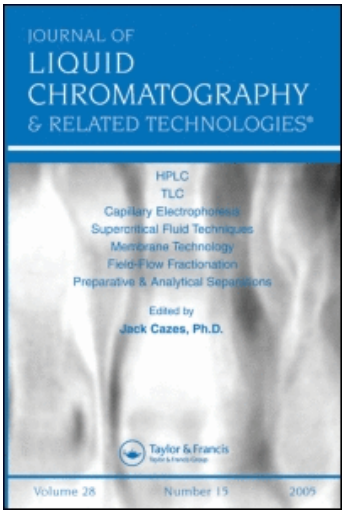

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Assay of Aminocaproic Acid in Dosage Forms by Reversed Phase High Performance Liquid Chromatography with Dansylation

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ASSAY OF AMINOCAPROIC ACID IN DOSAGE FORMS BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH DANSYLATION

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ABSTRACT

A HPLC method with precolumn derivatization is described for the assay of aminocaproic acid in solid and liquid dosage forms. Tablets are extracted into or liquid dosage forms are diluted with water, and the aqueous extracts are reacted with dansyl chloride in an alkaline solution containing the internal standard tranexamic acid. After removal of the excess of dansyl chloride with monoethanolamine, the mixture of dansyl derivatives is analyzed on an Econosphere C_{18} reversed phase column, with a methanol-water-acetic acid-triethylamine (60:38:1.5:0.5) mobile phase delivered at the rate of 1.5 mL/min, and a detection wavelength of 335 nm. Detector responses were rectilinearly related to on column concentrations of aminocaproic acid between 0.05 and 2 μg. The proposed method was found suitable for the analysis of commercial tablets, injections and syrups, and of samples from tablet dissolution tests. Assay results by this method were in close agreement with those obtained using assay methods of the USP XXII.

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INTRODUCTION

Aminocaproic acid (6-aminohexanoic acid) is a synthetic amino acid which is used as a hemostatic and antifibrinolytic agent (1), as well as a starting material for the synthesis of nylon polymers (2-7).

Several analytical methods have been described for the assay of this compound in industrial polymers (2-7), biological fluids (8-16), tissues (10,14) and pharmaceutical samples (17- 20). Because of the inherent structural features of this compound, many of these assay methods have relied on titrimetric (3,19,20), electrochemical (2,4,5) and gas chromatographic (15) approaches. An existing HPLC method (17) is only recommended for the drug substance and its injections. Alternatively, this drug has been analyzed by spectrophotometric (18,20) and by planar (7-11,17) and open column (12) chromatographic techniques after the formation of chromophores or fluorophores between the free ϵ-amino group and suitable reagents.

This report describes a simple and rapid reversed phase HPLC method for the assay of aminocaproic acid in pharmaceutical samples. Conversion of the drug to a dansyl derivative facilitated its detection by the photometric mode. The proposed method is also of utility in the

assay of aminocaproic acid in commercial liquid and solid dosage forms, and in samples collected during tablet dissolution tests.

EXPERIMENTAL

Samples

a. Dosage forms - Samples of aminocaproic acid tablets (500 mg), injections (250 mg/mL) and syrup (1.25 g/5 mL) were obtained from various commercial sources.

b. Standards - Aminocaproic acid, anhydrous, and tranexamic acid (Sigma Chemical Co.) were dried to constant weight and used as received.

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.

c. Internal standard solution - An accurately weighed quantity of tranexamic acid (about 40 mg), was placed in a 100 mL volumetric flask, dissolved in about 50 mL of basic solution, diluted to volume with basic

solution, and mixed. This solution was stored in an amber glass bottle.

Sample Preparations

a. Aminocaproic acid standard preparation - An accurately weighed quantity of aminocaproic acid (about 50 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 aminocaproic acid tablets was weighed and reduced to a fine powder. A portion of the powder, equivalent to about 50 mg of aminocaproic acid, 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 through a 0.45 μm membrane filter.

c. Liquid (injection or oral solution) preparation - An accurately measured volume of injection or syrup, equivalent to about 250 mg of aminocaproic acid, was transferred to a 100 mL volumetric flask, diluted to volume with water, and mixed. A 5.0 mL aliquot of this solution was transferred to a 25 mL volumetric flask, diluted to volume with water, and mixed.

d. Synthetic aminocaproic acid tablet preparation - To a 100 mL volumetric flask, an accurately weighed quan-

tity of aminocaproic acid (about 50 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, diluted to volume with water, mixed, and filtered through a 0.45 μm membrane filter.

e. Synthetic aminocaproic acid injection preparation - Prepared in a volumetric flask, by dissolving an accurately weighed quantity of aminocaproic acid in water to a concentration of 0.5 mg/mL.

f. Synthetic aminocaproic acid syrup preparation - Prepared in a volumetric flask, by dissolving an accurately weighed quantity of aminocaproic acid in simple syrup to a concentration of 0.5 mg/mL.

g. Tablet dissolution test preparation - Six tablets from each lot were individually subjected to the USP XXII dissolution test using apparatus 1, 900 mL of water as the medium, and 100 rpm stirring for 45 min. A portion of the dissolution medium was then filtered through a 0.45 μm membrane filter.

Dansylation Method

a. Without internal standard - To a 50 mL volumetric flask, 5.0 mL of sample preparation, 10.0 mL of DNS-Cl solution and 10.0 mL of basic solution were added in succession, and mixed with gentle swirling. After stoppering and allowing the mixture to stand at room tempera-

ture and in the dark for at least 30 min, 2 drops of monoethanolamine were added. The flask was stoppered, and its contents were mixed with swirling. After a 15 min standing period, the mixture was brought to volume with acetone-water (1:1), mixed, and injected into the liquid chromatograph.

b. With internal standard - The procedure was carried out as described under Dansylation (a), except that the basic solution was replaced by an equivalent volume of internal standard solution.

HPLC Method

a. Apparatus - An isocratic HPLC system consisting of a constant flow solvent pump, high pressure injection valve with 20 μL sample loop, variable wavelength photometric detector set at 335 nm and strip chart recorder (Perkin-Elmer Corporation). Peak areas were obtained using an electronic integrator (Hewlett-Packard). Separations were achieved on an Econosphere C_{18}, 15 cm x 4.6 mm i.d., 5 μm, column (Alltech), protected with a C_{18} cartridge guard column (Alltech).

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 (60:38:1.5:0.5), filtered and degassed prior to use. The flow rate was 1.5 mL/min.

c. Calculations - The quantity of aminocaproic acid in the sample preparation analyzed was calculated by one of the following equations:

$$\text{mg/tablet} = (Rsp/Rst) \times C \times (T/W) \times 100$$

$$\text{mg/mL} = (Rsp/Rst) \times (C/2)$$

$$\text{mg dissolved} = (Ru/Rs) \times 0.555 \times V$$

where Rsp and Rst = ratios of aminocaproic acid/internal standard peak responses in the sample preparation and the standard preparation, respectively; Ru and Rs = peak responses of aminocaproic acid in the tablet preparation and standard preparation, respectively; C = quantity of aminocaproic acid in the standard preparation, mg; T = average tablet weight, mg; W = quantity of tablet sample used in the assay, mg; and V = total volume of dissolution medium used in the assay, mL.

RESULTS AND DISCUSSION

Experimental conditions for the dansylation step were optimized for pH, reagent concentration, and solvent composition as previously described for the HPLC analysis of aminophylline (21). As shown in Figure 1, both aminocaproic acid and the internal standard tranexamic acid reacted with DNS-Cl rapidly at ambient temperature, with the reaction reaching completion in less than 30 min. After its dilution with acetone-water (1:1), the reaction

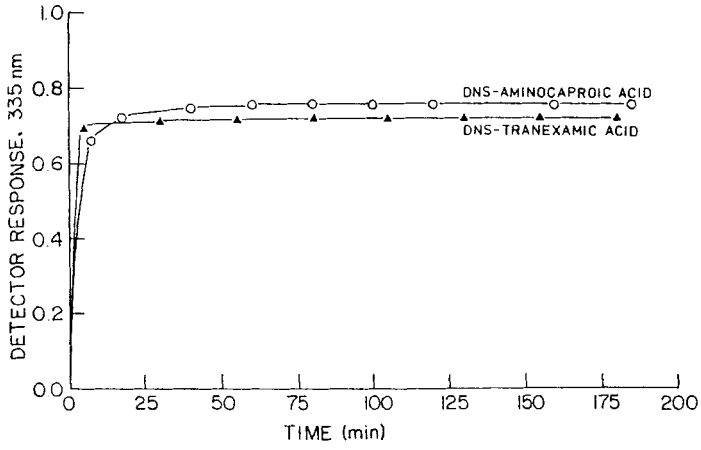

Figure 1. Time courses of the dansylation reactions of aminocaproic acid and the internal standard, tranexamic acid.

mixture containing the DNS-derivatives was directly injected into the chromatograph without the need for a preliminary isolation step.

As seen in Figure 2A, the chromatogram of a derivatized aminocaproic acid standard preparation contained an additional peak eluting at about 23 min, which corresponded to residual DNS-Cl. The analysis time was shortened considerably by treating the reaction mixture with a few drops of monoethanolamine so as to remove the DNS-Cl as a fast eluting (about 3 min) DNS-monoethanolamine peak (Figure 2B).

Using mobile phases that contained methanol-water in the range of proportions 50:48 to 65:33, peak resolution,

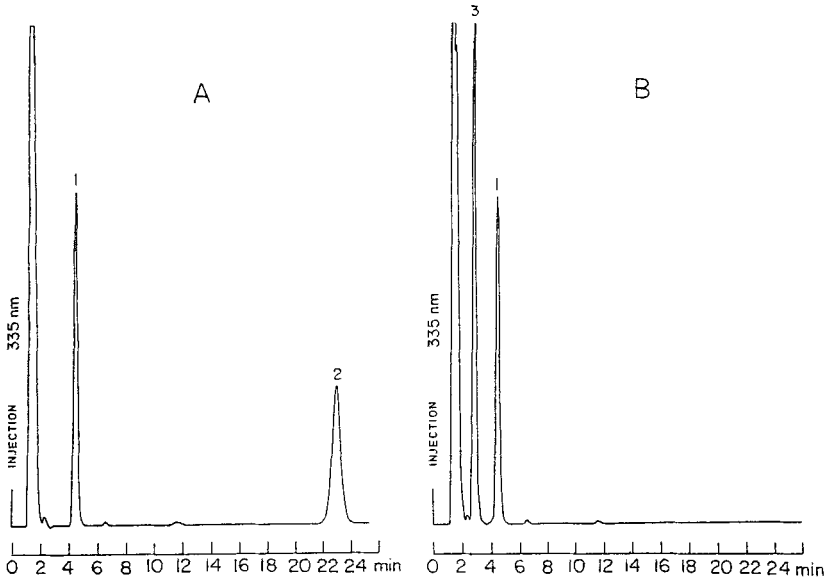

Figure 2. High performance liquid chromatograms of a derivatized aminocaproic acid standard preparation showing : (A) the slow eluting DNS-Cl peak, and (B) the removal of the DNS-Cl peak by a treatment with monoethanolamine. Key: 1, DNS-aminocaproic acid; 2, DNS-Cl; 3, DNS-monoethanolamine.

R, for a mixture of DNS-aminocaproic acid and DNS-tranexamic acid was found to vary between about 5.5 and less than 2.0. The recommended mobile phase (i.e, methanol-water 60:38) represents a compromise between a satisfactory resolution (R ≈2.0) and reasonable analysis time (<8 min). Typical retention times for DNS-aminocaproic acid and DNS-tranexamic acid in a standard preparation were 4.5 and 6.5 min, respectively (Figure 3).

Using an aminocaproic acid standard solution containing 1 mg/mL, calibrations curves covering the range 10

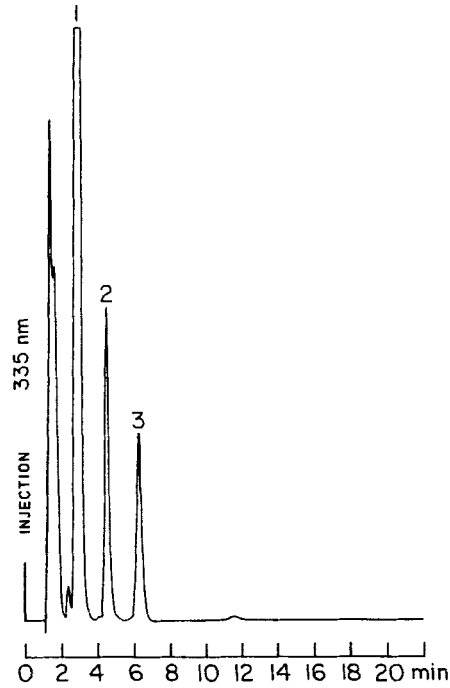

Figure 3. High performance liquid chromatogram of a derivatized standard preparation: 1, DNS-monoethanolamine; 2, DNS-aminocaproic acid; 3, DNS-tranexamic acid.

to 100 μg/mL, were constructed with and without the addition of the internal standard. In both cases, detector responses of the DNS-derivatives (peak heights or peak areas) were rectilinearly related to on column aminocaproic acid concentrations between 0.05 and 2 μg (r=0.99), with the curve passing through the origin. Based on these results, assays were routinely conducted using sample preparations that contained about 0.5 mg/mL of analyte. The reproducibility of the method was assessed on

Table 1

Results of recovery studies on synthetic dosage forms by the DNS-HPLC method with or without the addition of an internal standard

	Amount found, % of added					
	With internal std.			Without internal std.		
Dosage form	1	2	Mean	1	2	Mean
Tablet	100.5	100.2	100.3	102.9	100.2	101.5
Injecn.	100.3	100.3	100.3	100.3	100.3	100.3
Syrup	100.4	100.4	100.4	100.4	100.4	100.4

the basis of both peak area and peak height measurements for a set of six consecutive injections of a dansylated standard aminocaproic acid preparation. The RSD for DNS-aminocaproic acid based on peak areas and peak heights were 0.39% and 0.59%, respectively; based on the ratios of peak areas and peak heights of drug to internal standard, these values were 0.38% and 0.35%, respectively. To verify the accuracy of the proposed method, synthetic formulations simulating tablets, injections and syrups were spiked with known amounts of aminocaproic acid and put through the derivatization procedure. As shown in Table 1, mean recovery values (n = 2) of aminocaproic

acid from the synthetic tablet were 100.35 and 101.55% of the added amount, depending on whether the internal standard had been added or not, respectively. Recoveries from the synthetic injection and synthetic syrup were virtually the same in the presence or absence of the internal standard, and amounted to 100.3% and 100.4% of the added amount, respectively.

Table 2 summarizes the results of the assay of commercial dosage forms encompassing tablets, injections and syrups, by the proposed method. The values shown represent the mean of duplicate analyses. These results were validated against both the official colorimetric method for tablet dissolution testing and official HPLC method for the drug substance and injections (20). In general, the assay values obtained using the DNS-HPLC method were in close agreement with those obtained using the official methods. Maximum intermethod differences amounted to about 2.60% of labeled (vs. USP HPLC method) for tablets, 4.58% of labeled (vs. USP colorimetric method) for injections, and 6.70% of labeled (vs. USP colorimetric method) for the syrup. The tendency of the official colorimetric method to yield higher values than the proposed HPLC method may stem from the additional contributions by other syrup ingredients. Overall, however, all the samples conformed to the drug contents specified by USP XXII (20). In contrast to the USP XXII colorimetric method,

Table 2

Determination of aminocaproic acid in commercial dosage forms by DNS-HPLC and two alternative compendial methods[a]

	Amount found, % of declared			
	DNS-HPLC		USP XXII	
Sample No.	With IS[b]	Without IS No.	Colorim.[c]	HPLC[d]
	Tablets, 500 mg/tablet			
1	102.12	99.30	100.00	99.52
2	103.10	103.90	102.20	100.40
3	101.05	101.10	101.50	102.16
	Injection, 250 mg/mL			
1	102.90	101.50	101.90	100.80
2	103.00	101.33	99.23	101.70
3	104.20	103.00	99.62	102.50
	Syrup, 1.25 g/5 mL			
1	106.70	104.70	111.40	104.40

[a]USP XXII ranges: tablets = 05.0-105.0%; injections = 95.0-107.5%; syrups = 95.0-115.0%.
[b]IS = internal standard.
[c]Colorimetric method for tablet dissolution test
[d]HPLC method for the drug substance and injections.

Table 3

Comparison of assay results (% found) for tablet dissolution test samples by DNS-HPLC method and USP XXII colorimetric method[a]

Tablet No.	Lot 1		Lot 2		Lot 3	
	HPLC	USP	HPLC	USP	HPLC	USP
1	94.6	95.4	100.3	97.1	101.2	97.0
2	100.3	94.2	101.2	96.3	99.8	101.0
3	96.5	98.5	100.3	103.2	102.0	103.1
4	100.3	92.2	97.5	97.7	99.4	95.0
5	97.4	102.2	96.5	93.2	98.4	96.5
6	88.9	102.2	97.0	102.2	100.3	100.1
Mean	96.33	97.45	98.80	98.28	100.18	98.80
SD	3.89	3.84	1.85	3.44	2.17	2.83

[a]USP XXII tablet dissolution method with sample withdrawals at 45 min.

the proposed HPLC method does not entail the use of an unstable chromogenic reagent or of a closely controlled heating step. At variance with the USP HPLC method, the DNS-HPLC method does not require a mobile phase containing a high salt concentration; it does not detect the presence of syrup preservatives such ascorbic acid and benzoic acid (which in the compendial method elute as a pair of partially resolved peaks after 25 min); and it permits the monitoring of the effluent with a greater

sensitivity and at a more convenient wavelength of detection, i.e., 335 nm vs. 210 nm.

The suitability of the proposed DNS-HPLC method for assessing drug release during tablet dissolution studies was also investigated. For this purpose, three different tablet lots of six tablets each, were subjected to the dissolution test of USP XXII, and the results of this study were compared with those obtained using the compendial colorimetric assay. As seen in Table 3, intermethod agreements were generally close, and no interference from tablet excipients were apparent. In view of its good reproducibility, the proposed method did not require the addition of the internal standard for this purpose, thus simplifying the analysis.

SUMMARY

The DNS-HPLC method is a simple and straightforward means of assaying aminocaproic acid in pharmaceutical dosage forms with a minimum of reagents and procedural steps. Moreover, it is also useful for studying the extent of drug release from tablets undergoing dissolution testing. Therefore, the proposed method represents a convenient and unified approach to verifying conformance of the various types of aminocaproic acid samples to compendial requirements.

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