0.5% chrysotile. The result from the assay in Fig. 1 indicates 1.1 mg chrysotile in a 200-mg sample, or 0.55%. All tests for precision of the assay were done on spiked samples. A limited number of determinations on a sample containing 0.5% chrysotile indicated a standard deviation of ±0.06%.

To eliminate the possibility that a peculiarity of the chrysotile sample used was responsible for the dye adsorption, another sample was obtained[5]. This sample gave substantially the same adsorption behavior as the first chrysotile sample.

SUMMARY

A determination of chrysotile impurity in talc USP was developed. The assay is useful at least at the level of 0.5% chrysotile. An internal standard method is used which eliminates the need for a standard calibration curve. The assay depends on the adsorptive property of the chrysotile for a sulfonphthalein dye.

[5] Geology Department, Indiana University.

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Selective Determination of Phenylpropanolamine Hydrochloride in Pharmaceutical Dosage Forms by Reaction with Ninhydrin

D. BURKE, V. S. VENTURELLA ˣ, and B. Z. SENKOWSKI

Abstract □ A colorimetric method for the determination of phenylpropanolamine hydrochloride, based on the reaction with ninhydrin, was applied to various forms of pharmaceutical products. The method is applicable to pharmaceutical products that do not contain other primary or secondary amines. The determination is dependent upon the combined hydroxyl and primary amino moieties. It is postulated that phenylpropanolamine hydrochloride reacts with ninhydrin under the experimental conditions by a mechanism similar to that for amino acids. A comparison of the molar absorptivities of phenylpropanolamine hydrochloride with those of amino acids, such as glycine and phenylalanine, clearly demonstrates the similarity of the reaction mechanism to the classic ninhydrin mechanism.

Keyphrases □ Phenylpropanolamine hydrochloride formulations—colorimetric analysis, ninhydrin reagent □ Colorimetry—analysis, phenylpropanolamine hydrochloride in mixed formulations □ Ninhydrin reagent—used to determine phenylpropanolamine hydrochloride in mixed formulations

In the past few years, phenylpropanolamine hydrochloride has been used widely in many different pharmaceutical preparations as an adrenergic agent. This popularity can be attributed to the fact that it produces vascular and bronchial effects with little, if any, central effects. It is used in asthma and as a nasal decongestant, both locally and orally (1). Phenylpropanolamine hydrochloride can be found in many formulations including tablets, capsules, syrups, elixirs, suspensions, nasal jellies, and nasal solutions.

Recently, because of the increased use of phenylpropanolamine hydrochloride in complex mixtures, it has become necessary to develop a specific and rapid method for its determination. The method described in this report is dependent upon a colorimetric response to concentrations and, therefore, does not require prior separation from other active ingredients present in many allergenic and cold preparations. By contrast, many reported methods require long and tedious separations prior to quantitative determination.

A review of the literature (2–14) showed that a great variety of methods had been used to determine the phenylpropanolamine hydrochloride content in pharmaceutical formulations quantitatively. The methods used were diatomaceous earth[1] separation followed by spectrophotometry, GLC, extraction and titration, anion-exchange chromatography followed by titration with hydrochloric acid, and periodate oxidation to benzaldehyde.

EXPERIMENTAL[2]

Reagents—*Citrate Buffer, pH 5.0*—Dissolve 21.008 g of citric acid USP in 200 ml of water. Add 200 ml of 1.0 *N* sodium hydroxide solution and dilute to 500 ml with distilled water. Adjust the pH with sodium hydroxide or hydrochloric acid, if necessary.

Potassium Cyanide—Accurately weigh 0.1628 g of potassium cyanide, transfer to a 250-ml volumetric flask, and dilute to volume with water.

[1] Celite.

[2] The sample preparation given for layered tablets, capsules, and combination tablets is typical of that required for most dosage forms.

Table I—Constituents of Color Development Vessels

Added Solution	Volume Added, ml		
	Blank	Sample	Standard
Working sample solution	—	1.0	—
Working standard solution	—	—	1.0
Citrate buffer	0.5	0.5	0.5
Ninhydrin color reagent	1.2	1.2	1.2
Water	1.0	—	—

Table II—Analysis of a Layered Tablet Formulation[a] with Aspirin

Sample	Assay, mg/Tablet	Percent Claim
1	6.26	100.3
2	6.40	102.3
3	6.27	100.3
4	6.36	101.8
5	6.45	103.1

Mean 6.35 mg/tablet
$\sigma \pm 0.08$ mg/tablet
(ts:95%) $\pm$ 0.22 mg/tablet

[a] Phenylpropanolamine hydrochloride, 6.25 mg/tablet, present as an adsorbate.

Ethanol—Use 60% (v/v) ethanol.

Potassium Cyanide-2-Methoxyethanol Solution—Dilute 1 ml of the potassium cyanide reagent to 25 ml with 2-methoxyethanol[3].

2-Methoxyethanol-Ninhydrin Solution (5.0%)—Accurately weigh 1.25 g of ninhydrin (triketohydrindene hydrate), transfer to a 25-ml volumetric flask, and dilute to volume with 2-methoxyethanol.

Potassium Cyanide-Ninhydrin-2-Methoxyethanol Solution—Mix 5 ml of the 2-methoxyethanol-ninhydrin reagent with 25 ml of the potassium cyanide-2-methoxyethanol reagent.

Standard Reference Solution—Weigh accurately approximately 188 mg of phenylpropanolamine hydrochloride and transfer to a 1-liter flask. Dissolve in, and dilute to volume with, distilled water. Pipet 25 ml of this solution into a 50-ml volumetric flask and add 20 ml of citrate buffer. Adjust to pH 5.0 with sodium hydroxide or hydrochloric acid and dilute to volume with pH 5.0 buffer.

Sample Solution for Layered Phenylpropanolamine Hydrochloride (Adsorbate) and Aspirin Tablets—Weigh accurately the equivalent of approximately 37.5 mg of phenylpropanolamine hydrochloride from a ground, representative tablet mass. Transfer the powder to a blender[4] and add 100 ml of 0.2 N sodium hydroxide. Blend for 2.5 min at a speed suitable to prevent splashing. Let stand for 5 min and repeat the procedure.

Transfer the blender contents quantitatively to a 200-ml volumetric flask with the aid of 0.2 N sodium hydroxide. Dilute to volume with the same reagent. Centrifuge approximately 80 ml of the mixture for 5 min at 6000 rpm. Filter the centrifugate through fluted filter paper. Transfer exactly 25 ml of the filtrate to a 50-ml beaker, and titrate with 0.4 N hydrochloric acid to pH 5.0 $\pm$ 0.2, measured with a suitable pH meter. To a second 25 ml of solution contained in a 50-ml volumetric flask, add exactly the same volume of titrant and dilute to volume with pH 5.0 buffer. Use this prepared solution for the final colorimetric reaction.

Sample Solution for Phenylpropanolamine Hydrochloride Capsules and for Tablets Containing Phenylpropanolamine Hydrochloride, Atropine Sulfate, Chlorpheniramine Maleate, and Acetaminophen—Determine the average weight of 20 tablets or capsules and reduce them to a fine powder. Weigh accurately a portion of ground sample equivalent to about 37.5 mg of phenylpropanolamine hydrochloride, and transfer to a 200-ml volumetric flask. Add 100 ml of 0.10 N hydrochloric acid to the flask and shake mechanically for 30 min. Dilute to volume with 0.10 N hydrochloric acid and filter. Pipet 25 ml of the filtrate into a 50-ml volumetric flask. Add 20 ml of pH 5.0 buffer. Adjust the solutions to pH 5.0 $\pm$ 0.2 before diluting to volume.

[3] Methyl Cellosolve.
[4] Waring.

Table III—Data Obtained for a Combination Tablet[a]

Sample	Assay, mg/Tablet	Percent Claim
1	24.87	99.7
2	24.82	99.5
3	25.30	101.2
4	25.90	103.6
5	25.90	103.6

Mean 25.36 mg/tablet
$\sigma \pm 0.53$ mg/tablet
(ts:95%) $\pm$ 1.47 mg/tablet

[a] Composition: phenylpropanolamine hydrochloride, 25 mg; atropine sulfate, 0.08 mg; chlorpheniramine maleate, 1.33 mg; and acetaminophen, 300 mg.

Table IV—Assay of Phenylpropanolamine Hydrochloride in a Sulfonamide Preparation and a Timed-Release Formula

Sample	Active Formula	Phenylpropanolamine Hydrochloride Found	Percent Claim
1	Trisulfapyrimidines USP (500 mg), acetaminophen (120 mg), and phenylpropanolamine hydrochloride (12.5 mg)	12.9 mg/5 ml[a]	103.2
2[b]	Chlorpheniramine maleate (8 mg), phenylpropanolamine hydrochloride (50 mg), and isopropamide iodide (2.5 mg)	50.55 mg/capsule[c]	101.1

[a] Results of duplicate assays. [b] Timed-release formula. [c] Results of triplicate assays.

Colorimetry—Into separate test tubes of 1.8 cm i.d. (about 30 ml capacity), add, by pipet, sample, standard, buffer, and color reagent as given in Table I.

Transfer the tubes to a 1-liter beaker containing 750 ml of vigorously boiling water. Add several glass beads to each tube and allow the color to develop for 30 min. Remove the tubes from the bath and cool immediately under cold running water for 5 min. Add 10 ml of 60% (v/v) ethanol. Transfer the contents to individual 25-ml volumetric flasks and dilute to volume with the same solvent. Filter the solutions and determine the absorbance of the clear filtrates at 570 nm *versus* the blank in the reference cell.

The content of phenylpropanolamine hydrochloride per capsule or tablet can be found using Eq. 1:

$$\text{phenylpropanolamine hydrochloride (milligrams)} = \frac{A_u}{A_s} \times \frac{W_s \times W_0}{W \times 5} \quad \text{(Eq. 1)}$$

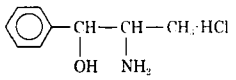
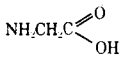
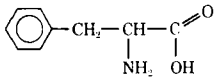
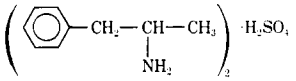
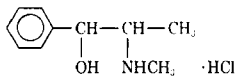
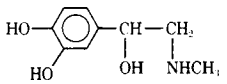
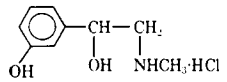
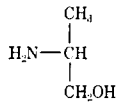
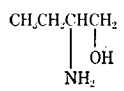
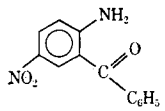
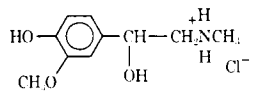
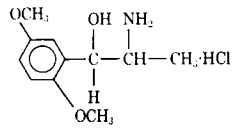
where:

A_u = absorbance of sample solution
A_s = absorbance of standard reference solution
W_s = weight of standard phenylpropanolamine hydrochloride (milligrams)
W = weight of sample (milligrams)
W_0 = average capsule or tablet weight (milligrams)
5 = dilution factor

RESULTS

Analyses of tablet formulations containing phenylpropanolamine hydrochloride adsorbate and aspirin (layered) clearly demon-

Table V—Molar Absorptivity Values for Phenylpropanolamine Hydrochloride, Related Amino Acids, and Amines in 60% Ethanol

Compound	Structure	Concentration, moles/liter × 10^5	A_{570}	ε
Phenylpropanolamine hydrochloride		2.00 2.61	0.387 0.516	19,510[a]
Glycine		2.49	0.492	19,759
Phenylalanine		2.44 2.16	0.425 0.400	17,986[a]
Amphetamine sulfate		1.94	Negligible	—
Ephedrine hydrochloride		2.16 6.53	0.106 0.311	4,628[a]
Epinephrine		8.56	0.179	2,009
Phenylephrine hydrochloride		7.80 9.04	0.200 0.220	2,552[a]
2-Aminopropanol		7.00	0.327	4,671
3-Aminopropanol		6.74	0.592	8,776
1-Amino-2,3-propanediol		2.16 1.93	0.302 0.262	13,778[a]
2-Aminobutanol		14.04 7.00	0.822 0.396	5,751[a]
2-Amino-5-nitrobenzophenone		2.06	Negligible	—
4-Hydroxy-3-methoxy-α-(methylaminomethyl)-benzyl alcohol hydrochloride		3.10 2.70 9.48	0.082 0.067 0.261	2,627[a]
Methoxamine hydrochloride		2.01 1.98	0.406 0.403	20,151[a]

[a] Average of all determinations.

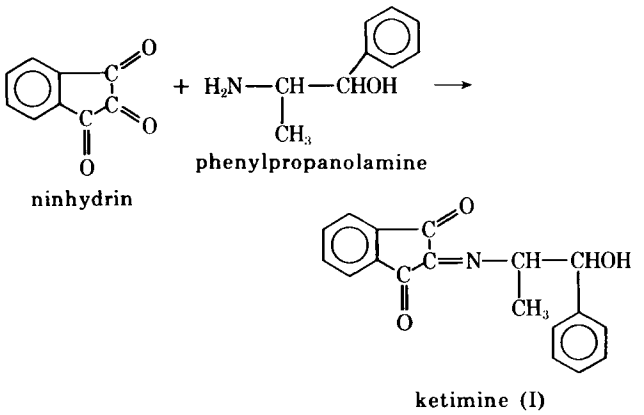
strated that aspirin does not interfere with the procedure (Table II). With formulations of this type, the adsorbate first is released with alkali before the ninhydrin reaction is performed.

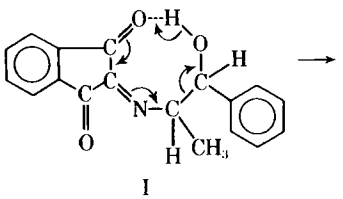
Phenylpropanolamine hydrochloride analyzed in capsule formulations contained the drug as the sole active agent. It was found that capsule diluents do not interfere with the ninhydrin reaction. The usual tablet and capsule diluents, including lactose, talc, starch, and magnesium stearate, did not contribute to the absorbance.

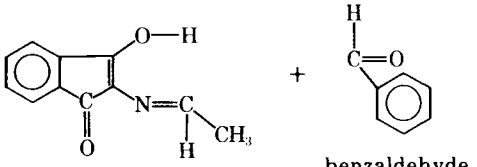
Additionally, phenylpropanolamine hydrochloride was analyzed in a tablet formulation containing acetaminophen (amide), atropine sulfate, and chlorpheniramine maleate (tertiary amine) (Table III). No interference was noted from either the amide or the tertiary amines present. As noted, the preparations are quite variable, which confirms the relative specificity of the ninhydrin reaction as given in this report. Data collected for the assay of other available formulations appear in Table IV.

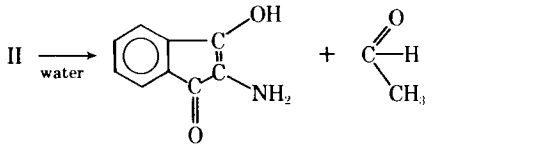
DISCUSSION

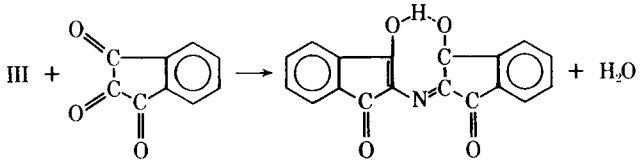
The reaction of several representative types of amino alcohols with ninhydrin was investigated in which the effect of the hy-



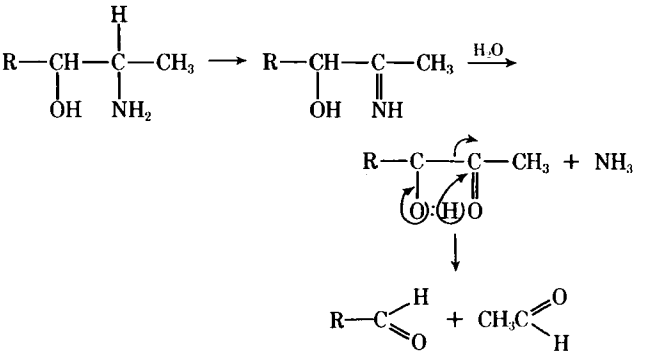








Scheme I

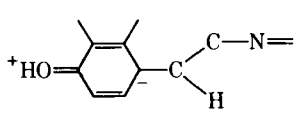


Scheme II

droxyl and the phenyl moieties on the molar absorptivity was examined. The similarity between the reaction of phenylpropanolamine hydrochloride and amino acids with ninhydrin is clearly demonstrated by a favorable comparison of their molar absorptivities as well as the similarity in the reaction conditions (15–18). This includes the fact that both the amine and amino acids give a maximum reaction with ninhydrin at about pH 5.0.

By using the procedure given for the preparation of the standard reference solution, 14 amines were analyzed in pure form to compare the absorbance (A_{570}) and molar absorptivity values. As noted in Table V, of those amino alcohols and amino acids tested, only glycine, phenylalanine, phenylpropanolamine hydrochloride, and methoxamine hydrochloride resulted in a molar absorptivity of similar magnitude (2×10^4), which is typical of a solution of Ruhemann's purple. Glycine and phenylalanine were used as reference models.

It is assumed that the mechanism of chromophore formation



V

with phenylpropanolamine hydrochloride and methoxamine hydrochloride is identical to that for glycine and phenylalanine, which follows the classic ninhydrin scheme, since the molar absorptivities are the same. A mechanism was clearly established and proposed by Neuzil *et al.* (19) for the reaction of primary amino aliphatic alcohols with ninhydrin. It was stated that primary amines having a hydroxyl group on the same carbon chain react with ninhydrin to form the Schiff base diketohydrindylidene–diketohydrindamine or Ruhemann's purple (Table V). The maximum reaction occurs at pH 5, and the maximum reaction for amino acids with ninhydrin occurs at the same pH.

Since phenylpropanolamine hydrochloride, methoxamine hydrochloride, and the amino acids tested have the same range of molar absorptivity, it can be postulated that the mechanism is similar. It also can be theorized that both compound types react *via* a Schiff-base intermediate. By comparing the molar absorptivity obtained for amphetamine sulfate (absence of hydroxyl) and ephedrine (secondary amine) with the molar absorptivity of the two adrenergic amines given, the hydroxyl group and primary amino group appear to be substituents for the proposed mode of reaction, as the carboxyl and amino groups are for the amino acids. By comparing the molar absorptivity of 2-aminopropanol with phenylpropanolamine hydrochloride, it can be seen that the addition of the phenyl group increases the molar absorptivity by a factor of about five. The inductive and conjugative effect of the phenyl group further promotes ease of formation of the aldehyde (Scheme I). Amphetamine, having a phenyl group but no hydroxyl group, has a molar absorptivity significantly less than phenylpropanolamine hydrochloride and, therefore, must be assumed to react in a manner that is not consistent with the amino acid mechanism.

Both the primary amino and alcoholic group are required for the complete reaction *via* the ketimine. Furthermore, it is necessary that cleavage of the ketimine occurs specifically to form an

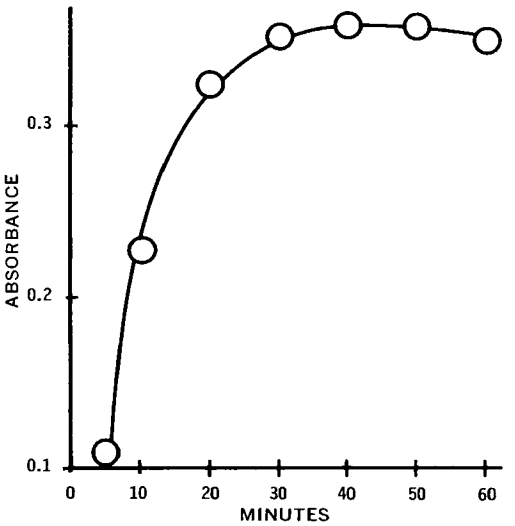


Figure 1—*Heating time–response curve for phenylpropanolamine hydrochloride–ninhydrin reaction.*

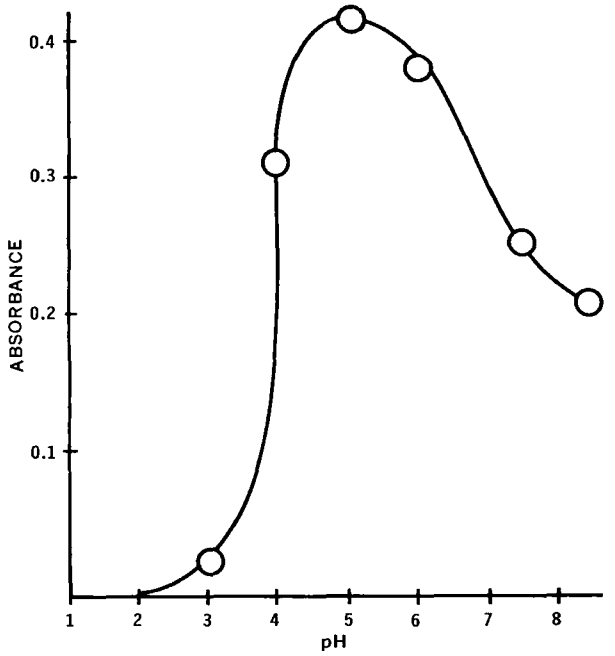


Figure 2—*The pH–response curve for phenylpropanolamine hydrochloride–ninhydrin reaction.*

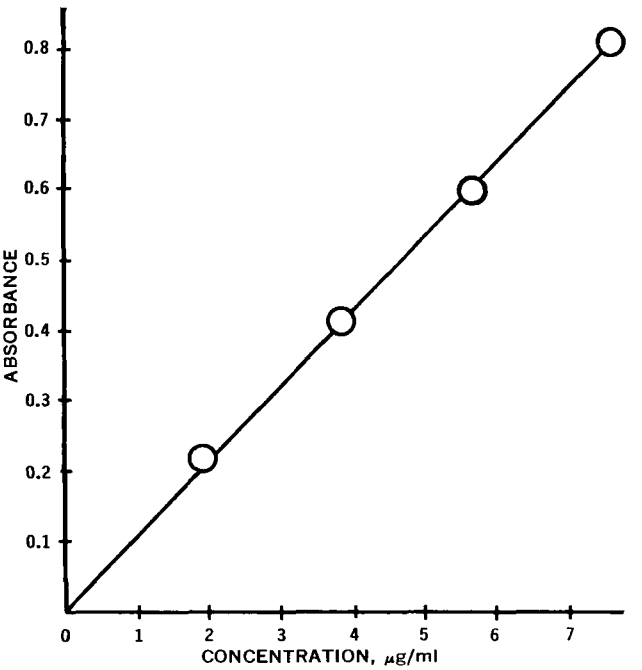


Figure 3—*Concentration–absorbance plot for phenylpropanolamine hydrochloride–ninhydrin reaction.*

aldimine and a carbonyl compound. This occurs to produce the intermediate species capable of reacting with a second mole of ninhydrin. It can be seen from the molar absorptivity values (Table V) that unless all of these conditions are met, complete conversion to Ruhemann's purple cannot occur and a molar absorptivity substantially lower than that for the amino acids is obtained. It is also apparent that the secondary amines or primary amino alcohols with strong deactivation at the aromatic apical center do not give a quantitative conversion. In the case of the secondary amines, the formation of the ketimine is retarded. In the deactivated primary amino alcohols, the deactivation retards cleavage of the ketimine to aldimine by the presence of Structure V. In aromatic secondary amino alcohols, little difference occurs regardless of the changes in nuclear substitution because the retarding influence is based upon the available amine protons.

An alternate mechanism for amino alcohols could be based upon the formation of an imino alcohol followed by formation of ammonia according to Scheme II. The liberated ammonia then reacts with ninhydrin to form III which then follows the latter part of Scheme I.

This alternative is logical since secondary amines (such as ephedrine) do not readily form imino derivatives and thus would not enjoy facile oxidative deamination. In addition, these would not release ammonia and, therefore, would necessarily yield lower molar absorptivity values.

During a study of the variables of the method, it was established that reproducibility largely depended upon heating time and pH. It was found that 30–35 min was required for the reaction to reach its maximum level (Fig. 1) and that the reaction had to be immediately quenched by cooling for 5 min. It also was established that the maximum reaction occurred at about pH 5 (Fig. 2).

The concentration–absorbance plot (Fig. 3) shows that the colorimetric response is linear with concentration in the range of 1.91–7.64 μg/ml. This result is consistent with that originally described by Yem and Cocking (15) for the amino acid reaction with ninhydrin.

SUMMARY

A specific reaction for the determination of phenylpropanolamine hydrochloride is reported. This method can be used to determine phenylpropanolamine hydrochloride in complex mixtures without prior separation. A reaction of phenylpropanolamine hydrochloride with ninhydrin is involved. A reaction mechanism is presented and compared with the classical reaction of amino acids with ninhydrin. The molar absorptivity of a number of amines is listed to show the functional group relationship of phenylpropanolamine hydrochloride to the listed amino acids and to other amines, not yielding a large absorptivity value.

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