Gas Chromatographic Analysis of Amine Mixtures in Drug Formulations

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Abstract □ By employing a derivatization scheme, phenylpropanolamine hydrochloride, phenylephrine hydrochloride, phenyltoloxamine citrate, and chlorpheniramine maleate can be separated by means of a single injection in a suitable flame-ionization gas-liquid chromatograph. Formulated mixtures of the same components can be analyzed quantitatively in the same way. Precision at the 95 percent confidence level for the four compounds is 4.4 to 7.9 percent. Accuracy with respect to label claim is good.

Keyphrases □ Amine mixtures in tablets, syrups—analysis □ GLC—analysis □ Tribenzylamine—internal standard, amine analysis

Phenylpropanolamine, phenylephrine, phenyltoloxamine, and chlorpheniramine have been chromatographed singly and in certain combinations, but the simultaneous quantitative analysis of all four by GLC has not been reported.

Brochmann-Hanssen and Svendsen (1) showed that phenylpropanolamine can be chromatographed on silicone rubber[1] as the free base or as the acetone condensation product. Derivatization required 5 hr. They found phenolic amines such as phenylephrine difficult to handle because of adsorption effects. MacDonald and Pflaum (2), Kazyak and Knoblock (3), and Fontan *et al.* (4) successfully separated antihistamines by gas chromatography, although their reported work was essentially qualitative. Celeste and Polito (5) were able to determine mixtures of antihistamines in commercial tablet formulations which also included phenylpropanolamine and/or phenylephrine. Chromatograms of the latter two were not shown. Phenylephrine was presumably not measured, because no data were reported and no reference was made to its quantitation. In a paper concerned with the gas chromatography of biologically important amines, Capella and Horning (6) reported the elution of the condensation product of phenylephrine with acetone and with cyclobutanone.

The analysis of all four components by combined liquid chromatography and spectrometry was published by Smith (7). In the authors' case, the ease and speed of the gas chromatographic method made this technique more desirable. The problem of preparing suitable derivatives was solved by silylating the hydrochlorides or the trifluoroacetate salts.

EXPERIMENTAL

Equipment—The chromatographic system[2] with a flame-ionization detector, was fitted with a 2-m. glass U-tube (4 mm. i.d.) packed with 0.1% silicone oil (DC-710) on 60-80 mesh, dimethyldichlorosilane-treated glass beads.[3] The column was preconditioned 16 hr. at 200° with helium flow at 35 ml./min. Injector and detector were held at 300 and 260°, respectively; helium, hydrogen, and air flow rates were 80, 80, and 450 ml./min., respectively.

Reagents—Bistrimethylsilylacetamide (BSA) was used.[4] All other reagents were certified ACS grade. Isopropyl alcohol containing 4.5 mg. HCl/ml. was prepared by bubbling the dry gas through the alcohol until the appropriate weight change was recorded.

Preparation of Standards—Phenylpropanolamine·HCl, phenylephrine·HCl, phenyltoloxamine dihydrogen citrate, and chlorpheniramine maleate were dissolved in 100 ml. of water to give final concentrations of about 3.37, 0.84, 1.20, and 0.42 mg./ml., respectively. Tribenzylamine (internal standard) was dissolved in pyridine, previously dried over molecular sieves, to give a concentration of 2 mg./ml.

Standardization—Shake 15 ml. of standard solution with 4.5 g. of Na_2CO_3; add 4.0 g. each of NaCl and Na_2SO_4 and shake again. Extract three times with 15-ml. portions of isopropyl alcohol, and dilute the combined extracts to 100 ml. with more alcohol. Discard the aqueous layer. Centrifuge the alcohol to obtain a clear solution, and pipet a 5.00-ml. aliquot into a 7.5-ml. serum vial. Add 0.16 ml. of alcoholic HCl to combine completely with the amines, evaporate to dryness under a gentle stream of nitrogen, and redissolve the residue in 0.4 ml. of internal standard solution. Add 0.1 ml. BSA, and stir 20 min. at room temperature. Inject 2.0 μl. into the chromatograph, and program from 100–200°C at 10°/min.

Sample Preparation for Tablets[5]—Dissolve four tablets containing about 40 mg. of phenylpropanolamine hydrochloride, 10 mg. of phenylephrine hydrochloride, 15 mg. of phenyltoloxamine dihydrogen citrate, and 5 mg. of chlorpheniramine maleate in 50 ml. of distilled water by heating in a water bath at 100°. Shake the solution mechanically until cool, centrifuge, and treat a 15.00-ml. aliquot exactly as described under Standardization.

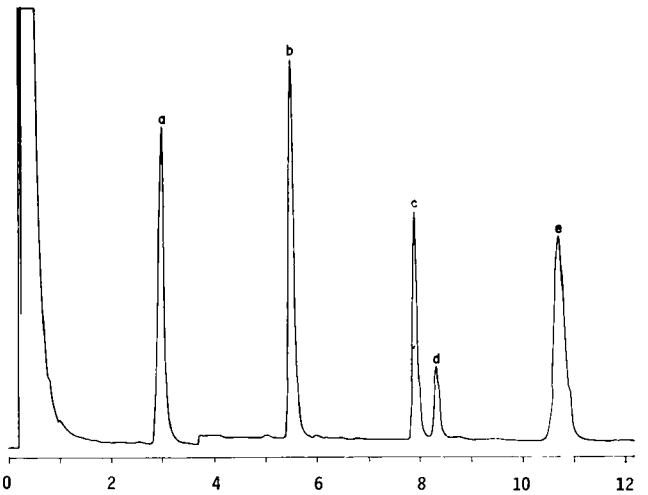

Figure 1—*Representative chromatogram, trifluoroacetic acid procedure. Key:* a, *phenylpropanolamine;* b, *phenylephrine;* c, *phenyltoloxamine;* d, *chlorpheniramine;* e, *tribenzylamine.*

[1] SE-30.
[2] Microtek model 220.
[3] Corning Code 0202.
[4] Applied Science Co.
[5] Directions given are for Naldecon, trade-name of Bristol Laboratories antihistaminic, decongestant tablets and syrup.

Table I—Four Active Ingredients Determined in the Tablet Preparation of One Lot

	mg./tablet			
	Phenyl-propanol-amine	Phenyl-ephrine	Phenyl-tolox-amine	Chlor-phenir-amine
Extract 1	41.1	9.6	13.9	5.0
	39.0	9.7	13.7	4.6
	38.9	9.6	13.7	4.7
Extract 2	39.6	9.9	13.2	5.0
	39.4	10.2	14.0	4.9
	41.0	10.1	14.0	5.1
Label claim	40	10	15	5
SD, % (95% confidence)	5.0	4.9	4.4	7.9
Deviation from label claim, %	−0.5	−1.5	−8.3	−2

Sample Preparation for Syrup—Dilute exactly 10 ml. of a syrup preparation to 15 ml. with distilled water, and shake with 4.5 g. of Na_2CO_3. Treat the sample as described under *Standardization*, except that 10 μl. of trifluoroacetic acid is added to the sample and standard aliquots in place of the 0.16 ml. of HCl solution. (The trifluoroacetic acid was later found to work equally well for the tablet preparations.) Inject a 2.7-μl. aliquot for the syrup sample only.

RESULTS AND DISCUSSION

A typical chromatogram is shown in Fig. 1. All peaks are well-resolved and symmetrical; adsorption effects appear to be absent.

Chromatographic response factors for replication of individual components in a standard solution were run at concentrations equivalent to 70 and 105% of expected sample levels using the trifluoroacetic acid procedure. Standard deviations at the 95% confidence level ranging between 4.3% for chlorpheniramine to 6.5% for phenylpropanolamine indicated good precision and linearity of response for the analytical procedure.

Table II—Duplicate Determinations on Three Syrups and One Tablet Lot TFA Addition Method

	Phenyl-propanol-amine	Phenyl-ephrine	Phenyl-tolox-amine	Chlor-phenir-amine
Syrup A (1)	20.7	5.3	7.8	2.7
(2)	19.9	5.2	7.5	2.6
Syrup B (1)	20.3	5.3	7.0	2.4
(2)	19.6	5.1	7.5	2.6
Syrup C (1)	20.0	4.9	7.7	2.6
(2)	20.6	5.2	7.9	2.7
Label claim	20	5	7.5	2.5
Relative *SD* of syrups, % (95% confidence)	4.7	5.9	6.3	7.6
Deviation from label claim, %	1.0	3.4	0.9	4.0
Tablet A (1)	39.6	10.0	13.6	5.0
(2)	39.5	9.6	13.7	4.8
Label claim	40	10	15	5

Table III—Other Commercial Preparations, Duplicate Determinations

Product	Phenyl-propanolamine		Phenylephrine		Chlor-pheniramine	
	Measured	Claimed	Measured	Claimed	Measured	Claimed
A	—	—	9.3	10	1.9	2.0
	—	—	9.2	—	2.0	—
B	13.8	15	14.1	15	—	—
	13.3	—	14.9	—	—	—
C	45.7	50	—	—	7.7	8
	49.2	—	—	—	8.1	—

Table I gives data for the four components of the antihistaminic, decongestant tablets, including results from duplicate extractions and triplicate evaporation residues. Precision is 4.4 to 7.9% over all replications at the 95% confidence level. Accuracy with respect to label claim ranges from −0.5 to −8.3%. Hydrochloric acid was the salt-forming agent in these cases.

Additional results for one tablet lot and data for three syrups, all prepared *via* the trifluoroacetic acid route, are shown in Table II. Precision for the three sets of syrup duplicates is 4.7 to 7.6% (95% confidence), and the accuracy with respect to label claim is 0.9 to 4.0%.

Table III gives data on other commercial tablet and capsule preparations which contain combinations of the same amines. Trifluoroacetic acid was used in each case. It is evident that the same method applies equally well to these products.

The use of HCl to form salts prior to the silylation step is satisfactory for tablets and capsules. When applied to syrups, the same procedure led to incomplete silylation and extraneous chromatographic peaks. The proportion of HCl to active component is also critical. When more than 110% of theoretical is used, phenylephrine degrades; with less than 90%, phenylpropanolamine degrades. The substitution by trifluoroacetic acid not only solved these problems, but conferred greater stability on the evaporation residues. The general procedure now in use in our laboratory employs trifluoroacetic acid for all samples.

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