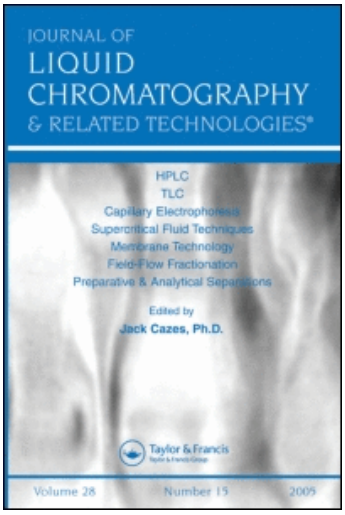

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Perez-Martinez, I. , Sagrado, S. and Medina-Hernández, M. J.(1996) 'Determination of Theophylline in Pharmaceuticals by Micellar Liquid Chromatography and Spectrophotometric Detection', Journal of Liquid Chromatography & Related Technologies, 19: 12, 1957 — 1966
To link to this Article: DOI: 10.1080/10826079608014019
URL: http://dx.doi.org/10.1080/10826079608014019

J. LIQ. CHROM. & REL. TECHNOL., 19(12), 1957–1966 (1996)

DETERMINATION OF THEOPHYLLINE IN PHARMACEUTICALS BY MICELLAR LIQUID CHROMATOGRAPHY AND SPECTROPHOTOMETRIC DETECTION

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ABSTRACT

An HPLC procedure for the determination of theophylline in pharmaceutical preparations is described. A Spherisorb octadecylsilane ODS-2 C_{18} analytical column and spectrophotometric detection at 273 nm were used. Adequate retention was achieved with a mobile phase containing 0.05 M sodium dodecylsulphate (SDS) and 3% propanol at pH 7. The reproducibilities were 1.2 % and 1.7 % for 3.8 and 7.6 μg/mL theophylline concentrations, respectively.

The determination of theophylline in six pharmaceutical preparations gave recoveries, with respect to the values declared by the manufacturers, which usually ranged between 83-97 % and 85-104% using peak heights and peak areas, respectively.

INTRODUCTION

Theophylline (1,3-dimethylxanthine), is a bronchodilator agent mainly used in the treatment of chronic asthma, bronchitis, emphysema and apnea in newborn children. It has a narrow therapeutic range and serum-theophylline concentrations should be monitored during therapy.

Adverse effects commonly affect the gastro-intestinal tract and central nervous system. Following overdosage tremor, delirium, convulsions and death may occur.[1]

Several analytical techniques have been applied to the determination of theophylline in pharmaceuticals, spectrophotometry,[2] phosphorimetry,[3] gas chromatography[4] and capillary electrophoresis.[5] However, high performance liquid chromatography (HPLC) now seems to be the most frequently used technique.

In these procedures, a C_{18} stationary phase, a mixture of acetonitrile-water or methanol-water with acetate and phosphate buffers as mobile phases and UV detection was usually used.[6-7]

Micellar liquid chromatography (MLC) is an alternative of reversed phase liquid chromatography, which employs aqueous solutions of surfactants above the micellar critical concentration as the mobile phases. Procedures for the evaluation of diuretics,[8,9] anabolic steroids,[10] and catecholamines[11] in pharmaceuticals, have been developed.

The main advantages to using a micellar solution, instead of a conventional hydroorganic mobile phase, in reversed phase liquid chromatography, are the lower cost and toxicity, the biodegradability of the solvent, the performance of elution gradients of surfactant without the need of reequilibration of the column,[12] and the easy solubilization of analytical samples, which allows the determination of drugs in physiological fluids without the need of a previous separation of the proteins present in the samples.[13]

In a previous paper, a micellar liquid chromatographic procedure for the determination of caffeine, theophylline and theobromine in urine samples was described.[14] Maximum resolution was achieved with a 0.075 M sodium dodecylsulphate+ 1.5% propanol eluent.

In this paper, investigations on the chromatographic behaviour of theophylline with micellar eluents, are reported and a rapid analytical procedure for the determination of this compound in pharmaceutical formulations is developed.

MATERIALS AND METHODS

Apparatus

A Hewlett-Packard HP 1050 chromatograph with a quaternary pump, a UV-visible detector and an HP 3396A integrator was used (Palo Alto, CA, USA). Data acquisition was made with the Peak-96 software from Hewlett-Packard (Avondale, PA, USA). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 μL loop. A Spherisorb octadecylsilane ODS-2 C_{18} column (5 μm, 120 x 4.6 mm) and a guard column of similar characteristics (35 x 4.6 mm) (Scharlau, Barcelona, Spain) were used. The mobile phase flow rate was 1 mL min^{-1}. The detection was performed in UV at 273 nm. All the assays were carried out at room temperature.

Reagents and standards

The micellar mobile phases were prepared by mixing aqueous solutions of sodium dodecylsulphate (99%, Merck, Darmstadt, Germany) with an alcohol to obtain the working concentration. The alcohols studied were methanol (HPLC, Panreac, Barcelona, Spain) and 1-propanol (analytical reagent, Panreac). The pH of the micellar eluent was adjusted with 0.01 M phosphate buffer, prepared with disodium hydrogen phosphate and phosphoric acid (analytical reagent, Panreac).

Stock standard solutions of theophylline (Fluka, Buchs, Switzerland, > 99%) were prepared by dissolving 10 mg of the compound in 100 mL of 0.05 M SDS solutions and they were stored in the dark at 4°C. Under such conditions, solutions were stable at least for one month. Working solutions were prepared by dilution of the stock standard solution.

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph, were vacuum-filtered through 0.45 μm and 0.22 μm Nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

Sample preparation

For the analysis of tablets, five tablets were weighed and ground in a mortar. A portion was taken, weighed and dissolved in 0.05 M SDS in an ultrasonic bath. The solutions were filtered trough a n° 4 sintered glass plate and diluted in a calibrated flask. Capsules were dissolved in 0.05 M SDS, by immersion in an ultrasonic bath. An adequate volume of the drops was taken and diluted with 0.05 M SDS. Other dilutions were made with 0.05 M SDS. In all cases, triplicate or quintuplicate determinations were performed.

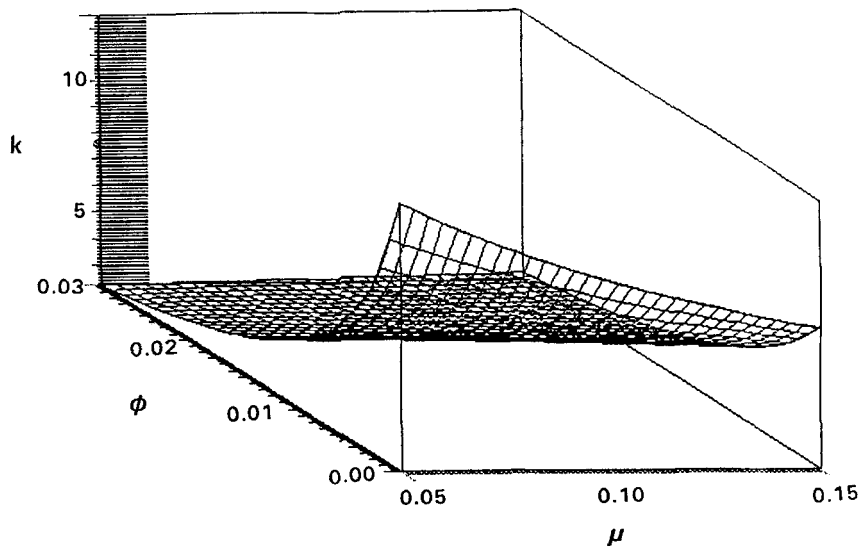

Figure 1. Retention surface for theophylline as a function of the concentration of surfactant, μ, and propanol, ϕ, in the mobile phase (pH = 7).

Table 1

Capacity Factors and Efficiency of Theophylline Obtained with Different SDS Mobile Phases

SDS, M	Modifier, v/v %	k	N
0.1	Methanol, 5%	4.0	66
	Propanol, 3%	2.0	147
0.05	None	12.6	27
	Propanol, 1.5%	4.0	144
	Propanol, 3%	2.2	475
0.1	Propanol, 1.5%	3.6	115
0.15	None	7.6	31
	Propanol, 1.5%	3.0	136
	Propanol, 3%	2.5	237

RESULTS

Chromatographic Behaviour of Theophylline

A study to select the composition of the mobile phase (pH, concentration of SDS, and nature and concentration of modifier), for the adequate retention of theophylline was performed.

No significant changes in the retention of theophylline were observed in the 3.5-6.9 pH range at a fixed 0.1 M SDS concentration, as can be expected owing the protonation constants of the compound in aqueous solutions ($\log K_1 = 8.6$ and $\log K_2 = 3.5$). Table 1 shows the capacity factors and efficiency values of the peaks of theophylline obtained with different mobile phases. The retention of theophylline decreased when the SDS concentration in the mobile phase increased. In a purely micellar medium, the peaks of theophylline, obtained for different SDS concentrations, were asymmetrical and the values of efficiency were very low and slightly modified with the mobile phase composition.

In MLC, the addition of an alcohol to the mobile phase produces, for most solutes, a decrease in retention and an improvement in the efficiency. A short-chain alcohol (methanol, 5% and propanol, 3%) was added to the 0.1 M SDS eluent. As can be observed, the addition of propanol to the 0.1 M SDS mobile phase produced adequate retention and improvement of the efficiency of the chromatographic peaks with respect to the use of methanol. As a consequence, propanol was selected.

In order to select the composition of the mobile phase (SDS and propanol concentrations), the equation of the retention of theophylline was obtained in agreement with the suggestions reported by Torres Lapasió et al.[15] The capacity factors of theophylline for the selected mobile phases (Table 1) were adjusted to an equation of the type:

$$\frac{1}{k'} = A\mu + B\phi + C\mu\phi + D \qquad (1)$$

where μ is the total concentration of surfactant, ϕ is the volume fraction of alcohol and A, B, C and D fitting parameters. The fitting parameters for theophylline calculated using multiple regression analysis were: 0.5204, 14.284, -35.588 and 5.3779×10^{-2}, respectively.

In this equation, the term $(B+C\mu)$ is a measure of the eluent strength of modifier in the presence of a constant concentration of the surfactant. On the other hand, the term $(A+C\phi)$ indicates the eluent strength of surfactant in the presence of a constant concentration of the modifier. High values of these terms can be

Table 2

Regression Statistics for the Calibration Curves of Theophylline

Parameter	(1) Peak Area	(2) Peak Height
Slope	38.2	1.896
C.I. slope	37.3 - 39.1	1.860 -1.932
S.E. slope	0.4	0.016
Intercept	28	0.75
C.I. intercept	23, 33	0.71 - 0.79
S.E. intercept	2	0.10
Standard error	3.9	0.165
r	0.9982	0.9995
F	413	14086
N	16	16

* C.I. = Confidence intervals (95%);
S.E. = standard error;
r = correlation coefficient;
F = the ratio between the residual variance and the variance modelled by regression;
N = number of points

interpreted as high eluent strengths of the modifier and the surfactant, respectively. For theophylline the eluent strength of propanol was 16.063, 10.725 and 8.9454 for 0.05, 0.1 and 0.15 M SDS concentration, respectively, and the eluent strength of surfactant was + 0.5204, - 0.0134 and - 0.5473 for 0, 1.5 and 3% propanol concentration, respectively. These data should be interpreted in the following way. The eluent strength of propanol is significantly larger than the eluent strength of SDS.

Only for a purely aqueous SDS mobile phase (no propanol added) the SDS shows an appreciable eluent strength. If propanol is added to the mobile phase, the eluent strength of SDS is negligible.

Figure 1 shows the retention surface of theophylline as a function of the SDS and propanol concentrations. As can be observed, for a 0.05 M SDS mobile phase, an increase in propanol concentration from 0 to 3.0 % leads to a drastic decrease of retention. In the presence of a 3% propanol concentration, the increase of the SDS concentration in the mobile phase, practically, did not produced a decrease of the retention. Adequate retention and efficiency was achieved with a 0.05 M SDS + 3% propanol eluent and was selected for further experiments.

Table 3

Analysis of Pharmaceutical Preparations

Preparation (presentation), Source	Declared	Found (Peak Areas)	Found (Peak Heights)
Dexa-bronchisan (tablets), Boehringer Mannheim	Theophylline 50 mg Dexamethasone 0.5 mg. Diphenhidramine HCL 10 mg Ephedrine HCL 25 mg Calcium lactate 70 mg Excipient	36.3 ± 1.1	39. 6 ± 1.1
Eufilina retard 175 (covered tablets), Elmu	Theophylline 140.9 mg Ethylenediaminedichlorohydrate 75.5 mg Excipient	123 ± 2	125 ± 3
Elixifilin (syrup), Morrith S.A.	Theophylline 5.33 mg/mL Potassium iodure 8.66 mg/mL Saccharin sodium 0.0 mg/mL Sacharose 150 mg/mL Excipient and ethanol	5.23 ± 0.17	4.91 ± 0.08
Muco-teolixir (syrup), Carulla-Vekar S.A.	Theophylline 4 mg/mL N-acetyl-DL-homocysteine Thiolactone 0.04 mg/mL Sodium benzosulfimide 0.1 mg/mL Excipient	3.9 ± 0.1	3.86 ± 0.13
Teolixir compositum (syrup), Biogalenica S.A.	Theophylline 5.33 mg/mL Prednisolone 0.333 mg/mL Guaifenesin 6.66 mg/mL Saccharin sodium 1 mg/mL Ethanol 0.2 mL/mL Saccharose 225 mg/mL Excipient	5.20 ± 0.13	4.94 ± 0.06
Pulmeno (capsules), Sandoz Pharma S.A.	Theophylline anhydrous 200 mg Excipient	183 ± 4	179 ± 5

Analytical Data

The calibration curve of theophylline were obtained by triplicate injection of standard solutions with a varying concentration of the theophylline in the range 2-10 μg/mL. Peak heights and peaks areas were used as dependent variables.

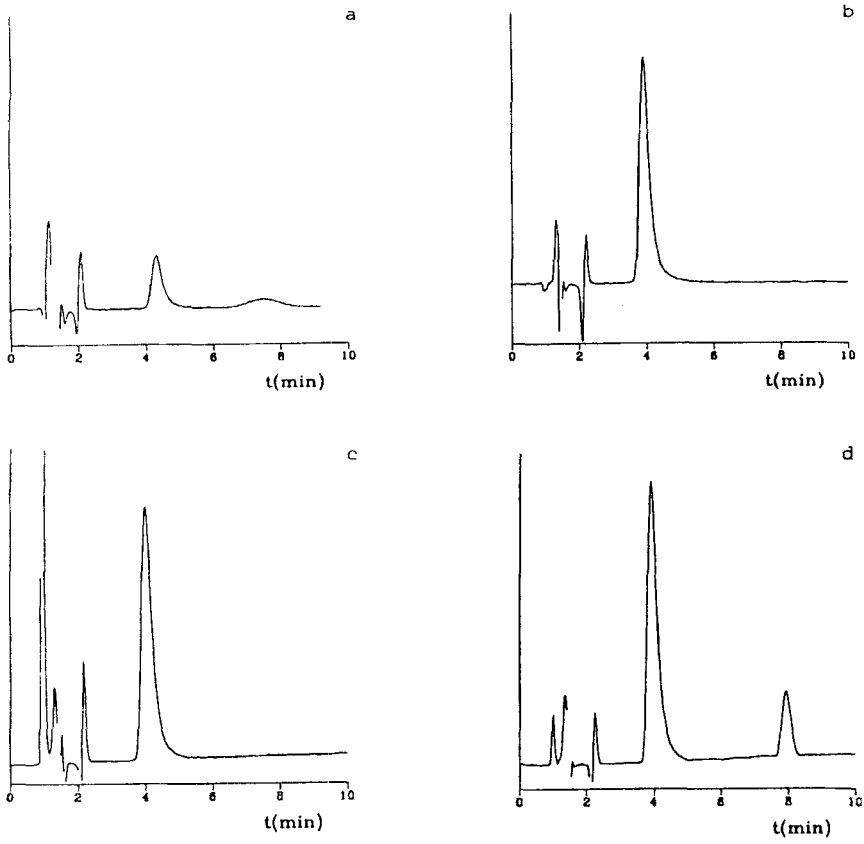

Figure 2. Chromatograms of some pharmaceutical preparations: a) Dexabronchisan; b) Eufilin retard; c) Mucoteolixir; d) Teolixir.

The presence of outliers, normality of residuals (Kolmogoroff test), homogeneity of variances (Cochran and Bartlett tests) and validity of the linear model (lack-of-fit test) were studied in agreement with the suggestions reported by Sarabia and Ortiz[16]

In all cases the significance levels found assures the validity of the regression models. Table 2 shows regression statistics for the calibration curves of theophylline.

The calibration curves showed adequate regression coefficients with peak areas and heights over the working interval. The value of the residual variance to the variance modelled by regression ratio (F) indicated that the use of peak height as dependent variable is preferable.

The reproducibility was evaluated from two series of five aliquots of theophylline. The coefficient of variation was 1.7% at a 3.8 μg/mL concentration level, and 1.2% for 7.6 μg/mL.

Analysis of Pharmaceutical Formulations

The procedure was applied to the determination of theophylline in six pharmaceutical preparations found in the spanish market, which contain theophylline together with a number of other components (Table 3). Some chromatograms are shown in Figure 2. As can be observed, the peaks of the others components in the samples did not overlap with the peak of theophylline.

The theophylline content was obtained by taking three aliquots of each three or five independent dissolved formulations, and injected into the chromatograph. The results were reproducible and the recoveries with respect to the values declared by the manufacturers were in the 85-104 % range using peak areas and between 83-97% using peak heights, except for Dexa-bronchisan (73% and 79%, respectively).

The proposed procedure for the determination of theophylline is rapid (five minutes per sample), reliable and free of interferences.

ACKNOWLEDGEMENTS

The authors are grateful to M. Cuenca, A. Montesinos and Y. Lopez for their collaboration in this work.

REFERENCES

1. J.E.F. Reynolds, **Martindale. The Extra Pharmacopeia,** 30th ed., The Pharmaceutical Press, London, (1993).

2. M. A. Abuirjeie, M. S. El-Din, I. I. Mahmoud, J.Liq. Chromatog., **15**, 101-125 (1992).

3. A. D. Campiglia, J. J. Laserna, A. Berthod, J. D. Winefordner, Anal. Chim. Acta, **244**, 215-222 (1991).

4. H. W. A. Teeuwen, E. L. Elbers, J. M. Van-Rossum, Mol. Biol. Rep., **15**, 1-7 (1991).

5. X. Q. Dang, L. Yang, Z. P. Sun, D. K. Ling, J.Chromatogr., **630**, 363-369 (1993).

6. M. Korman, J. Vindevogel, P. Sandra, Electrophoresis, **15**, 1304-1309 (1994).

7 Z. Budvari-Barany, G. Radeczky, G. Szasz, A. Shalaby, Acta Pharm. Hung., **61**, 1-7 (1991).

8. E. Bonet Domingo, M. J. Medina Hernández, G. Ramis Ramos, M. C. García Alvarez-Coque, Analyst, **117**, 843-847 (1992).

9. E. Bonet Domingo, M. J. Medina Hernández, G. Ramis Ramos, M. C. García Alvarez-Coque, J. Pharm. Biomed. Anal., **11**, 711-716 (1993).

10. S. Torres Cartas, M. C. García Alvarez-Coque, R. M. Villanueva Camañas, Anal. Chim. Acta, **302**, 163-172 (1995).

11. R. M. Villanueva Camañas, J. Mª Sanchis Mallols, J .R. Torres Lapasió, G. Ramis Ramos, Analyst, **120**, 1767-1772 (1995).

12. M. G. Khaledi, J. G. Dorsey, Anal. Chem., **57**, 2190-2196 (1985).

13. M. J. Rosen, **Surfactants and Interfacial Phenomena**, Wiley, New York, 1978.

14. I. Perez-Martinez, S. Sagrado, M. J. Medina-Hernández, Anal. Chim. Acta, **304**, 195-201 (1995).

15. J. R. Torres Lapasió, R. M. Villanueva Camañas, J. M. Sanchis Mallols, M. J. Medina-Hernández, M. C. García Alvarez-Coque, J. Chromatogr., **639**, 87-96 (1993).

16 L. Sarabia, M. C. Ortiz, Trends Anal. Chem., **13**, 1 (1994).

Received November 24, 1995
Accepted December 20, 1995
Manuscript 4046