Determination of Phenylpropanolamine Salts in Dosage Forms through Fluorescent Derivative Formation

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Abstract □ Phenylpropanolamine was determined by measuring its fluorescent fluorescamine derivative. The method is rapid, sensitive, and easily automated. Statistics are presented for an effervescent cold product, and recovery data are presented for other commercially available products.

Keyphrases □ Phenylpropanolamine—fluorometric analysis in dosage forms □ Fluorometry—analysis, phenylpropanolamine in dosage forms □ Adrenergics—phenylpropanolamine, fluorometric analysis in dosage forms

Phenylpropanolamine can be determined by several different methods (1–7). Although they provide acceptable results, these methods are time consuming because of their complexity or the manipulations required. The need arose in this laboratory for a simple, rapid, and sensitive method for the determination of phenylpropanolamine bitartrate in a combination effervescent tablet containing aspirin and chlorpheniramine maleate. None of the existing methods was considered fast enough.

The fluorescent derivative of fluorescamine[1], 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (I), has been used (8, 9) to determine quantitatively primary amines in various media. This paper reports the application of this technique to the analysis of phenylpropanolamine in dosage forms. This approach is a valuable alternative to existing procedures, especially where simplicity and rapidity are important.

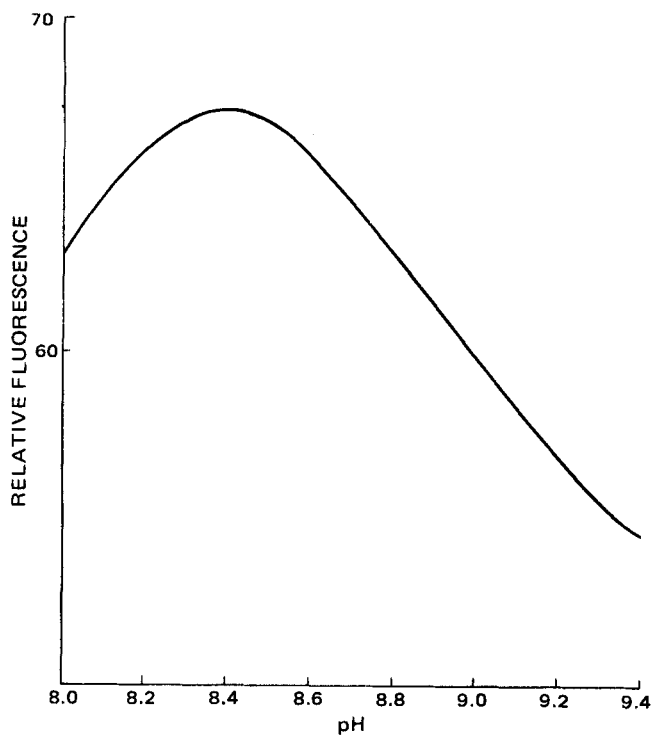

Figure 1—*Effect of pH on the intensity of fluorescence.*

[1] Fluram, Roche Diagnostic, Nutley, N.J.

Table I—Statistical Evaluation of Proposed Method

Level	Recovery, %	*CV*, %
1 (−20%)	97.3	4.52
2	96.2	1.78
3 (+20%)	97.9	1.66
Overall	97.3	2.81

EXPERIMENTAL

Reagents—To prepare borate buffer, 6.2 g of reagent grade boric acid was dissolved in 500 ml of water and the pH was adjusted to 8.4 with 10% NaOH.

A 0.3% solution of I was prepared in reagent grade acetone. The standard solution was an aqueous solution containing an accurately known concentration of phenylpropanolamine salt equivalent to approximately 2 μg of free base/ml.

Procedure—A representative sample was dissolved and diluted to obtain a final concentration of approximately 2 μg/ml as the free base. Exactly 2 ml of standard, sample, and water (blank) was transferred to separate cells or 19 × 150-mm test tubes. To each tube, 2.0 ml of borate buffer was added. Then, while mixing on a vortex mixer, 1.0 ml of I was added rapidly. Within 35 min, the fluorescence was determined at 480 nm while exciting at 398 nm.

RESULTS AND DISCUSSION

The relation of the subsequent fluorescence intensity *versus* the test medium pH is shown in Fig. 1. Although the pH effect was not pronounced, the greatest sensitivity and reproducibility were obtained at pH 8.4. The fluorescence of the reagent blank was pH independent.

The suggested concentration range for I is 0.015–0.030%[2]. In this case, the highest concentration was used since greater precision resulted, as evidenced by a 3.3% decrease in the coefficient of variation.

The fluorescence was stable under normal benchtop conditions for at least 35 min, while limited data suggested constancy over several hours. Extended intervals were not investigated since 35 min was considered ample to obtain readings on normal batch runs.

The intended use of the proposed assay required that it be stability indicating. Stressed samples of a typical formulation were assayed by periodate (5) and ninhydrin (7) methods as well as by the proposed method. Highly precise first-order data were obtained by the fluorometric method over three half-lives. Although the data of the other two methods were more variable, the results confirmed the fluorometric data. Claims have been made for the stability-indicating capabilities of the periodate method (5), and these data support the conclusion that the fluorometric method is stability indicating.

The range of 0–3 μg/ml as the free base was selected since the fluorescence obtained at this level could be read using the midrange sensitivity of the spectrofluorometer. A linear calibration curve was obtained over this range. Some indications suggest that this linearity extends over a much broader range. However, evidence of quenching was noticed in samples that were an order of magnitude higher in concentration.

It is desirable to operate at peak excitation and emission wavelengths, but good results were obtained with much simpler filter instruments[3].

A statistical analysis was accomplished by assaying three sets of five

[2] Roche Diagnostics package insert issued May 1973.

[3] Coleman 12 c photofluorometer with filters 12-221 and 14-221.

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Table II—Assay Results of Commercial Products

Product	Phenylpropanolamine Hydrochloride		Additional Active Ingredients[a]
	Labeled	Found	
Uncoated tablet	12.5 mg/tablet	13.1	2, 4
Syrup	37.5 mg/5 ml	34.6	4, 5
Film-coated tablet	18.75 mg/tablet	19.9	2, 4
Capsule	25 mg/capsule	25.5	3, 4
Spray	0.2%	0.224	6, 7, 9
Capsule	18 mg/capsule	19.0	1
Uncoated tablet	25 mg/tablet	24.1	2
Sugar-coated tablet	18.75 mg/tablet	17.7	2, 4
Uncoated tablet	25 mg/tablet	24.2	1, 8
Press-coated tablet	25 mg/tablet	25.2	2, 3, 4

[a] 1 = acetaminophen, 2 = aspirin, 3 = caffeine, 4 = chlorpheniramine maleate, 5 = dextromethorphan hydrobromide, 6 = naphazoline, 7 = phenylephrine hydrochloride, 8 = phenyltoloxamine citrate, and 9 = pyrilamine maleate.

synthetic samples (Table I). One set contained phenylpropanolamine at the level of interest while the other two sets contained levels 20% above and below that level.

To demonstrate the utility of the proposed method, several commercially available products were assayed (Table II). An attempt was made to select products representing a wide variety of dosage form types as well as accompanying active ingredients. Duplicate analyses were carried out on two individual unit dose samples or, with liquids, on two aliquots containing between 12 and 25 mg of phenylpropanolamine hydrochloride. The object was merely to illustrate the applicability of the assay and not to establish accurate phenylpropanolamine hydrochloride levels for the various products.

For dosage forms where phenylpropanolamine is the only primary amine present, this method provides a convenient, specific, sensitive, and easily automated alternative to existing methods. It has been employed routinely and successfully in this laboratory for more than 2 years.

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Thimerosal Determination by High-Pressure Liquid Chromatography

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Received December 30, 1977, from *Allergan Pharmaceuticals, Inc., Irvine, CA 92713.* Accepted for publication March 24, 1978.

Abstract □ A sensitive and useful high-pressure liquid chromatographic method for the determination of intact thimerosal was developed. This method is extremely fast and reliable, and its inherent specificity makes it a breakthrough over other common wet chemical methods.

Keyphrases □ Thimerosal—high-pressure liquid chromatographic analysis in aqueous pharmaceutical preparations □ High-pressure liquid chromatography—analysis, thimerosal in aqueous pharmaceutical preparations □ Anti-infectives, topical—thimerosal, high-pressure liquid chromatographic analysis in aqueous pharmaceutical preparations

Thimerosal, ethyl (sodium *o*-mercaptobenzoato)mercury (I), is a relatively stable organomercurial that has long been used as both a topical antiseptic and a preservative. It is the preservative of choice for soft contact lens care solutions. Its stability, compatibility, and low toxicity account for its wide use.

BACKGROUND

The low concentrations of thimerosal typically used (10–50 ppm) have led to difficulties in development of suitable assays. Early analysis methods for organic mercurials involved the decomposition of the metallo-organic compounds (1) and detection with a complexing agent such as diphenylthiocarbazone (dithizone) or diphenylcarbazone (2, 3). More specificity was obtained by the use of better extraction procedures (4) or column chromatography (5). More sophisticated analytical methods such as atomic absorption (6), neutron activation (7), and GLC (8) detect degradation products as well as intact thimerosal.

Thimerosal degrades in aqueous solution to form ethylmercury salts and thiosalicylic acid (9, 10). Therefore, techniques based on either total mercury or total organic mercury do not reflect accurately the amount of intact thimerosal present in solution. Since some degradation products may have a higher toxicity potential than the original thimerosal (11, 12), accurate analytical techniques are essential.

Quantitative high-pressure liquid chromatography (HPLC) has been suggested as a simple, specific method that meets these objectives (13).

The HPLC method described here is a rapid, specific determination of intact thimerosal at 5–25 ppm (μg/g) in aqueous samples. In many cases, no sample cleanup is needed before determination. However, interferences are sometimes encountered because of components of the formulation. In such cases, a simple extraction procedure can be used.

To facilitate the handling of large numbers of samples, the assay is designed for the smallest retention time consistent with good chromatography. Thimerosal content of several commercial soft contact lens formulations was determined using this method to verify the usefulness of the procedure.

EXPERIMENTAL

Apparatus—The HPLC system consisted of a pump[1], a UV detector[2], and an automatic sampler[3].

Reagents—All reagents were analytical reagent grade unless noted otherwise. The mobile phase was 0.1 *M* ammonium carbonate with the pH adjusted to 7.9 with acetic acid. To prepare pH 4 acetate buffer, 410

[1] Waters Associates model 6000 pump.
[2] Chromatonix model 260 UV detector (254 nm).
[3] DuPont Instruments model 834 autoinjector with a 50-μl loop.

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