ELSEVIER

Journal of Chromatography B, 736 (1999) 289–293

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

High-performance thin-layer chromatographic determination of theophylline in plasma

Padma V. Devarajan[a,*], Poonam N. Sule[a], D.V. Parmar[b]

[a]Pharmaceutical Division, Department of Chemical Technology, University of Mumbai, Matunga, Mumbai 400019, India
[b]Clinical Pharmacology Laboratory, Pharmacology Department, Grant Medical College, Sir J.J. Group of Hospitals, Byculla, Mumbai 400008, India

Received 22 June 1998; received in revised form 27 July 1999; accepted 28 July 1999

Abstract

A high-performance thin-layer chromatographic method for quantification of theophylline from plasma is described. The calibration curves of theophylline in methanol and in plasma were linear in the range 20–100 ng. The correlation coefficients were 0.9971±0.0011 and 0.9955±0.0003 for standard curves in methanol and in plasma, respectively. The limit of quantitation of theophylline in human plasma (assay sensitivity) was 20 ng and no interference from endogenous compounds was observed. The recovery of theophylline from human plasma using the described assay procedure was 89%. The mean relative standard deviations for intra- and inter-day analyses were 1.67% and 2.34% for 50 ng and 2.25% and 3.14% for 75 ng theophylline concentration, respectively. The method was utilized to monitor plasma concentration of theophylline post-administration of sustained release tablets in human patient volunteers. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Theophylline

1. Introduction

Theophylline(1,3-dimethyl xanthine) [1] is a widely used bronchodilator for the treatment of asthma and chronic obstructive pulmonary disease (COPD) [2]. To study the bioavailability of theophylline in animals and humans various analytical methods have been developed [3]. Various gas–liquid chromatography (GLC) methods for quantitative determination of theophylline have been reported [4–6], but a most of these require derivatization. Numerous high-performance liquid chromatography (HPLC) and immunoassay procedures with varying sensitivities, utilizing small plasma samples, have also been reported [7–23]. These methods, although sensitive, are relatively uneconomical especially in terms of speciality gases/solvents/immunoreagents that are required for routine application. High-performance thin-layer chromatography (HPTLC) facilitates automatic application and scanning in situ and has been successfully used in the analysis of pharmaceuticals. Moreover, a large number of samples can be run simultaneously using small quantities of solvents unlike HPLC and GLC [24,25]. Hence, a HPTLC procedure for the determination of theophylline from plasma has been developed.

This paper describes a rapid, simple, economical and specific HPTLC method for measuring theo-

*Corresponding author. Fax: +91-22-4145-614.

0378-4347/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(99)00354-0

phylline in human plasma. The application of the method for analysis of plasma concentration of theophylline in human patient volunteers is also described.

2. Experimental

2.1. Reagents and chemicals

Theophylline was supplied by German Remedies (Mumbai, India). Chromatographic-grade solvents were purchased from Ranbaxy (Gurgaon, India). All other reagents were of analytical-grade and were used without further purification.

2.2. Instrumentation

A Remi cyclomixer and centrifuge was used for mixing and centrifuging the samples. The samples were spotted on E-Merck thin-layer chromatography (TLC) aluminum plates precoated with Silica gel 60 F254 (layer thickness 250 μm). Using the Camag Linomat Model IV, the samples were applied as narrow bands of 4 mm width at a constant rate of 10 μl/s under a nitrogen atmosphere. The length of chromatogram run was 8 cm and the time required for each run was approximately 15 min. The separation was carried out in a Camag twin trough chamber (10×10 cm) using ascending mode of development. The separation was visualized by irradiation of the plates with a short-wavelength (254 nm) ultraviolet lamp. Densitometric analysis of the separated components was carried out using a Camag TLC scanner II Model 1988 in the absorbance mode at 272 nm. Scanning speed was kept at 4 mm/s. Integration of chromatogram was performed using a Camag TLC LCI-100 scanner/integrator system.

2.3. Assay procedure

2.3.1. Extraction of theophylline from plasma

Aliquots of stock solution of theophylline in methanol (0.2–1.4 μg) were pipetted in glass stoppered centrifuge tubes. Methanol was evaporated at 45°C under a gentle stream of nitrogen gas. Control plasma (drug-free) (1 ml) was added to each centrifuge tube and the tubes were vortexed on a cyclomixer for 5 min. Appropriate blank was prepared simultaneously. Sodium hydroxide (1 *M*) (1 ml) and 72% (v/v) perchloric acid (0.3 ml) were added to each of the centrifuge tubes. The capped tubes were again vortexed for 5 min. The drug was then extracted in 5 ml of diethyl ether–dichloromethane–isopropanol (6:4:1, v/v) [16,17]. The tubes were then centrifuged at 3000 *g* for 10 min. The organic layer was separated by aspiration into 75×10 mm glass tubes and evaporated at 45°C under a stream of nitrogen gas. The residue obtained was reconstituted with 0.1 ml methanol and 10 μl of this solution was applied as a band to obtain theophylline amounts in the range 20–140 ng on plate. In the case of volunteer plasma samples, the reconstituted sample was diluted 5–10-fold and 10 μl spotted, so that the amount of theophylline on plate was within the linear range of the assay.

2.3.2. Selection of mobile phase

Various solvent systems reported in literature [3,26,27] for TLC analysis of theophylline were tried. Subsequently, a modified solvent system constituting chloroform–methanol (9:1) was chosen [27].

2.3.3. Standard curve for theophylline

Stock solution of theophylline (1 μg/μl) was prepared in methanol/plasma. This solution was diluted to yield a 0.01 μg/μl working stock solution. Aliquots of 2, 4, 6, 8 and 10 μl of this working stock solution were applied as bands to obtain 20, 40, 60, 80 and 100 ng theophylline on the plate.

2.4. Recovery and repeatability studies

Recovery of theophylline from the sample by the isolation procedure was demonstrated by external standardization. Twelve sample tubes were taken. To six of these tubes 50 ng of theophylline (stock solution equivalent to 50 ng theophylline) was added. The contents of the tubes were evaporated to dryness. To each of the 12 tubes 1-ml of drug-free plasma was added. Further processing was done as described before in Section 2.3.1. The supernatant from each of tubes 1–6 was poured into empty 75×10 mm glass tubes while contents from tube seven to twelve were poured into analogous tubes

containing 50 ng of theophylline in methanol. The contents of all the 12 tubes were evaporated to dryness under a stream of nitrogen gas at 45°C. The residue was reconstituted in 100 μl of methanol, 10 μl applied as a band, and the area under the curve determined. The ratio of the mean of the areas under the curves of tubes 1–6 divided by the mean of the areas under the curves of tubes 7–12 multiplied by 100 indicated the percent recovery of theophylline. A similar experiment was carried out at a theophylline concentration of 75 ng.

3. Results and discussion

3.1. Selection of mobile phase

Reports in the literature state that a system optimized for TLC can be adapted for HPTLC by carrying out modifications in the solvent strength and selectivity. The selection of mobile phase was therefore attempted by modification of TLC system reported for identification and purity testing of theophylline from bulk and pharmaceuticals. The solvent system consisting of chloroform–methanol (9:1, v/v) which gave dense and compact bands with appropriate R_F values for both theophylline and its potential metabolites in plasma (Fig. 1) was selected.

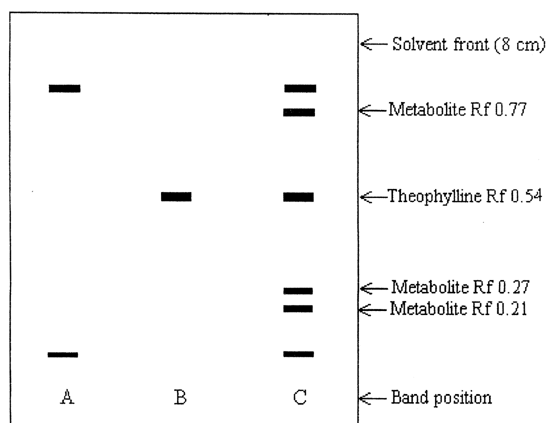

Fig. 1. Schematic representation of the TLC plate after the development of bands. (A) Blank (drug-free) plasma, (B) plasma spiked with theophylline, and (C) human patient plasma dosed with theophylline preparation.

3.2. Standard curve of theophylline in methanol and plasma

A series of standard curves (six) of theophylline were prepared in methanol and in plasma. All standard curves were linear over the range 20–100 ng. The mean values of intercept, slopes and coefficient of correlation were 0.1251±0.0254, 0.4980±0.0034 and 0.9971±0.0011 for standard curves in methanol and 0.6186±0.0340, 0.4430±0.0017, and 0.9955±0.0003 for standard curves in plasma, respectively.

The intercept values were 1.05% (for standard curve in methanol) and 5.11% (for standard curve in plasma) of the area under the curve obtained, for the lowest points of the standard curve, i.e., 20 ng. The range of reliable quantitation was set at 20–100 ng as no significant difference was observed in the slopes of the standard curves in this range (analysis of variance, ANOVA; $P>0.05$). Beyond 100 ng theophylline on plate, the standard curves deviated from linearity.

The limit of quantitation for theophylline in plasma was observed to be 20 ng on plate (0.2 μg/ml theophylline in plasma). This was the lowest concentration of theophylline in plasma that was accurately detected and integrated by the instrumentation used. The relative standard deviation (RSD) was 1.09% ($n=6$). Below this concentration the spot for theophylline was not clearly visible. No interference from endogenous compounds in plasma was observed.

3.3. Recovery and repeatability study

This study was undertaken to document the extraction efficiency of the method. The results indicated that the recovery of theophylline from human plasma using the described extraction was 89±3.25% at the 50 ng level (low theophylline concentration) and 89±4.11% at the 75 ng level (high theophylline concentration). The results also confirmed the repeatability of the method.

Accuracy and repeatability of the assay was tested at 50 ng and 75 ng level of theophylline. The results shown in Table 1 revealed excellent accuracy and high precision of the assay method.

The intra-day repeatability of the assay was as-

Table 1
Accuracy and repeatability of theophylline HPTLC assay

Theophylline amount on plate (ng)		Difference (%)	RSD (%)
Experimental[a]	Theoretical		
49.79	50	0.42	1.53
74.62	75	0.49	1.67

[a] $n=6$.

sessed by assaying 12 plasma samples corresponding to theophylline concentrations of 50 and 75 ng, 12 times, within one day. The stability of frozen aliquots of human plasma containing 50 and 75 ng of theophylline was examined by analysis of extracted samples ($n=6$) of each concentration for 12 days. The results are presented in Table 2. The low RSD indicated good intra- and inter-day repeatability of assay. There was no indication of compound instability as a result of freezing and thawing over a period of 12 days.

3.4. Plasma levels of theophylline in COPD patients

The utility of the analytical method was assessed by determining the plasma concentration of theophylline following single oral administration of sustained release tablet of theophylline (200 mg) in six patient volunteers suffering from mild COPD.

Table 2
Intra- and inter-day repeatability of the assay

	Intra-day repeatability		Inter-day repeatability	
	Theoretical amount (ng)			
	50	75	50	75
	Actual amount (ng)			
Mean (ng)	50.24	74.87	50.37	74.98
Range (ng)	48.43–52.01	71.22–78.43	47.81–52.85	70.33–79.87
RSD (%)	1.68	2.25	2.34	3.15

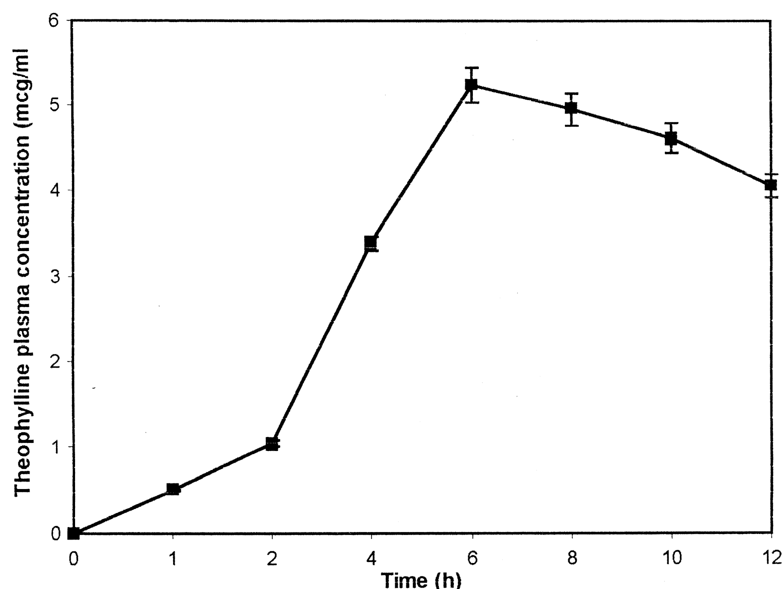

Fig. 2. Average theophylline plasma levels after the administration of a single oral sustained release marketed tablet of theophylline.

Fig. 2 shows the mean plasma concentration versus time curve. A mean peak plasma level of 5.24±0.522 μg/ml was observed at 6 h post-administration. Mean elimination rate constant was found to be 0.063±0.008/h. Plasma drug disappearance half life was 10.9±1.27 h. Mean area under the plasma concentration time curve was 42.2±3.92 μg h/ml.

4. Conclusion

The method described herein is rapid, selective and economic. It allows rapid analysis of theophylline in plasma at a sensitivity which is suitable for the plasma levels of drug reported in biopharmaceutical and therapeutic analysis.

References

[1] Merck Index, 10th ed., Merck, Rahway, NJ, 1983, p. 9114.
[2] Physicians Desk Reference, 49th ed., Medical Economics Data Production Company, Montwale, NJ, 1995, p. 1287.
[3] K. Florey, in: Analytical Profiles of Drug Substances, Vol. 4, Academic Press, New York, 1975, p. 466.
[4] G.F. Johnson, W.A. Dechtriaruk, H.M. Solomon, Clin. Chem. 21 (1975) 144.
[5] D. Perrier, E. Lier, Clin. Chem. 22 (1976) 898.
[6] H.A. Schwertner, T.N. Ludden, J.E. Wallace, Anal. Chem. 48 (1976) 1875.
[7] L.A. Broussard, Clin. Chem. 27 (1981) 1931.
[8] K.T. Muir, J.H. Jonkman, D.S. Tang, M. Kunitani, S. Riegelman, J. Chromatogr. 221 (1980) 85.
[9] I.N. Papadoyanis, V.F. Samanidou, Anal. Lett. 26 (1993) 851.
[10] J. Moncrieff, J. Chromatogr. 568 (1991) 177.
[11] L. Stanley, M. Galina, J. Liq. Chromatogr. 12 (1989) 1851.
[12] B.E. Kushkenbaeva, A.P. Arzamastev, E.T. Gneushev, Khim-Farm. Zh. 22 (1988) 501.
[13] A.V. Sokolov, E.G. Brezhneva, Khim-Farm. Zh. 22 (1988) 252.
[14] R.F. Meatherall, Ther. Drug Monit. 10 (1988) 101.
[15] R.E. Venkata, R.G.R. Raghuveer, S.P. Khadgapathi, Indian J. Pharm. Sci. 49 (1987) 180.
[16] N. Emmanuel, F. Bernard, A. Charles, P. Michel, J. Chromatogr. 419 (1987) 177.
[17] R. Chien, R.J. Stubbs, W.F. Bayne, J. Chromatogr. 422 (1987) 281.
[18] S. Subramaniam, S.K. Garg, R. Dhand, S.K. Malik, P.L. Sharma, Bull. Post-Grad. Inst. Med. Educ. Res. 20 (1986) 63.
[19] L. Qisui, Z. Yanru, Zhongguo Yixue Kexueyuan Xuebao 9 (1987) 55.
[20] C.E. Cook, M.E. Twin, Res. Commun. Chem. Path. Pharmacol. 13 (1976) 497.
[21] N. Takashi, S. Masayuki, K. Hiroaki, Chem. Pharm. Bull. 27 (1979) 893.
[22] J.B. Gushaw, M.W. Hu, P. Singh, J.G. Miller, R.S. Schneider, Clin. Chem. 23 (1977) 144.
[23] A.L. Nesse, L.F. Soyka, Clin. Pharmacol. Ther. 21 (1977) 633.
[24] J.C. Touchstone, S. Ahuja (Eds.), Ultratrace Analysis of Pharmaceutical and Other Compounds of Interest, Wiley InterScience, New York, 1986.
[25] V.P. Shah, S. Riegelman, J. Pharm. Sci. 63 (1974) 1283.
[26] A.C. Moffat (Ed.), Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Material, 2nd ed., Pharmaceutical Press, London, 1986.
[27] L.J. Kraus, E. Dumont, J. Chromatogr. 48 (1970) 96.