(8) M. Rink, R. Lux, and E. Franken, *ibid.*, **98**, 660(1958); through *Anal. Abstr.*, **6**, 1885(1959).
(9) T. Higuchi and J. Concha, *J. Amer. Pharm. Ass., Sci. Ed.*, **40**, 173(1951).
(10) H. Ellert, T. Jasinski, and K. Marcinkowska, *Acta Pol. Pharm.*, **17**, 29(1960); through *Chem. Abstr.*, **54**, 11806(1960).
(11) M. Hadicke and K. Howorka, *Pharm. Zentralhalle*, **99**, 312(1960); through *Anal. Abstr.*, **8**, 773(1961).
(12) J. Vacek and J. Kracmar, *Cesk. Farm.*, **5**, 80(1956).
(13) G. Goldstein, O. Menis, and D. L. Manning, *Anal. Chem.*, **33**, 266(1961).
(14) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1968, pp. 858, 865.

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Quantitative GLC Determination of Codeine in Plasma

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Abstract □ A sensitive and accurate GLC method for quantitating codeine in plasma at levels of 50 ng/ml, with limits of detection as low as 5 ng/ml, is described.

Keyphrases □ Codeine—GLC analysis, *n*-butyl chloride extraction, plasma □ GLC—analysis, codeine in plasma

Codeine has long been used as an analgesic in pharmaceutical preparations. Because of the recent interest in drug efficacy and bioavailability, a sensitive method for determining low nanogram quantities in plasma is needed. Several methods for the analysis of codeine in urine have been developed for the study of its metabolism and for forensic purposes (1–3). However, the levels found in urine are many times greater than those found in plasma, so these methods are generally not applicable in plasma analysis.

Schmerzler *et al.* (4) reported a GLC method for codeine in serum (and urine) of adequate sensitivity. This method has two serious drawbacks: the tendency for chloroform to form emulsions, especially with serum or plasma, and a variable recovery of codeine in the extraction procedure.

In this study, *n*-butyl chloride, which does not readily form emulsions with plasma, was used in place of chloroform. Dihydrocodeinone was added to the plasma to serve as an internal standard for both the extraction procedure and GLC quantitation.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] with a flame-ionization detector was used.

Reagents—*n*-Butyl chloride[2] saturated with water was used for all extractions. Dihydrocodeinone was synthesized in this laboratory from thebaine by a two-step procedure (5). The water used to prepare aqueous reagents was checked to ensure low GLC background; all other solvents and chemicals were reagent grade.

Glassware was oven cleaned at 400° (6), silanized with 5% dimethyldichlorosilane in toluene, and rinsed with toluene and methanol. Immediately before use, the glassware was rinsed with chloroform to maintain a low GLC background. Chromatographic grade carbon disulfide[3] was used for GLC.

Procedure—To 2.0 ml of plasma was added 1 μg of dihydrocodeinone internal standard in aqueous solution. The plasma was made alkaline with 0.10 ml of 1 *N* NaOH and extracted twice with 2 ml of butyl chloride on a reciprocal shaker at 60 cpm for 20 min. The organic fractions were combined, a 0.5-ml aliquot of 0.05 *N* H_2SO_4 was added, and the solution was shaken for 20 min. Then the organic layer was discarded, and the aqueous phase was washed with an additional 1 ml of butyl chloride. The aqueous layer then was made alkaline with 0.05 ml of 1 *N* NaOH and extracted twice with 1 ml of butyl chloride.

The organic layers were combined in a conical vial, and the solvent was evaporated immediately before GLC analysis. The residue was taken up in 10 μl of carbon disulfide, and 2 μl was injected for GLC analyses. The column used was a 1.8-m (6-ft), 2.5-mm i.d., silanized glass column with 2% (w/w) XE-60 on acid-washed Anakrom A (90–100 mesh).

The temperatures were: injector, 250°; column, 230°; and detector, 250°. The nitrogen carrier gas flow rate was 20 ml/min. The ratio of the codeine to dihydrocodeine peak areas was compared to a standard curve; peak areas were measured by peak height times width at half-height.

Table I—Recoveries of Codeine Added to Plasma

Codeine Added to Plasma, ng/ml	Codeine Found[a], ng/ml
0	ND[b]
50	48 ± 2 (n = 2)
100	105 ± 7 (n = 3)

[a] Corrected for the calculated extraction recovery. [b] Not detected.

RESULTS AND DISCUSSION

The objective of this study was to devise a method that was accurate and precise without requiring inordinate amounts of time for evaluation of recoveries. Several codeine analogs were considered as possible internal standards. Of these, morphine and norcodeine could not be used since they are major metabolites of codeine (7). Dihydrocodeine and codeine were unresolved by GLC on OV-17 or XE-60. Since dihydrocodeinone was well resolved on both columns and had a relative retention time of 1.67 (codeine = 1.0) on the XE-60 column, it was the choice as an internal standard. With the use of dihydrocodeinone, this procedure is nearly

[1] Fisher Victoreen series 4400.
[2] Burdick and Jackson.
[3] Fisher.

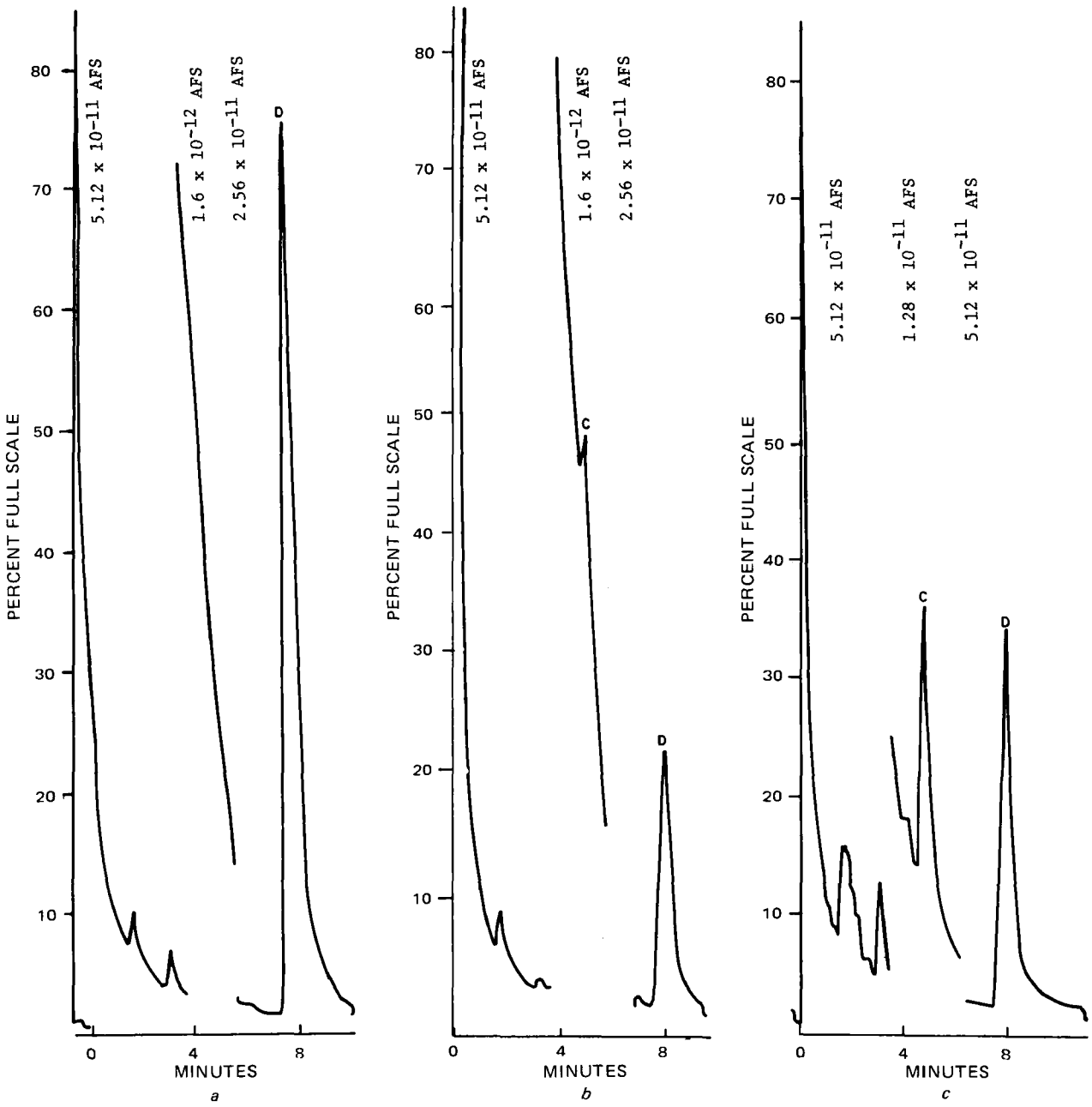

Figure 1—*Gas–liquid chromatograms of plasma extract. Key:* a, *blank;* b, *10 min after oral dose of codeine (14 ng/ml codeine);* c, *40 min after oral dose of codeine (181 ng/ml codeine); C, codeine; and D, dihydrocodeinone.*

independent of recovery so long as sufficient material is available for GLC detection.

The distribution coefficient for codeine between *n*-butyl chloride and aqueous base was found to be 2. On this basis, a theoretical maximum recovery of 85% would be predicted from the described extraction procedure. In a similar manner, a 99.5% recovery would be predicted for dihydrocodeinone (partition coefficient = 14). Recoveries of codeine relative to the internal standard are calculated to be 86% based on the partition coefficients; this value correlates well with the observed recoveries (averaging 87.6 ± 7.8%) obtained by GLC analysis (Table I). Quantitation was possible at the 50-ng/ml level as indicated, but limits of detection as low as 5 ng/ml were obtained when detector noise was optimal.

Although the amount of internal standard used is larger than the amounts of codeine encountered in plasma samples, the validity of this approach was demonstrated by the linearity of the calibration curve obtained from standard mixtures of codeine and dihydrocodeinone (r = 0.99997). An intercept for the calibration curve at 5 ng/ml correlated well with the observed limit of detection. Over the concentration range from 50 to 250 ng/ml plasma, the slope of the calibration curve including the factor for the calculated recovery was 0.000815 (peak area ratio *versus* nanograms of codeine per milliliter of plasma). This standard curve was used for the determination of the samples. Standard deviations for duplicate injections averaged 3% for eight standard mixtures in the codeine concentration range between 50 and 250 ng/ml. Standards chromatographed over 8 days gave standard deviations between days of not greater than 3.1%. Table I lists the results of the analysis of codeine added to plasma.

Figure 1 shows GLC tracings of codeine in the plasma of a subject 10 and 40 min after an oral dose of two tablets containing 65 mg of codeine as well as the plasma blank. The plasma codeine levels as a function of time for this subject are shown in Fig. 2. The standard deviations indicated are for duplicate injections of the same sample.

Some practical benefits are obtained using *n*-butyl chloride as the extraction solvent, including lower background, greatly reduced tendency to form emulsions, cleaner solvent–water interfaces, and the fact that only the upper phase must be transferred. This added convenience permits a set of 20 samples to be prepared

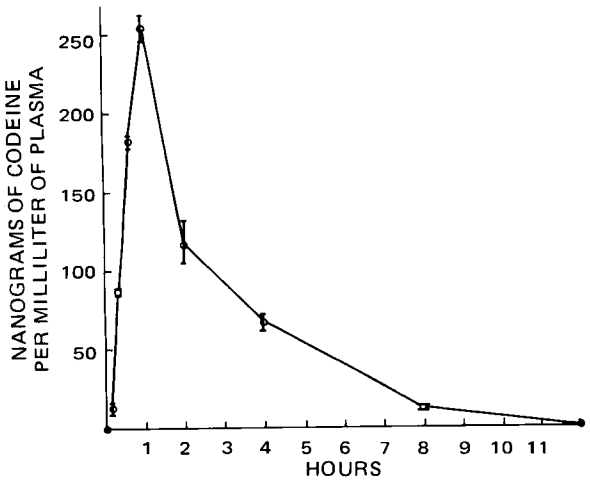

Figure 2—*Plasma codeine levels after an oral dose of two tablets containing 65 mg of codeine.*

for GLC analysis in an 8-hr day. The chromatography of the samples requires approximately the same time. Precision for codeine determination was 7% for levels of 50 ng/ml or greater.

REFERENCES

(1) D. W. Cornish, D. M. Grossman, A. L. Jacobs, A. F. Michaelis, and B. Salsitz, *Anal. Chem.*, **45**, 221R(1973).

(2) J. W. Sutherland, D. E. Williamson, and J. G. Theivagt, *ibid.*, **43**, 206(1971).

(3) B. S. Finkle, *ibid.*, **44**, 18A(1972).

(4) E. Schmerzler, W. Uy, M. I. Hewitt, and I. J. Greenblatt, *J. Pharm. Sci.*, **55**, 155(1966).

(5) K. W. Bentley, "The Chemistry of the Morphine Alkaloids," Oxford University Press (Claverdon), Oxford, England, 1954.

(6) S. Kushinsky and W. Paul, *Anal. Biochem.*, **30**, 465(1969).

(7) H. Yoshimura, M. A. Mori, K. Oguri, and H. Tsuhamoto, *Biochem. Pharmacol.*, **19**, 2353(1970).

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Aqueous Solubility of ^{14}C-Triamcinolone Acetonide

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Abstract □ The blue tetrazolium colorimetric assay has often been employed in the analysis of corticosteroids. However, the reaction between blue tetrazolium and corticosteroids partly depends on the apparent pH of the system. In an earlier study of the temperature dependence of the solubility and dissolution of triamcinolone acetonide in aqueous dissolution media (distilled water and 0.2–1.0 *M* potassium chloride solutions), pH was not considered. To determine the reliability of the data previously reported, the solubility of ^{14}C-triamcinolone acetonide was determined by a radioisotopic method. The results of the analyses, using a liquid scintillation counting system, indicate that the steroid solubility is generally about 20% lower than that reported previously.

Keyphrases □ Triamcinolone acetonide, ^{14}C-labeled—synthesized, aqueous solubility determined, radioisotopic method □ Radioisotopic methods—determination of aqueous solubility of ^{14}C-triamcinolone acetonide □ Glucocorticoids—^{14}C-triamcinolone acetonide, synthesis, aqueous solubility determined, radioisotopic method □ Solubility, aqueous—^{14}C-triamcinolone acetonide, radioisotopic method

In a previous report from these laboratories (1), data were presented for the dissolution and solubility of triamcinolone acetonide in aqueous media. The data were obtained from the colorimetric determination of steroid concentration. The pH of the aqueous dissolution systems employed was not considered.

Graham and Kenner (2) studied the parameters affecting the chemical reaction involved in the colorimetric assay of triamcinolone acetonide and indicated a dependence of absorbance on the apparent pH of the system. Thus, the colorimetric process employed might have involved some error, particularly when the measured absorbance was relatively low. To define more precisely the solubility of the steroid in aqueous media, ^{14}C-labeled triamcinolone acetonide was synthesized and its aqueous solubility was determined by measuring the associated radioactivity.

EXPERIMENTAL

Materials—The following were used as received: concentrated perchloric acid (70%)[1], spectrophotometric grade acetone[2], anisole[2], ethanol[2], ether[2], dioxane[2], 1,4-bis[2-(5-phenyloxazolyl)]-benzene[2], potassium chloride[2], 2,5-diphenyloxazole[2], sodium bicarbonate[2], 1,3-^{14}C-acetone[3], ^{14}C-benzoic acid[4], triamcinolone acetonide[5], triamcinolone alcohol[5], 1,2-dimethoxane[6], recrystallized blue tetrazolium[2], chloroform[2], toluene[2], sodium hydroxide[2], and methanol[2].

Solutions—A stock solution of 1,3-^{14}C-acetone was diluted with acetone to yield a solution with a specific activity of 3.02×10^7 dpm/ml. A liquid scintillation solution was prepared by dissolving 12 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene in 750 ml of dioxane, along with 125 ml of anisole and 125 ml of 1,2-dimethoxane. A standard solution of ^{14}C-benzoic acid was prepared to provide suitable specific activity.

[1] Allied Chemicals, General Chemical Division, Morristown, N.J.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] ICN Chemical and Radioisotope Division, Irvine, Calif.
[4] New England Nuclear Corp., Boston, Mass.
[5] Squibb Institute for Medical Research, E.R. Squibb and Sons, New Brunswick, N.J.
[6] Eastman Organic Chemicals, Rochester, N.Y.