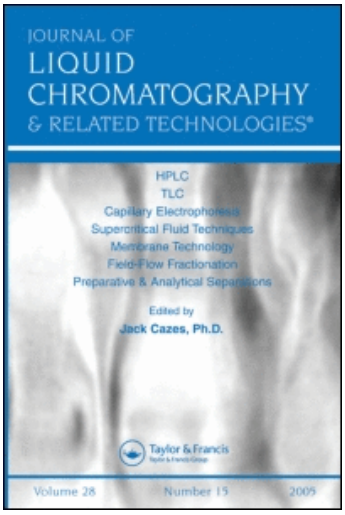

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A REVERSED PHASE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF CODEINE IN BIOLOGICAL FLUIDS WITH APPLICATIONS

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ABSTRACT

A rapid, simple and sensitive high performance liquid chromatographic method is described for the routine determination of codeine, a narcotic analgesic and antitussive drug, in biological fluids. The chromatographic system involves the use of a cyanopropylsilane column with KH_2PO_4/acetonitrile (83:17%) as a mobile phase. Fluorescence detection is performed at 285 and 345 nm excitation and emission wavelengths, respectively. Codeine and the internal standard are extracted from alkalinized plasma using a mixture of hexane/methylene chloride (2:1). The results show that the method is linear ($r^2 \geq 0.995$), accurate, and reproducible (CV $\leq$ 5.4% for concentrations $\geq$ 50 ng/ml and 17.8% for 10 ng/ml). The complete analysis time takes less than 10 minutes where concentrations as low as 5 ng/ml of codeine can be detected. The method was applied to the determination of codeine pharmacokinetic parameters in a healthy volunteer.

INTRODUCTION

Various analytical methods have been described for the determination of the analgesic and antitussive drug codeine. Gas-chromatographic procedures involving flame-ionization detection for the determination of codeine in various media (1-4), combined chromatography-mass spectrometry (5,6), and radioimmunoassay procedures (7) have all been reported.

Several high performance liquid chromatographic methods with normal phases (8,9), reversed phases (10,11), ion-pairing (12), ultraviolet (13) and fluorescence (14) detection have also been described.

Some of these procedures are either not sufficiently sensitive or selective for the measurement of codeine at therapeutic concentrations, involve tedious sample preparation procedures which are time consuming or require sophisticated equipment or highly specialized techniques which are not available in many clinical laboratories; moreover, some of these techniques are not suitable for the analysis of a large number of samples.

The objective of the presented studies was to develop an HPLC method which is simple, rapid, sensitive and selective for the routine determination of codeine in biological fluids. The method was applied for the evaluation of the pharmacokinetic parameters of codeine in a healthy human volunteer.

EXPERIMENTAL

Chemicals

Codeine phosphate, USP reference standard, was supplied by Boots Pharmaceuticals Inc. (Shreveport, LA). The internal standard, isopropylnorcodeine HCl was synthesized by the Department of Medicinal

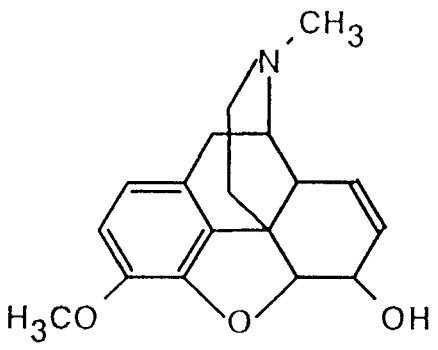

FIGURE 1 Chemical structure of codeine.

Chemistry at the University of Florida by the alkylation of norcodeine and purification before use. Hexane, methylene chloride and acetonitrile, HPLC grade, were purchased from Fisher Scientific Co., (Fair Lawn, NJ). 1-octanesulfonic acid sodium salt was obtained from Eastman Kodak Company, (Rochester, NY). All other chemicals were analytical grade and purchased from Fisher Scientific Co.

Instrumentation

The HPLC system used consisted of a high pressure pump (Constametric III G LDC/Milton Roy, Riviera Beach, FL), an auto sampler (Model ISS-100, Perkin Elmer Corp., Analytical Instruments, Norwalk, CT) provided with a 50 µl loop, a cyanopropylsilane column (Zorbax CN 15 cm x 4.6 mm, 5 µm particle size, Dupont Instruments, Wilmington, DE), a guard column filled with packing material (Pellicular Cyano, PN 650-69, Keystone, FL) placed before the analytical column, a fluorimetric detector with a xenon lamp (Model 650-10 S, Perkin Elmer Corp.), and an integrator (Model 3392 A, Hewlett Packard, Palo Alto, CA). Heparinized vacutainer tubes (Vacutainer Systems, Rutherford, NJ) were used for collecting blood samples.

Chromatographic Conditions

Reversed-phase chromatography was performed at ambient temperature. The mobile phase consisted of 0.05 M KH_2PO_4 and acetonitrile (83:17%, pH 4.9). Sodium octanesulfonate (0.005 M) was added as ion-pairing reagent. The mobile phase was filtered through a 0.2 μm filter and degassed before use. The flow rate was set at 1.2 ml/min with a back pressure of about 1500 psi. Fluorescence detection was performed with excitation and emission wavelengths at 285 nm and 345 nm, respectively.

Extraction Procedure

Plasma (1 ml) was placed into a 15 ml screw top centrifuge tube. The specified amounts of codeine and internal standard were added. The mixture was vortexed for 30 seconds. One ml of 0.05 M phosphate buffer (pH 8) was added and the mixture was vortexed. The mixture was extracted twice with 6 ml portions of hexane/methylene chloride (2:1) by mechanically shaking for 5 minutes and centrifuging. The organic phases were combined and extracted with 1 ml of 0.05 M acetate buffer (pH 3) and discarded. The aqueous phase was alkalinized with 1 ml of 0.1 M NaOH and again extracted with 6 ml of hexane/methylene chloride mixture. The back extraction procedure was performed to remove any potentially interfering substances. The organic phase was transferred to a 10 ml conical test tube and evaporated under gentle stream of nitrogen to about 1 ml. The inside wall of the tube was washed with 1-2 ml of hexane/methylene chloride and the entire content of the tube were evaporated to dryness. The residue was dissolved in 100 μl of the mobile phase and vigorously vortexed. 50 μl were injected into the system. A schematic diagram of the extraction procedure is shown in Figure 2.

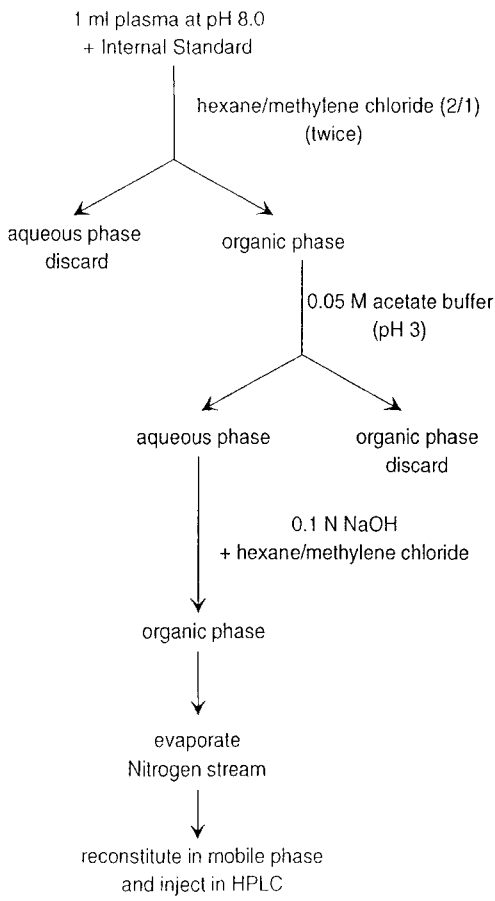

FIGURE 2 Schemetic diagram for the extraction procedure.

Application

To determine the applicability of the developed method, codeine (60 mg) was administered orally to a human volunteer. Blood samples were collected at different time intervals over a period of 12 hours. The samples were centrifuged immediately; plasma was separated and analyzed for codeine. Pharmacokinetic parameters of codeine were determined and compared with those reported in the literature.

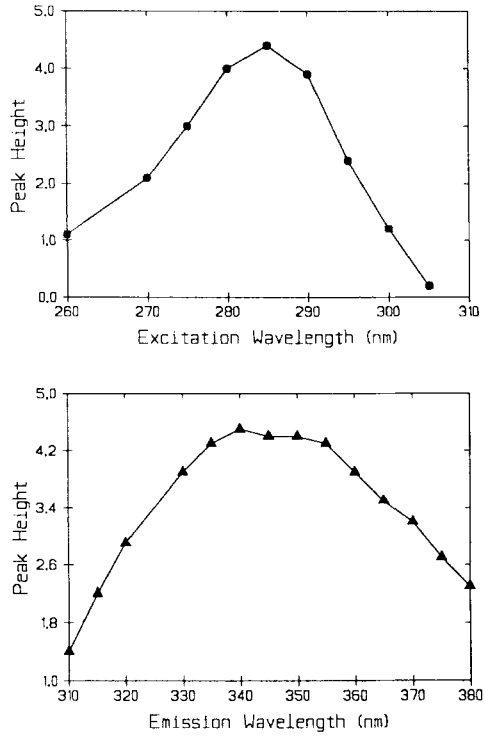

FIGURE. 3 Excitation (top panel) and emission (bottom panel) spectra of the fluorescence of codeine.

RESULTS AND DISCUSSION

Determination of Optimum Detection Conditions

Fluorimetric detection was used since codeine and internal standard are naturally fluorescing. Comparison of fluorescence and UV detections revealed that the former is more sensitive than the latter. Excitation and emission wavelengths were chosen from the maxima of the excitation and emission spectra of codeine shown in Figure. 3. Fluorescence of codeine solution was maximum at excitation and emission wavelengths of 285 and 345 nm,

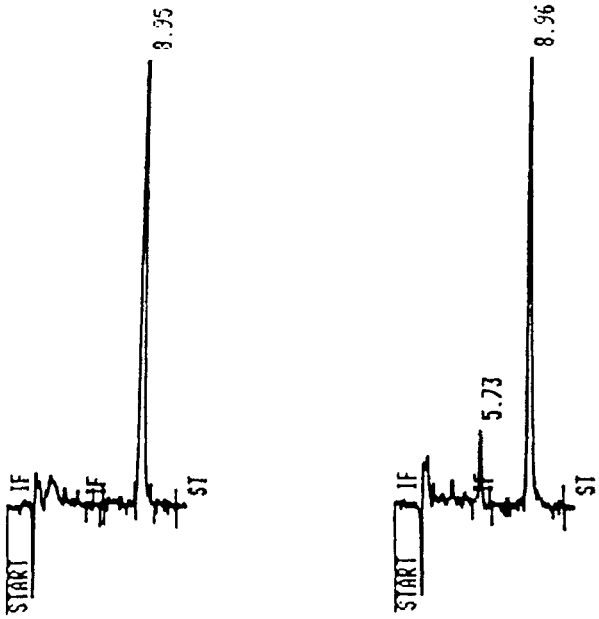

FIGURE 4 Chromatograms obtained from the analysis of: (A) drug-free plasma spiked with 100 ng/ml internal standard and (B) drug-free plasma spiked with 10 ng/ml of codeine and 100 ng/ml of internal standard. (retention times are 5.7 minutes for codeine and 9 minutes for the internal standard).

respectively. Few endogenous substances fluoresce under the described assay conditions. As a result, by using fluorescence detection it was possible to increase the sensitivity to nanogram/ml range.

Chromatographic Separation

Sodium octanesulfonate was added to the mobile phase to form an ion-pair with codeine. This decreased the polarity of the eluate leading to a reasonable retention time and an improved peak shape. Isopropylnorcodeine was selected as internal standard due to its structural similarity to codeine and

its natural fluorescing characteristics. Codeine and the internal standard were extracted similarly and resolved well under the described chromatographic conditions. Typical chromatograms obtained from the analysis of plasma spiked with 100 ng/ml of internal standard and plasma spiked with 10 ng/ml of codeine and 100 ng/ml of isopropylnorcodeine are shown in Figure. 4. The retention times for codeine and internal standard were 5.7 and 9 minutes, respectively. As can be seen in Figure. 4, very good separation between codeine and isopropylnorcodeine was achieved with no interfering peaks from endogenous materials or metabolites.

Linearity and Precision

Calibration curves obtained by analyzing plasma samples spiked with codeine in the concentration range of 10 to 400 ng/ml were subjected to regression analysis. The data indicated linearity ($r^2 \geq 0.995$) and reproducibility of the assay in the specified concentration range. For further validation of the chromatographic procedure, intraday precision and accuracy were investigated. Drug-free plasma was spiked with five different concentrations of codeine and five replicates of each concentration were assayed. The results illustrated in Table 1 indicate the good precision and accuracy of this method.

Recovery and Sensitivity

The recovery of assayed codeine was determined by comparing the peak area ratios obtained from the extraction of plasma samples spiked with known amounts of codeine with the peak area ratios obtained by the direct injection of the same amount of codeine in phosphate buffer (pH 7.4). The recovery determined using this method was greater than 85%. Samples as low as 5 ng/ml, based upon signal-to-noise ratio, can be analyzed using this procedure.

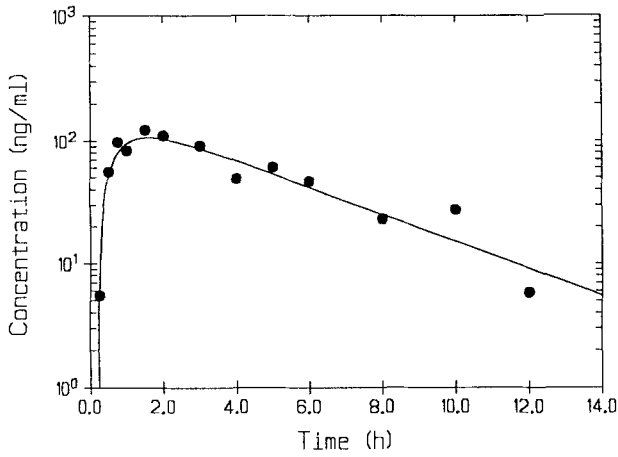

FIGURE 5 Plasma concentration-time profile of codeine following oral administration of 60 mg codeine to a human volunteer.

Figure 5 shows the fitted log plasma concentration-time profile for a human volunteer after the ingestion of 60 mg of codeine sulfate. The maximum plasma concentration (123.4 ng/ml) was reached 1.5 hours after drug administration. A total area under plasma concentration-time curve was found to be 613 ngh/ml. The rate of elimination of codeine indicated a half-life of 2.38 hours. These pharmacokinetic parameters are in good agreement with those reported in the literature (15).

In summary, the HPLC method described here provides a simple, sensitive and precise method for the routine determination of codeine in biological fluids. The method was successfully applied for the determination of the pharmacokinetic parameters of codeine in a healthy volunteer. The method required a simple extraction procedure with a total chromatographic running time of no longer than 10 minutes.

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