REFERENCES

(1) F. J. Sena, J. T. Piechocki, and K. L. Li, *J. Pharm. Sci.*, **68**, 1465 (1979).

(2) V. D. Gupta, *J. Pharm. Sci.*, **69**, 110 (1980).

(3) W. O. McSharry and I. V. E. Savage, *J. Pharm. Sci.*, **69**, 212 (1980).

(4) L. Carnevale, *J. Pharm. Sci.*, **72**, 196 (1983).

(5) A. G. Ghanekar and V. D. Gupta, *Am. J. Hosp. Pharm.*, **34**, 651 (1977).

(6) J. R. Koziol, J. T. Jacob, and R. G. Achari, *J. Pharm. Sci.*, **68**, 1135 (1979).

(7) M. K. Chao, I. J. Holcomb, and S. A. Fusari, *J. Pharm. Sci.*, **68**, 1463 (1979).

(8) E. J. Kubiak and J. W. Munson, *J. Pharm. Sci.*, **69**, 1380 (1980).

(9) V. D. Gupta and A. G. Ghanekar, *J. Pharm. Sci.*, **66**, 895, (1977).

(10) A. G. Ghenekar and V. D. Gupta, *J. Pharm. Sci.*, **67**, 873 (1978).

(11) D. R. Heidemann, *J. Pharm. Sci.*, **70**, 820 (1981).

ACKNOWLEDGMENTS

The work was supported in part by Ohm Laboratories, North Brunswick, N.J.

Determination of Codeine in Human Plasma by Reverse-Phase High-Performance Liquid Chromatography

V. NITSCHE *x and H. MASCHER

Received January 1, 1983, from the *Pharmakologische Untersuchungsgesellschaft m.b.H., A-1171 Vienna, Austria.* Accepted for publication November 16, 1983. * Present address: Biokinet, A-1080 Vienna, Austria.

Abstract □ A high-performance liquid chromatographic (HPLC) method for the determination of codeine in human plasma is described. The specific, precise, and sensitive method can be used to determine plasma codeine levels after administration of therapeutic doses of codeine. After purification on a C_{18} extraction column, codeine in the form of hydrochloride is eluted. After addition of the internal standard, the codeine is separated on a reverse-phase C_{18} column using a slightly alkaline mobile phase and is then determined by UV detection. The analysis takes 3.5 min per run; the limit of detection is ~3 μg/L for a 50-μL loop and 800 μL of plasma. The absolute recovery is 98.4 ± 6.7% (n = 14) in the 10-300-μg/L range. Within the range, the calibration curve is linear.

Keyphrases □ Bioavailability—codeine, human plasma, HPLC □ Codeine—bioavailability, HPLC □ HPLC—bioavailability of codeine

Numerous methods for the detection of codeine are reported in the literature, including GC (1-5), GC-MS (6-9), TLC (10-12), RIA (3, 13-15), radioactive labeling (16, 17), and several high-performance liquid chromatographic (HPLC) methods. However, with one exception (18), these have not been used to detect codeine in plasma (19-21).

Some of the detection methods are not sufficiently sensitive or selective and others require sophisticated equipment (GC-MS) or specialized techniques (RIA). Our objective was to develop an HPLC method which is well suited for routine determination of codeine in plasma.

EXPERIMENTAL SECTION

Material and Methods—A liquid chromatograph[1] equipped with a UV detector[2] was used. The loop volume was 50 μL, but could be increased to 150 μL without affecting separation performance. Membrane filters[3] (pore size, 0.45 μm) and a 10-μL syringe[4] were also used.

Codeine hydrochloride[5] and diazepam[5] (internal standard) were used as supplied. Methanol[6] and ammonium carbonate[7] were AR grade. The C_{18} extraction columns[6] had a volume of 1 mL. Solutions of 0.1 M HCl, 1 M ammonium hydroxide, methanol-0.1 M ammonium hydroxide (20:80; mixture A), and methanol-0.1 M HCl (50:50; mixture B) were prepared. The internal standard solution was ~200 μg of diazepam/20 mL of methanol.

The buffer (pH 8.9) was made with 80.5 mL of 0.1 M borate solution (1.237 g of boric acid in 10 mL of 0.1 M NaOH) and 19.5 mL of 0.1 M HCl. Distilled water was used throughout.

The mobile phase was methanol-0.1 M ammonium carbonate (70:30). The mixture was used within 2-3 d, because tailing at the codeine peak was sometimes observed with older mixtures.

The chromatographic conditions included a 250 × 4-mm column[8], filled in-house[9] with Polygosil C 18, 7.5 μm; the flow rate was 2.0 mL/min; the column oven temperature was 45°C; the detector wavelength was 220 nm, 0.01 AUFS, time constant 1 min; the injection volume was 50-150 μL by loop; and the recorder advance was 10 mm/min.

Sample Preparation—Plasma (citrate anticoagulant) was filtered through a 0.45-μm membrane filter and 0.8 mL was mixed with 0.3 mL of buffer (pH 8.9). The mixture was quantitatively transferred into a C_{18} extraction column[6] and centrifuged at 500×g for 1-2 min; the plasma passed completely through the tube. To eliminate interfering substances, the extraction column was washed with 1 mL of 0.1 M HCl, 1 mL of mixture A, and 0.3 mL of 0.1 M HCl (each wash was followed by a 1-min centrifugation at 500×g). The eluates were discarded. Codeine was then eluted with 300 μL of mixture B. With commonly used centrifuges, 10-40 extraction columns can be eluted at the same time, which shortens the analysis time. The ammonium hydroxide solution (~30 μL, pH >8) and 5 μL of the internal standard solution were added to the eluate. After thorough mixing, the loop was rinsed and filled with ~100 μL (~180 μL if a 150-μL loop was used) of this solution.

Standard Preparation—Samples (2, 8, 20 and 40 μL) of a solution containing 4 ng of codeine base/μL (weighed as codeine hydrochloride, dissolved in water and diluted accordingly) were added to 0.8-mL plasma blanks. The remainder of the procedure is as described in *Sample Preparation*.

The quotients of the peak areas (codeine-internal standard) of the samples were compared with a calibration curve.

Bioavailability Study—In a randomized crossover study, bioavailability was determined with six healthy male volunteers (mean age, 46.8 ± 15.2; mean weight, 74.1 ± 9.7 kg; and mean height 178.3 ± 6.25 cm). The subjects underwent medical examinations and laboratory tests which showed them to be in good health. All subjects had been informed of the investigation procedures and had expressed their agreement in writing.

The subjects received two different oral codeine-doxycycline preparations on different days. The test preparations were 100 mg of doxycycline with 30 mg of codeine base as the phosphate in diffusion pellets (preparation 1)[10] and 100 mg of doxycycline with 30 mg of codeine base as resinate (preparation 2)[11]; both were sustained-release preparations. The dose was two capsules per

[1] Model 8000; Spectra Physics, Santa Clara, Calif.
[2] Spectromonitor III; Laboratory Data Control, Riviera Beach, Fla.
[3] Sartorius; Göttingen, F.R.G.
[4] Hamilton, Bonaduz, Switzerland.
[5] Gerot Pharmazeutika, Vienna, Austria.
[6] Baker, Deventer, The Netherlands.
[7] Merck.
[8] Hibar RT; Merck.
[9] Machery/Nagel, Düren, F.R.G.
[10] Batch 4PA-222, Doxitussin; Gerot Pharmazeutika.
[11] Batch 810238, Vibratussal; Pfizer, Karlsruhe, F.R.G.

0022-3549/84/1100-1556$01.00/0

Table I—Reproducibility of Codeine Determination in Blank Plasma Spiked with Codeine

Codeine Added, μg/L	Codeine Found, μg/L (mean ± *SD*)
303	304.8 ± 3.70[a]
152	147.3 ± 6.24[b]
19	16.9 ± 1.94[c]

[a] $n = 4$. [b] $n = 5$. [c] $n = 3$.

person (60 mg of codeine). A 1-week wash-out phase passed between the examination days. The subjects ingested the preparations with liquid in the morning after fasting overnight. Two hours after administration they had a light breakfast.

Blood samples were taken at 0, 1, 2, 3, 4, 5, 7, and 24 h after administration. The plasma obtained from the blood samples was frozen until assayed. The samples were analyzed by the method described above. A Student's *t* test for dependent samples was used to statistically compare the areas under the curves (AUC). Concentrations in the plasma after 24 h were usually at the limit of detection and could not be accurately determined; therefore, the values have not been included.

RESULTS

Recovery and Reproducibility—Codeine was added to the plasma and assayed according to specifications. The results were compared with those of codeine added to 300 μL of distilled water (plus internal standard and ammonium hydroxide) directly injected into the chromatograph. The recovery was 98.4 ± 6.6% (mean value ± *SD*; $n = 16$) (Table I).

Extracts of samples from the bioavailability study were injected twice within 15 h. The *SD* was 0.3–5.9 with a *CV* of 0.6–10%. Repeated extraction and injection of several samples (20–155 μg/L of plasma) gave an *SD* of 0.14–11 with a *CV* of 0.8–12.9%.

We found a linear regression ($r = 0.9989$; $n = 14$; $k = 0.0038596$; $d = 0.01646$) of the calibration curve in the 10–300-μg/L range by adding codeine to blank plasma (Table I). The limit of detection with 0.8 mL of plasma and a 50-μL loop is 3 μg/L of plasma. If a 150-μL loop and greater volume of plasma are used, the limit of detection can be reduced to <1 μg/L of plasma. There are no interfering substances present in the chromatogram; the limit is due to UV absorption constraints.

Bioavailability Study—Representative chromatograms of plasma samples are shown in Fig. 1. The individual and mean (±*SD*) values obtained in the bioavailability study are given in Tables II and III and are graphically represented in Fig. 2. The AUC value, a measure of relative bioavailability, and the half-life calculated from each plasma level curve are shown in Table III.

DISCUSSION

The method outlined above offers advantages over GC, GC–MS, and TLC with regard to handling and analysis duration. Moreover, the rate of recovery is extremely high.

The only substantial obstacle encountered in the development of this assay

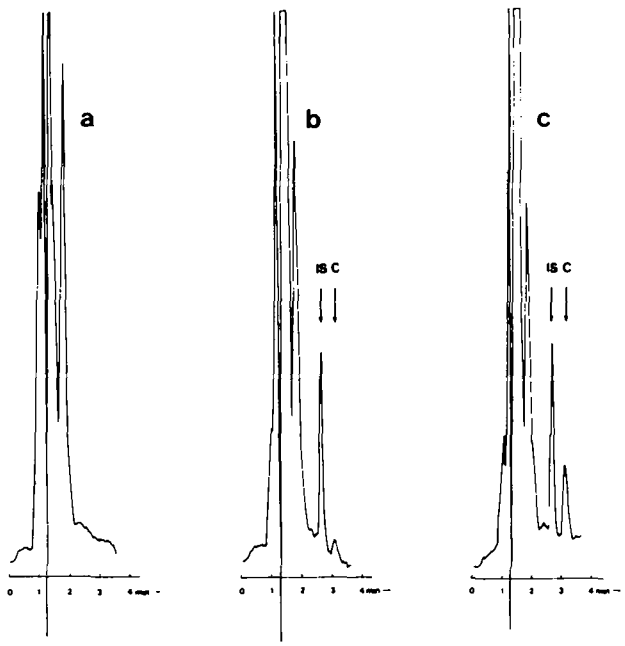

Figure 1—*Chromatograms of blank plasma (a), plasma with 13 μg/L of codeine (b), and plasma with 59 μg/L of codeine (c). Key: (IS) internal standard; (C) codeine.*

method was the multitude of endogenous substances detected in the codeine range at a wavelength of 220 nm. The problem was solved by selective prepurification of the plasma on a C_{18} extraction column[6]. Any neutral substances and acids that could interfere with the determination of codeine in the chromatogram with an alkaline mobile phase were retained by the extraction column or eluted during the washing; codeine was eluted quantitatively in the form of its salt. No interference was found with frequently coadministered drugs, such as benzodiazepines (*e.g.*, oxazepam, diazepam, flurazepam, and nitrazepam), doxycycline, bromhexine, amoxicillin, and ethambutol, using this procedure. The metabolites of codeine, its glucuronide, and some other narcotics do not interfere with codeine (retention times: codeine, 3.1 min; codeine glucuronide, 1.4 min; norcodeine, <1.6 min; morphine, 1.7 min; nalorphin, 1.3 min).

The results of bioavailability studies using this HPLC method agree with results obtained by other methods with similar dosages. After administration of 60 mg of codeine sulfate, a maximum level of 100–120 μg/L and an elimination half-life of 3.0–4.2 h (4) were obtained by GC. After administration of 65 mg of codeine phosphate, a maximum of 117 μg/L and an elimination half-life of 3.60 ± 0.15 ($n = 6$) were found by RIA (12). With a 25-mg dose of labeled codeine phosphate, a maximum level of ~40 μg/L and elimination half-lives of 2.2 h (1.8–2.8; $n = 4$) for tablets and 4.2 h (3.2–5.2; $n = 4$) for slow-release capsules were obtained (14). TLC indicates a maximum level of 90 μg/L after administration of 70 mg of codeine phosphate in the form

Table II—Plasma Concentrations After Oral Administration of 60 mg of Codeine

Subject	Preparation	Plasma Concentration, μg/L[a] 1 h	2 h	3 h	4 h	5 h	7 h
A	1[b]	34.00	55.00	65.00	58.00	52.00	39.00
	2[c]	16.00	59.00	77.00	87.00	76.00	50.00
B	1	59.00	62.00	64.00	66.00	50.00	26.00
	2	10.00	21.00	33.00	60.00	50.00	37.00
C	1	93.00	187.00	155.00	133.00	115.00	88.00
	2	22.00	125.00	181.00	158.00	133.00	77.00
D	1	60.00	109.00	80.00	64.00	49.00	38.00
	2	29.00	108.00	94.00	42.00	30.00	20.00
E	1	38.00	115.00	89.00	82.00	59.00	45.00
	2	14.00	68.00	96.00	100.00	83.00	57.00
F	1	8.00	38.00	87.00	73.00	54.00	34.00
	2	19.00	65.00	71.00	54.00	72.00	42.00
Mean	1	48.67	94.33	90.00	79.33	63.17	45.00
SD		±28.93	±54.76	±33.57	±27.55	±25.64	±21.98
Mean	2	18.33	74.33	92.00	83.50	74.00	47.17
SD		±6.65	±37.17	±49.17	±42.38	±34.88	±19.30

[a] Plasma concentrations measured at 0 and 24 h were 0 μg/L. [b] Doxycycline (100 mg) in 30 mg of codeine base as the phosphate. [c] Doxycycline (100 mg) in 30 mg of codeine base as the resinate.

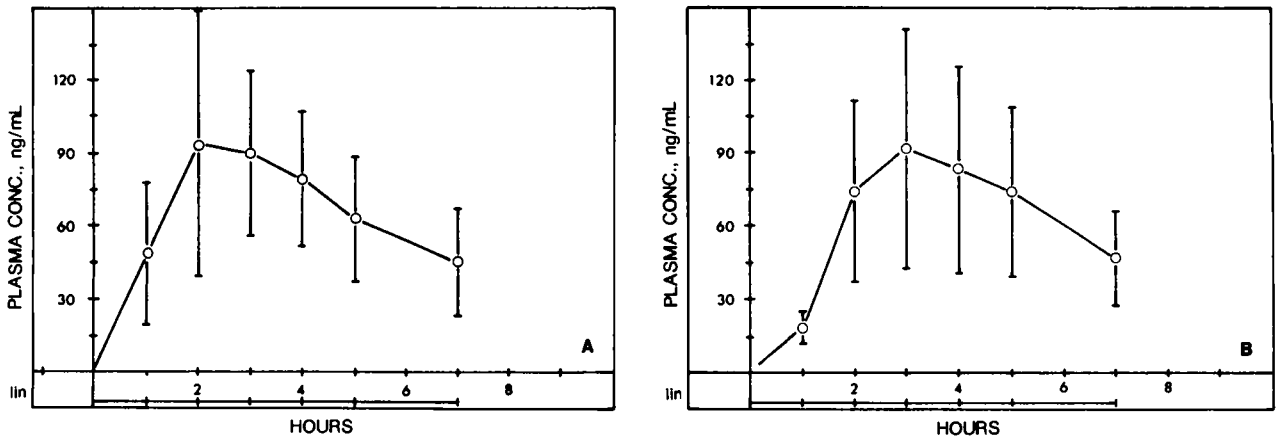

Figure 2—*Mean plasma concentration of six volunteers after ingestion of 60 mg of codeine in two different preparations.*

Table III—Area Under the Curve (AUC) and Half-Life ($t_{1/2}$) After Oral Administration of 60 mg of Codeine

Subject	AUC		$t_{1/2}$	
	Preparation 1[a]	Preparation 2[b]	Preparation 1	Preparation 2
A	329.00	403.00	5.42	3.68
B	352.00	236.00	2.22	4.34
C	828.50	762.50	2.89	3.20
D	424.50	338.00	4.65	1.90
E	457.50	459.50	3.30	3.70
F	321.00	359.00	3.68	—
Mean	452.1	426.3	3.69	3.36
SD	±192.3	±180.7	±1.07	±0.82

[a] Doxycycline (100 mg) in 30 mg of codeine base as the phosphate. [b] Doxycycline (100 mg) in 30 mg of codeine base as the resinate.

of a slow-release preparation, with a maximum level of ~95 μg/L and an elimination half-life of 2.2–3.9 h after administration of 35 mg of codeine phosphate in tablet form (9). This rapid HPLC method is, therefore, well suited for pharmacokinetic and clinical detection of codeine in plasma.

REFERENCES

(1) R. A. Zweidinger, F. M. Weinberg, and R. W. Handy, *J. Pharm. Sci.*, **65,** 427 (1976).

(2) B. Dahlström, L. Paalzow, and P. O. Edlund, *Acta Pharmacol. Toxicol.*, **41,** 273 (1977).

(3)N. J. Jain, T. C. Sneath, W. J. Leung, and R. D. Budd, *J. Pharm. Sci.*, **66,** 66 (1977).

(4) M. K. Brunson and J. F. Nash, *Clin. Chem. (Winston-Salem, N.C.)*, **27,** 1956 (1975).

(5) G. Brugaard and K. E. Rasmussen, *J. Chromatogr.*, **147,** 476 (1978).

(6) G. Lhoest and M. Mercier, *Pharm. Acta Helv.*, **55,** 316 (1980).

(7) D. Pearce, S. Wiersema, M. Kuo, and C. Emery, *Clin. Toxicol.*, **14,** 161 (1979).

(8) E. J. Cone, C. W. Gorodetzky, S. Y. Yeh, W. D. Darwin, and W. F. Buchwald, *J. Chromatogr.*, **230,** 57 (1982).

(9) W. O. R. Ebbighausen, J. H. Mowat, P. Vestergaard, and N. S. Kline, *Adv. Biochem. Psychopharmacol.*, **7,** 135 (1973).

(10) R. D. Budd, D. F. Mathis, and W. J. Leung, *Clin. Toxicol.*, **16,** 61 (1980).

(11) P. Haefelfinger, *J. Chromatogr.*, **124,** 351 (1976).

(12) H. Fischer, H. G. Eulenhöfer, and R. Kraft, *Drug Res.*, **21,** 1692 (1971).

(13) J. A. W. Findlay, R. F. Butz, and R. M. Welch, *Clin. Pharmacol. Ther.*, **22,** 439 (1977).

(14) J. W. A. Findlay, R. F. Butz, and R. M. Welch, *Life Sci.*, **19,** 389 (1976).

(15) J. W. A. Findlay, E. C. Jones, R. F. Butz, and R. M. Welch, *Clin. Pharmacol. Ther.*, **24,** 60 (1978).

(16) W. D. Bechtel and E. Sinterhauf, *Drug Res.*, **28,** 308 (1978).

(17) K. Sinterhauf and W. D. Bechtel, *Drug Res.*, **30,** 1012 (1980).

(18) I. W. Tsina, M. Fass, J. A. Debban, and S. B. Matin, *Clin. Chem. (Winston-Salem, N.C.)*, **25,** 1137 (1982).

(19) C. Olieman, L. Maat, K. Waliszewski, and H. C. Beyerman, *J. Chromatogr.*, **133,** 382 (1977).

(20) C. Y. Wu, and J. J. Wittick, *Anal. Chem.*, **49,** 359 (1977).

(21) J. O. Svensson, A. Rane, J. Säwe, and F. Sjögnvist, *J. Chromatogr.*, **230,** 427 (1982).

ACKNOWLEDGMENTS

The authors thank H. Schütz for technical assistance, Gerot Pharmazeutika for providing the codeine and diazepam, and Dr. Wagner Löffler for filling the HPLC column.