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GLC Analysis of Caffeine and Codeine Phosphate in Pharmaceutical Preparations

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Abstract □ A procedure for the determination of caffeine and codeine phosphate in pharmaceutical preparations was developed. It depends upon a one-step extraction followed by GLC analysis of the concentrated extract.

Keyphrases □ Caffeine and codeine phosphate—GLC analysis in pharmaceutical products □ Codeine phosphate and caffeine—GLC analysis in pharmaceutical products □ GLC—analysis, caffeine and codeine phosphate

Caffeine and codeine phosphate are used singly or in combination with other drugs in various pharmaceutical preparations. Quality control requirements (1) have made it mandatory to determine both the caffeine and codeine phosphate content uniformity. James (2) reported a procedure for the determination of the codeine and caffeine contents of individual aspirin, phenacetin, caffeine, and codeine phosphate tablets based upon a fluorometric–UV spectroscopic assay. While accurate, this method and other spectroscopic assays (3, 4) involve time-consuming multiple extractions which are liable to inherent errors.

The reported GC methods are either not amenable to single-tablet assay, do not include codeine, or are rather involved (2, 5).

The developed GLC procedure is simple, and the sample preparation involves only two extractions per tablet. This process represents a 77% reduction in the extraction requirements when compared with the compendial method (1) with no loss in accuracy. Caffeine, codeine phosphate, and phenacetin are extracted from the tablet matrix into a common solvent, and no further separation is needed.

EXPERIMENTAL

Equipment—A gas chromatograph[1] equipped with a flame-ionization detector was used. The detector signal was fed into a 1-mv recorder[2] operated with a chart speed of 1.3 cm (0.5 in.)/min. One-microliter samples were injected with a 10-μl syringe[3].

Materials—High purity helium was the carrier gas. Purified hydrogen and compressed air were used in the detector. The stationary phase was 3%, OV-17 on Chromasorb W[4] (80–100 mesh), acid washed and silanized, packed in a 1-m × 0.64-cm o.d. glass column. All chemicals employed were spectrograde. All sample and standard materials were of NF or USP grade.

Operating Conditions—The column was operated isothermally at 240°. Both the detector and the injector port were held at 250°. The carrier gas flow rate was 28 ml/min at an inlet pressure of 40 psig. The initial electrometer range and attenuation settings were 10^2 and 32, respectively. After the elution of the solvent and the first two components, the range setting was 10 and the attenuation setting was 8 for the duration of the run.

Preparation of Standard Solutions—Two standard solutions were prepared, an internal standard and the working standard.

Internal Standard—Three hundred milligrams of nortriptyline hydrochloride was dissolved in 20 ml of 15% sodium chloride solution in a 125-ml separator. The solution was made basic by the addition of 5 ml of 2 *N* NaOH and extracted with 2 × 15 ml of chloroform. The extracts were filtered through filter paper[5] containing 5 g of anhydrous sodium sulfate, and the extracts were combined in a 50-ml volumetric flask. The filter was washed with 15 ml of chloroform, and the washings were added to the combined extracts. Chloroform was used to bring the contents to volume.

Working Standard—In a 125-ml separator, 234 mg of aspirin, 150 mg of phenacetin, 30 mg of caffeine, and 15–60 mg of codeine phosphate were combined. The exact amount of codeine phosphate varied depending upon its concentration in the tablet preparation to be analyzed. The mixture was treated in the same way as the internal standard preparation, except that the extracts and the

Table I—GLC Data

Compound	Retention Time, min	Relative Retention Time, min
Phenacetin	1.2	0.276
Caffeine	1.95	0.448
Nortriptyline	4.35	1.000
Codeine	8.60	1.970

Table II—Standard Samples

Ingredient	Mixture 1, mg	Found, mg	Mixture 2, mg	Found, mg
Aspirin	234	—	234.7	—
Phenacetin	151	—	150.8	—
Caffeine	31.6	31.0	30.6	30.3
Codeine phosphate	15.0	14.9	30.4	30.2

[1] Hewlett-Packard model 402.
[2] Hewlett-Packard model 7127-A.
[3] Hamilton No. 701.
[4] Johns-Manville Corp.
[5] Whatman No. 41.

Table III—Analysis of Caffeine and Codeine Phosphate in Prepared Aspirin, Phenacetin, Caffeine, and Codeine Phosphate Tablets

Tablet Number	Codeine Phosphate, mg		Caffeine, mg	
	Claim	Found	Claim	Found
A-1	30	33.4	30	30.0
A-2	30	28.9	30	27.3
A-3	30	32.9	30	30.7
B-1	60	60.4	30	31.9
B-2	60	60.4	30	31.9
B-3	60	60.5	30	30.9
C-1	15	15.0	30	30.5
C-2	15	16.0	30	29.7
C-3	15	14.8	30	31.3

washings were collected in a 50-ml erlenmeyer flask and evaporated to dryness on a steam bath under nitrogen flow. The dried extract was redissolved in 3 ml of the internal standard solution prior to use.

Preparation of Sample Solutions—One tablet, or one tablet weight, of aspirin, phenacetin, caffeine, and codeine phosphate was put into a 125-ml separator. Twenty milliliters of 15% sodium chloride solution was added, and the contents were shaken until the tablet disintegrated. The mixture was then treated exactly as described for the working standard beginning with: "The mixture was treated in . . . ."

Calculations—For the standard chromatogram, the ratio, R-1, of the areas of the nortriptyline/caffeine peaks and the ratio, R-2, of the areas of the nortriptyline/codeine peaks were obtained. For the sample chromatogram, the ratio, R-3, of the caffeine/nortriptyline peak areas and the ratio, R-4, of the codeine/nortriptyline peak areas were obtained. Then:

$$(R\text{-}1) \times (R\text{-}3) \times Cf = \text{mg caffeine in sample} \quad \text{(Eq. 1)}$$

where Cf is the milligrams of caffeine in the working standard, and:

$$(R\text{-}2) \times (R\text{-}4) \times Cc = \text{mg codeine phosphate in sample} \quad \text{(Eq. 2)}$$

where Cc is the milligrams of codeine phosphate in the working standard.

RESULTS AND DISCUSSION

Nortriptyline hydrochloride served as the internal standard in the compendial procedure for the analysis of codeine phosphate (1). In this study, it was equally satisfactory for the quantitative evaluation of both caffeine and codeine phosphate. Figure 1 illustrates a typical chromatogram. Area ratios were used rather than peak height ratios, since the area ratios yielded analytical data without repetitive chromatograms. As the figure shows, isothermal operation of the column at the temperature and carrier gas flow rate employed resulted in excellent resolution of the compounds. Table I presents the associated GLC data.

The accuracy of the method is evident from the data given in Table II. Each of the two simulated preparations contained the four active ingredients in amounts similar to the sample tablet preparations. Each mixture was subjected to the entire analytical procedure. Use of an integrator to measure the peak areas would enhance the accuracy further. The present error is estimated to be about 2%.

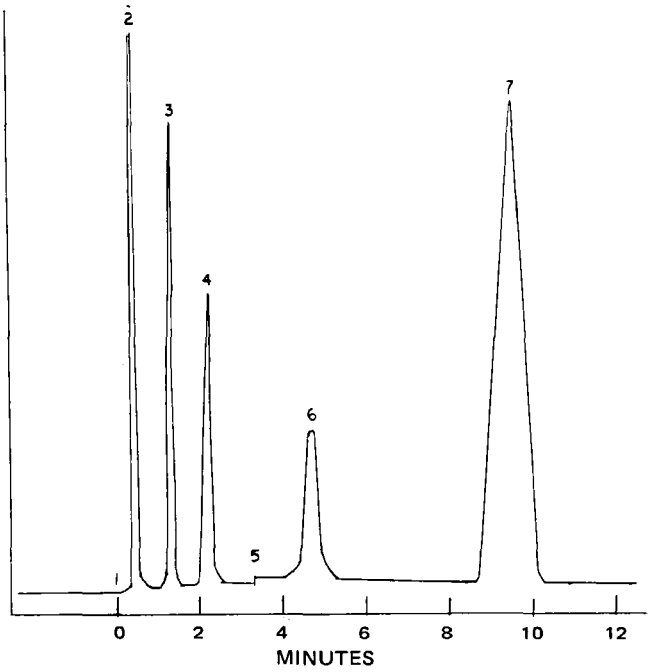

Figure 1—*Gas chromatogram of a sample extract with internal standard. Key: 1, inject; 2, solvent; 3, phenacetin; 4, caffeine; 5, attenuation change; 6, nortriptyline (internal standard); and 7, codeine.*

Application of the procedure to the analysis of tablet preparations is given in Table III. By first extracting the mixture with chloroform, then making the solution basic, and then extracting again with chloroform, all four ingredients are present in the combined extracts. In the present case, the aspirin remains in the aqueous phase as the sodium salt. Temperature programming would allow all active ingredients to be analyzed, but now only phenacetin, caffeine, and codeine phosphate can be analyzed.

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