Table II—Plasma Levels[a] of Acetaminophen (Micrograms per Milliliter) after Oral Administration of Acetaminophen Either Alone or in Combination with Choline Salicylate

Time Postdrug, min.	Acetaminophen, 10 mg./kg.	Acetaminophen, 10 mg./kg., plus Choline Salicylate, 2.0 mg./kg.
0	0	0
5	9.5 ± 0.9	11.4 ± 1.0[b]
10	9.0 ± 0.6	11.6 ± 1.3[b]
15	11.3 ± 2.8	8.5 ± 1.2
30	4.4 ± 0.3	4.5 ± 0.6
45	2.5 ± 0.3	3.5 ± 0.7
60	1.5 ± 0.2	1.5 ± 0.2
90	0.6 ± 0.1	0.6 ± 0.1
120	0.1 ± 0.03	0.3 ± 0.1

[a] Average of 10 individual experiments ± *SE*. [b] $0.05 < p < 0.1$ (by two-tail *t*-test).

forming enzyme system would be metabolized to a greater extent than one with a lower affinity, then salicylate appears to have a greater affinity and extent of glucuronide formation than acetaminophen in the rat.

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DRUG STANDARDS

Individual Tablet Analysis for Codeine and Caffeine in Codeine-Aspirin-Phenacetin-Caffeine Tablets

THERON JAMES

Abstract □ A procedure is reported for determining the codeine and caffeine content of individual codeine-aspirin-phenacetin-caffeine tablets. Codeine is determined fluorometrically after extraction into dilute sulfuric acid; caffeine is extracted from a chloroform solution of the remaining ingredients with phosphoric acid and determined by UV spectroscopy. Average recoveries with a synthetic mixture were 99.7 and 98.9% for codeine and caffeine, respectively. Assay results are reported for codeine [1–65 mg. (1/65–1 grain)/tablet] and caffeine [32 mg. (1/2 grain)/tablet] in several different commercial samples. The proposed procedure is also compared with official methods.

Keyphrases □ Codeine with aspirin-phenacetin-caffeine tablets—individual tablet analysis for codeine and caffeine, compared to official methods □ Aspirin-phenacetin-caffeine with codeine tablets—individual tablet analysis for codeine and caffeine, compared to official methods □ Spectrophotofluorometry—analysis, codeine in aspirin-phenacetin-caffeine with codeine tablets □ UV spectrophotometry—analysis, caffeine in aspirin-phenacetin-caffeine with codeine tablets □ Analgesic formulations—analysis of codeine and caffeine in individual aspirin-phenacetin-caffeine with codeine tablets

The NF XIII monograph (1) for tablets containing codeine and aspirin-phenacetin-caffeine (1) requires content uniformity tests for codeine and caffeine. The methods involve a GC determination for codeine, while caffeine is determined by UV spectroscopy after column chromatographic separation. Utilization of these procedures for content uniformity necessitates two separate analyses. Consequently, a single tablet is not tested for *both* drugs (*i.e.*, codeine is determined in one group of tablets while caffeine is determined in

another). In addition, the assay procedures have several disadvantages when they are used for individual tablet analysis:

1. The original method for codeine (1) uses propoxyphene hydrochloride as an internal standard. Erratic results are often obtained because of the thermal decomposition of this compound.

2. The revised method for codeine (2) uses another internal standard but includes a lengthy *system suitability* procedure.

3. Assay results on tablets containing less than 30 mg. codeine are questionable because errors inherent in multiple extractions, transfers, and evaporations are greatly magnified when the active ingredient is at a low concentration.

4. Applying the assay procedure (1) for I to individual tablet analysis requires the acquisition and preparation of at least 10 chromatographic columns. With the accessory glassware needed as well, the method becomes both lengthy and cumbersome.

Obviously, a utilitarian method for determining both drugs in the same tablet would have more appeal. Heuermann and Levine's (3) method for determining I with other drugs has been used to determine codeine and caffeine. Codeine is trapped on a strong acid column and subsequently eluted with triethylamine in chloroform. Incomplete elution of codeine is common because of difficulty in completely basifying the strong acid column. Furthermore, the triethylamine must be completely removed prior to the determinative step and be of sufficient purity so as not to leave any UV-absorbing residues. Compound I passes through this acid column, and caffeine is determined as previously described by Levine (4). A procedure reported by Siegmund (5) is also capable of assaying both drugs. Its application to individual tablet analysis, however, is unwieldy because it requires *two* chromatographic columns per analysis. Parenthetically, Siegmund also reported incomplete separation of codeine and caffeine if the sample is not ground sufficiently.

The sensitivity and separation properties inherent in GLC offer another plausible alternative; however, reported procedures (6–9) are not apposite for individual tablet analysis. The methods of Hoffman and Mitchell (6) and Haefelfinger *et al.* (7) do not include codeine, and the method reported by Dechene *et al.* (8) involves a preliminary separation and temperature programming. Retention data reported by Rader and Aranda (9) indicate good separation between codeine and I. However, most parameters in their procedure (*e.g.*, sample extraction, instrument operating conditions, and retention times) need modification for individual tablet analysis.

The quest for a more practical approach generated this investigation. It was determined experimentally that caffeine can be quantitatively extracted from chloroform and effectively separated from phenacetin by using phosphoric acid. This discovery, coupled with the easy extractability of codeine by dilute acids, led to the development of a practical, facile method for individual tablet analysis for codeine and caffeine in common analgesic tablets. In the proposed method, a single tablet is dispersed in dilute sulfuric acid and extracted with chloroform. The acid layer retains codeine, which is determined fluorometrically after filtration and proper dilution. Caffeine is extracted from a portion of the chloroform solution with phosphoric acid and subsequently determined by UV spectroscopy.

EXPERIMENTAL

Apparatus—Fluorescence measurements were made using a recording spectrophotofluorometer[1] with 1-cm. cells. The following instrument parameters were employed: xenon lamp; meter multiplier, 0.03; sensitivity, 40–50; 1P21 photomultiplier; slit arrangement No. 4; excitation wavelength, about 285 nm.; and emission wavelength, about 355 nm.

UV spectra were obtained using a recording spectrophotometer[2] with 1-cm. cells.

Reagents—Reagent grade chloroform and ether and approximately 0.1 *N* ether-saturated sulfuric acid were used. A phosphoric acid solution was prepared by mixing equal volumes of distilled water and reagent grade phosphoric acid.

Standard Solutions—The following were used:

1*a*. Codeine phosphate stock solution, 0.13 mg./ml. in 0.1 *N* ether-saturated sulfuric acid.

1*b*. Codeine phosphate working solution, 13 mcg./ml. Dilute 10.0 ml. stock solution to 100 ml. with 0.1 *N* ether-saturated sulfuric acid.

2*a*. Caffeine stock solution, 1 mg./ml. in chloroform.

2*b*. Caffeine working solution, 0.01 mg./ml. Pipet 1.0 ml. stock solution into a 100-ml. volumetric flask and evaporate to dryness. Then add 30 ml. phosphoric acid solution to the residue, swirl to dissolve, and dilute to volume with distilled water.

Sample Preparation—Place one tablet in a separator containing about 30 ml. 0.1 *N* ether-saturated sulfuric acid and allow to disintegrate by swirling or standing. Extract with four 20-ml. portions of chloroform, collecting the extracts in a 100-ml. volumetric flask. Dilute to volume with chloroform and save for the caffeine determination.

Codeine Determination—Wash the acid solution in the separator with one 50-ml. portion of ether, and filter acid layer through a wetted filter into a 100-ml. volumetric flask. Rinse separator and filter with several portions of 0.1 *N* ether-saturated sulfuric acid, adding rinsings to flask. Finally, dilute to volume with 0.1 *N* ether-saturated sulfuric acid and mix. Using 0.1 *N* ether-saturated sulfuric acid, dilute an appropriate aliquot to a codeine phosphate concentration of 10–13 mcg./ml.

With the codeine phosphate working standard, at an excitation wavelength of 285 nm., adjust the spectrophotofluorometer to about 70% relative fluorescence intensity at 355 nm. Scan the sample and standard solutions from 285 to 375 nm., reading the maximum at about 355 nm. Use 0.1 *N* ether-saturated sulfuric acid as a blank.

Caffeine Determination—Pipet 3.0 ml. of the chloroform solution from the sample preparation into a separator containing 20 ml. chloroform. Add 30 ml. of the phosphoric acid solution and extract by shaking vigorously for 2 min. Allow the layers to separate and discard the chloroform. Backwash the acid layer with another 20 ml. of chloroform and discard wash. Rinse the stopper with distilled water and add about 25 ml. distilled water to the acid solution. Mix and filter through a wetted filter into a 100-ml. volumetric flask. Rinse separator and filter with several portions of distilled water, and finally dilute to volume with distilled water. Compare the absorbance of this solution to that of the caffeine working standard at 269 nm., using 30% phosphoric acid solution in distilled water as a blank.

RESULTS AND DISCUSSION

Caffeine is sparingly soluble in aqueous solutions, while phenacetin is only very slightly soluble. However, because phenacetin is present in relatively large amounts and because of its high absorptivity, an essentially complete separation is necessary for an ac-

[1] Aminco-Bowman spectrophotofluorometer, American Instrument Co., Silver Spring, Md.

[2] Cary model 15 spectrophotometer, Applied Physics Corp., Monrovia, Calif.

Table I—Recovery of Codeine and Caffeine from a Synthetic Mixture of Codeine–Aspirin–Phenacetin–Caffeine

Codeine Recovery, %	Caffeine Recovery, %
100.4	99.1
100.0	98.2
100.0	99.4
99.3	98.2
98.8	97.6
99.8	97.9
99.4	98.2
99.6	100.3
99.5	99.4
100.0	100.3
Average = 99.7	Average = 98.9
SD = 0.46	*SD* = 0.98

Table II—Comparison of Assay Methods for Codeine and Caffeine in Commercial Tablets of Codeine–Aspirin–Phenacetin–Caffeine

Sample	Codeine Found, mg.[a] Proposed Method	Codeine Found, mg.[a] AOAC Method[b]	Caffeine Found, mg.[a] Proposed Method	Caffeine Found, mg.[a] NF XIII Method[c]
1	1.04	1.03	33.24	33.11
2	7.91	7.91	33.44	33.37
3	17.43	17.50	32.79	33.05
4	62.73	62.53	31.56	31.88

[a] Each assay result is the average of four determinations. [b] *Reference 12.* [c] *Reference 1.*

curate caffeine determination. Previously, it was generally thought impossible to separate caffeine from phenacetin by liquid–liquid partitioning in a separator. The organic phase must be chloroform to prevent significant amounts of phenacetin from extracting into the aqueous phase, but sulfuric acid, the commonly used aqueous phase, will not quantitatively extract caffeine from chloroform. The switch to phosphoric acid proved eminently successful. Under the experimental conditions used, caffeine is completely recovered in a *single* phosphoric acid extraction. Traces of phenacetin in the acid extract are effectively removed with an additional chloroform wash, thereby permitting UV determination of caffeine.

Although the natural fluorescence of codeine was previously reported (10, 11), it was thought to be too nonspecific and insensitive for clinical or biological work and its application to pharmaceutical analysis has been largely overlooked. Fluorescence was chosen in this instance because the UV spectra for codeine in some commercial samples showed interferences and because the sensitivity of the fluorescence permitted easy assay of low level codeine preparations [1 and 8 mg. ($^1/_8$ grain)]. However, at the sensitivity used, it was determined empirically that ether-saturated aqueous solvents contribute to the fluorescence of the solute. Consequently, all codeine solutions were prepared in ether-saturated sulfuric acid.

A synthetic mixture was prepared to contain the usual combination of I [*i.e.*, 227 mg. ($3^1/_2$ grains), 162 mg. ($2^1/_2$ grains), and 32 mg. ($^1/_2$ grain), of aspirin, phenacetin, and caffeine, respectively] plus 16 mg. ($^1/_4$ grain) codeine, about 16% starch, and 2% magnesium stearate. Ten sample weights, each approximating one theoretical tablet, were assayed by the proposed procedure (Table I). The average recoveries were 99.7 and 98.9% for codeine and caffeine, respectively.

The proposed procedure was compared to official methods for codeine (12) and caffeine (1) by assaying composites of four different commercial preparations (Table II). The method shows excellent agreement with the existing methods.

Table III shows individual tablet data. The samples represent five different manufacturers. The time required for analysis of five tablets was about 1.5 hr.

With minor modification, the described procedure may be adaptable to the determination of aspirin and phenacetin also. The chloroform washes from the caffeine determination contain aspirin and phenacetin. A single extraction with 5% sodium bicarbonate will remove aspirin. The chloroform solution can then be evaporated, and the phenacetin residue can be determined by UV spectroscopy after dissolution in chloroform and isooctane. Aspirin can be reextracted into chloroform after acidifying the bicarbonate solution and also determined by UV spectroscopy. Cursory experiments indicate that this approach is quite feasible, but an extensive investigation was not undertaken.

Table III—Individual Tablet Analysis for Codeine and Caffeine in Commercial Samples

Sample	Codeine, mg. Declared	Codeine, mg. Found	Caffeine, mg. Declared	Caffeine, mg. Found
A	1	0.964	32.40	31.88
		1.11		33.44
		1.06		30.65
		1.07		34.34
		1.08		33.44
B	16.20	16.07	32.40	32.72
		15.16		31.10
		15.55		32.14
		16.07		31.95
		15.55		32.66
C	16.20	15.68	32.40	31.95
		16.20		32.21
		15.55		32.40
		15.62		31.49
		15.68		32.53
D	32.40	31.04	32.40	33.70
		32.33		34.67
		32.01		29.81
		30.33		27.54
		30.97		33.44
E	32.40	30.84	32.40	30.52
		31.69		31.30
		30.91		30.07
		31.43		32.21
		32.66		31.49
F	64.80	59.94	32.40	29.09
		57.87		29.87
		57.87		29.55
		58.71		29.48
		64.35		29.68

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