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Gas chromatographic analysis of acetylsalicylic acid, phenacetin, caffeine, and codeine in APC and codeine tablets

EARL B. DECHENE, L. H. BOOTH AND (MISS) M. J. CAUGHEY

The control laboratories of Merck-Frost Laboratories, Montreal, Quebec, Canada

All active ingredients in APC and codeine tablets are determined by gas chromatographic procedures after separation of acetylsalicylic and phenacetin from caffeine and codeine.

Hoffman & Mitchell (1963) described a gas chromatographic method for determining in a single run the active ingredients in APC tablets. In our hands their method gave satisfactory assays of acetylsalicylic acid and phenacetin, in tablets containing acetylsalicylic acid 0·22 g, phenacetin 0·16 g, caffeine 0·016 g, codeine phosphate 0·008 to 0·032 g. It was not reliable for determining caffeine due to the high ratio of phenacetin to caffeine, and the small difference in their retention times.

Haefelfinger, Schmidli & Ritter (1964) described a gas chromatographic procedure for determining caffeine and phenacetin combined with other active ingredients in tablets. Again we encountered difficulty with this method in obtaining satisfactory assays for caffeine because of interference from phenacetin.

We established in model assays that the chromatographic system described by Haefelfinger could reliably determine caffeine and codeine alone or in combination.

The Hoffman procedure uses chloroform to extract the active ingredients from the tablets whereas the Haefelfinger method uses acetone. Neither of these solvents is suitable for extracting codeine phosphate. We investigated a number of extraction procedures, and developed a procedure that quantitatively extracted caffeine and codeine from tablets while separating them from phenacetin and acetylsalicylic acid. We were able then, to reliably determine caffeine and codeine in combination by the Haefelfinger procedure. We used a higher column temperature to decrease the retention time of codeine. We have not as yet found satisfactory "internal standards" for use in these assay procedures.

EXPERIMENTAL

Apparatus. A linear programmed temperature gas chromatograph, Perkin Elmer Model 880, equipped with a flame ionization detector was used. The detector signal was supplied to a Leeds Northrum Speedomax Model G recorder with chart speed of ½ inch/min, connected to a Perkin Elmer Printing Integrator Model 194B. The chromatograph was operated with dual columns.

Materials. Nitrogen was the carrier gas. The columns were prepared with the materials and by the procedures described by Hoffman & Mitchell (1963), and with the materials and by the procedures described by Haefelfinger & others (1964).

Method for determining aspirin and phenacetin

Two (dual columns) 6 ft × ⅛ inch o.d. stainless steel tubing filled with 2% Dow

Corning Fluid No. 200 absorbed on Haloport F-80 mesh (supplied by F & M Scientific Corp., Avondale, Penn., USA). Gas pressures: air 44 lb/inch^2, hydrogen, optimized, nitrogen 60–65 lb/inch^2; nitrogen flow: 50 ml/min; operating temperature: injection port 300°, detector 200°, column 100°–180°, programmed at 10°/in.

Procedure for sample preparation. Weigh an amount of powdered tablets to yield about 450 mg of acetylsalicylic acid and 325 mg of phenacetin. Place the powder in a 25 ml volumetric flask containing about 20 ml of chloroform AR. Loosely stopper the flask, and shake periodically while maintaining it at 50° for 15 to 20 min. Cool and dilute to volume with chloroform AR. Filter the solution, and discard the first 5 ml of filtrate. Use 2·0 μl of the clear filtrate for each assay. Determine on at least 2 injections the areas of the peaks with retention times corresponding to those shown by the standard preparation.

Procedure for standard preparation. Add 1·80 g of acetylsalicylic acid B.P. and 1·30 g of phenacetin B.P. in a 100 ml volumetric flask. Add 80 ml of chloroform AR, shake until solids dissolve and make to volume with the same solvent. Use 2·0 μl of this solution for each assay. On at least 2 assays, determine the peak areas showing retention times at 4·1 min for acetylsalicylic acid and at 9·6 min for phenacetin.

Method for determining caffeine and codeine phosphate

Two (dual columns) 6 ft × $\frac{1}{8}$ inch o.d. stainless steel tubing filled with 10% SE-30 (Chromatographic Specialties, Brookville, Ont.) absorbed on Acid Washed Chromasorb W (F. & M. Scientific Div., Avondale, Penn.). Gas pressures: air 44 lb/inch^2, hydrogen, optimized, nitrogen 60–65 lb/inch^2; nitrogen flow: 50 ml/min; operating temperature: injection port 300°, detector 270°, column 195° for 6 min followed by temperature programming at 48°/min to 260°.

Procedure for sample preparation. Place an amount of powdered tablets to yield about 100 mg of caffeine and 50 mg of codeine phosphate in a 50 ml stoppered centrifuge tube. Add 30 ml of 1% aqueous ammonium hydroxide, stopper the tube, shake for 30 min and centrifuge. Transfer 25·0 ml of the clear supernatant to a beaker, and bring the solution to pH 10 by the addition of concentrated ammonia. Transfer the solution quantitatively to a 125 ml separatory funnel using water to wash, and extract with 4 × 10 ml of chloroform. Combine the chloroform extracts in a small beaker. To the aqueous phase add 3·5 ml of 40% aqueous sodium hydroxide, mix and extract with 2 × 10 ml of chloroform AR. Add these chloroform extracts to those obtained previously, and evaporate the combined chloroform extracts to dryness on a steam bath. Transfer the residue with chloroform AR to a 5 ml volumetric flask, and make to volume with the same solvent. Use 2·0 μl of this solution for each assay, and determine on at least 2 assays the average of the peak areas with retention times corresponding to the standard preparation.

Procedure for standard preparation. Place 162·0 mg of anhydrous caffeine B.P. and 56·0 mg of anhydrous codeine alkaloid B.P.C. in a 10 ml volumetric flask. Dissolve the solids in chloroform AR, and make to volume with the same solvent. Use 2·0 μl of this solution for each assay, and determine on at least 2 assays the average of the peak areas showing retention times of 5 min for caffeine and 10·5 min for codeine.

Table 1. *Results from replicate determinations of standard solutions*

	Recovery (%)			
Ingredient	Aspirin	Phenacetin	Caffeine	Codeine phosphate
High value	102·05	102·62	103·37	106·03
Low value	98·10	97·37	95·40	96·08
Average	100·10	99·99	100·18	100·92
s.d.	±1·46	±1·81	±2·44	±4·05
Number replicates	11	11	18	18

Table 2. *Results of applying the assay procedure to tablets of APC with codeine taken from the same batch*

	Recovery (%)			
Ingredient assay	Aspirin	Phenacetin	Caffeine	Codeine phosphate
Average of 10 assays ..	98·30	100·77	100 00	102·88
High value	103·30	106·20	105·04	109·60
Low value	93·76	95·46	97·92	94·06
s.d.	±2·77	±3·26	±1·16	±4·83
B.P. or B.P.C. limit ..	95–105	95–105	93–107	92·5–107·5
Number of assays outside limits	1	1	0	2

RESULTS AND DISCUSSION

The results obtained in replicate determinations of "standard" solutions are shown in Table 1.

The results obtained by the assay procedures described above in replicate determinations on tablets of APC with codeine taken from the same batch, are shown in Table 2.

The standard deviation values for the replicate assays of the "standard" solutions and of the tablets indicate that no significant increase is contributed by the performance of the assay procedures.

The number of assay values outside the "official limits" for the individual components is considered acceptable relative to the rapidity and accuracy of the assay procedures for routine application.

The gas chromatographic procedures described have been used satisfactorily for the routine analysis of over 200 batches of commercially prepared APC and codeine tablets. An experienced technician can conveniently assay 10 samples of tablets for all active ingredients in 8 h.

REFERENCES

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