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Spectrophotometric Determination of Theophylline Formulations

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Abstract □ Minophylline (theophylline ethanoate of piperazine) and aminophylline (theophylline ethylenediamine) were determined spectrophotometrically in dosage forms without interference from excipients and/or preservatives. A mixture of minophylline, in about 30-fold concentration, with phenobarbital was assayed for both components with good accuracy and high reproducibility.

Keyphrases □ Minophylline—spectrophotometric analysis in pharmaceutical formulations □ Aminophylline—spectrophotometric analysis in pharmaceutical formulations □ Spectrophotometry—analyses, minophylline and aminophylline in pharmaceutical formulations □ Diuretic-vasodilators—minophylline, spectrophotometric analysis in pharmaceutical formulations □ Relaxants, smooth muscle—aminophylline, spectrophotometric analysis in pharmaceutical formulations

The assay of binary mixtures in pharmaceutical formulations is challenging. One example is minophylline[1] and phenobarbital mixtures, especially when the latter component is present in small amounts. The interference of excipients and/or preservatives increases the severity of the problem.

BACKGROUND

The various methods dealing with the correction of interfering absorbances were reviewed (1, 2). The correction of linear interferance can be carried out graphically (3) or algebraically (4-7). By applying the algebraic version to the correction of linear impurity absorption, the concentration, C, can be determined from:

$$C = \frac{A_1(\lambda_2 - \lambda_3) - A_2(\lambda_1 - \lambda_3) + A_3(\lambda_1 - \lambda_2)}{E_1(\lambda_2 - \lambda_3) - E_2(\lambda_1 - \lambda_3) + E_3(\lambda_1 - \lambda_2)} \quad \text{(Eq. 1)}$$

in which A_1, A_2, and A_3 are the absorbances at λ_1, λ_2, and λ_3, respectively; E_1, E_2, and E_3 are the corresponding 1-cm path length absorbances of a 1% solution. Dividing both numerator and denominator by $(\lambda_1 - \lambda_3)$ and substituting h for $(\lambda_2 - \lambda_3)/(\lambda_1 - \lambda_3)$ give the following equation after simple rearrangement:

$$A_2 - hA_1 - (1-h)A_3 = C[E_2 - hE_1 - (1-h)E_3] \quad \text{(Eq. 2)}$$

Substitution of the left-hand term by corrected A (A_c) and the second term in the right-hand side by K yields:

$$A_c = CK \quad \text{(Eq. 3)}$$

A linear relationship is obtained by plotting A_c *versus* C.

Another method for the correction of interfering absorbances is Glenn's method of orthogonal function (8), in which absorbance A is replaced by the coefficient of the orthogonal function, p_j. This coefficient is proportional to concentration. To extract the coefficient of a given polynomial from an absorption curve, it is necessary to obtain absorbances at a number of equally spaced wavelengths. Thus, to extract the coefficient of the quadratic polynomial p_2, for example, six absorbance measurements at six equally spaced wavelengths are needed. By plotting the p_2 at different intervals *versus* λ_m (the mean set of wavelengths), a convoluted absorption curve is obtained (9).

The present paper reports the determination of minophylline in the presence of the tablet base, sweetening agent, coloring agent, and preservatives usually existing in pharmaceutical preparations; the determination of aminophylline in ampuls containing benzyl alcohol as a preservative; and an assay for a minophylline-phenobarbital mixture in syrup. Determination of phenobarbital in this mixture is difficult since it is present in a small amount.

EXPERIMENTAL

Materials—Minophylline[2] and aminophylline[3] standard solutions were at a concentration of 1 mg/ml in 0.1 N H_2SO_4. Phenobarbital sodium[4] standard solution was 1 mg/ml in water. Minophylline tablets[2], Batch 7, contained 250 mg/tablet; minophylline ampuls[2], Batch 29, contained 200 mg/2 ml.

Minophylline-phenobarbital[2], Batch 101,004, contained 2.0 g of minophylline and 0.06 g of phenobarbital/100 ml. Aminophylline ampuls[5], Batch S/52D, contained 500 mg of aminophylline/2 ml and 0.04 ml of benzyl alcohol as the preservative.

Reagents—Analytical grade 0.1 N H_2SO_4, 0.5 N NaOH, 0.25 M Na_2CO_3 (anhydrous), 0.25 M $NaHCO_3$, and alcohol were used.

Instruments—A photoelectric spectrophotometer[6] with 1-cm silica cells was used.

Procedures—*Standard Curves for Minophylline and Aminophylline Using* A_c *Method*—Different solutions containing 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mg % minophylline were prepared by dilution with 0.1 N H_2SO_4. The absorbance of each solution was measured at λ_1 246 nm, λ_2 274 nm, and λ_3 295 nm.

For aminophylline, the concentrations prepared were 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, and 2.1 mg %; λ_1, λ_2, and λ_3 were 242, 270, and 287 nm, respectively. The A_c for each concentration of minophylline or aminophylline was calculated.

Standard Curve for Minophylline Using p_2 *Method*—The absorbances of the same solutions were measured at 266, 270, 274, 278, 282, and 286 nm. The coefficient p_2 for each concentration was calculated.

Standard Curve for Phenobarbital Applying ΔA *Method*—Two sets of solutions were prepared so that each contained 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mg % phenobarbital. One set was prepared in 0.1 N NaOH (Solution A), and the other was prepared in a mixture of 0.025 M Na_2CO_3 (anhydrous) and 0.025 M $NaHCO_3$ (Solution B). The absorbance of Solution B was measured at 238 nm using Solution A as a blank. Then Solution A was measured at 260 nm using Solution B as a blank. The $\Sigma\, \Delta A_{238}$ and ΔA_{260} for each concentration were calculated.

Assay for Pharmaceutical Preparations—*Minophylline Tablets*—From powdered tablets (10 tablets were powdered and mixed), an

[1] The theophylline ethanoate of piperazine. The International Nonproprietary Name is acefylline piperazine.

[2] Alexandria Company for Pharmaceutical and Chemical Industries.
[3] Boehringer Ingelheim, Germany.
[4] VEB Chemische Werk, Germany.
[5] Burroughs Wellcome and Co.
[6] Prolabo, Paris, France.

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Table I—Assay Results for Theophylline Formulations

Preparation	n	Mean Percentage ± CV, %: A_c Method	A Method	p_2 Method
		Minophylline Tablets and Ampuls		
Tablets[a]	12	99.65 ± 0.78 (2.27)[b]	100.47 ± 0.98	99.64 ± 1.49 (3.07)
Commercial tablets	12	101.77 ± 0.82 (2.66)	102.66 ± 0.79	101.17 ± 1.70 (2.71)
Ampuls	8	93.56 ± 0.61 (7.94)	96.79 ± 1.03	93.00 ± 1.55 (6.12)
		Aminophylline Ampuls		
Solution[c]	5	99.94 ± 0.79 (9.41)	104.73 ± 0.78	—
Ampuls	5	100.31 ± 0.34 (13.25)	105.57 ± 0.78	—
		Minophylline–Phenobarbital Mixture		
Minophylline	11	101.15 ± 0.71 (17.85)	107.49 ± 0.87	99.30 ± 1.16 (18.29)
		ΔA_{238} Method	ΔA_{260} Method	ΔA_T Method
Phenobarbital	8	92.75 ± 2.91	103.90 ± 3.00	98.36 ± 1.21

[a] The tablet powder was prepared in the laboratory by weighing 250 mg of minophylline and adding 0.5 g of commercial lactose. [b] The figures in parentheses are the calculated t values with reference to the A method; theoretical t (α = 0.05) = 2.306 (for df 8), 2.145 (for df 14), 2.086 (for df 20), and 2.074 (for df 22). [c] A volume of 10 ml of aminophylline solution (250 mg/ml) to which 0.2 ml of benzyl alcohol was added.

accurately weighed quantity equal to about 0.7 g was extracted with three 30-ml portions of 0.1 N H_2SO_4 and suitably diluted for spectrophotometric measurement.

Minophylline Ampuls—The contents of five ampuls were mixed together in a dry conical flask. A measured volume was suitably diluted with 0.1 N H_2SO_4 for spectrophotometric measurement.

Aminophylline Ampuls—This assay was as described for minophylline ampuls.

Minophylline–Phenobarbital Syrup—Minophylline was determined as described for minophylline ampuls by suitably diluting a measured volume with 0.1 N H_2SO_4. Phenobarbital was assayed by transferring a measured volume to a separator. The solution was acidified with dilute sulfuric acid and extracted with four 25-ml portions of chloroform. The extract was evaporated on a water bath, and the residue was dissolved in ethanol and quantitatively transferred to a volumetric flask (50 ml).

Two similar volumes were transferred into 50-ml measuring flasks, one containing 5 ml of 1 N NaOH (Solution C) and the other containing a mixture of 5 ml of 0.25 M Na_2CO_3 and 5 ml of 0.25 M $NaHCO_3$ (Solution D). The contents were diluted to volume. The absorbance (ΔA_{238}) of Solution D was measured at 238 nm using Solution C as a blank, followed by measurement of Solution C against Solution D at 260 nm.

RESULTS AND DISCUSSION

With the conventional spectrophotometric method, the absorbances of the prepared solutions in 0.1 N H_2SO_4 were measured at λ_{max} 274 nm for minophylline and at 270 nm for aminophylline. Beer's law was valid within concentration range of 0.3–1.8 mg % for minophylline and of 0.3–2.1 mg % for aminophylline. The calibration curves can be described by the following regression equations:

$$A_{274} = -0.0002 + 0.0290C \text{ (for minophylline)} \quad \text{(Eq. 4)}$$

$$A_{270} = -0.002 + 0.4251C \text{ (for aminophylline)} \quad \text{(Eq. 5)}$$

On application of the A method, a high mean percent recovery (Table I) was obtained. The contribution of irrelevant absorbance led to high results.

The absorbances of interfering substances, *e.g.*, sweetening agents, binders, diluents, and fillers, varied linearly with wavelength (10). To correct the linear impurity absorbance, the absorbances of the minophylline solution were measured at λ_1 246 nm, λ_2 274 nm, and λ_3 295 nm (Fig. 1). For the aminophylline solution, λ_1, λ_2, and λ_3 were 242, 270, and 287 nm, respectively.

The A_c can be calculated from the following formulas:

$$A_c = A_{274} - (21/49)A_{246} - (28/49)A_{295} \text{ (for minophylline)} \quad \text{(Eq. 6)}$$

and:

$$A_c = A_{270} - (17/45)A_{242} - (28/45)A_{287} \text{ (for aminophylline)} \quad \text{(Eq. 7)}$$

Within a concentration range of 0.3–1.8 mg % for minophylline and of 0.3–2.1 mg % for aminophylline, A_c *versus* C showed a linear relationship. The corresponding calibration curves can be described from the following regression equations:

$$A_c = 0.0010 + 0.1697C \text{ (for minophylline)} \quad \text{(Eq. 8)}$$

$$A_c = 0.0040 + 0.3030C \text{ (for aminophylline)} \quad \text{(Eq. 9)}$$

With the orthogonal function method, the absorbances of minophylline solution were measured over the 266–286-nm wavelength range at 4-nm intervals. The quadratic coefficient was calculated by:

$$p_2 = [(+5)A_{266} + (-1)A_{270} + (-4)A_{274} + (-4)A_{278} + (-1)A_{282} + (+5)A_{286}]/84 \quad \text{(Eq. 10)}$$

The numbers between brackets are given in standard texts (11, 12), and the divisor 84 is the normalizing factor. Within a concentration range of 0.3–1.8 mg %, p_2 *versus* C showed a linear relationship. The calibration curve can be described by:

$$p_2 \times 10^3 = -0.1410 - 8.1465C \quad \text{(Eq. 11)}$$

The wavelength range (Fig. 1) of 266–286 nm (λ_m 276) at 4-nm intervals was chosen as the analytical set, because the p_2 value is maximum and q_2 (where $q_2 = p_2\sqrt{N}$ and N is the normalizing factor 84) for a solution of 1.9 mg % (w/v) minophylline in 0.1 N H_2SO_4 was found to exceed 0.140[10].

The results of the assay for different pharmaceutical preparations are presented in Table I. The following conclusions were made.

The mean percentage from results of the A method is either slightly or distinctly higher than that of the A_c and p_2 methods. These data were

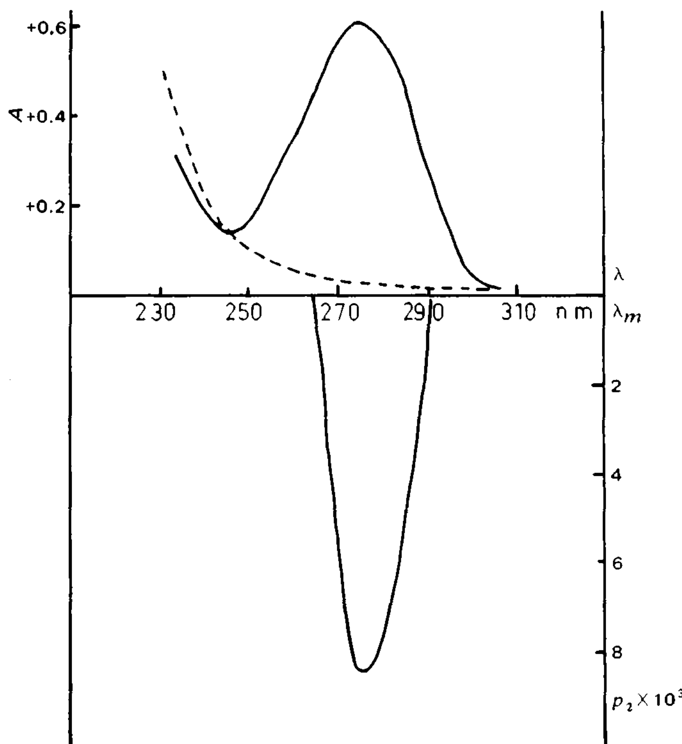

Figure 1—*Spectra of minophylline (—) (1 mg %) and convoluted curve therefrom and phenobarbital (- - -) (2 mg %). (The solvent was 0.1* N *H_2SO_4.)*

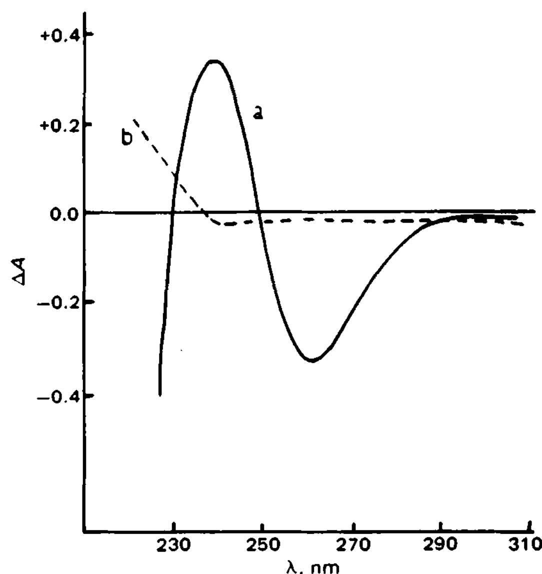

Figure 2—*Curve of phenobarbital (a) (2 mg %) and minophylline (b) (0.5 mg %).*

subjected to statistical analysis. Since the calculated t value (α = 0.05) is higher than the theoretical value (Table I), the null hypothesis is rejected (13) and the results of the A_c and p_2 methods are considered more accurate. Therefore, the irrelevant absorbance due to excipients in pharmaceutical formulations can be corrected by using the A_c and p_2 methods.

The irrelevant absorbance due to benzyl alcohol is corrected by applying the A_c method, although the spectrum of benzyl alcohol exhibits typical benzenoid structure. It exhibits maxima at 254 ($A^{1\%}_{1cm} \simeq 40$) and 260 ($A^{1\%}_{1cm} \simeq 34$) nm. Because of the low absorptivity and relatively small concentration (*i.e.*, in a ratio of ~1:12.5 to aminophylline) of benzyl alcohol, canceling of its irrelevant absorbance by application of the A_c method is possible.

The coefficient of variation from the results of the p_2 method is always high compared with the A and A_c methods. Such error in the p_2 method can be attributed to wavelength-setting errors since extinction measurements are usually made on the slopes of the absorption curves (14). Therefore, for its simplicity and high reproducibility, the A_c method is preferable to the p_2 method.

The presence of minophylline and phenobarbital in a ratio of about 30:1 in syrup necessitates the separation of phenobarbital prior to its estimation. In the assay of minophylline in the presence of phenobarbital, there is no problem since the latter absorbs minimally. Furthermore, the absorbance of phenobarbital in an acid medium is small and varies linearly with wavelength (Fig. 1). Such absorbance was treated as irrelevant absorbance, *i.e.*, corrected by the A_c and p_2 methods (Table I).

Phenobarbital was determined by the application of the ΔA method (15) at λ_{238} (ΔA_{238}) and λ_{260} (ΔA_{260}) nm. The contribution of the differential absorbance of minophylline (that could be extracted with phenobarbital) is negligible (Fig. 2). For both ΔA_{238} and ΔA_{260} methods, Beer's law is valid within a 0.5–5-mg % concentration range. The regression equations are:

$$\Delta A_{238} = 0.0119 + 0.1605C \quad \text{(Eq. 12)}$$

$$\Delta A_{260} = 0.0243 + 0.1530C \quad \text{(Eq. 13)}$$

$$\Delta A_T = 0.0363 + 0.3135C \quad \text{(Eq. 14)}$$

where ΔA_T is ($\Delta A_{238} + \Delta A_{260}$).

The results obtained from ΔA_{238}, ΔA_{260}, and ΔA_T are presented in Table I.

The ΔA_{238} method gave lower results than the ΔA_{260} method while ΔA_T gave a mean value for both. The low results of ΔA_{238} are attributed to the differential absorbance of minophylline (Fig. 2), *i.e.*, negative error is obtained. Such error becomes positive on reversing the cells in the ΔA_{260} method. On summing ΔA_{238} and ΔA_{260}, these errors cancel each other. Therefore, it is not surprising that ΔA_T results are more accurate and give lower coefficients of variation. Moreover, on summing ΔA_{238} and ΔA_{260}, a higher slope value is obtained, which renders ΔA_T more sensitive.

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