Separation and Spectrophotometric Determination of Theophylline and Hydroxyethyltheophylline in a Pharmaceutical Syrup

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Abstract □ A simple chromatographic procedure was developed for the separation and determination of theophylline and hydroxyethyltheophylline. An aliquot of diluted syrup was pipetted into a chromatographic column containing a strong anion-exchange resin, which retains theophylline and allows the hydroxyethyl derivative to pass through it. Theophylline was subsequently eluted with 1 *N* HCl and determined by difference spectrophotometry at 284 nm; its derivative was determined at 272 nm.

Keyphrases □ Theophylline—chromatographic separation from hydroxyethyl derivative in pharmaceutical syrup, spectrophotometric analyses □ Hydroxyethyltheophylline—chromatographic separation from theophylline in pharmaceutical syrup, spectrophotometric analyses □ Chromatography, ion exchange—separation of theophylline and hydroxyethyl derivative in pharmaceutical syrup □ Spectrophotometry—analyses, theophylline and hydroxyethyl derivative in pharmaceutical syrup

Theophylline (I) is widely used for the treatment of asthma. Because of its low solubility, oral dosage forms are sometimes formulated with a more soluble derivative to enhance theophylline availability for prompt therapeutic effect. Although several methods were reported for the determination of theophylline alone and in combination with other drugs (1–5), few reports concerned the assay of its mixture containing hydroxyethyltheophylline (etofylline) (II). Kaniewska and Zyzynoky (6) analyzed I in the presence of II using a spectrophotometric titration in which the absorbance of argentometrically determined theophylline was subtracted from the total absorbance of both compounds and the content of II was calculated.

A titrimetric procedure also was reported (7) in which the determination of II was carried out by precipitating theophylline with excess mercuric acetate. The excess was then determined in a portion of filtrate by titration with ammonium rhodanide; another portion of filtrate was treated with excess 0.1 *N* iodide to determine II. Stuchlik *et al.* (8) described an electrophoresis method for the purification of II and hydroxypropyltheophylline from the unchanged starting substances. These procedures, although giving the required separation and determination of the two active ingredients in other formulations, were unsuitable for the syrup, where the weight ratio of theophylline to II was 8:1. Consequently, a new analytical method was needed.

EXPERIMENTAL

Apparatus—A recording spectrophotometer[1] with matched UV 1-cm cells was used. The glass column was 35 × 1 cm with a fritted glass disk and polytef stopcock.

Reagents—The following were used: 100–200-mesh ion-exchange resin[2], 6 *N* HCl in 50% (v/v) ethanol, 1 *N* HCl in water, 4 *N* NaOH in water, and 1 *N* NaOH in water.

[1] Pye Unicam SP-1800.
[2] Amberlite CG-400 (OH), British Drug Houses.

Table I—Recovery of I and II Mixed Standard in 50% (v/v) Ethanol

Mixture	I Amount Taken, mg	I Recovery, %	II Amount Taken, mg	II Recovery, %
a	79.3	98.3	9.7	101.0
b	80.5	97.9	9.3	100.6
c	81.1	99.0	9.8	99.7
d	79.9	98.7	10.1	98.8
e	79.7	97.9	9.7	101.3
f	81.4	97.8	9.9	98.7
g	80.7	98.3	9.4	99.1
h	80.1	99.1	10.5	98.8
Average, %	—	98.4	—	99.8
SD, %		±0.51		±1.07

Standard Solutions—Theophylline USP (anhydrous powder), 0.064 mg/ml, is prepared by dissolving about 64 mg, accurately weighed, in 100 ml of 1 *N* HCl and diluting stepwise with the same solvent. Hydroxyethyltheophylline (Aust. Phar. grade), 0.008 mg/ml, is prepared by dissolving about 80 mg, accurately weighed, in 100 ml of distilled water and diluting stepwise with the same solvent.

Column Preparation—Slurry 3 g of resin with 20–35 ml of water. Decant off the floating materials until the supernate is clear. Wash once with ethanol and then with water. Transfer to a column with the stopcock closed and let the resin settle by gravity. Drain the water and wash the resin with five 10-ml portions of 6 *N* HCl. Stir gently after each addition and then rinse twice with 50-ml portions of water. Add 20 ml of 4 *N* NaOH to the column and stir gently for a few seconds. Wash with water until the eluate is pH 4–7. Finally, top with a small pledget of glass wool and drain to level.

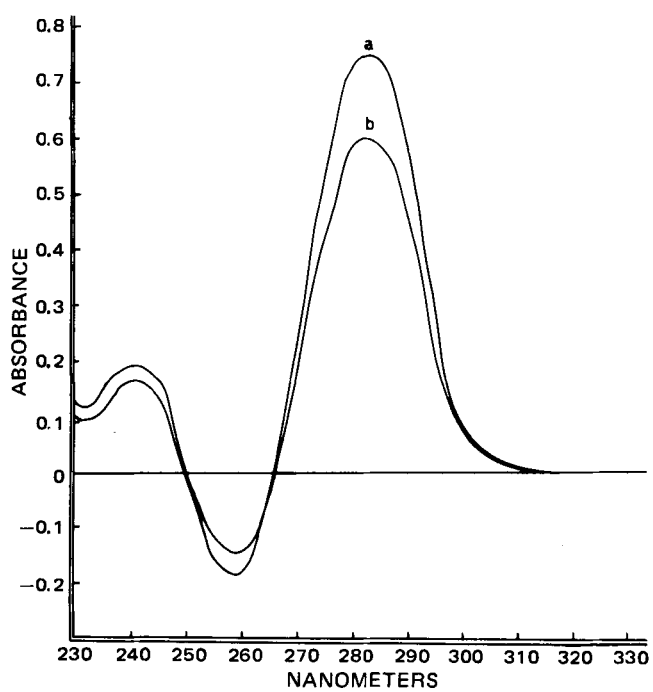

Figure 1—*Differential absorption spectra of 0.0190 mg of theophylline/ml (a) and 0.0152 mg of theophylline/ml plus saccharin sodium (b).*

Table II—Recoveries of I and II in the Two Laboratory Prepared Syrups

	Syrup 1				Syrup 2			
	I		II		I		II	
Test	Taken, mg	Found, %	Taken, mg	Found, %	Taken, mg	Found, %	Taken, mg	Found, %
1	81.0	98.5	10.5	97.9	80.0	101.3	9.5	100.6
2	80.9	97.9	11.6	100.5	79.9	100.0	9.3	98.8
3	80.1	99.8	12.0	100.9	75.6	98.8	10.4	97.8
4	79.8	99.9	9.5	98.5	77.6	97.8	9.1	99.3
5	81.2	100.3	9.7	97.9	81.3	99.1	9.6	98.8
6	82.0	98.1	10.5	99.3	82.0	98.6	11.4	101.5
7	75.0	99.4	10.3	101.6	79.9	98.3	12.0	100.7
8	79.9	100.7	11.6	100.3	79.8	99.3	9.1	98.6
Average, %	—	99.3	—	99.6	—	99.2	—	99.5
SD, %		±0.78		±1.42		±1.09		±1.27
CV		0.79		1.43		1.10		1.28

Repeat the treatment after each analysis.

Sample Treatment—Using a 10-ml pipet, draw the syrup[3] just to the meniscus and transfer to a 25-ml volumetric flask, allowing 5 min for drainage. Dilute to volume with distilled water and mix.

Determination—*Compound II*—Pipet 3 ml of diluted syrup into the prepared column and let the sample solution pass through at a rate of 1 ml/min. Collect the eluate in a 100-ml volumetric flask placed beneath the column. Add more water (80 ml) divided into portions, allowing each portion to sink into the resin. Remove the flask, dilute to volume with water, and mix. This solution contains II. Scan the sample and the standard solutions between 230 and 310 nm in 1-cm cells against water. Determine the absorbance at the maximum, about 272 nm, and calculate the concentration of II in the sample.

Theophylline—Drop the level of liquid in the column just above the glass wool pledget and then elute the column with 1 *N* HCl at a rate of 2 ml/min, collecting the eluate into a 100-ml volumetric flask to the mark. Pipet a 4-ml aliquot of this solution into each of two 50-ml volumetric flasks. To one flask, add water to volume (Solution A); to the other, add 4.1 ml of 1 *N* NaOH and then water to volume (Solution B). Measure the absorbance maximum of Solution B at 284 nm in 1-cm cells against Solution A. Compare the absorbance of the sample with that of the standard solution treated in a like manner (excluding the chromatographic step) and calculate the theophylline concentration in the syrup.

RESULTS AND DISCUSSION

Results for synthetic mixtures containing the two active ingredients alone are reported in Table I. The mixtures were dissolved in 50% (v/v) ethanol and then treated as the sample. The method was subjected to a test of the effect of excipients[4] by using excipient mixtures similar to the commercial product under study. The recoveries of two laboratory prepared syrups containing I, II, and excipients are shown in Table II. These simulated syrups were used for calculating the average, standard deviation, and coefficient of variation. Each syrup was assayed eight times.

The close agreement between the amount of each component taken and the determined value suggests that the procedure is unaffected by the excipients. Coloring material in this pharmaceutical preparation was held on the resin, and the other ingredients that passed through the column did not affect the direct spectrophotometric determination of II. A direct spectrophotometric determination of theophylline at about 271 nm would have been affected by the presence of saccharin sodium. This ingredient was held by the resin and eluted together with theophylline.

[3] The analysis was applied to a syrup containing 80 mg of I and 10 mg of II/15 ml.

[4] Excipients used were alcohol 95% BP, sucrose BP, saccharin sodium BP, amaranth BPC, and apricot flavor Naarden.

Table III—Analyses of Commercial Products

Sample	Component	Amount Declared, mg/5 ml	Amount Found, mg/5 ml	Percent of Declared Amount
A	I	80.0	81.5	101.9
	II	10.0	10.7	107.0
B	I	80.0	79.3	99.1
	II	10.0	9.6	96.0
C	I	80.0	82.3	102.9
	II	10.0	10.3	103.0
D	I	80.0	83.2	104.0
	II	10.0	10.5	105.0
E	I	80.0	76.8	96.0
	II	10.0	9.8	98.0

To cancel this interference, differential spectrophotometry was adopted for theophylline. Figure 1 shows the differential spectra of two prepared mixtures, one containing theophylline with all ingredients, including saccharin sodium, and the other without saccharin sodium. This technique was possible since theophylline, as a weak acid, can undergo a pH-induced shift. Although the conventional spectral change of solutions containing theophylline in acid and alkaline media are apparently minor (about 4 nm), useful difference spectra, with two maxima at about 240 and 284 nm and two isosbestic points, are produced, recording base against acid. An accurate adjustment of pH was not necessary to have theophylline, with a single ionizable group, directly conjugated to a chromophore. It was important to have the solutions of theophylline (acidic and alkaline) at least 2 pH units above and below the pK value to ensure that each species is in equilibrium at 99% spectral purity. Analyses of commercial products are shown in Table III.

This method is rapid and simple and allows the determination of the therapeutic ingredients from a single sample aliquot.

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