% ± 5.3%.

TABLE I

REPRODUCIBILITY OF THE MEASUREMENT OF CODEINE AT VARIOUS PLASMA CONCENTRATIONS

Codeine (ng/ml)		*n*	± S.D. (ng/ml)	Coefficient of variation (%)	Recovery (%)
Added	Found				
10	10.9	7	0.9	8.3	109.0
20	18.6	7	1.7	9.1	93.0
40	38.9	7	1.9	4.9	97.3
80	81.0	7	4.0	4.9	101.3
120	118.8	7	4.6	3.9	99.0
160	158.6	7	5.0	3.2	99.1

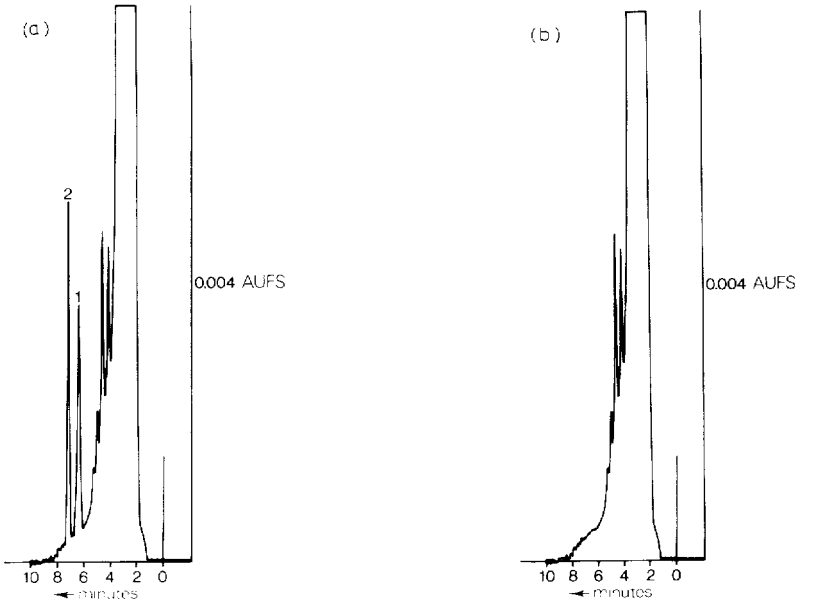

Fig. 1. (a) HPLC separation of 100 ng of codeine (1) and 320 ng of methadone (2) after extraction from plasma. (b) Chromatogram of a blank human plasma after extraction.

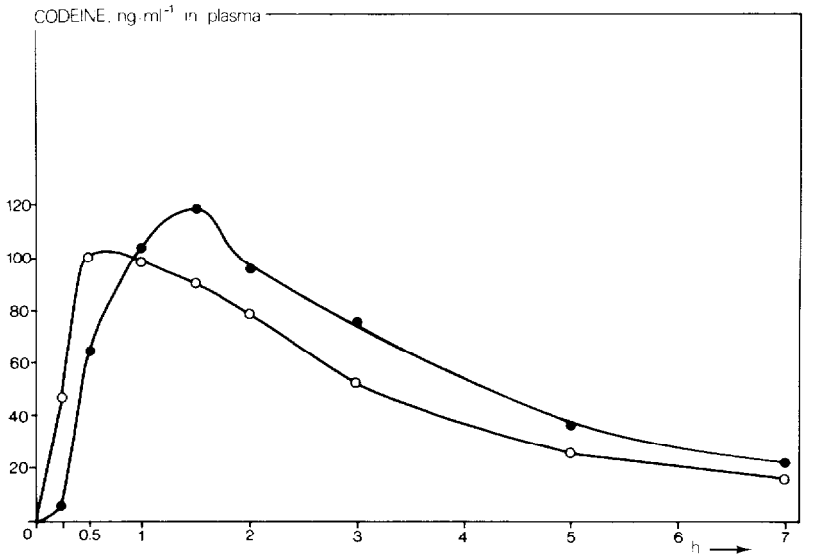

Fig. 2. Typical plasma concentration curves of codeine after administration of 60 mg of codeine phosphate in one subject as an oral solution (○) and as a rectal suppository (●).

It is important to note that the accuracy and precision depends on the volume to which the dichloromethane layer is reduced by evaporation. This volume should not be less than 200 μl, since the methadone peak in that case is reduced significantly.

Although it is possible to detect morphine concentrations in the same run

(retention time is about 15 min), the plasma concentrations of this active metabolite produced after an oral dose of 60 mg of codeine phosphate are too low (< 10 ng/ml) to allow quantitative determination, since the detection limit of morphine following the described procedure is about 50 ng/ml of plasma.

Fig. 2 shows typical plasma concentration curves for codeine after oral and rectal administration of 60 mg of codeine phosphate to a human subject. Subsequent experiments with seven volunteers have been carried out to determine the absorption profiles after administration of various dosage forms [11].

The methods of Zweidinger et al. [1] and Tsina et al. [9] are also useful for measuring therapeutic plasma concentrations of codeine. However, these procedures require 2-ml plasma samples. Besides, HPLC with UV detection is probably the most widely used technique available in clinical laboratories.

In conclusion, it can be said that our HPLC method is a simple, fast and accurate procedure and can be used to study biopharmaceutics of codeine in man.

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