Gas Chromatographic Determination of Codeine in Serum and Urine

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A method for gas chromatographic detection of codeine in serum is described. An application to a clinical drug evaluation study is given. A method for simultaneous detection of free and total codeine and its metabolites, norcodeine and morphine, in urine is also presented.

USE OF GAS-LIQUID chromatography as a means of comparing serum levels in subjects receiving oral codeine dosages has been investigated. Although an efficient SE30 column enabled detection of nanogram quantities, codeine was not resolved from norcodeine on this nonpolar phase, as previously reported by Anders and Mannering (1). Elliot *et al.* (2) used the acetates formed inside the column (1) to detect higher levels present in urine of narcotic addicts. The authors found small interfering peaks present in blanks which precluded the use of codeine acetate for reliable quantification of low levels present in serum. However, there is a great difference in reaction rate between codeine and norcodeine under mild acetylation conditions. This was used to remove norcodeine before injection on SE30 columns. The specificity and accuracy of the results were checked, using an XE60 column on which free codeine and norcodeine are well resolved.

A method for the estimation of codeine and its metabolites in urine is also described.

METHODS

Codeine in Serum or Urine.—To 2.5 ml. of serum or urine in a 15-ml. glass-stoppered conical centrifuge tube is added 9 ml. of 10% butanol in freshly distilled chloroform (3). The tube is hand shaken 5 sec. Then 0.12 ml. of 16 *N* KOH is added (0.05 ml. for urine). Again mix by hand 5 sec., then mechanically shake in 10 min. After centrifuging, the aqueous serum and jell layers are aspirated. If jell formation is excessive, the aqueous layer is aspirated and the jell broken by vigorous reshaking before recentrifuging. A 7-ml. aliquot of the organic layer is transferred to a clean centrifuge tube. Care must be taken not to transfer any of the strongly basic aqueous layer. A quantity of 1.5 ml. of 0.01 *N* H_2SO_4 is added. Shake 10 min. Mechanical shaking for 3 min. is followed by centrifuging and transfer of the acid layer to a new 15-ml. tube. Quantities of 0.04 ml. of 16 *N* KOH and 8 ml. of distilled chloroform are added. Shake 10 min. After centrifuging, remove the aqueous layer. Dry the chloroform extract with anhydrous sodium sulfate. Evaporate to dryness with a stream of nitrogen at 60° in 2-ml. conical centrifuge tubes. Twenty microliters (50 μl. for urine) of an internal standard solution, 0.1 mg./ml. of cholesterol acetate in ethyl acetate, is added just prior to injection of 2 μl. of this mixture.

On SE 30, codeine and norcodeine are not resolved and peaks observed represent any contributions from either compound. To determine codeine alone, a trace amount of acetic anhydride is added

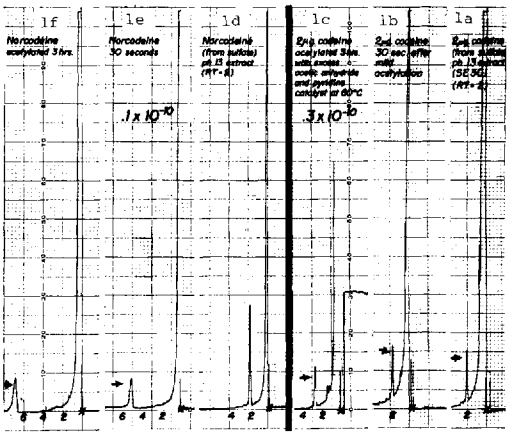

Fig. 1.—Norcodeine removal technique (SE30). Key: (1a) codeine; (1b) codeine injected 30 sec. after mild acetylation; (1c) codeine after acetylating 3 hr. at 60° with pyridine catalyst; (1d) norcodeine; (1e) norcodeine after 30 sec. acetylation; and (1f) norcodeine after 3 hr. acetylation. Note the retention time of codeine = norcodeine = 2 min.

TABLE I.—RETENTION OF RESINATED AND NONRESINATED PREPARATIONS

Retention Times	XE60 (240°) min.	SE30 (240°) min.
Codeine	2.2	2.0
Norcodeine	2.8	2.0
Codeine acetate	2.8	2.6
Morphine diacetate	5.1	3.4
Norcodeine-*N*-acetamide	16.0	5.0
Norcodeine-*N*-acetamide-6-acetate	19.0	6.8
Cholesterol acetate	6.7	16.0
Cholestane	1.6	5.6

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TABLE II.—TOTAL CODEINE AND NORCODEINE, RELATIVE UNITS (SE30)

Subject and Age	15 mg. Resinated Codeine Time, hr. 2	5	7	9	11	15 mg. Nonresinated Codeine Time, hr. 2	5	7	9	11
A, 37	3.6	1.6	0.5	0.3	0.1	1.4	1.6	0.5	0.4	0.2
B, 21	6.4	3.6	2.4	0.4	0.1	6.4	4.4	3.6	2.0	0.4
C, 23	0.8	5.0	1.2	0.6	0.4	3.2	2.0	1.6	0.4	0.1
D, 24	4.8	1.6	0.5	0.5	0.3	5.6	1.2	0.5	0.2	0.1
E, 46	5.2	2.7	0.9	0.6	0.1	4.8	4.0	1.9	1.0	0.6
Av.	4.2	2.9	1.1	0.5	0.2	4.3	2.6	1.6	0.9	0.3

TABLE III.—SERUM CODEINE (mcg./100 ml.) AFTER NORCODEINE REMOVAL ON SE30

		Codeine				Codeine	
		Time, hr.				Time, hr.	
Subject	Medication	2	5	Subject	Medication	2	5
B	Resinated	2.8	1.2	*B*	Nonresinated	3.3	2.2
E	Resinated	3.6	1.2	*E*	Nonresinated	2.6	1.3

TABLE IV.—SERUM CODEINE (mcg./100 ml.) ON XE60

		Codeine				Codeine		
		Time, hr.				Time, hr.		
Subject	Medication	2	5	9	Subject	Medication	2	5	9
B	Resinated	3.6	1.2	0.1	*B*	Nonresinated	3.6	2.8	0.4

(1 μl. of a 1/50 solution of acetic anhydride in ethyl acetate for serum or 1/10 for urine). The mixture is stirred and 2 μl. injected into the chromatograph within 30 sec. (Fig. 1). Norcodeine may be estimated by difference if an injection before acetylation is made first. Suitable standards and blanks are included with each analysis.

On XE60 the 2 alkaloids are resolved as free compounds, and acetylation is unnecessary.

Codeine and Metabolites in Urine.—Three milliliters of urine is brought to pH 9 with 0.3 Gm. of sodium bicarbonate and 0.3 ml. of 10 *N* sodium hydroxide. Nine milliliters of 10% butanol in chloroform is added and, at this point, the procedure continues as described above through the extraction at pH 2 into dilute sulfuric acid. The acid layer is adjusted to pH 13 with 0.05 ml. of 10 *N* sodium hydroxide. Codeine and norcodeine are extracted into 8 ml. of distilled chloroform. The aqueous layer containing morphine sulfate is completely transferred to another tube, adjusted to pH 9 with 0.2 Gm. of sodium bicarbonate and extracted 10 min. with 8 ml. of chloroform containing 1% ethanol. After centrifuging, the aqueous layer is discarded. Both organic layers are dried with anhydrous sodium sulfate and evaporated to dryness under nitrogen at 60° in 2-ml. centrifuge tubes. The codeine and norcodeine may be chromatographed by dissolving the extract in 50 μl. of internal standard solution and injecting 2 μl. on the XE60 column.

Free morphine tails excessively on XE60 or SE30 and must be acetylated completely to chromatograph well. Acetylation of the pH 9 extract containing free morphine is carried out by adding 0.1 ml. of a mixture of acetic anhydride, pyridine, and ethyl acetate (1:1:1) and warming about 2 hr. at 60°. The solution is evaporated to dryness and dissolved in 50 μl. of internal standard solution.

Total codeine, norcodeine, and morphine are obtained by first hydrolyzing with acid. Concentrated HCl (10% by volume) is added to 3 ml. of urine and the sample placed in a boiling water bath for 1 hr. (4). As a check, or if the presence of occasional interfering compounds compel it, the codeine and norcodeine in the pH 13 extract may be completely acetylated as for morphine.

Gas chromatographic data were obtained on a Jarrel-Ash instrument model 28-710.

SE30 column: 6-ft., 4-mm. i.d., silanized glass column with 5% (w/w) SE30 on acid washed 80/100 mesh Gas-Chrom P. Temperatures: injector, 260°; detector, 260°; column, 240°. Nitrogen carrier flow rate = 70 ml./min. measured at room temperature. Theoretical plates = 1600.

XE60 column: 4 ft., 4-mm. i.d., silanized glass column with 4% (w/w) XE60 on acid washed 90/100 mesh Anakrom A. Temperatures: injector, 260°; detector, 260°; column, 240°. Nitrogen flow = 70 ml./min. measured at room temperature. Theoretical plates = 1500.

The liquid phases were applied by filtration technique (5).

RESULTS

The objective was to determine if a 15-mg. resinated oral codeine preparation[1] would show either slower absorption or last longer in human blood when compared with a nonresinated codeine preparation of the same composition except for absence of resin complexing. Five healthy male volunteers were tested. After 10 days, those individuals who received the resinated material then received the nonresinated material in the same dosage and vice versa. (Table I.)

All 10 blood series were examined for total (unresolved) codeine and norcodeine values on an SE30 column. The peaks were calculated, arbitrarily, using codeine standards in micrograms per 100 ml. of serum. In Table II, these results are recorded

[1] Strional which contains 15 mg. of codeine base as cation exchange resin complex, 10 mg. of methaqualone as cation exchange resin complex, 162 mg. of acetylsalicylic acid U.S.P. Strional was supplied by the Strasenburgh Laboratories, Rochester, N. Y.

TABLE V.— URINARY CODEINE, NORCODEINE, AND MORPHINE ON XE60 (SUBJECT *I*)

Time, hr.	Vol.	pH	Codeine		Norcodeine		Morphine	
			Free	Total	Free	Total	Free	Total
			mcg./100 ml.		mcg./100 ml.		mcg./100 ml.	
4	35	7	40	70	70	170	5	40
11	320	7.5	30	90	75	330	20	100

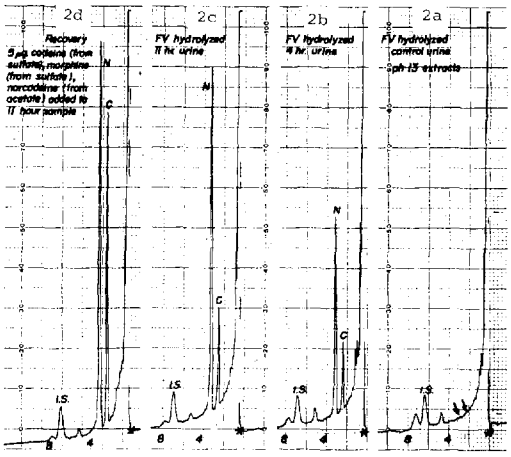

Fig. 2.—Shows hydrolyzed (FV, subject *I*) urine extracted at pH 13 for total codeine (*C*) and norcodeine (*N*). Cholesterol acetate = internal standard (*I. S.*). Key: (2a) control; (2b) 4 hr. after ingestion of resinated codeine; (2c) 11 hr.; (2d) recovery. (XE60.)

in relative units. The averages obtained using resinated or nonresinated preparations are about the same. The specific determination of codeine at key time periods was made in 2 of the individuals (*B* and *E*) by the technique of norcodeine removal on SE30 (Table III). In one case values for free codeine were also checked on an XE60 column (Table IV). Codeine levels for these individuals show the same essential hourly pattern as for the combined alkaloids. In all methods, results were read from standard curves, since these were not quite linear at serum levels. Recoveries of added codeine ranged from 70–140% and averaged 105%.

Codeine Metabolites in Urine.—The method for codeine and metabolites was used to study the urine of a female patient (Table V). After 11 hr., the conjugated forms of all 3 alkaloids are present in greater concentrations than at 4 hr. Figure 2 shows chromatograph for total codeine and norcodeine in this individual.

The urines of 4 patients receiving resinated codeine and 1 patient receiving free oral codeine preparation were examined after 3.5 hr. by norcodeine removal technique on SE30. The data presented in Table VI indicate wide variations in the ratio of codeine to norcodeine.

TABLE VI.—URINARY CODEINE AND NORCODEINE BY NORCODEINE REMOVAL TECHNIQUE ON SE30

Subject	Medication	Codeine, mcg./100 ml.	Norcodeine,[a] mcg./100 ml.
F	Resinated	570	70
G	Resinated	160	90
H	Resinated	220	70
I	Resinated	30	65
J	Nonresinated	260	100

[a] Calculated using standards made by dissolving authentic norcodeine in chloroform. Results based on norcodeine salts carried through extraction procedure give results about 10 times greater.

SUMMARY

Methods are presented for gas chromatographic analysis of codeine in serum and its metabolites in urine. In a comparison of 2 oral codeine preparations, no significant difference in serum levels between resinated and nonresinated codeine was found. Over-all precision of the methods average 100 ± 20% in serum with better precision at the higher levels in urine.

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