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Chromatographic Analysis of Chlorpheniramine, Pyrilamine, and Methapyrilene in Combination

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Abstract □ A partition chromatographic method is presented for the separation and quantitation of chlorpheniramine, methapyrilene, and pyrilamine in combination. A solution of *p*-toluenesulfonic acid and potassium chloride on a column of diatomaceous earth is used to effect the separation. The isolated chlorpheniramine is determined by UV spectrophotometry. The methapyrilene and pyrilamine are separated and determined by GLC.

Keyphrases □ Chlorpheniramine, pyrilamine, methapyrilene combination—analysis □ Column chromatography—separation □ UV spectrophotometry—analysis □ GLC—analysis

Pharmaceutical preparations containing chlorpheniramine, methapyrilene, and pyrilamine in combination have been on the market for a number of years. However, the literature does not cite a method for the analysis of this combination. GLC has been used successfully to separate and analyze a large number of antihistamines (1–4). However, with these chromatographic procedures the separation of methapyrilene and chlorpheniramine is not complete enough to permit their analysis.

The separation and analysis of pharmaceutical amines based on ion-pair partition chromatography has been reported by Levine *et al.* (5–7). They have shown that *p*-toluenesulfonic acid (tosic acid) is effective in extracting a wide variety of these amines.

In the present study, a tosic acid-potassium chloride partition column is used to separate chlorpheniramine from methapyrilene and pyrilamine. Chlorpheniramine is determined by UV spectrophotometry; methapyrilene and pyrilamine are determined by GLC.

EXPERIMENTAL

GLC Column Preparation—The packing support was prepared and conditioned in the following manner (4): 200 mg. of cyclohexanedimethanol succinate[1] and 2.0 g. of methylphenyl silicone[2] were weighed into a 600-ml. beaker and dissolved in 350 ml. of benzene–toluene (1:2) by heating on the steam bath with mixing. To this solution, 20 g. of 80–100-mesh diatomaceous earth[3] was added. The solvent was removed by evaporation on a steam bath with frequent stirring. The packing material was slowly added to a 1.8-m. (6-ft.) × 4-mm. i.d. glass coiled column, using vacuum and tapping. The column was conditioned overnight at 250° with a 50 ml./min. nitrogen flow.

Apparatus—A gas chromatograph[4] equipped with a flame ionization detector and a 1-mv. recorder was used. The operating conditions were: column temperature, 250°; inlet temperature, 280°; detector temperature, 250°; voltage, 250; carrier gas, nitrogen at 100 ml./min.; sensitivity, 1×10^{-9} amp. full scale.

A recording UV spectrophotometer was used with 1-cm. silica cells.

Reagents—*Tosic Acid-KCl Solution*—Dissolve 10 g. of *p*-toluenesulfonic acid and 11.2 g. of KCl in 35 ml. of distilled water with heating. Cool, dilute to 50 ml., and mix.

Diatomaceous Earth[5]—Acid wash as outlined by USP XVII (8).

Ether and Chloroform—Reagent Grade—Wash twice with equal volumes of water.

Standard Solutions—Pyrilamine maleate NF reference standard and methapyrilene HCl,[6] 1 mg./ml. in methanol; chlorpheniramine maleate USP reference standard, 0.02 mg./ml. in 0.1 *N* H_2SO_4.

Column Preparation—*Column I*—Mix 2.0 g. of diatomaceous earth with 1.0 ml. of 1 *N* NaOH, transfer to a chromatographic column[7] containing a pad of glass wool, and tamp. Weigh a portion of finely ground sample equivalent to 5 mg. of pyrilamine maleate into a 100-ml. beaker. Add 2.0 ml. of 1 *N* NaOH and 3.0 g. of diatomaceous earth. Mix until uniform; quantitatively transfer to column and tamp. Cover with a pad of glass wool.

Column II—Mix 3.0 g. of diatomaceous earth and 2.0 ml. of 1 *M* $NaHCO_3$, transfer to a second column containing a pad of glass

[1] HI-EFF-8BP, Applied Science Laboratories, Inc., State College, PA 16801
[2] SE-52, Analabs, Inc., Hamden, Conn.
[3] Gas Chrom Q, Applied Science Laboratories, Inc., State College, PA 16801
[4] Packard model 7621S, Packard Instrument Co., Downers Grove, IL 60515
[5] Celite 545, Johns-Manville Corp., New York, N. Y.
[6] K & K Laboratories, Plainview, N. Y.
[7] Cat. No. 420300, 250 mm. × 22 mm. i.d., Kontes Glass Co., Vineland, N. J.

Table I—Analysis of Chlorpheniramine Maleate, Methapyrilene HCl, and Pyrilamine Maleate in Commercial and Synthetic Drug Preparations

Sample	Chlorpheniramine Maleate, mg./tablet			Methapyrilene HCl, mg./tablet			Pyrilamine Maleate, mg./tablet		
	Declared	Found	%	Declared	Found	%	Declared	Found	%
1[a]	2	1.98	99	12	12.0	100	12	12.7	106
2[a]	1	0.95	95	6	6.07	101	6	5.71	95
3	1.25	1.22	98	6	6.33	105	6	6.10	102
4[b]	0.65	0.68	105	—	—	—	4.38	4.28	98
5	1	0.98	98	7.5	7.83	104	10	10.3	103
6[c]	1	1.03	103	7.5	7.75	103	10	10.5	105
Synthetic 1	1.4	1.44	103	6	6.04	101	6	5.97	99
Synthetic 2	0.99	0.95	96	7.56	7.54	100	9.97	10.1	101

[a] Sample also contained, by label declaration, hesperidin, ascorbic acid, caffeine, phenylpropanolamine HCl, salicylamide, and aluminum aspirin. [b] Sample also contained, by label declaration, carbetapentane tannate, glyceryl guaiacolate, salicylamide, ephedrine sulfate, atropine sulfate, and caffeine. [c] Sample also contained, by label declaration, calcium lactate and phenylpropanolamine HCl.

wool, and tamp. Pipet 3.0 ml. of the tosic acid-KCl solution into a beaker containing 4.0 g. of diatomaceous earth. Mix and transfer to the column in two equal portions. Tamp first portion before adding the second portion. Cover with a pad of glass wool.

Procedure—Mount Column I over Column II, and pass 100 ml. of ether through the columns. Discard Column I. Wash Column II with two 10-ml. portions of ether, discarding the eluate. Elute methapyrilene and pyrilamine from Column II with 120–125 ml. of chloroform, collecting the eluate in a 250-ml. beaker. Evaporate to approximately 1 ml. on a steam bath, using a small current of air. (Do not evaporate to dryness.) Quantitatively transfer to a 5-ml. volumetric flask with methanol. Dilute to volume and mix (Sample Solution 1). Elute chlorpheniramine from Column II with 120 ml. of 1% acetic acid in chloroform into a 250-ml. glass-stoppered conical flask. Evaporate just to dryness on a steam bath, using a current of air. Dissolve residue in a known volume of 0.1 *N* H_2SO_4 to give an approximate concentration of 0.02 mg./ml. (Sample Solution 2).

To quantitatively determine the methapyrilene and pyrilamine in Sample Solution 1, inject 5–6 μl. into the gas chromatograph (4). Compare the peak heights to those obtained with the respective standard solutions.

For chlorpheniramine, record the UV spectrum of Sample Solution 2 from 350–240 mμ, using 0.1 *N* H_2SO_4 as the reference. Determine the absorbance at 263 mμ and calculate by comparison to a standard.

If the UV spectrum of Sample Solution 2 shows interference, use the following clean-up procedure.

Pipet 10 ml. of the sample solution into a 125-ml. separatory funnel, make the solution alkaline with 1 *M* $NaHCO_3$, and extract with five 20-ml. portions of chloroform. Transfer each chloroform phase to a second separatory funnel and shake with 25 ml. of 1 *M* $NaHCO_3$. Combine the chloroform phases in a third separatory funnel containing 10.0 ml. of 0.1 *N* H_2SO_4 and shake vigorously for 2 min. Determine the UV absorbance of the aqueous layer as above.

RESULTS AND DISCUSSION

It was found that increasing the concentration of tosic acid on the column improved the separation of chlorpheniramine from methapyrilene and pyrilamine when chloroform was used as the eluting solvent. A 1 *M* tosic acid column gave approximately 95% separation. However, the higher concentrations produced excessive tailing, which prevented better separation of the antihistamines. The addition of KCl reduced this tailing and increased the retention of chlorpheniramine on the column, resulting in quantitative separation from methapyrilene and pyrilamine. The mechanism involved in the effect of tosic acid and KCl concentration on the ion-pair is currently being investigated.

No more than 6 mg. of methapyrilene HCl or pyrilamine should be added to the column; with larger amounts the elution bands become too broad to give adequate separation from chlorpheniramine. Chloroform alone eluted chlorpheniramine, but a greater elution volume was required than with 1% acetic acid in chloroform.

In the chlorpheniramine assay, an absorbance at 310 mμ is due to a trace of methapyrilene which is not removed by the clean-up procedure. However, this absorbance will give no interference if it does not exceed 0.050.

The results of the analysis of six commercial samples and two synthetic samples are listed in Table I. Two of the commercial samples contained only the antihistamines; the other samples contained additional ingredients. The synthetic samples were prepared by adding starch, calcium phosphate, talc, and magnesium stearate to the ingredients specified on the label of Sample 2 (Synthetic 1) and Sample 6 (Synthetic 2). Recoveries ranged from 95–106% of declared for the commercial drug preparations and from 96–103% for the synthetic preparations.

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