Table I—Absorbance of Known Hydrogen Peroxide Solutions

Concentration of H_2O_2, g./100 ml.	Absorbance at 304.0 nm.
4.335	0.988
3.902	0.890
3.468	0.790
3.034	0.691
2.601	0.593
2.167	0.495

The USP XVIII standard for hydrogen peroxide solution (7)[6] is that 100 ml. of solution contains not less than 2.5 g. and not more than 3.5 g. of H_2O_2.

Determinations of seven random samples of hydrogen peroxide solutions were performed by both spectrophotometric and titrometric procedures. Agreement between both methods was within ±2 parts per 1000. The error introduced by working on a steep area of the absorption band is small when compared to the errors encountered using dilution techniques. Also, an appreciable saving in time, labor, and materials results by direct measurement of the hydrogen peroxide solution. An accurate calibration of the wavelength (either with a mercury emission spectrum or a solution of known hydrogen peroxide concentration) is essential for the reproducibility of this method[7]. By locking the controls of the spectrophotometer once it has been calibrated, many samples of hydrogen peroxide can then be assayed in a very brief period.

[6] Preservatives in hydrogen peroxide solution USP XVIII total not more than 0.05%. The stabilizer did not interfere with the spectrophotometric determination reported here.

[7] Calibration of the spectrophotometer using a sample of hydrogen peroxide whose concentration was found titrometrically requires more time but gives more accurate results.

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Fluorometric Determination of Amphetamines with 3-Carboxy-7-hydroxycoumarin

J. T. STEWART and D. M. LOTTI

Abstract □ A fluorometric method of analysis for amphetamines based on the interaction between aliphatic and/or cyclic amines and 3-carboxy-7-hydroxycoumarin to yield highly fluorescent coumarin–amine salts was investigated. The procedure was applied to the analysis of several amphetamines and amphetamine mixtures. Comparison of the coumarin method to other existing fluorometric methods for amines in the analysis of amphetamines was performed.

Keyphrases □ Amphetamines—analysis □ 3-Carboxy-7-hydroxycoumarin–cyclic amine reaction—fluorescence □ Fluorometry—analysis

The objective of this investigation was to evaluate a fluorometric method of analysis for amphetamines based on the interaction between aliphatic and/or cyclic amines and 3-carboxy-7-hydroxycoumarin to yield highly fluorescent coumarin–amine salts. The assay procedure applied to aliphatic and cyclic amines was reported previously from this laboratory (1). Use of the method permits determination of trace quantities of amines based upon fluorometric measurement of coumarin–amine salt, even in the presence of excess fluorescent coumarin reagent.

In this study, the method was applied to the analysis of several amphetamines and amphetamine mixtures. A study demonstrating the usefulness of the procedure to commercial dosage forms containing amphetamines was made. Comparison of this procedure to other existing fluorometric methods for amines was performed.

EXPERIMENTAL

Apparatus—Fluorescence spectra and measurements were made with a spectrophotofluorometer[1]. Clear, fused quartz cells (12.5 × 47 mm.) were used as sample cells.

Reagents and Chemicals—Powdered samples of dextroamphetamine sulfate[2], methamphetamine hydrochloride[3], benzphetamine hydrochloride[4], chlorphentermine hydrochloride[5], methylphenidate hydrochloride[6], phenmetrazine hydrochloride[7], and phendimetrazine tartrate[8] were used in the analytical procedure for preparation of standard solutions. 3-Carboxy-7-hydroxycoumarin was synthesized according to the procedure of Woods and Sapp (2). All other chemicals used were the highest grade of the commercially available materials.

Solutions of amphetamine salts (8×10^{-5} *M*) and 3-carboxy-7-hydroxycoumarin (6.8×10^{-4} *M*) were prepared by dissolving

[1] Aminco-Bowman equipped with slit arrangement No. 3.
[2] Smith Kline & French Laboratories, Philadelphia, Pa.
[3] Mann Research Laboratories, New York, N. Y.
[4] The Upjohn Laboratories, Kalamazoo, Mich.
[5] Warner-Lambert Research Institute, Morris Plains, N. J.; research affiliate of Warner-Chilcott Laboratories.
[6] Ciba Pharmaceutical Co., Summit, N. J.
[7] Geigy Pharmaceuticals, Ardsley, N. Y.
[8] Ayerst Laboratories, Inc., New York, N. Y.

Table I—Analysis of Amphetamines

Amphetamine	Range[a], mcg./25 ml.
Amphetamine sulfate	7.4–118.4
Methamphetamine hydrochloride	3.7–59.2
Benzphetamine hydrochloride	5.5–88.0
Chlorphentermine hydrochloride	4.4–70.4
Methylphenidate hydrochloride	5.4–86.4
Phenmetrazine hydrochloride	4.3–68.8
Phendimetrazine tartrate	27.3–109.2

[a] Range observed for linear response extending through the origin.

weighed amounts of the amphetamine salts in water and 3-carboxy-7-hydroxycoumarin in ethanol.

Procedure—A quantity of amphetamine salt, indicated in Table I, dissolved in 1 ml. of water was placed in a 30-ml. separator followed by the addition of 3 drops of 0.1 *N* sodium hydroxide solution. The solution was extracted once with 5 ml. of diethyl ether, and the extract was washed with two 1-ml. portions of water. The ether extract was then transferred to a 25-ml. volumetric flask and evaporated to near dryness on a steam bath. Two milliliters of ethanol was added, followed by the addition of 1 ml. of an ethanolic solution of 3-carboxy-7-hydroxycoumarin. Samples, together with control solutions of reagent, were shaken at room temperature for 5 min. and diluted to volume with ethanol. The increases in fluorescence of reaction mixtures compared to their corresponding reagent blanks were measured using activation and emission wavelength settings of 420 and 450 nm., respectively.

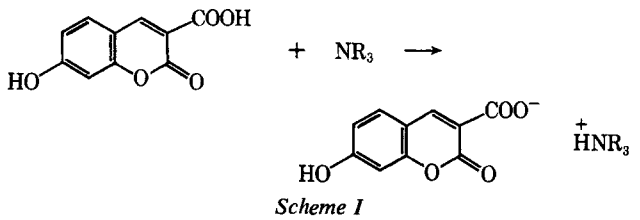
RESULTS AND DISCUSSION

In the analytical procedure, an amphetamine interacts with 3-carboxy-7-hydroxycoumarin to yield a highly fluorescent coumarin–amine salt (Scheme I). This interaction results in an increase in fluorescence and is accompanied by a change in the activation wavelength of the reaction mixture. The use of 420 and 450 nm. as the excitation and emission wavelengths, respectively, for the procedure can be correlated with known amphetamines run as analytical solutions and corresponds to pure synthesized coumarin–amine salts formed from various aliphatic and cyclic amines in the original method (1). The analysis is applicable to organic bases having pKa values of approximately 8 or greater. Reproducibility of the procedure can be shown by the standard deviation of the mean, which is 0.87%.

The method permits detection of fluorescence of the final product and correlation of fluorescence to concentration of drug even in the presence of a 2- to 32-fold excess of fluorescent coumarin reagent.

Analytical results for amphetamines successfully examined by this method are shown in Table I. The analysis of unknown concentrations of these drugs in this procedure requires direct comparison of the fluorescence of an unknown to that of a known concentration under the same conditions. The linear fluorescence-to-concentration range indicates the range over which samples could be compared.

Quantitative data from several systems shown in Table II reveal that use of this procedure permits the determination of amphetamines in the presence of various medicinals found in combination with them in commercial dosage forms. Earlier studies showed that primary, secondary, and/or tertiary aromatic amines, aromatic heterocycles, aromatic and aliphatic amides, and carbonyl-containing compounds also do not interfere with this method (1). However, due to the nature of the assay procedure, the presence of other extractable strongly basic organic compounds can significantly interfere with the fluorescent readings of the various amphetamines.



Scheme I

Table II—Analysis of Known Amphetamine Mixtures for Amphetamine

Mixture, Concentration of 3.2×10^{-6} *M*	Amphetamines—Found[a] $\times 10^{-6}$ *M*	Amphetamines—Percent of Theory
Amphetamine sulfate–amobarbital	3.15	98.4
Methamphetamine hydrochloride–nicotinic acid–thiamine mononitrate	3.16	98.8
Benzphetamine hydrochloride–aspirin–phenacetin	3.14	98.0
Chlorphentermine hydrochloride–chlorpromazine hydrochloride	3.20	100.0
Methylphenidate hydrochloride–meprobamate	3.15	98.4
Phendimetrazine tartrate–ascorbic acid–riboflavin	3.07	96.0

[a] Values represent average of four determinations.

Generally, it has been shown in this laboratory that inorganic acids and bases and organic acids interfere with the analytical determination unless the amphetamine can be successfully removed from them with the use of a suitable separation technique. In this study, solvent extraction of the free amphetamine bases with ether was sufficient to permit determination of these substances in mixtures containing various acidic and basic components (Table II).

No heating of the analytical solution is required to ensure a more complete reaction. Maximum fluorescence development and, hence, yield of product can be obtained upon shaking the solution at room temperature for 3–5 min. Repeated readings for a series of different samples indicated that the fluorescence of the solutions was stable for periods up to 1 day. Great care was taken to prevent overexposure of the samples to the xenon source of the fluorometer as well as to prevent contamination of solutions and cells used in the analysis.

The method of analysis of amphetamines by the 3-carboxy-7-hydroxycoumarin approach was performed on various dosage forms; comparative analyses were conducted using other known fluorometric procedures for aliphatic and/or cyclic amines (3, 4). The literature does not reveal that any of these existing fluorometric methods have been used for the analysis of amphetamines. The coumarin method possesses the advantages that: (*a*) only one re-

Table III—Comparative Determinations of Various Dosage Forms Containing Amphetamines by the 3-Carboxy-7-hydroxycoumarin and Other Known Fluorometric Methods

Amphetamine	Mean Percent of Labeled Amount—3-Carboxy-7-hydroxycoumarin Method	Acetylacetone Method[a]	Aconitic Anhydride Method[b]	Dansyl Chloride[d] Method[b]
Amphetamine sulfate[c]	97.7	95.7	—[d]	96.0
Methamphetamine hydrochloride[e]	100.0	—	—	95.3
Benzphetamine hydrochloride[f]	97.0	—	97.3	—
Methylphenidate hydrochloride[g]	100.0	—	—	90.0
Phenmetrazine hydrochloride[h]	96.3	—	—	93.3
Phendimetrazine tartrate[i]	96.1	—	—	—

[a] *Reference 3.* [b] *Reference 4.* [c] Benzedrine tablets, Smith Kline & French. [d] Blank denotes method not applicable to particular type of amine listed. [e] Desoxyn tablets, Abbott Laboratories. [f] Didrex tablets, The Upjohn Laboratories. [g] Ritalin tablets, Ciba Corp. [h] Preludin tablets, Geigy Pharmaceuticals. [i] Plegine tablets, Ayerst Laboratories.

agent is necessary for a wide variety of amines, and (*b*) concentration sensitivity is comparable and in most cases better than the other procedures listed (1).

Four determinations using the various methods were performed on each tablet. The mean percent of labeled amount for each drug is shown in Table III for all the methods.

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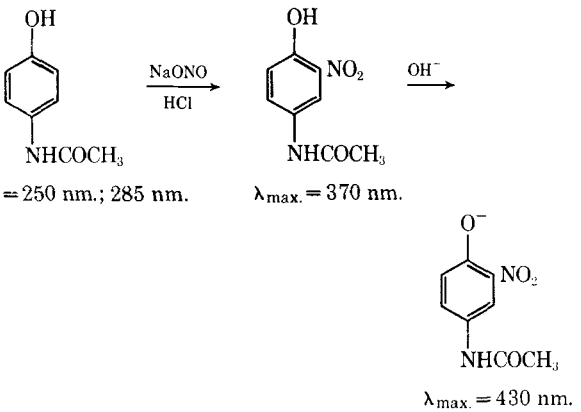
Selective Colorimetric Determination of Acetaminophen

LESTER CHAFETZ, ROBERT E. DALY, HERBERT SCHRIFTMAN, and JOSEPH J. LOMNER

Abstract □ A method for the quantitative determination of acetaminophen as 2-nitro-4-acetamidophenol, formed by the reaction of nitrous acid with the drug in aqueous solution, was compared with methods using this reaction reported by others. Phenacetin causes only negligible interference in the assay method; however, salicylamide forms a chromophore which interferes in the determination of acetaminophen. No other interferences were encountered. The procedure is stability indicating with respect to hydrolysis, and it appears to be adaptable to automated apparatus. Good recovery and precision data were obtained on application of the procedure to conventional and sustained-action tablet formulations declaring acetaminophen, phenacetin, phenylpropanolamine HCl, and phenyltoloxamine dihydrogen citrate.

Keyphrases □ Acetaminophen in dosage forms—analysis □ Nitrous acid–acetaminophen reaction—analysis method □ Colorimetric analysis—spectrophotometer

Most colorimetric methods (1–6) described for acetaminophen require preliminary hydrolysis of it to *p*-aminophenol. A significant contribution to methods for acetaminophen was provided by Le Perdriel *et al.* (7). They discovered that acetaminophen and nitrous acid react under mild conditions to form 2-nitro-4-acetamidophenol, which can be measured by its color in alkaline solution (Scheme I). They found no interference from structurally similar drugs such as phenacetin (acetaminophen *O*-ethyl ether) or acetanilide. Unlike the colorimetric method described by Brockelt (8), where the same chromophore is produced by nitration with nitric acid, there is no interference from *p*-aminophenol; thus the reaction of Le Perdriel *et al.* (7) is stability indicating for acetaminophen with respect to hydrolysis. Independently and later, Inamdar and Kaji (9) reported use of the chromophore formed by the reaction of nitrous acid with acetaminophen for dosage form assay; however, they measured the yellow color in acid solution instead of the orange-red color of the phenolate ion, and they attributed the chromophore to nitroso derivatives.



Scheme I

This report describes further investigations on the reaction of acetaminophen with nitrous acid and extends the observations of previous workers (7, 9). A modified procedure is proposed, and results obtained in the assay of some acetaminophen dosage forms are described.

EXPERIMENTAL

Equipment and Supplies—Acetaminophen NF, salicylamide NF, phenacetin USP, 6 *N* hydrochloric acid, 10% sodium nitrite, 15% sulfamic acid, and 10% sodium hydroxide were used. Spectra were determined in 1-cm. silica cells in a Cary model 14 recording spectrophotometer or in a Beckman DU fitted with the Gilford model 222 modification.

Proposed Method—*Standard Preparation*—Accurately weigh about 100 mg. of acetaminophen reference standard NF, dissolve it in water, and dilute to 100 ml. in a volumetric flask. Further dilute 10.0 ml. to 100 ml. with water to obtain a standard concentration of about 100 mcg./ml.

Assay Preparation—Weigh and finely powder not less than 20 tablets. Transfer a portion of the powder equivalent to about 100 mg. of acetaminophen to a glass-stoppered 250-ml. conical flask, add exactly 100.0 ml. of water, shake the mixture mechanically for 10 min., and filter. Dilute 10.0 ml. of the filtrate to 100 ml. with water in a volumetric flask.

Procedure—Transfer 10.0-ml. portions of the assay preparation and the standard preparation to 50-ml. volumetric flasks. Add successively to each flask 2.0 ml. of 6 *N* hydrochloric acid and 5.0 ml. of 10% sodium nitrite. Mix, let stand 15 min., and then destroy excess nitrous acid with 5.0 ml. of 15% sulfamic acid. After nitro-