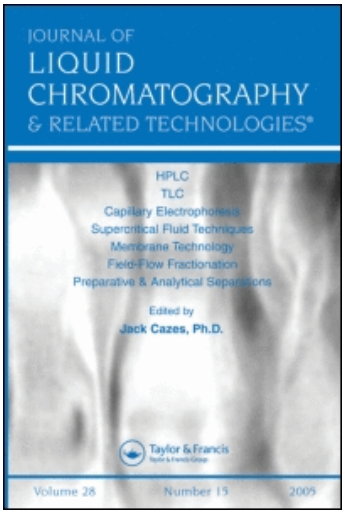

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ANALYSIS OF AMINOPHYLLINE IN THIGH CREAM FORMULATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Aminophylline is a 2:1 mixture of theophylline and ethylenediamine which is widely used as a bronchorelaxant for the treatment of asthma. More recently, it has been claimed that formulations of aminophylline in topical creams are useful for the reduction of lower body fat. As part of a study of the efficacy of such formulations, we have developed a chromatographic method for the analysis of aminophylline in cream preparations. The method involves dissolving the creams in an acetone-water solvent, derivatizing the aminophylline components with dansyl chloride and analyzing the resulting mixtures by reversed-phase high performance liquid chromatography (HPLC). Because other components of the creams are separated from the aminophylline components during the HPLC analysis, no extractions of aminophylline from the creams are required. However, vigorous

stirring of the reaction mixture during the dansylation is required to ensure that all of the aminophylline components are derivatized. Several commercial aminophylline-based thigh cream formulations were analyzed by this method. It was found that these formulations varied significantly in the amount of aminophylline present.

INTRODUCTION

Aminophylline (1H-purine-2,6-dione-3,7-dihydro-1,3-dimethyl-1,2-ethane diamine) is a bronchorelaxant used primarily in the treatment of asthma. It consists of two components, theophylline and ethylenediamine, in a 2:1 molar ratio. As a bronchial dilator, aminophylline is commercially available in several dosage forms. These include both single-dose and sustained-release capsules and tablets, solutions and suppositories.

A number of methods have been reported for the quantitative determination of aminophylline in biological and pharmaceutical samples. The majority of these methods, however, measure theophylline content only. Some of the earliest methods were based on extraction of theophylline with organic solvents, precipitation of a theophylline salt, and gravimetric determination of the residue.[1] The official USP method is based on the titration of silver theophyllate with ammonium thiocyanate.[2] Other volumetric methods include the titration of theophylline with alkali to a potentiometric or colorimetric endpoint,[3,4] the titration of ethylenediamine with a strong acid (e.g., HCl),[5] and the complexation of theophylline with mercuric acetate and subsequent titration of excess mercury ions with ammonium thiocyanate.[6] Alternatively, cupric acetate has been used followed by back-titration with EDTA.[7] There is also a nonaqueous titration procedure, utilizing sodium methoxide or acetous perchloric acid as the titrant, which allows for the determination of both components in a single titration procedure.[8,9]

Several spectroscopic methods have been developed in which the UV absorbance of theophylline is measured at 275 nm. Isolation of theophylline from its matrix is achieved in several ways. For pharmaceutical and many biological samples, extraction with an organic solvent is often used.[10,11] For serum samples, extraction with a mixture of ammonium sulfate, chloroform, and hexane followed by back-extraction of theophylline into aqueous borate buffer (pH 9) has been reported.[12] Charcoal extraction has also been used in which theophylline is adsorbed on charcoal and eluted with an organic solvent.[13] In another application, the theophylline present in blood samples was analyzed by oxidation with potassium dichromate in an acidic medium.[14]

The oxidation product was then isolated by steam distillation and its absorbance measured at 257 nm. Finally, spectral subtraction methods were utilized to determine aminophylline content from the UV absorbances of samples containing benzyl alcohol as a preservative, as well as mixtures of aminophylline and phenobarbitol.[15]

Many methods have been developed for the analysis of theophylline in biological samples based on high performance liquid chromatography (HPLC) and gas chromatography (GC).[16] Chromatographic conditions must be developed on a case-by-case basis to prevent interference from other drugs and/or theophylline metabolites. Ethylenediamine may also be analyzed by GC or HPLC although, until recently, its prior derivatization and separate analysis from theophylline was required.

A few years ago, Lau-Cam and Roos developed a chromatographic method which allowed for the simultaneous determination of both theophylline and ethylenediamine in solid and liquid dosage forms, specifically tablets and solutions.[17] The method is based on the HPLC separation of the two components as their dansyl derivatives (i.e., dansyl-theophylline and bis-dansyl-ethylenediamine). It involves extraction of tablets into water or dilution of liquid dosage forms, reaction of the extract with dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride) in an alkaline medium, and analysis of the resulting mixture by reversed-phase HPLC. Separation is achieved on a standard octadecylsilica column using a quaternary mobile phase. Excellent precision, accuracy and recovery were obtained on the analyses of a variety of dosage forms.

Recently, aminophylline has become available as an over-the-counter cream. It has been marketed by the cosmetic industry as an agent for the reduction of lower body fat. For asthma patients, aminophylline functions by dilating the bronchioles.[18] The receptors upon which it acts are also found in fat cells, with an especially high concentration occurring in the femoral regions of women. It has, therefore been hypothesized that when applied topically, these creams are absorbed into the thigh where aminophylline serves to dilate the fat cells and thus facilitate the breakdown of resistant fat storage.[19]

The topic of thigh creams and the debate as to whether or not they actually work has received much media attention within recent years with articles appearing in countless women's magazines and even in a national news publication.[20] There are currently over 50 different aminophylline-based thigh cream preparations on the market. At costs ranging from $10-$30 per bottle, a year's supply of this product can cost the consumer up to $900.

There are other concerns, however, besides cost. Aminophylline is known to occasionally produce serious cardiopulmonary side effects in the dosages administered to asthma patients. Although, it has been reported that when applied topically, no trace of the drug has been found in blood chemistry tests,[21] there have been no studies published to date that actually monitored cardiopulmonary functioning in response to acute and/or chronic use of such creams.

We have recently begun a study to determine the efficacy and cardiopulmonary effects associated with regular use of these creams. Since the actual dosing contained in these creams is not readily available to the consumer, it is conceivable that they may contain far less than the reputed effective dose of 2% aminophylline. Quantifying the percent active ingredient in the cream preparations used in this study is, therefore, necessary.

Despite the extensive list of methods previously developed for the quantitative analysis of aminophylline (or at least its theophylline component), no one to date has done such an analysis on a cream formulation. The newest analytical method, that of Lau-Cam and Roos, was developed specifically for pharmaceuticals in solid and liquid dosage forms. A cream matrix presents a more formidable challenge. The purpose of this study, therefore, is to develop a means of quantitatively analyzing aminophylline in cream dosage forms through modification of Lau-Cam and Roos method.

EXPERIMENTAL

Materials

The thigh cream products were purchased from various commercial sources, as described later. Aminophylline standard was obtained from Sigma Chemical Co. (St Louis, MO). Dansyl chloride was obtained from Lancaster Synthesis, Inc. (Windham, NH). Chromatographic-grade methanol, other solvents and sodium carbonate (used to adjust pH) were obtained from Fisher Scientific, Inc. (Fair Lawn, NJ).

Instrumentation and Conditions

All analyses were performed on an HPLC system consisting of a Perkin-Elmer Series 410 solvent delivery system, a Rheodyne Model 7125 injector (10 μL loop), and a Perkin-Elmer Model LC-135 diode array detector set at

254 nm. Separations were achieved on a Microsorb-MV C-18 column, 15 cm x 4.6 mm i.d., 5 μm particle size (Rainin Instrument Co., Inc., Woburn, MA) Chromatograms were recorded and processed on a Perkin-Elmer Omega data system.

The isocratic mobile phase consisted of 69% methanol, 29% water, 1.5% acetic acid, and 0.5% triethylamine. The flow rate was 1.0 mL/min.

Preparation of Samples

Standard solutions were prepared from a stock solution containing 1 mg/mL aminophylline in water. 1-5 mL of the stock solution were mixed with 10 mL of dansyl chloride solution (5 mg/mL in acetone) and 10 mL of sodium carbonate solution (0.9 mg/mL in 50% aqueous acetone, by volume). The mixtures were allowed to stand at room temperature in the dark for 12 hours, then brought to a volume of 50 mL and analyzed by HPLC under the conditions described above.

Cream sample solutions were prepared by adding 10 mL of 50% aqueous acetone to Erlenmeyer flasks containing an accurately weighed quantity of cream (100-400 mg) as described in Table 10. The suspensions were then stirred for several minutes to dissolve the cream. Dansylation of the resulting solutions was performed in a similar manner as the standards under various conditions of reagent concentration, pH, and temperature as described later.

Further Preparation of Creams by Extraction

Removal of the organic, water-insoluble cream components prior to dansylation was attempted in the following manner: a 10 mL aliquot of cream solution prepared as described above was first transferred to a 50 mL centrifuge tube. 10 mL of chloroform was then added to the tube and the mixture was shaken vigorously for 2 minutes. After centrifuging at 3000 rpm for 5 minutes, the aqueous layer was removed, dansylated as described above, and finally, analyzed by HPLC.

Standard Addition Analysis

A total of 20 mL of a dansylated aminophylline standard (0.1 mg/mL in water) was added to an equal volume of a previously analyzed cream sample and rechromatographed.

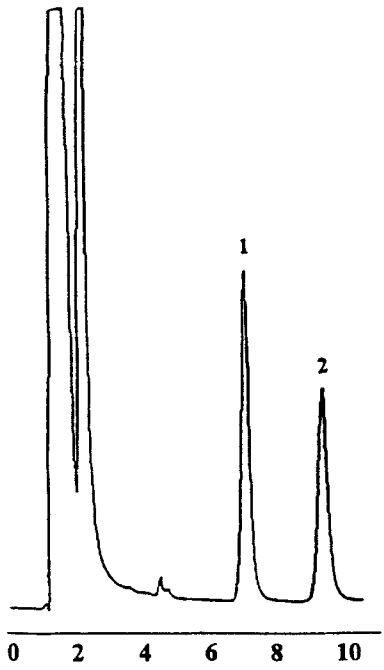

Figure 1. Chromatogram of a standard solution of aminophylline after dansylation. Identified peaks correspond to (1) dansyl-theophylline and (2) bis-dansyl ethylenediamine.

Recovery Experiments

A mass of aminophylline weighing 13 mg was added to 1.30 g of a previously analyzed thigh cream formulation and stirred to form a homogenous mixture. 130 mg of this mixture was then prepared for analysis as outlined above.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram obtained from the analysis of a standard aminophylline solution using the method developed by Lau-Cam and Roos. The retention times for dansyl-theophylline and bis-dansyl-

Table 1

Results of Thigh Cream Analysis Under Initial Derivatization Conditions

Sample No.	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	4.97	0.128	13:1
2	3.74	0.132	9:1
3	4.87	0.147	11:1
4	5.51	0.124	15:1
5	5.64	0.143	13:1
6	5.42	0.153	12:1

ethylenediamine were 7.01 min and 9.21 min, respectively. At 254 nm, the theophylline component gave a similar detector response as bis-dansyl-ethylenediamine, corresponding to the 2:1 molar ratio present in aminophylline samples.

All of our preliminary data on the cream samples was obtained using University Medical's Original Thigh Cream (University Medical Products, Newport Beach, CA). Due to the complex nature of its inactive ingredients, we perceived this cream to be the most difficult to analyze, and on this basis, chose it for all of the initial method development. The results shown in Table 1 were obtained when the cream samples were prepared and dansylated in exactly the same manner as the standards (i.e., identical conditions of reagent conc., pH, and temperature).

Figure 2A shows a typical chromatogram obtained from this analysis. In the presence of the other cream components, both theophylline and ethylenediamine exhibited a slight decrease in retention time. Ethylenediamine was most affected. More importantly, bis-dansyl-ethylenediamine exhibited a much lower response than did dansyl-theophylline, resulting in molar ratios of theophylline to ethylenediamine between 5 and 8 times greater than the expected 2:1 molar ratio (see Table 1).

Cream is a very complex matrix. Since the aminophylline in the cream was analyzed in the presence of this matrix, it appeared likely that the anamolous quantitative results were caused by interference from the cream.

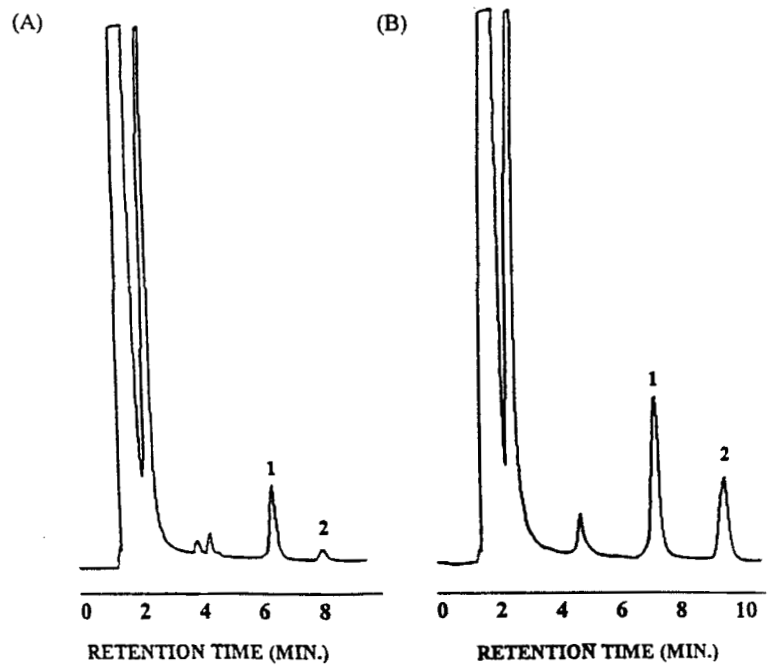

Figure 2. Chromatogram of cream formulation under (A) initial dansylation conditions and (B) optimized dansylation conditions. Peak identities are the same as that described in Figure 1.

Two modes of interference were possible:

1. The cream matrix could be distorting the chromatographic separation and/or relative responses of the two components.

2. The cream components could be inhibiting the dansylation of one or both of the compounds in the aminophylline in an unequal manner.

Investigations were conducted to determine the source of the anamolous results and to minimize them, as discussed below.

Investigation of Possible Chromatographic Interference

As stated above, the possibility existed of cream components interfering with the quantitative data. A standard addition experiment was conducted to

Table 2

Standard Addition Experiment

	Dansyl-theophylline Peak Area	Bis-Dansyl-ethylenediamine Peak Area
Before Addition	52279984	7908328
After Addition	95216896	51262802
Δ Peak Area	42936912	43354476

investigate this possibility in which an aliquot of derivatized standard was added to a previously analyzed sample and then rechromatographed. Table 2 shows the corresponding peak areas obtained. After addition of the derivatized standard, the peak areas increased by a similar amount for each component. The nearly identical increase in response indicates that the cream matrix did not affect the quantitative chromatographic results for either component. Therefore, it can be concluded that incomplete and/or unequal dansylation of the target compounds in the presence of the cream was the source of the anamolous results.

Attempted Extraction of Interfering Cream Components

The second possibility of the cause for the anomolous ratio of theophylline to ethylenediamine was interference of cream components (e.g., various paraffins, herbal extracts and oils) in the dansylation process. To minimize these effects, we attempted to extract the potential interfering compounds into chloroform and away from the reaction mixture prior to dansylation.

Table 3 shows data obtained on four duplicate cream samples. As the number of extractions increased, the molar ratio was observed to decrease, approaching the theoretical value of 2:1.

These results, however, were apparently caused by a decrease in the amount of theophylline detected (see Table 3) rather than an overall improvement in the dansylation. Theophylline was most likely lost by back-extraction into the chloroform layer.

Table 3

Extraction of Cream Samples with Chloroform

Number of Extractions	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	3.71	0.35	3.5:1
2	3.13	0.30	3.4:1
3	2.95	0.38	2.6:1
4	1.89	0.37	1.7:1

Table 4

Extraction of Aminophylline Standard with Chloroform

Number of Extractions	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
None	0.0200	0.0034	2.0:1
4	0.0039	0.0034	0.4:1

To confirm the occurrence of this theophylline loss, a 0.025 mg/mL solution of aminophylline was analyzed once with, and once without extraction into chloroform. The data presented in Table 4 shows that after four extractions only one-fifth of the original theophylline content remained in the aqueous layer removed for analysis. This confirmed the occurrence of back-extraction of the theophylline into the organic phase.

Optimization of Reaction Conditions

The demonstrated loss of theophylline during the attempted extraction of cream components prior to dansylation necessitated our reverting to performing the derivatization and analysis in the presence of the cream. Since the apparent problem with the analysis appeared to be interference by cream components in the dansylation process, we attempted to optimize the derivatization by a systematic variation of reaction conditions. Table 5 describes these variations and the resulting analytical data.

Table 5

Optimization of Dansylation Conditions

Condition Varied	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
Reagent Conc. (increased 3-fold)	13.26	0.55	8:1
pH (raised to 3.5)	13.32	0.36	12:1
Temperature (heated to 60°C)	9.87	0.39	8:1
Vigorous Stirring (magnetic stirrer at 300 rpm)	8.92	1.35	2:1

As shown there, each variation produced an increase in the amount of both components detected compared to that obtained in initial experiments (Table 1). However, in most cases, the molar ratios were still higher than the expected 2:1 molar ratio, indicating incomplete dansylation of the target compounds. Only under conditions of vigorous stirring of the reaction mixture was the expected 2:1 molar ratio achieved.

Method Validation

Table 6 shows the optimized derivatization conditions used in the remainder of this study. Figure 2B shows a chromatogram of a cream formulation derivatized under these optimized conditions. It exhibits the expected relative response for the two aminophylline components. System precision data, shown in Table 7, were obtained from successive injections of a single cream solution after derivatization. The relative standard deviation (RSD) of results obtained for the ethylenediamine component is somewhat greater than that obtained for theophylline but still is under 3%. The precision of the entire method was evaluated by derivatizing and analyzing six separate samples of the same cream formulation. The results shown in Table 8 demonstrate good analytical reproducibility.

Table 6

Optimized Reaction Conditions

Dansyl Chloride Concentration	pH	Temperature	Special Conditions
2 mg/mL	2.2	ambient	in the dark stirred virorously

Table 7

System Precision

Injection	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	8.69	1.32	2.19:1
2	8.59	1.28	2.24:1
3	8.60	1.36	2.11:1
mean	8.63	1.32	2.18:1
RSD	0.52%	2.47%	2.46%

Table 8

Method Precision

Sample	Theophylline (mg/g)	Ethylenediamine (mg/g)	Molar Ratio
1	8.52	1.32	2.15:1
2	8.88	1.26	2.34:1
3	8.72	1.23	2.36:1
4	9.20	1.30	2.36:1
5	9.10	1.28	2.37:1
6	8.69	1.32	2.19:1
mean	8.85	1.28	2.29:1
RSD	2.9%	2.8%	4.3%

Table 9

Recovery Experiment

Component	Amount Added, mg	Amount Recovered, mg	Percent Recovery	Corrected Recovery
Theophylline	1.07	1.02	95%	97%
Ethylenediamine	0.178	0.157	88%	94%

A recovery experiment was also conducted in which a weighed amount of aminophylline was added to a cream formulation prior to extraction, derivatization and analysis. Subtracting the amounts of each component which were initially found in the unspiked formulation gave the results shown in Table 9. However, the percent recovery determined in this way underestimates the actual recovery of the drug from a single analysis of such a formulation, since these results are based on extraction and analyses of two cream formulation samples, one spiked and one unspiked. Employing a propagation-of-errors approach, a better estimate of recovery from a single extraction and analysis was obtained by calculating a "corrected recovery", which is essentially the square root of the recovery based on the two analyses. The results, also shown in Table 9, indicate that at least 94% of each of the drug components were extracted and analyzed by this method.

Additional Analyses of Commercial Creams

Three commercial creams were analyzed by this method. Results are shown in Table 10. Amounts of creams used for the analysis needed to be varied to obtain similar chromatographic responses. These results indicate that the percent active ingredient (calculated by adding the percentages of the two aminophylline components) varies widely among the commercial formulations.

CONCLUSION

In this study we have shown that the dansylation of both theophylline and ethylenediamine in thigh cream formulations is adversely affected by other components of these creams. This interference, which complicates the analysis of these components by reversed-phase HPLC, has been minimized through optimization of dansylation conditions. With this new method we have demonstrated excellent system and method precision as well as acceptable

Table 10

Aminophylline Content in Several Cream Preparations

	Amount of Cream Used For Analysis (mg)	Theophylline (mg)	Ethylenediamine (mg)	Percent Amino-phylline
University Medical Orig.[a]	260	2.30	0.33	1.00
Thinny Thighs[b]	400	1.05	0.18	0.31
Thigh High[c]	130	2.31	0.39	2.08

[a] University Medical Products, Newport Beach, CA.
[b] Winning Solutions, Inc., Westport, CT.
[c] Faneuil Companies, Scottsdale, AZ.

recovery. It appears that analyzing such complicated matrices requires a careful investigation and optimization of reaction and analytical conditions, especially when derivatization is involved. The analytical results show that there is a wide variation in the percent aminophylline present in various brands of thigh creams. This may have important consequences in conducting clinical studies of the safety and efficacy of these formulations.

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