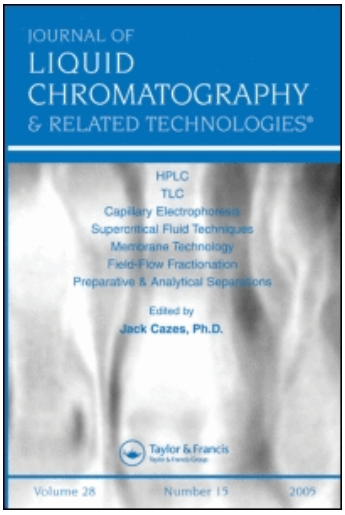

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Simultaneous High Performance Liquid Chromatographic Determination of Theophylline and Ethylenediamine in Aminophylline Dosage Forms as Their Dansyl Derivatives

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SIMULTANEOUS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF THEOPHYLLINE AND ETHYLENEDIAMINE IN AMINOPHYLLINE DOSAGE FORMS AS THEIR DANSYL DERIVATIVES

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ABSTRACT

A simple HPLC method has been developed for the assay of the components of aminophylline (theophylline: ethylenediamine 2:1) in solid and liquid dosage forms. Following the extraction of tablets into or the dilution of a liquid dosage form with water, the aqueous extract was reacted with dansyl chloride in an alkaline medium. The mixture of the dansyl derivatives of theophylline and ethylediamine was analyzed on an reversed phase μBondapak C_{18} column, using a methanol-water-acetic acid-triethylamine (60-65:33-38:1.5:0.5) mobile phase delivered at the rate of 1.5 mL/min, and a detection wavelength of 254 nm. In addition to exhibiting excellent accuracy and precision, the method yielded detector responses that were linearly related to concentrations of theophylline and ethylenediamine in aminophylline of up to 7 mg of theophylline, and of up to 10 mg

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of ethylenediamine. The proposed method was found to be equally applicable to the assay of tablets, injections and oral solutions. Assay values were validated by a direct HPLC method (theophylline) and by the titrimetric method of USP XXII (ethylenediamine), and the inter-method agreements were found to be close.

INTRODUCTION

Aminophylline, a 2:1 mixture of theophylline and ethylenediamine, is a widely used bronchorelaxant which is commercially available in a variety of solid and liquid dosage forms.

The official (USP XXII) assay method for aminophylline powder, tablets and oral solutions requires two separate titrations and a lengthy and multistep sample preparation for the theophylline part (1). In contrast, that for the determination of the theophylline content of enemas and individual tablets is based on UV spectrophotometry (1).

Although numerous methods have been reported for the assay of aminophylline in parmaceutical samples (2-8), the majority of them measure only theophylline (2-5), whereas the remaining few measure exclusively ethylenediamine (6-8). Assay methods for the two components of aminophylline appear to be limited to a differential nonaqueous potentiometric titration procedure (9), to the separate titration of each drug after their resolu-

tion on an anion-exchange resin (10), and to a HPLC method requiring the prior derivatization of ethylenediamine and its separate analysis from theophylline (11). The simultaneous analysis of both theophylline and ethylenediamine in aminophylline tablets has been accomplished by proton NMR spectroscopy (12), but this approach is not applicable to liquid dosage forms.

The alternative assay method described in this report permits the simultaneous measurement of the two components of aminophylline in commercial tablets, injections and oral solutions. The method is based on the HPLC separation of the dansyl derivatives of theophylline and ethylenediamine on a reversed phase C_{18} column, using a quaternary mobile phase and photometric detection. In this manner, the assays can be accomplished with a minimum of reagents and procedural steps, and with the required linearity, accuracy and precision.

EXPERIMENTAL

Reagents

a. Dansyl chloride (DNS-Cl) solution - Prepared by dissolving DNS-Cl (Aldrich Chemical Co.) in reagent grade acetone and filtering. This solution, containing 5 mg/mL, was stored in an amber glass bottle and in the refrigerator.

b. Basic solution - Prepared by dissolving 550 mg of anhydrous sodium carbonate in 300 mL of water, adding 300 mL of reagent grade acetone, and mixing. This solution was stored in an amber glass bottle.

Standard

Anhydrous aminophylline (Sigma Chemical Co.), assayed for theophylline content by a direct HPLC method (13), and for ethylenediamine content by the titrimetric method of USP XXII.

Dosage Forms

Aminophylline tablets (100 and 200 mg), injections (25 mg/mL) and oral liquid (21 mg/mL) were obtained from various commercial sources.

Sample Preparations

a. Aminophylline standard preparation - An accurately weighed quantity of aminophylline (about 100 mg) was transferred to a 100 mL volumetric flask, dissolved in about 50 mL of water, brought to volume with water, and mixed.

b. Tablet preparation - A group of 20 aminophylline tablets was weighed and finely powdered. A portion of the powder, equivalent to about 100 mg of aminophylline, was transferred to a 100 mL volumetric flask, mixed with about 50 mL of water, and sonicated for about

15 min. After bringing to volume with water and mixing, the solution was filtered.

c. Liquid (injection or oral solution) preparation - An accurately measured volume of injection or oral solution, equivalent to about 100 mg of aminophylline, was transferred to a 100 mL volumetric flask, diluted to volume with water, and mixed.

d. Synthetic aminophylline tablet preparation - To a 100 mL volumetric flask, an accurately weighed quantity of aminophylline (about 100 mg), 100 mg of starch and 100 mg of lactose were added in succession. After the addition of about 50 mL of water, the mixture was sonicated for 15 min. The solution was brought to volume with water, mixed, and filtered.

e. Synthetic aminophylline liquid preparation - Prepared in a volumetric flask, by dissolving aminophylline in water to a concentration of 25 mg/mL. Then, 4.0 mL of this solution was diluted with water to 100 mL in a volumetric flask.

Dansylation Method

To a 50 mL volumetric flask, 5.0 mL of sample preparation, 10 mL of DNS-Cl and 10 mL of basic solution were added in succession. After mixing with gentle swirling and stoppering, the mixture was allowed to stand at room temperature and in the dark for at least 12 hr. After bringing to volume with acetone-water

(1:1) and mixing, the solution was injected into the chromatograph.

HPLC Method

a. Apparatus - An isocratic HPLC system consisting of constant flow solvent pump, high pressure injection valve with 20 µL sample loop, variable wavelength photometric detector and strip chart recorder (Perkin-Elmer Corporation). Separations were achieved on a µBondapak C_{18}, 30 cm x 3.9 mm i.d., 10 µm, column (Waters Associates), protected by a Co:Pell ODS, 7 cm x 2.1 mm i.d., guard column (Whatman Inc.).

b. Mobile phase - Prepared from HPLC grade solvents and reagent grade chemicals (J.T. Baker), consisting of a mixture of methanol-water-acetic acid-triethylamine (60-65:33-38:1.5:0.5, parts per 100), filtered and degassed prior to use. The flow rate was 1.5 mL/min. For the direct HPLC assay of theophylline, the mobile phase was methanol-water-acetic acid-triethylamine (30:68:1.5:0.5).

c. Detection - 254 nm.

d. Calculations - Obtain the quantities of theophylline and ethylenediamine in the aminophylline dosage form from the following equations:

Theophylline:

$$mg/tablet = (R_{sp}/R_{st}) \times W \times (T/100) \times (A/S)$$

$$mg/mL = (R_{sp}/R_{st}) \times W \times (T/100) \times (1/V)$$

Ethylenediamine:

$$\text{mg/tablet} = (R_{sp}/R_{st}) \times W \times (E/100) \times (A/S)$$

$$\text{mg/mL} = (R_{sp}/R_{st}) \times W \times (E/100) \times (1/V)$$

where R_{sp} and R_{st} = peak responses (heights or areas) of the dosage form preparation and aminophylline standard preparation, respectively; W = total weight of aminophylline in the aminophylline standard preparation, mg; T = percentage of theophylline in the aminophylline standard preparation; A = average tablet weight, mg; S = quantity of sample taken for the assay, mg; V = volume of sample taken for the assay, mL; and E = percentage of ethylenediamine in the aminophylline standard preparation.

RESULTS AND DISCUSSION

The feasibility of being able to simultaneously analyze the two components of aminophylline by HPLC is hampered by the absence in ethyelenediamine of a functionality with sufficiently high molar absorptivity to permit the detection of this component with a photometric or fluorescence detector. One possible way of circumventing this problem is to convert ethylenediamine to a UV-visible absorbing or fluorescence emitting derivative prior to its introduction into the chromatograph. This approach, however, may complicate the HPLC analysis if the amine were the only component of aminophylline to

undergo derivatization. This is the case with the method of Ishiguro et al. (11), in which ethylenediamine, but not theophylline, is converted to a stable UV-absorbing dibenzo[f,h]quinoxaline derivative; hence this method requires two separate sample injections and different chromatographic conditions for each component.

The use of DNS-Cl as the derivatizing reagent was found ideally suited for the purpose on hand: it reacted with both ethylenediamine and theophylline to form DNS-derivatives that were stable, these derivatives did not require their preliminary isolation from the reaction mixture and, more importantly, they were chromatographically resolvable and detectable under the same experimental conditions. The conditions for the reaction of aminophylline with DNS-Cl were optimized for pH, reagent concentration and solvent composition, using published data on the reaction of this reagent with primary aliphatic amines as a guide (14,15). Although ethylenediamine and theophylline started to react with DNS-Cl within 30 minutes, completion of the reaction with the former compound required a longer time than with the latter one. For this reason, the dansylation reaction was allowed to proceed overnight (at least 12 hours) to ensure that all of the aminophylline in the sample had undergone derivatization and that the excess of reagent, observable in the chromatograms as a large

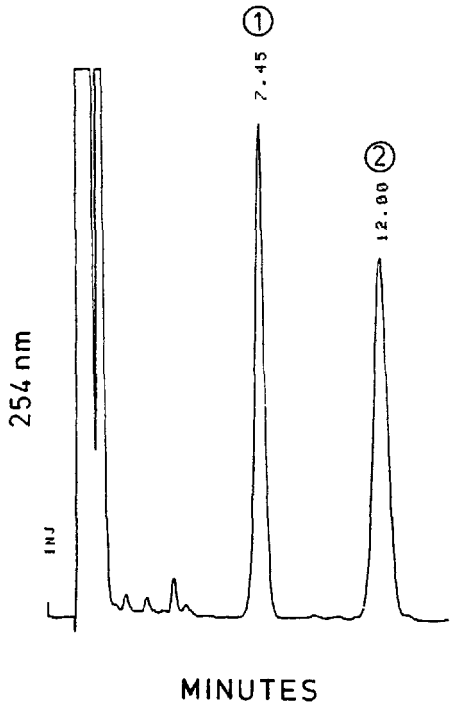

Figure 1. Typical HPLC separation of (1) DNS-theophylline and (2) bis-DNS-ethylenediamine from an aminophylline sample. Mobile phase: methanol-water-acetic acid-triethylamine (60:38:1.5:0.5) at 1.5 mL/min.

and very late eluting peak, had disappeared through spontaneous degradation.

A standard solution containing the DNS-derivatives of theophylline and ethylenediamine was effectively resolved into its components by using the recommended chromatographic conditions (Figure 1). To speed up peak elutions and, thus, shorten the analysis time, one may

adjust either the flow rate or the concentration of methanol-water in the mobile phase. However, if the latter approach is elected, one finds that not only the retention times are altered but also the resolution factor (R). For example, when the methanol-water ratio was (60:38), the approximate retention times for DNS-theophylline and bis-DNS-ethylenediamine were 7.45 min and 12.00 min, respectively with R = 4.73 (Figure 1). On the other hand raising the methanol-water ratio to (65:33) shortened the retention times to 6.50 min and 7.80 min, respectively, but with a concomitant loss in resolution (R = 2.22) (Figure 2).

HPLC separations were routinely monitored at 254 nm, a wavelength at which theophylline, at the concentrations found in aminophylline samples, yielded a DNS-derivative that gave the same or a slightly greater detector response than bis-DNS-ethylenediamine. Alternatively, one may monitor the effluents at 335 nm, in which case the detector response for bis-DNS-ethylenediamine is greater than that for DNS-theophylline. These differences are ascribable to differences in the contributions of the various chromophores present in the analytes to radiation absorption at each of the two wavelengths, namely the DNS moiety and purine nucleus.

Using an aminophylline stock solution containing 2 mg/mL, a calibration curve was constructed in the

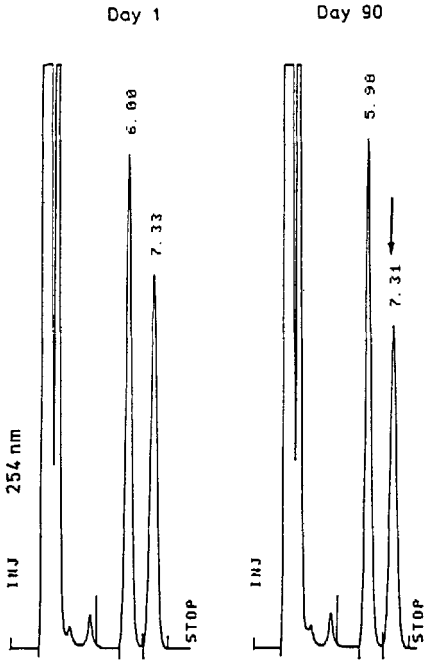

Figure 2. Application of the proposed DNS-HPLC method to the stability study of an aminophylline dosage form. After 90 days at 45°C and a relative humidity of 75%, the ethylenediamine component of 200 mg tablet was found to decrease in concentration (arrow). Mobile phase: methanol-water-acetic acid-triethylamine (65:33:1.5:0.5) at 1.5 mL/min.

range 0-10 mg of aminophylline added to the dansylating mixture. Detector responses were linearly related to amounts of aminophylline added over the concentration range of up to 7 mg of theophylline, and of up to 10 mg of ethylenediamine, with the curve passing through the origin. Assays were conducted at a concentration of aminophylline of about 5 mg. The reproducibility of the

proposed method was determined on the basis of both peak area and peak height measurements for a set of five consecutive injections of a standard dansylated aminophylline preparation. The RSD for DNS-theopylline and bis-DNS-ethylenediamine were 0.45% and 0.53%, respectively, based on peak areas; and 0.67% and 1.35%, respectively, based on peak heights.

To verify the accuracy of the proposed method, synthetic formulations simulating tablets and a liquid product were spiked with known amounts of aminophylline and put through the derivatization procedure. Mean recovery values (n = 2) of theophylline and ethylenediamine from the synthetic tablet were 100.5% and 99.9% of added, respectively. Those from the synthetic liquid preparation were 99.6% and 99.7% of added, respectively.

Commercial dosage forms of aminophylline, encompassing tablets, injections and oral solutions, were assayed for theophylline and ethylenediamine contents by the proposed method with the results shown in Tables 1 and 2. All the values represent the mean of duplicate analyses. The results for theophylline were validated by a direct HPLC method (13); those for ethylenediamine were validated by the titrimetric method of USP XXII (1). In the case of theophylline, close intermethod agreements were noted for the assay values of the vari-

Table 1

Determination of theophylline in aminophylline dosage forms by DNS-HPLC and direct HPLC methods[a,b]

Sample	Aminophylline declared	Theophylline found			
		DNS-HPLC	HPLC	DNS-HPLC	HPLC
Tablets:	mg/tab	mg/tab	mg/tab	%	%
1	100	78.8	78.1	99.9	99.1
2	100	80.8	80.2	102.5	101.8
3	100	80.4	79.5	102.1	100.9
4	100	81.2	80.3	103.1	101.9
5	200	154.4	154.2	98.0	97.8
6	200	152.8	154.5	96.9	98.1
7	200	162.7	158.3	103.3	100.4
Injections:	mg/mL	mg/mL	mg/mL	%	%
1	25	20.4	20.5	103.6	104.3
2	25	19.7	19.5	100.0	99.1
3	25	19.8	20.2	100.5	102.4
4	25	19.6	19.7	99.5	100.1
5	25	19.5	19.7	99.0	99.8
Oral solution:	mg/mL	mg/mL	mg/mL	%	%
1	21	18.0	17.6	100.1	97.9

[a]USP range for tablets and injections = 93.0-107.0%; for oral solution = 90.0-110.0%.

[b]Chromatographic conditions as for DNS-HPLC, except that mobile phase consisted on methanol-water-acetic acid-triethylamine (30:68:1.5:0.5). All samples were prepared as described under Sample Preparations.

Table 2

Determination of ethylenediamine in aminophylline dosage forms by DNS-HPLC and USP XXII methods[a,b]

Sample	Aminophylline declared	Ethylenediamine found			
		DNS-HPLC	USP XXII	DNS-HPLC	USP XXII
Tablets:	mg/tab	mg/tab	mg/tab	mg/g theoph. found	
1	100	13.8	13.9	174.9	177.7
2	100	13.8	13.5	170.2	168.7
3	100	12.1	12.5	150.2	156.9
4	100	13.9	14.9	171.7	185.3
5	200	24.7	24.2	159.8	157.1
6	200	24.1	24.3	157.6	159.0
7	200	25.3	25.9	155.3	163.8
Injections:	mg/mL	mg/mL	mg/mL	mg/g theoph. found	
1	25	3.7	3.7	181.4	178.1
2	25	3.7	3.6	187.8	184.8
3	25	3.7	3.7	186.9	185.0
4	25	3.6	3.7	184.4	187.5
5	25	3.7	3.7	187.6	190.2
Oral solution:	mg/mL	mg/mL	mg/mL	mg/g theoph. found	
1	21	3.7	3.7	205.9	207.8

[a]USP range (mg of ethylenediamine/g of theophylline found in the assay) for tablets = 152-178; for injections = 166-192; for oral solution □ 176-221. Values shown were calculated from the data reported in Table 1.

[b]USP XXII method is titrimetry with 0.1 N HCl to methyl orange end point.

ous dosage forms (Table 1), with the maximum intermethod difference for the theophylline component in tablets, injections and oral solution being 2.8, 1.9 and 2.2%, respectively, and with all of the samples yielding results that fell within the acceptable range of USP XXII. Regarding the assay values for the ethylenediamine part, close intermethod agreements were noted for all but one of the various dosage forms, with the maximum intermethod differences for tablets, injections and oral solution being 2.3, 2.7 and 0.0 %, respectively, and with all but one of the samples conforming to the compendial requirements (Table 2). However one of the tablet samples (sample 4) was found to exceed the compendial requirements when assayed by the titrimetric method of USP XXII. Since such a problem was not encountered with the proposed DNS-HPLC method, the discrepancy is interpreted as arising from interference of the titration by a tablet excipient. A similar problem was reported by Medwick and Schiesswohl (9) during the nonaqueous titrimetric assay of aminophylline tablets.

The suitability of the proposed DNS-HPLC method for monitoring the stability of aminophylline in dosage forms was investigated by subjecting a group of 200 mg tablets to an accelerated stability study (temperature, 45°C; relative humidity, 75%; duration, 90 days). As indicated by the ratio of the peak height of DNS-theo-

phylline to that of bis-DNS-ethylenediamine, it was verified that only the ethylenediamine part experienced a significant drop in potency during the course of the study (Figure 2). Based on the assay values for theophylline and ethylenediamine, which corresponded to 154.4 and 24.7 mg/tablet, respectively, on day 1, and to 152.6 and 21.1 mg/tablet, respectively, on day 90, the drop in ethylenediamine content amounted to about 15%.

SUMMARY

The DNS-HPLC method reported here represents a convenient means of simultaneously assaying the two components of aminophylline in a simple, straightforward and specific manner. In addition, the method is found to be of utility in the analysis of both solid and liquid dosage forms, and in the monitoring of the stability of aminophylline in pharmaceutical samples.

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