Colorimetric Determination of Acetaminophen

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Abstract □ A rapid method for routine determination of acetaminophen is presented which makes use of vanillin, which reacts with the hydrolytic product, *p*-aminophenol, to produce an intense yellow color suitable for photometric measurement. In typical cold capsules, many time-consuming separations are circumvented.

Keyphrases □ Acetaminophen dosage forms—analysis □ Vanillin—color reagent □ Colorimetric analysis—spectrophotometer

Vanillin is known to react with aromatic amines to produce stable yellow solutions of azomethine compounds (1). Since acetaminophen (APAP) is readily hydrolyzed to *p*-aminophenol (2), the color produced with a vanillin reagent should be utilizable in estimating acetaminophen.

Often, acetaminophen is compounded with several other ingredients in medicinal dosage forms such that its separation is difficult and time consuming.

Investigation was undertaken to see if separation might be circumvented and if the vanillin reaction would lend itself to a rapid quantitative method for acetaminophen in the complex mixture of cold capsules containing acetaminophen, salicylamide, ascorbic acid, phenylephrine hydrochloride, caffeine, pyrilamine maleate, and chlorpheniramine maleate.

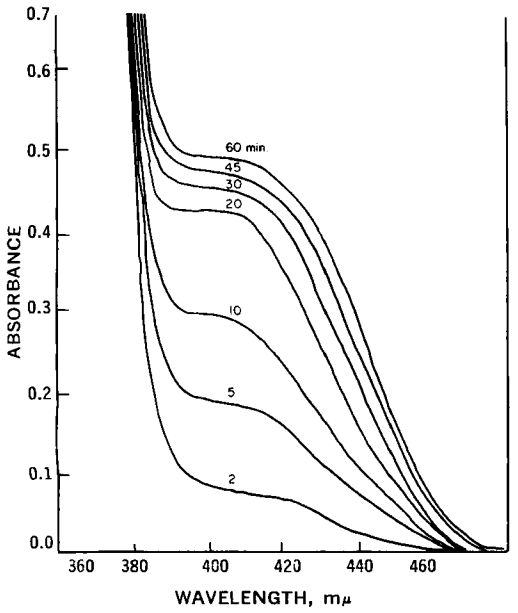

Figure 1—*Acetaminophen hydrolysis with time. Absorbance of azomethine compound after hydrolysis at 100°C. in 1* N *HCl. Concentration: 1.2 mg. acetaminophen/50 ml.*

Table I—Results of a Series of Determinations on an Accurately Weighed Mixture of Typical Capsule Components Containing 120 mg. APAP

Found, mg.	%
118.4	98.7
120.0	100.0
118.4	98.7
120.0	100.0
120.0	100.0
120.8	100.7
120.0	100.0
119.3	99.4
120.8	100.7
119.3	99.4
Mean, 119.7	Mean, 99.8

EXPERIMENTAL

Limited hydrolysis of acetaminophen occurs in the presence of strong acids at room temperature. It was found that 1 *N* hydrochloric acid resulted in a satisfactory degree of hydrolysis within a few minutes at elevated temperatures (Fig. 1). This hydrolysis may be accomplished for analytical purposes by subjecting acetaminophen to 1 *N* hydrochloric acid in a test tube in a beaker of boiling water. Complete hydrolysis with 1 *N* hydrochloric acid is not attained even after 45 min. Thus, in the interest of keeping the method short in time, 10 min. in boiling water was chosen and found satisfactory. When vanillin is added to the hydrolytic product, *p*-aminophenol, a stable, intense yellow color is produced, which shows a prominent absorbance peak near 395 mμ. Time and temperature of hydrolysis were found to be critical indicating the need for running a standard in parallel.

The hydrolysis or color production were not interfered with by the presence of salicylamide, pyrilamine maleate, chlorpheniramine maleate, ascorbic acid, phenylephrine hydrochloride, or caffeine. To these ingredients, in quantities expected in cold capsules, plus starch, tartaric acid, and magnesium stearate, 1 *N* HCl was added. Filtrate aliquots subjected to a boiling water bath showed negligible absorbance readings at 395 mμ when vanillin reagent was present.

Standard Curve—*Reagents*—Vanillin reagent, 5% in isopropanol m.p. 82–83.5°[1]; standard solution, 120 mg. acetaminophen (NF Reference Standard) in 100 ml. 1 *N* hydrochloric acid; Hydrochloric acid, 1 *N;* Blank, 6 ml. 1 *N* HCl, 10 ml. vanillin reagent, and enough water to make 50 ml.

Transfer by means of pipets, 0.5, 1.0, and 1.5 ml. of the standard solution to three test tubes. Add 5 ml. 1 *N* HCl. Place the tubes in a beaker of boiling water for 10 min. Allow to cool. Transfer tube contents quantitatively to 50-ml. volumetric flasks. Add 10 ml. of vanillin reagent to each flask and add water to the mark. Mix.

[1] Matheson Coleman & Bell.

Table II—Results of Duplicate Determinations on a Series of Commercial Products

Product	Label, mg./Tablet	Found, mg./Tablet
A	250	234
B	325	319
C	150	151
D	300	310
E	325	327
F	120	131

Measure the absorbance in 1-cm. cells against a proper blank in a spectrophotometer set to 395 mμ. The absorbances obtained plot into a straight line.

Procedure—Weigh contents of 10 capsules. Weigh an aliquot of the powder to correspond to 120 mg. acetaminophen. Transfer it to a suitable container and add about 50 ml. 1 *N* hydrochloric acid. Agitate with mechanical shaker for 20 min. Filter through paper quantitatively into a 100-ml. volumetric flask and bring to the mark with 1 *N* hydrochloric acid. Mix. Transfer 1.0 ml. to a test tube. Add 5 ml. of 1 *N* hydrochloric acid and heat for 10 min. in boiling water. Treat a standard solution of acetaminophen in the same fashion. Allow tubes to cool. Transfer quantitatively to 50-ml. volumetric flasks. Add 10 ml. vanillin reagent to each. Bring to the mark with water and mix. Measure the absorbances in 1-cm. cells against a blank in a spectrophotometer set to 395 mμ. To calculate milligrams of acetaminophen in aliquot of powder weighed, divide absorbance of sample by absorbance of standard and multiply by 120.

Remarks—The satisfactory application of the procedure was shown by determination of known synthetic powder mix in Table I and a number of commercial pharmaceutical products, Table II.

SUMMARY

A rapid method for the determination of acetaminophen in typical cold capsule medications is outlined. Because of the specificity of the reaction involved, many tedious separations may be circumvented, and its application to routine control testing has been demonstrated.

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Application of Absorbance Ratios to Analysis of Pharmaceuticals V: Analysis of Tetracycline Hydrochloride in Presence of Anhydrotetracycline and Epianhydrotetracycline

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Abstract □ The principles inherent in the absorbance ratio technique are applied to the analysis of mixtures containing tetracycline HCl, anhydrotetracycline, and epianhydrotetracycline. The analysis is carried out without prior separation of the components of the mixture and is applicable to commercial preparations containing these substances.

Keyphrases □ Tetracycline HCl tablets—analysis □ Epi- and anhydrotetracycline presence—tetracycline HCl analysis □ Absorbance ratio technique—tetracycline HCl analysis □ UV spectrophotometry—analysis.

Titrimetric (1, 2), polarographic (3, 4), chromatographic (5–9), spectrophotometric (10–14), and microbiological (15, 16) methods of analysis have been used to determine the tetracyclines. The precision of the latter method is rarely more than 15% (17) and accuracy is limited by the presence of biologically active tetracycline-like substances in the samples being analyzed. Spectrophotometric procedures tend to be more accurate, but are based on a prior separation by TLC (5, 6, 17, 18) or on a conversion of the antibiotic to an anhydro compound (13, 19, 20).

An examination of the spectrophotometric characteristics of tetracycline (TC), anhydrotetracycline (ATC), and epianhydrotetracycline (EATC) in 0.1 *N* hydrochloric acid solution indicates that these substances may be analyzed by using the absorbance ratio method of analysis (21, 22). This method is based on the linear relationship between absorbance ratio values (Q values) and the fraction of one of the components in a mixture. Q values are calculated from absorbance values at a wavelength of maximum absorption and at an isosbestic point. Conversion of the parent substance to secondary compounds and prior isolation procedures are, therefore, unnecessary.

EXPERIMENTAL

Apparatus—UV Spectrophotometer.[1,2]

[1] Beckman Model DU

[2] Bausch & Lomb Spectronic 505.