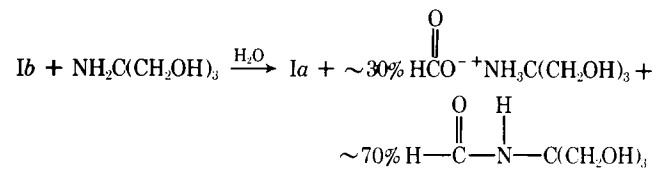

Scheme I

The rate of hydrolysis of cefamandole nafate to cefamandole upon the addition of sodium carbonate may be studied conveniently by NMR. The NMR spectrum of cefamandole nafate (Fig. 3A) differs from that of cefamandole (Fig. 3C) in the position of the α-phenylmethine proton peaks, at δ 6.2 and 5.3, respectively, and in the presence of a formyl proton peak at δ 8.2 in the ester. In cefamandole, the formyl proton is absent but occurs as a formate anion peak, δ 8.5 (Fig. 3B). By determining either the ratio of the cefamandole nafate methine peak (δ 6.2) to the cefamandole methine peak (δ 5.3) or, more conveniently, the ratio of the formyl peak (δ 8.2) to the formate anion peak (δ 8.5), one may calculate the percent hydrolysis at a given time. Figure 3B represents ~28% hydrolysis upon the addition of 0.28 mole of sodium carbonate.

Figure 4 shows the percent conversion of cefamandole nafate to cefamandole by 0.28, 0.60, and 0.90 molar equivalents of sodium carbonate at 25 ± 1°. The error indicated represents the 95% confidence interval determined from five experiments.

The aqueous hydrolysis of cefamandole nafate with tromethamine (Scheme I) and ethanolamine was also examined by NMR. In each case, upon the addition of 1.0 molar equivalent of amine, hydrolysis was complete within the time necessary to obtain the NMR spectrum. The NMR spectrum of the products of the tromethamine reaction indicated two new kinds of formyl peaks at δ 8.04 and 8.15 in addition to ~30% formate anion at δ 8.5. These new formyl peaks were identified as the formyl proton of *N*-formyl tromethamine in the *trans*- and *cis*-configurations of the amide bond (7). An authentic sample of *N*-formyl tromethamine was prepared by the reaction of methyl formate and tromethamine.

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Quantitative Determination of Theophylline in Pharmaceutical Dosage Forms by Differential Spectrophotometry

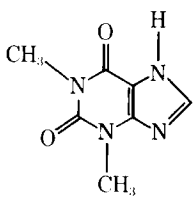
YOUNG SOON CHAE and WILLIAM H. SHELVER [x]

Abstract □ Theophylline was determined with good precision in tablets and capsules by differential spectrophotometry. Xanthines such as caffeine and theobromine did not interfere providing the total xanthine concentration was kept below 100 μg/ml. At a higher total xanthine concentration, nonlinearity occurred, presumably due to complex formation. This interference could be minimized by proper selection of the analytical wavelength.

Keyphrases □ Theophylline—analysis, differential spectrophotometry, pharmaceutical formulations □ Spectrophotometry, differential—analysis, theophylline in pharmaceutical formulations □ Aminophylline tablets and capsules—theophylline content analyzed by differential spectrophotometry

An argentimetric titration is described in the USP for the analysis of aminophylline and tablets containing aminophylline. The reactive component in the analysis is the theophylline (I) portion of aminophylline. The silver salt titration was compared with a spectrophotometric method, and some difficulties of the official method were discussed, particularly the effect of materials obscuring the end-point or interfering with filtration prior to titration (1). In addition, compounds with a structure similar to theophylline, such as theobromine (II), were reported (2) to interfere with the official method of analysis.

The direct spectrophotometric method, measuring the absorption peak in either acid (1) or base (3), is convenient and precise. However, it suffers from interference from other substances, such as theobromine and caffeine (III), absorbing in the same spectral region. Differential spectrophotometry offers the convenience and most of the precision of spectrophotometric



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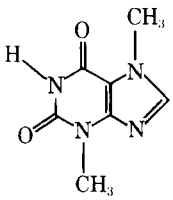

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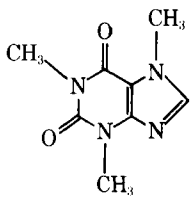

III

Table I—Percentage Recovery of Theophylline from Theophylline–Xanthine Mixtures Containing 50 μg of Total Xanthine/ml

Components	Analytical Wavelength of Differential Spectrophotometry[a] 281–290 nm	285 nm	290 nm	USP Method[b]	Direct UV Method[c]
Theophylline[a], 25 μg/ml	102.39 ± 2.32	102.73 ± 2.32	101.73 ± 2.14	99.18 ± 0.37	102.40 ± 4.44
Theophylline[a], 25 μg/ml, plus caffeine, 25 μg/ml	96.22 ± 0.74	96.04 ± 0.67	96.89 ± 0.64	81.99 ± 1.47	100.25 ± 3.40
Theophylline[a], 25 μg/ml, plus theobromine, 25 μg/ml	100.06 ± 1.85	99.56 ± 1.98	100.51 ± 2.49	197.88 ± 0.38	168.98 ± 2.99

[a] The values represent the mean of four determinations, two independent weighings with duplicate determinations from each. The error is the standard deviation of the four determinations. [b] The analyses were carried out utilizing the procedure described in USP XVIII. [c] The analyses were carried out using the Schack and Waxler method as described under *Experimental.*

methods and is much more specific (4). This report compares results from the application of the differential spectrophotometric method with those from the official analysis.

EXPERIMENTAL

Instrument—A double-beam spectrophotometer[1] was used for differential UV analysis, and incident radiation was maintained constant by utilizing the automatic slit programs for all determinations.

Reagents—Tromethamine buffer solutions of pH 7 and 9 were prepared according to the formula given in Ref. 5. All dosage forms were obtained commercially. Theophylline[2], caffeine[3], and theobromine[3] were the best quality commercially available. All other chemicals employed were analytical reagent grade and were used without further purification.

The dosage forms[4] analyzed were aminophylline tablets[5], 100 mg; aminophylline tablets[5], 200 mg; and capsules[6] containing 130 mg of aminophylline, 25 mg of ephedrine, and 25 mg of amobarbital.

USP Method—*Aminophylline Tablets*—The USP XVIII (6) method was followed.

Aminophylline Capsules—The contents of 12 capsules were removed as completely as possible and mixed with 50 ml of distilled water and 10 ml of ammonia TS in a 125-ml beaker. This mixture, including the capsules, was stirred on a magnetic stirrer for 30 min and then filtered by suction. The beaker and residue were washed with five 10-ml portions of distilled water. The filtrate was transferred into a 250-ml volumetric flask, the suction flask was washed with six 15-ml portions of distilled water, and the combined solution was diluted to volume. A 50.0-ml aliquot was used for the USP XVIII method (6).

Theophylline and Xanthine Mixtures—Two hundred milligrams of theophylline alone or mixed with 200.0 mg of caffeine or theobromine was used for the USP XVIII method.

Schack and Waxler Method—A procedure adopted from the original Schack and Waxler (3) method and utilized for the determination of theophylline in blood was used to test for potential interference of xanthine derivatives in the theophylline analysis. Theophylline, 30 μg, or theophylline, 30 μg, and either caffeine, 30 μg, or theobromine, 30 μg, in 1 ml of water were extracted with 15 ml of chloroform. After centrifuging, a 10-ml aliquot of the chloroform was removed and extracted with 5 ml of 0.1 *N* sodium hydroxide.

After centrifuging again, approximately 4 ml of the sodium hydroxide solution was heated in a boiling water bath for 30 min, cooled, and then read in a spectrophotometer at 310 and 274 nm. A set of theophylline standards at 15, 30, and 60 μg/ml was carried through the same procedure. The percent recovery of the theophylline is reported in Table I.

Differential UV Method—*Standard Curve*—Standard stock solutions of theophylline were prepared by dissolving 50.0 mg of anhydrous theophylline in sufficient tromethamine buffer solutions of pH 7 and 9 to make 500.0 ml. The stock solutions were diluted with each buffer solution to obtain the concentrations of 10, 20, 40, 50, and 60 μg/ml. The pH 7 solution was placed in the reference side, and the pH 9 solution was placed in the sample side.

All solutions were prepared in duplicate, and duplicate readings were obtained for all concentrations. The absorbances of the standard solutions were determined at 10 wavelengths (281–290 nm). The regression line of the absorbance–concentration curves through zero was determined with standard statistical procedures, calculated and plotted with a programmable calculator[7]. The regression analysis for the absorbance–concentration curves are shown in Table II.

Tablets—Twenty tablets of each preparation were accurately weighed to determine the average tablet weight. Then the tablets were crushed and ground to a fine powder with a mortar and pestle. A portion of each tablet powder, equivalent to 500.0 mg of theophylline, was accurately weighed in a 150-ml beaker, dissolved in 100 ml of distilled water, filtered through filter paper[8] into a 250-ml volumetric flask, and diluted to volume with distilled water through the filter paper. An aliquot of the filtrate was diluted to 100.0 ml with each buffer solution to obtain a theophylline concentration of 50 μg/ml. The absorbances were determined at 10 wavelengths (281–290 nm). Four different lots of 20 tablets were subjected to this procedure.

Capsules—The contents of 20 capsules (as one batch) were removed as completely as possible and mixed with 250 ml of distilled water. The mixture, including the capsules, was stirred on a magnetic stirrer for 30 min, filtered through filter paper into a 500-ml volumetric flask, and diluted to volume with distilled water passed through the filter paper. An aliquot of the filtrate was diluted to 100.0 ml with each buffer solution. The filtrates of four different batches of capsules were prepared, each with two separate aliquots, and the absorbances were determined at 10 wavelengths (281–290 nm).

Theophylline and Xanthine Mixtures—Five hundred milligrams of theophylline alone or with either caffeine or theobromine was accurately weighed and dissolved in distilled water to make 100.0 ml of each solution. Heating was necessary to dissolve theobromine. An aliquot of each solution was combined and diluted to 100.0 ml with each buffer solution to obtain a theophylline concentration of 25 or 50 μg/ml, mixed either with the same volume of distilled water or with the same concentration of caffeine or theobromine.

The aliquots of theobromine solution were taken while the solution was warm to prevent precipitation. Two separate weighings of each xanthine were subjected to this procedure, each with two dilutions. The absorbances were determined at 10 wavelengths (281–290 nm) and the percent recovery was calculated. The means and standard deviations were calculated with a programmable calculator[7].

RESULTS AND DISCUSSION

Differential spectrophotometry offers the convenience and most of the precision of direct spectrophotometric methods but is more specific. The method requires the component to exist in two forms that differ in their absorptivity. Most frequently, the forms are different ionization states; the ionization must affect the absorptivity so the two forms have different absorption spectra.

[1] Cary model 118C.
[2] Mann Research Laboratories.
[3] Eastman.
[4] Obtained from the proprietary laboratory, College of Pharmacy, North Dakota State University.
[5] Searle.
[6] Lilly Amsec.
[7] Hewlett-Packard model 10.
[8] Whatman No. 40.

Table II—Regression Analysis of Concentration–Absorbance Data for Theophylline[a]

Wavelength, nm	Slope[b]	95% Confidence Limits	SE of Slope
281	0.01063	0.01042–0.01083	0.000089
282	0.01069	0.01049–0.01089	0.000086
283	0.01075	0.01053–0.01096	0.000093
284	0.01078	0.01055–0.01102	0.000101
285	0.01079	0.01022–0.01055	0.000101
286	0.01073	0.01050–0.01097	0.000102
287	0.01061	0.01039–0.01082	0.000095
288	0.01035	0.01015–0.01055	0.000087
289	0.00968	0.00978–0.01015	0.000078
290	0.00948	0.00934–0.00961	0.000059

[a] The regression analysis was carried out utilizing a computer program, WHSR, written by W. H. Shelver. [b] The slopes are in terms of absorbance *versus* concentration in micrograms per milliliter. The slope was determined by utilizing duplicate measurements at 10, 20, 40, and 50 μg/ml.

Theophylline, with a pKa of 8.79 (7), undergoes a bathochromic shift (8) upon ionization and is well suited to analysis by differential spectrophotometry. After initiation of this study, Gupta and Lundberg (9) reported a method for the determination of theophylline in blood by differential spectrophotometry. These investigators utilized an acid solution prepared from 3 ml of 0.2 *N* sodium hydroxide and 0.5 ml of 2.5 *M* monobasic sodium phosphate in the reference cell and 0.2 *N* sodium hydroxide in the sample cell. To eliminate interference from either caffeine or theobromine, pH 7 tromethamine buffer was used in the reference cell and the pH 9 buffer was used in the sample cell.

Caffeine decomposes rapidly in strongly alkaline solutions, and the products differ in their absorption spectra, possibly causing changes in the difference spectra if a strongly alkaline solution is used in the sample cell. The rate of decomposition at pH 9 is slow enough so no noticeable effects are observed in the analysis. Theobromine, with a pKa of 9.95, is not appreciably ionized at pH 9 and produced little absorption under the analysis conditions. To obtain this specificity, some sensitivity is given up since the theophylline is not fully ionized under the analysis conditions.

Recovery experiments for theophylline alone and for mixtures of theophylline and caffeine and theophylline and theobromine are shown in Tables I and III. Observation of the spectral curves (Figs. 1 and 2) obtained from runs with relatively high total xanthine concentrations indicated the development of a strong peak at 274 nm and a slight decrease in the analytical peak at 285 nm, presumably from the formation of a complex (10). Consequently, two recovery experiments were run, one at high total xanthine concentrations and the other at low total xanthine concentrations. Observation of the spectral curves indicated that there was little interference at wavelengths above 290 nm, so the data were processed three ways: (*a*) averaging results from 10 wavelengths from 281 to 290 nm, (*b*) measurement at the peak at 285 nm, and (*c*) measurement at 290 nm where interference was at a minimum.

The USP method was also used to analyze the theophylline and theophylline–xanthine mixtures. The Schack and Waxler method utilized in many clinical laboratories for the determination of theophylline was applied to the analysis of theophylline and theophylline–xanthine mixtures. The recovery experiments run at low total xanthine concentrations (Table I) showed no appreciable difference between the methods of calculation utilized in the differential spectrophotometric method but demonstrated the superiority of this method to the USP method. As previously mentioned, theobromine interferes; the recovery experiment demonstrated that the USP method analyzes theobromine as if it were theophylline. Caffeine demonstrated a different type of interference in the USP method, lowering the recovery of theophylline by nearly 20%. The Schack and Waxler method also showed interference from theophylline but the caffeine removed both by extraction and heating in alkali showed no interference.

The recovery experiments run at high total xanthine concentrations

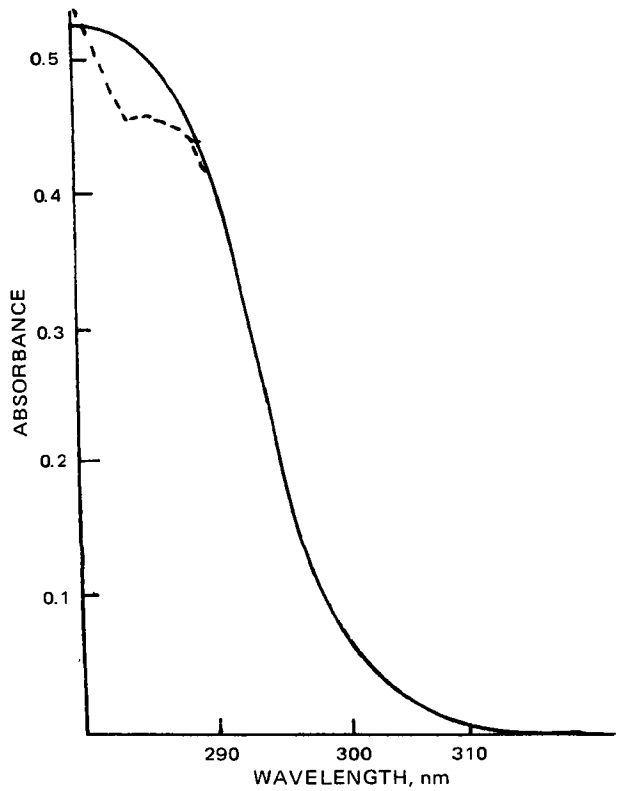

Figure 1—*Effect of increased nonabsorbing xanthine on the difference absorption spectrum of theophylline (pH 7 reference and pH 9 sample). Key: ——, 50 μg of theophylline/ml; and – – –, 50 μg of theophylline/ml plus 50 μg of caffeine/ml.*

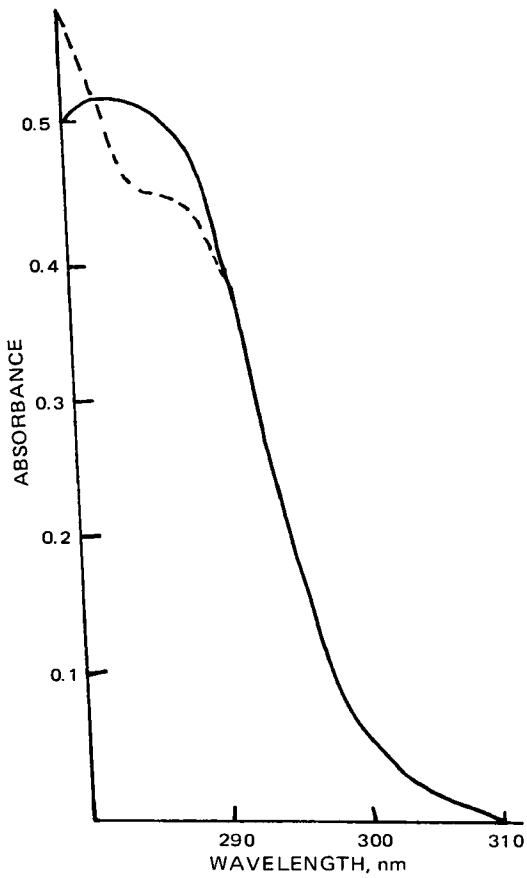

Figure 2—*Effect of increased nonabsorbing xanthine on the difference absorption spectrum of theophylline (pH 7 reference and pH 9 sample). Key: ——, 50 μg of theophylline/ml; and – – –, 50 μg of theophylline/ml plus 50 μg of theobromine/ml.*

Table III—Percentage Recovery of Theophylline from Theophylline–Xanthine Mixtures Containing 100 μg of Total Xanthine/ml

Components	Analytical Wavelengths of Differential Spectrophotometry[a]		
	281–290 nm	285 nm	290 nm
Theophylline, 50 μg/ml	98.50 ± 2.53	98.62 ± 2.55	97.99 ± 2.24
Theophylline, 50 μg/ml, plus caffeine, 50 μg/ml	89.83 ± 1.96	84.49 ± 2.21	91.87 ± 1.78
Theophylline, 50 μg/ml, plus theobromine, 50 μg/ml	93.00 ± 1.04	86.23 ± 1.27	95.01 ± 0.77

[a] The values represent the mean of eight determinations, four independent weighings with duplicate determinations for each. The error is the standard deviation of the eight determinations.

Table IV—Analysis of Theophylline in Dosage Forms

Dosage Form	281–290 nm	285 nm	290 nm	USP Method[b]
Tablet, 100 mg[a]	101.44 ± 1.27	101.55 ± 1.29	102.53 ± 0.69	—[c]
Tablet, 200 mg[a]	101.23 ± 0.71	101.89 ± 1.03	101.79 ± 0.89	100.54 ± 1.36
Capsule, 130 mg[a], plus ephedrine, 25 mg, plus amobarbital, 25 mg	98.35 ± 0.72	98.67 ± 0.77	99.05 ± 0.50	96.47 ± 1.31

[a] Each value represents the mean of four determinations and is expressed as the percent of the labeled amount. [b] The analyses were carried out utilizing the procedure described in USP XVIII. [c] The presence of coloring matter obscured the end-point of the USP method.

(Table III) demonstrated the interference observed in the spectral curves shown in Figs. 1 and 2. Calculation of the results in the high xanthine recovery experiments demonstrated the advantage of calculating results at 290 nm compared to using either an average of 10 wavelengths or the peak at 285 nm. The results from Tables I and III also indicate the desirability of keeping the concentration of total xanthine in the solutions being read below 50 μg/ml.

The results obtained in the analysis of pharmaceutical dosage forms are shown in Table IV. Three dosage forms were analyzed: a tablet containing 100 mg of aminophylline, a tablet containing 200 mg of aminophylline, and a capsule containing 130 mg of aminophylline, 25 mg of ephedrine, and 25 mg of amobarbital. The results were calculated by the same three methods already described and show no appreciable differences. The precision of the differential spectrophotometric method was comparable with the USP method. The USP analysis of the 100-mg tablets failed to give acceptable results, presumably because color change made observation of the end-point difficult. The 100- and 200-mg tablets were of obviously different formulations, even though produced by the same company, and no difficulty was observed in analyzing the 200-mg tablets by the USP method.

The results from the analyses of the capsule indicated no interference from either ephedrine or amobarbital. An attempt was also made to analyze a syrup containing theophylline without prior extraction of the theophylline. The analysis of this product showed obvious spectral interference when differential spectrophotometry was employed. The direct application of the USP method also failed to give satisfactory results because of the colored material in the syrup.

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