The application of the color reaction to the levodopa assay was considered, taking into account the linearity, precision, and accuracy of the method. The calibration graphs in Fig. 6 show that for pure levodopa concentrations ranging from 10 to 50 mcg./ml., the Lambert–Beer law is substantially obeyed, especially at 35°. The precision of the standard procedure was ascertained on six replicate analyses. The relative standard deviation was 0.59 (mean value of absorbance 0.503, range 0.006), and it was calculated according to the recommended guidelines (13). In practice, it was found that there is no need to check the calibration curve of the standard procedure unless the batch of I is changed. The accuracy trials were carried out on different samples of levodopa, at various purity levels, from natural (A) and synthetic (B) sources. Table I shows that the recovery data are satisfactory for both type of samples.

The influence of possible interfering substances in Assay Method I is summarized in Table II. Substances other than aminoacids, such as hydroquinone and resorcinol, were selected on the basis of their functional similarity to some by-products of natural levodopa (9–11). The potential interfering substances with levodopa of synthetic origin were those directly involved in the synthetic process, namely tyrosine and 3-aminotyrosine. For comparison purposes, Table II also reports the data, obtained on the same samples, using Method II (6). Method II was chosen due to its simplicity and accuracy. The examination of the data demonstrates that Method I is more specific. In fact, the only appreciable interference using Method I is for 1,4-hydroquinone; with Method II, interferences are more numerous and are particularly relevant for 3-aminotyrosine and resorcinol.

CONCLUSIONS

The chromophore produced in the reaction between levodopa and I in alkaline medium varies as a function of pH. Under acidic conditions the absorption maximum shifts from 475 to 355 nm. (Fig. 1). The color variation can be reverted after realkalinization of the medium. Investigations on the presence of a reversible equilibrium of the different forms of the chromophore and on the consequent presence of ionizable functions associated to it are the subject of further studies to acquire information for structure elucidation. Preliminary oxidation by atmospheric oxygen seems to be the necessary step for color formation (Fig. 4).

The application of the color reaction, as reported in the present paper, seems to be particularly suitable for assaying levodopa in routine analysis due to its precision and accuracy. The simplicity of the analytical procedure, moreover, allows this method to be used in automated analysis during the production process of levodopa for clinical uses (14).

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Acetaminophen Colorimetry as 2-Nitro-4-acetamidophenol

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Abstract □ A method previously proposed for assay of acetaminophen and its tablet formulations was found to give comparable results with three commercial elixir formulations. The procedure was adapted to an automated assay apparatus for analysis of the drug in tablets containing acetaminophen alone and formulated with other drugs. Relative standard deviations of the automated method were about 1.4%.

Keyphrases □ Acetaminophen tablets and elixir—colorimetric analysis, as 2-nitro-4-acetamidophenol □ 2-Nitro-4-acetamidophenol—colorimetric derivative of acetaminophen, analysis in tablets and elixir □ Colorimetry—analysis, acetaminophen in tablets and elixir, as 2-nitro-4-acetamidophenol

Chafetz *et al.* (1) described a simple and selective colorimetric assay for acetaminophen and its tablet preparations in which the drug is measured as 2-nitro-4-acetamidophenol after reaction with nitrous acid. Since the procedure requires only the successive addition of reagents, it was predicted that it would be easily adaptable to an automated assay apparatus. The realization of this prediction is described, along with data obtained by extending the colorimetric method to the assay of acetaminophen elixir.

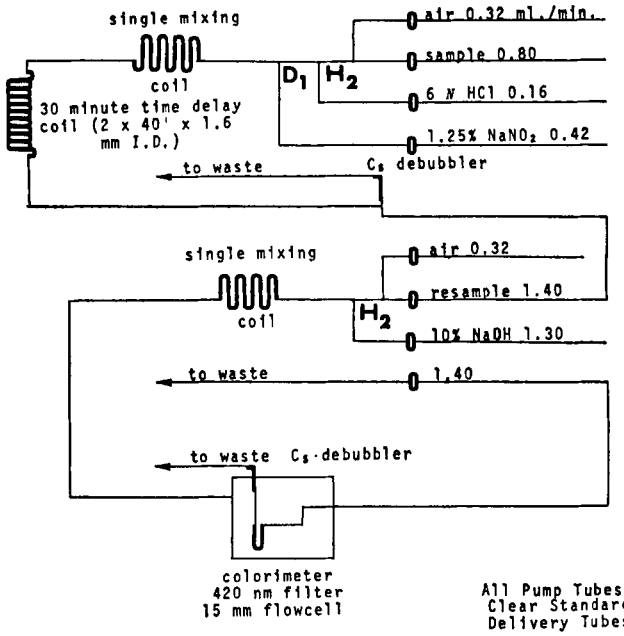

Figure 1—*Flow diagram for the automated colorimetric assay of acetaminophen.*

ACETAMINOPHEN ELIXIR

Experimental

Equipment and Supplies—Acetaminophen NF, NF acetaminophen reference standard, 6 *N* hydrochloric acid, 10% sodium nitrite, 15% sulfamic acid, and 10% sodium hydroxide were used. Three different commercial preparations of acetaminophen elixir NF were purchased for trial of the method, and recovery studies were performed by adding weighed amounts of drug to a placebo vehicle closely resembling one of them. Spectra were determined in 1-cm. cells in a spectrophotometer[1].

Assay—The elixir preparations were diluted quantitatively and stepwise with water to obtain concentrations of acetaminophen of about 100 mcg./ml. The standard preparation and procedure used were exactly those described previously for the assay of tablets (1).

Results and Discussion

Two commercial preparations with declared acetaminophen contents of 120 mg./5 ml. and one preparation with a declared

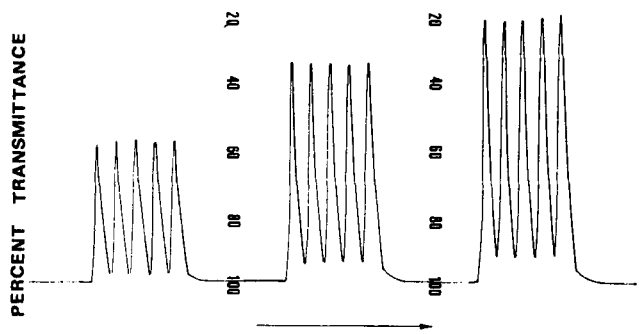

Figure 2—*Reproducibility of response of acetaminophen standards.*

[1] Cary model 14.

Table I—Assay of Acetaminophen Elixir Formulations

Product	Acetaminophen, mg./ml. Declared	Acetaminophen, mg./ml. Found (Average)	Relative Standard Deviation, %
A	24.0	24.3, 25.1, 24.4 24.2, 24.3, 23.8 (24.4)	1.78
B	24.0	24.5, 24.1, 23.3 23.8, 23.3, 23.5 (23.7)	1.95
C	100.0	100.3, 100.0, 102.8 102.2, 102.4, 102.1 (101.6)	1.16

content of 100 mg./ml. were assayed; the results are shown in Table I. Spectrophotometer recordings showed no difference between sample and standards, indicating no interference by the colorants. Moreover, samples of the elixir diluted 4 in 1000, as required in the assay of the 120-mg./5 ml. preparations, or 1 in 1000, as required for the other, showed no visible spectra. A placebo formulation closely resembling the 100-mg./ml. product was used in recovery experiments. The results obtained are presented in Table II.

AUTOMATED TABLET ASSAY

Experimental

Equipment and Supplies—Acetaminophen NF, NF acetaminophen reference standard, 6 *N* hydrochloric acid, 1.25% sodium nitrite, and 10% sodium hydroxide were used. Acetaminophen tablets prepared in these laboratories were used in trials of this method.

A standard apparatus[2] which included a sampler[3], one proportionating pump[4], and a flowthrough filter photometer[5] with a 420-nm. filter was employed. Connections in the apparatus were made with 4-mm. o.d. glass tubing, butt-jointed where necessary with polyvinyl chloride sleeves.

Assay—*Standard Preparation*—Accurately weigh about 60 mg. of NF acetaminophen reference standard, dissolve it in about 70 ml. of water by shaking mechanically for 15 min., and dilute the solution to 100 ml. Further dilute 10.0–50 ml. with water to obtain a concentration of about 120 mcg./ml.

Assay Preparation—Mechanically shake one tablet with water for 30 min., using 200 ml. of water for tablets declaring 150 mg. of acetaminophen and about 400 ml. of water for 325-mg. tablets. Dilute the mixtures with water to nominal concentrations of about 600 or 650 mcg./ml., respectively, and filter, discarding the first 50 ml. of filtrate. Dilute 10.0 ml. of filtrate to 50 ml. with water.

Procedure—Set up the apparatus as shown diagrammatically in Fig. 1. Allow the photometer to stabilize for at least 30 min., and charge the reagent lines. Insert the 20/hr. sample cam with a 1:4 sample–wash ratio, place 2-ml. sample cups in the sampler, and arrange so that duplicate assays are obtained for each tablet and each five assay preparations are bracketed with a standard preparation.

Results and Discussion

The principal changes made in adapting the manual method (1) to the automated apparatus were a reduction in the concentration

[2] Technicon AutoAnalyzer, Technicon, Inc., Tarrytown, N. Y.
[3] Sampler II.
[4] Proportionating pump I.
[5] AutoAnalyzer colorimeter.

Table II—Recovery of Acetaminophen Added to Placebo Elixir Vehicle

Acetaminophen added, mg.	101.1	101.6	102.0	100.6	101.1	100.6
Acetaminophen found, mg.	100.4	102.8	101.2	99.2	100.4	98.8
Percent recovered	99.3	101.2	99.2	98.6	99.3	98.2

of sodium nitrite from 10 to 1.25% and the elimination of sulfamic acid as a reagent. Excess nitrous acid was minimized because its gaseous decomposition products tended to disrupt the flow patterns. The concentration used was sufficient to nitrate the drug; however, the excess was dissipated readily in the time-delay coil. It was shown previously (1) that excess nitrous acid does not interfere at the analytical wavelength for the anion of 2-nitro-4-acetamidophenol. Glass tubing was used in the apparatus in place of the customary plastic lines, because the latter reacted with nitrous acid.

Acetaminophen tablets NF[6], declaring 325 mg./tablet, and two of its combination product tablets were assayed. One of these[7] declared 150 mg. of acetaminophen with phenacetin, phenyltoloxamine dihydrogen citrate, and phenylpropanolamine hydrochloride; the other[8] was similar except that phenyltoloxamine was omitted. Recoveries of added acetaminophen in six replicate assays of each formulation by the automated method were 99.3 ± 1.49% for acetaminophen tablets, 101.6 ± 1.47% for the combination product containing phenyltoloxamine, and 103.5 ± 1.30% for the other tablet. The reproducibility of this method with three standards is illustrated in Fig. 2 by typical recorder tracings.

[6] Accu-Med Division, Warner-Lambert Co.
[7] Sinutab tablets, Warner-Chilcott Laboratories.
[8] Sinutab II, Warner-Chilcott Laboratories.

SUMMARY AND CONCLUSIONS

A method for colorimetric determination of acetaminophen as its 2-nitro derivative (1) was extended to elixir formulations of the drug, and the assay of tablets by this reaction was adapted to an automated apparatus. In both cases, recovery data on added acetaminophen were excellent, and the procedures were shown to be applicable to commercial formulations of the drug.

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Rapid GLC Quantitation of Salicylic Acid in Multicomponent Codeine and Propoxyphene Analgesic Formulations

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Abstract □ A rapid GLC procedure for the precise estimation of salicylic acid in codeine- and propoxyphene-type capsule and tablet analgesic formulations is presented. The sample material is treated with diazomethane prepared in tetrahydrofuran solution, and the salicylic acid is eluted as its methyl ester. Methyl *o*-methoxybenzoate serves as the internal standard. Peak areas are quantitated by means of an electronic digital integrator of wide input signal range capacity. The results obtained by applying the method to the analysis of 25 commercial preparations were in excellent agreement with those given by the trap column spectrophotometric procedure, except at salicylic acid levels exceeding about 15%. The proposed GLC technique precludes some drawbacks of the column method and is superior in many respects.

Keyphrases □ Salicylic acid in analgesic formulations—GLC analysis □ Codeine–aspirin formulations—GLC analysis of salicylic acid □ Propoxyphene–aspirin formulations—GLC analysis of salicylic acid □ Aspirin and codeine or propoxyphene formulations—GLC analysis of salicylic acid □ GLC—analysis, salicylic acid in analgesic formulations

The superior qualities of aspirin as an antipyretic and general analgesic have resulted in the proliferation of a wide variety of commercial products in which aspirin is formulated with antihistaminic, sedative, tranquilizer, and other analgesic therapeutic agents. Despite this multiplicity of preparations, the long-standing and well-documented problem of aspirin stability in commercial formulations is of continuing concern to manufacturing firms and pharmacopeial commissions. For example, it has become apparent that aspirin–propoxyphene capsule preparations tend to be much less stable than the more common aspirin formulations; realization of this fact is reflected in the 3.0% free salicylic acid limit allowed for such preparations in the NF XIII (1). Recently, the USP XVIII (2) raised the salicylic acid limit from 0.15 to 0.30% in compressed aspirin tablets and from 0.75 to 3.0% in coated or buffered tablets.

Over the years, numerous studies have been directed to the elucidation of the mechanism and to the factors governing this degradation. The collective conclusion is that, given the clear prerequisite of sorption of moisture (3, 4), the aspirin molecule appears to be at the mercy of its chemical environment, although other factors no doubt come into play. Interreactions between aspirin and other active components such as codeine (5), phenyl-