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

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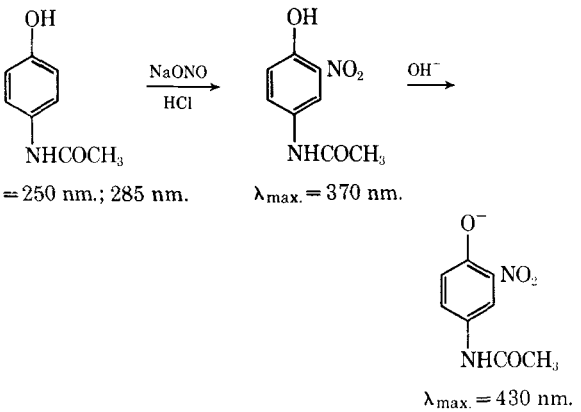
Abstract □ A method for the quantitative determination of acetaminophen as 2-nitro-4-acetamidophenol, formed by the reaction of nitrous acid with the drug in aqueous solution, was compared with methods using this reaction reported by others. Phenacetin causes only negligible interference in the assay 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-

Table I—Synopsis of Methods for Acetaminophen Colorimetry with Nitrous Acid

Method	Proposed	*Reference 7*	*Reference 9*
Acetaminophen, mcg./ml.	100	100	2000
Milliliters taken	10.0	1.0	1.0
Sample solvent	Water	Alcohol or water	1.0 ml. methanol, then 10 ml. water
HCl added	2.0 ml. 6 *N*	0.5 ml. 6 *N*	10 ml. 10%
10% NaONO added	5.0 ml.	1.0 ml.	1.0 ml.
Minutes of reaction	15	15	15
15% H_2NSO_3H added	5.0 ml.	Nil	Nil
Diluted with	15.0 ml. 10% NaOH	5 ml. 5% Na_2CO_3	5 ml. alcohol
Wait time (minutes)	To cool	Nil	1
Dilute to	50 ml. with water	–	100 ml. with water
Blank	Water	Reagent	Modified[a] reagent
Absorbance maximum	About 430 nm.	440–445 nm.	395 nm.

[a] See text under *Chemistry of the Reaction.*

gen evolution has ceased, add 15.0 ml. of 10% sodium hydroxide, cool under the tap, and dilute to volume with water. Concomitantly determine the absorbances of the solutions from the assay preparation and the standard preparation in 1-cm. cells in a suitable spectrophotometer at the wavelength of maximum absorbance at about 430 nm., using water as the blank. Calculate the quantity, in milligrams per tablet, of $C_8H_9NO_2$ by the formula $C(A_U/A_S)(TW/SW)$, where: C is the concentration, in micrograms per milliliter, in the standard preparation; A_U and A_S are the absorbances of the solutions from the assay preparation and the standard preparation, respectively; and TW and SW are the weights, in milligrams, of the average tablet and of the sample taken for assay, respectively.

Experiment on Phenacetin Interference—*Acetaminophen Stock Solution*—Dissolve an accurately weighed quantity of about 250 mg. of acetaminophen in water, and dilute the solution to 100 ml.

Acetaminophen Reference Solution—Transfer 10 ml. of alcohol to a 100-ml. volumetric flask. Add exactly 40.0 ml. of the acetaminophen stock solution, and dilute to volume with water. Further dilute 10.0 ml. with water to obtain a solution containing about 100 mcg./ml. of acetaminophen.

Acetaminophen–Phenacetin Test Solution—Dissolve an accurately weighed quantity of about 100 mg. of phenacetin in 10 ml. of alcohol in a 100-ml. volumetric flask. Add exactly 40 ml. of acetaminophen stock solution, and dilute the solution to volume with water. Further dilute 10.0 ml. to 100.0 ml. with water to obtain a solution containing about 100 mcg./ml. each of acetaminophen and phenacetin.

Procedure A—Transfer 10.0-ml. portions of the acetaminophen reference solution and the acetaminophen–phenacetin test solution 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 nitrogen evolution has ceased, dilute to volume with water. Concomitantly determine the absorption spectra of the solutions in 1-cm. cells in a suitable recording spectrophotometer, using a 1-in-100 solution of alcohol in water as the blank, and compare the spectrograms.

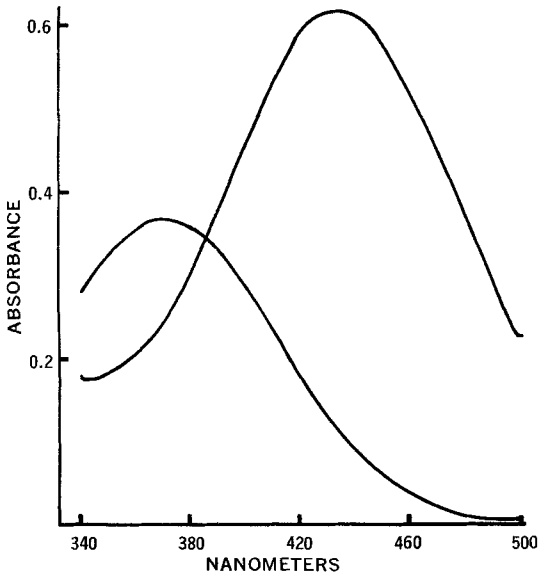


Figure 1—*Absorption spectra of acetaminophen chromophore in acid (low wavelength maximum) and in base.*

Procedure B—Follow Procedure A but change the directions following: "After nitrogen evolution has ceased,..." to "add 15.0 ml. of 10% sodium hydroxide, cool under the tap, and dilute to volume with water." Continue as directed under Procedure A with "Concomitantly determine...."

Experiment on Salicylamide Interference—*Acetaminophen Stock Solution and Reference Solution*—Use solutions described under *Experiment on Phenacetin Interference.*

Salicylamide Reference Solution—Dissolve about 300 mg. of salicylamide, accurately weighed, in 10 ml. of alcohol in a 100-ml. volumetric flask and dilute to volume with water. Further dilute 40.0 ml. to 100.0 ml. with water.

Acetaminophen–Salicylamide Test Solution—Dilute 40.0 ml. of acetaminophen stock solution to 100.0 ml. with water. Transfer 10.0-ml. portions of the dilution and of salicylamide reference solution to a 100.0-ml. volumetric flask; dilute the solution to volume with water to obtain a solution containing about 100 mcg./ml. of acetaminophen and about 120 mcg./ml. of salicylamide.

Procedure—Dilute 10.0 ml. of salicylamide reference solution to 100.0 ml. with water. Transfer 10.0-ml. portions of this dilution, of acetaminophen reference solution, and of acetaminophen–salicylamide test solution to separate 50-ml. volumetric flasks. Follow Procedure B under *Experiment on Phenacetin Interference* beginning with: "Add successively to each flask...."

Recovery Experiments—Add accurately weighed amounts of acetaminophen to portions of an analytically prepared blend of the remaining constituents of each of two commercial acetaminophen tablet formulations. Assay the samples by the method described under *Proposed Method.*

RESULTS AND DISCUSSION

Comparison of Methods—The similarities and differences among the proposed method and those described by Le Perdriel *et al.* (7) and Inamdar and Kaji (9) are evident in Table I.

Chemistry of the Reaction—Le Perdriel *et al.* (7) provided convincing evidence that 2-nitro-4-acetamidophenol is the reaction product—*viz.*, its spectra and that of its acid hydrolysis product are qualitatively and quantitatively superimposible on those of authentic 2-nitro-4-acetamidophenol and 2-nitro-4-aminophenol, respectively. Furthermore, TLC of a chloroform extract of the solution after color development provides a spot identical in R_f and reaction to that of the authentic compound. Moreover, nitration with nitrous acid is a well-known reaction of phenols (10). Rapoport *et al.* (11) determined the electronic absorption spectral constants for 2-nitro-4-acetamidophenol in 0.1 *N* sodium hydroxide and in 0.1 *N* hydrochloric acid, reporting maxima at 429 nm. (ϵ = 4170) in base and at 375 nm. (ϵ = 2250) in acid. Using the

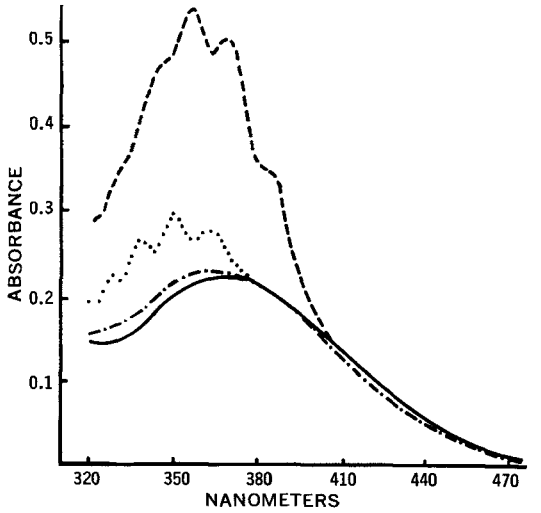


Figure 2—*Absorption spectra of acetaminophen chromophore prepared by the Inamdar and Kaji (9) method. Key: . . ., determined as in* Reference 9; - - -, *determined* versus *water;* —·—, *sulfamic acid added and water used as the blank; and* ——, *determined* versus *reagent blank.*

method proposed in this report, the acetaminophen reaction product shows maxima at about 432 nm. (ϵ = 3800) in base and at about 371 nm. (ϵ = 2010) in acid solution, *i.e.*, where no 10% sodium hydroxide is added after destruction of excess nitrous acid. Spectra of the chromophore are shown in Fig. 1. From the ratio of the absorptivities determined for the acetaminophen reaction product by the proposed method to those reported for the authentic compound (11), one may estimate that about 90% of the drug is converted to the chromophore.

Inamdar and Kaji (9) reported maxima at 375 and 395 nm., and they recommended use of the longer wavelength for determination of the acetaminophen reaction product. Their observations are at variance with the spectral data reported by Rapoport *et al.* (11). From the close similarity of the Le Perdriel *et al.* (7) and Inamdar and Kaji (9) methods evident in Table I, one would expect the chemistry to be identical. This was confirmed using the TLC system described by Le Perdriel *et al.* (7). Inamdar and Kaji (9) recommended a blank made by substituting water for the sample; *i.e.*, the sample solution contains 1 ml. of methanol, but the blank does not. They averred that addition of 95% alcohol destroys excess nitrous acid. In the present study, their method (9) was found to give spectra which showed nitrous acid peaks, and the wavelength designated by them for measurement lay on the slope of the curve (Fig. 2). Addition of sulfamic acid to destroy excess nitrous acid afforded a spectrum with a maximum at about 370 nm. (ϵ = 1570), indicating a reaction efficiency of about 70%. A similar curve was obtained when a true blank was used.

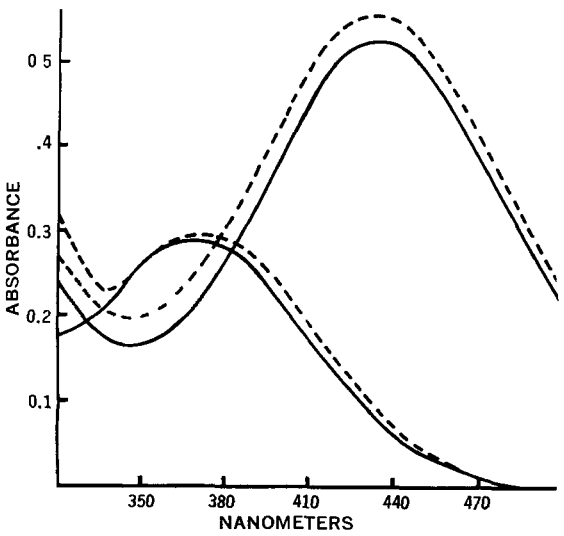


Figure 3—*Absorption spectra of acetaminophen chromophore formed in the presence of only acetaminophen* (——) *and in the presence of an equal concentration of phenacetin* (- - -).

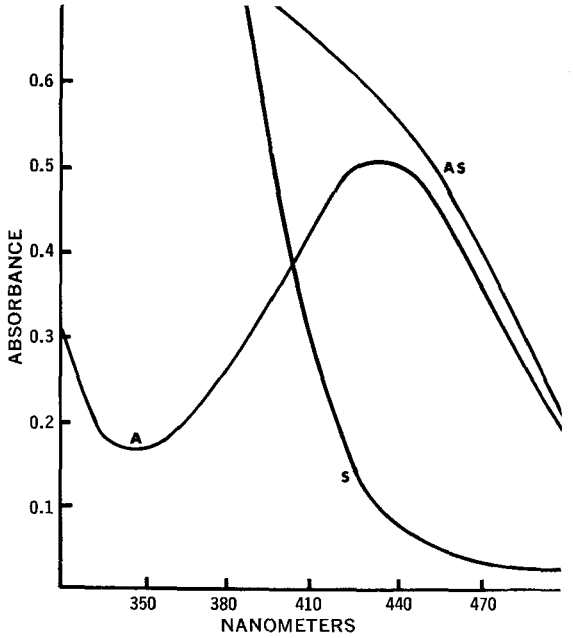


Figure 4—*A = absorption spectra of chromophore representing 20.5 mcg./ml. of acetaminophen. S = absorption spectra of chromophore representing 24.1 mcg./ml. of salicylamide. AS = absorption spectra from chromophore representing mixture of acetaminophen and salicylamide.*

Solvent Effect—Substituting methanol for ethanol in the Le Perdriel *et al.* (7) method lessened the absorptivity obtained by about 15%. The standard curve, using either ethanol or methanol, deviated from Beer's law at acetaminophen concentrations above about 35 mcg./ml. Using the wholly aqueous system of the proposed method, Beer's law conformance was obtained up to about 60 mcg./ml., the highest concentration tested, corresponding to an absorbance reading of about 1.47 units. An hypothesis that the difference in behavior of the aqueous solution from that of the alcohol solutions could be due to carbonyl compound impurities in the alcohols proved untenable: solutions of acetaminophen containing formaldehyde or acetone gave identical spectra with those prepared in water alone. The small percentage of alcohol in the solution determined served to displace the maximum to a slightly higher wavelength.

Selectivity of the Assay—Le Perdriel *et al.* (7) reported that aspirin, acetanilide, phenacetin, phenobarbital, tripelennamine, lidocaine, eucalyptol, glyceryl guaiacolate, sodium camphorsulfonate, *para*-hydroxybenzoate ester preservatives, and a number of tablet excipients did not interfere. The present authors have extended the list of drugs that do not interfere in the assay to include phenylpropanolamine and phenyltoloxamine.

Phenacetin Effect—Because nitration with nitrous acid is a reaction characteristic of phenols and phenolic ethers, the lack of

Table II—Recovery of Acetaminophen Added in Tablet Formulations

Formulation	mg. Acetaminophen Added per Average Tablet Weight	mg. Acetaminophen Found per Average Tablet Weight
Tablet A[a]	150.6	154.0, 151.8, 150.3 150.2, 148.7, 149.8
Tablet B[b]	300.0	299.3, 303.8, 304.9 296.2, 303.3, 299.4

[a] Marketed as Sinutab Tablets by Warner-Chilcott Laboratories, Division of Warner-Lambert Co. [b] Marketed as Sinubid Tablets by Warner-Chilcott Laboratories, Division of Warner-Lambert Co.

Table III—Assay of Acetaminophen in Commercial Tablet Formulations

Formulation	mg. Acetaminophen Claimed per Tablet	mg. Acetaminophen Found per Tablet	NF Assay
Tablet A	150.0	157.2,150.4,151.2 152.4,150.3,153.4	—
Tablet B	300.0	310.7,311.0,308.9 304.9,309.3,309.7	—
Acetaminophen tablets NF			
(Lot X)	325.0	324.2	319.0
(Lot Y)	325.0	320.8	319.1

interference of phenacetin, the *O*-ethyl ether of acetaminophen, would appear paradoxical. Recently, Hanegraaff and Chastagner (12) reported a study of the formation of nitro derivatives by acetaminophen, phenacetin, and a number of other derivatives of acetanilide using different nitrating agents—*viz.*, nitric acid, a mixture of sulfuric acid and sodium nitrite solution, a mixture of sodium nitrite and nitric acid, and a mixture of nitric acid and procaine. The last mixture did not effect nitration of any of the compounds, a fact they attributed to complete removal of nitrous acid by procaine. Using sodium nitrite and sulfuric acid, they produced a chromophore from acetaminophen with maximum absorbance at 375 nm. (ϵ = 630) and a chromophore from phenacetin with a maximum at 355 nm. (ϵ = 1880). They attributed the lack of interference by phenacetin in the Le Perdriel *et al.* (7) method to the fact that the alkaline pH used for measurement converts the acetaminophen chromophore to the phenolate ion, which absorbs at a higher wavelength than the phenacetin chromophore, which does not undergo a bathochromic shift in alkali. The conditions used by Hanegraaff and Chastagner (12), however, differ significantly from those used by Le Perdriel *et al.* (7) and in the method proposed here. The nitration mixture used by Hanegraaff and Chastagner (12) was 10 *N* with respect to sulfuric acid content, while it is about 0.7 *N* in hydrochloric acid content in the proposed method. A definitive test of the effect of phenacetin in the determination of acetaminophen, ignoring solubility considerations, was provided by comparing the colors formed by a mixture of equal concentrations of acetaminophen and phenacetin and by acetaminophen alone. Figure 3 shows that phenacetin interferes to a negligible extent in the acetaminophen determination by this method, even in acid solution. Moreover, the proposed method requires dissolution of the sample in water, in which phenacetin has less than 1 mg./ml. solubility. The excellent recovery values obtained for acetaminophen added to phenacetin-containing tablet formulations and the correspondence of assay values to label claim on commercial tablets provide further confirmation that phenacetin does not interfere in the method.

Salicylamide Interference—Trials of the proposed method on a proprietary analgesic formulation containing 194.4 mg. (3 gr.) of aspirin, 64.8 mg. (1 gr.) of caffeine, 97.2 mg. (1.5 gr.) of acetaminophen, and 129.6 mg. (2 gr.) of salicylamide per tablet provided an average assay value of 122.2 mg. acetaminophen per tablet. Figure 4 shows the effect of salicylamide in the reaction. At 430 nm., the salicylamide reaction product exhibits an absorptivity of about 0.9 l./g. cm., where the acetaminophen chromophore has an absorptivity of about 5.0 l./g. cm. Assuming that the tablets purchased contain the formula amounts of acetaminophen and salicylamide, the difference between the formula and determined values for acetaminophen can be accounted for by the salicylamide content. The structure of the salicylamide chromophore was not determined.

Recovery and Precision—The results of adding known amounts of acetaminophen to each of two analytically prepared commercial tablet formulations are shown in Table II.

One formulation tested (Tablet A) has a label declaration of 150 mg. each of acetaminophen and phenacetin, 22 mg. of phenyltoloxamine dihydrogen citrate (DHC), and 25 mg. of phenylpropanolamine HCl. The other (Tablet B) is a sustained-action tablet formula of the wax matrix type with a claim of 300 mg. each of acetaminophen and phenacetin, 66 mg. phenyltoloxamine DHC, and 100 mg. phenylpropanolamine HCl. The average recoveries were 100.1 ± 1.7% [relative standard deviation (*RSD*)] for the first and 100.4 ± 1.1% (*RSD*) for the sustained-action tablet.

Assay of Commercial Tablets—The proposed method was tested with the two marketed dosage forms described in the recovery experiments. Two different lots of acetaminophen tablets NF were assayed by the proposed method and the UV spectrometric method of NF XIII. The results are shown in Table III. The percent of declared amount and relative standard deviations obtained were 101.7 ± 1.57% for Tablet A, 103.3 ± 0.71% for Tablet B, and 99.7 and 98.4%, respectively, for the two lots of acetaminophen tablets NF.

SUMMARY AND CONCLUSIONS

A modified procedure for the colorimetric determination of acetaminophen was evaluated. The method is based on the observations of Le Perdriel *et al.* (7), who discovered that acetaminophen reacts with nitrous acid to form 2-nitro-4-acetamidophenol, which is orange-red in alkaline solution. A similar method proposed by Inamdar and Kaji (9), who measured the color in acid solution at its peak in the near UV, was found to have identical chemistry. The modified method employs a wholly aqueous system; the color thus obtained conforms to Beer's law, at least to an acetaminophen concentration of 64 mcg./ml. in the solution determined. Interference by phenacetin was found to be negligible; however, salicylamide forms a chromophore which interferes in the determination. Since the recommended method requires only the successive addition of reagents and dilution, it appears to be well suited to automated techniques.

Recovery of acetaminophen added in each of two different tablet formulations containing phenacetin, phenyltoloxamine, and phenylpropanolamine was close to theoretical. Relative standard deviations determined during recovery experiments and trials of the method on commercial products (6 trials each) ranged from ±0.71 to ±1.7%.

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