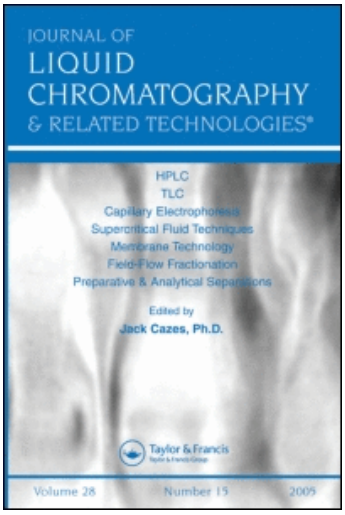

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A NEW HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) METHOD FOR DETERMINATION OF THEOPHYLLINE IN PLASMA

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ABSTRACT

A new high-performance thin-layer chromatography (HPTLC) method for the determination of theophylline in plasma has been developed. Plasma samples were prepared by the internal reference method. Denaturation was carried out through cold centrifugation.

Separation of the drug from the complex matrix was accomplished within a short 50 mm migration distance and 25 min migration time on silica gel 60 F 254 layers using toluene-isopropanol-acetic acid (80:20:5) as the mobile phase. The analyte was quantified by absorbance/reflectance densitometry using peak-area ratio analysis. Validation parameters were investigated considering the special features of the method.

INTRODUCTION

Theophylline, 1,3-dimethylxanthine, was introduced into medical use several decades ago for its bronchodilator properties. The therapeutic range of theophylline in plasma is, consequently, narrow and the management of the drug in clinical practice is complicated by several factors which influence the disposition of theophylline, e.g., age, smoking habits, diet, disease, obesity, and drugs all contribute to the wide inter-individual variability of theophylline clearance. As a consequence, the monitoring of plasma concentration in patients on theophylline treatment is now current practice.[1]

High performance liquid chromatography (HPLC) has been used as the suitable method for the determination of theophylline.[2-8] Since HPTLC is a powerful tool for the screening of drugs and other substances,[9,10] it may be regarded as a renaissance of this practical and effective method. Therefore, we have developed a more selective, sensitive, and cost effective HPTLC method for determination of theophylline in human plasma. Our method permits assay of at least 17 samples, using a three-point, in-system calibration on a single 10×20 cm pre-coated plate.

EXPERIMENTAL

Theophylline, caffeine, and acetaminophen (Sigma, St. Louis, USA); toluene, isopropanol, acetic acid, and chloroform (Merck, Darmstadt, Germany); ethyl acetate (Fluka AG, Bush, Switzerland); methanol (Riedel-de-Haen, Germany); plasma samples (Amiralmomenin Hospital, Tehran, Iran).

Method

Standard solutions

Stock solutions of theophylline and acetaminophen (1mg/mL) were prepared by dissolving accurately weighed amounts of the substances in

methanol using 10 mL, acid-washed, volumetric flasks. Required concentrations were made through appropriate dilutions. Internal reference standard acetaminophen stock solution was added to each diluted solution. These solutions were used for calibration graph construction.

Sample solutions

Samples were prepared using 2 mL of plasma in 10 mL, acid-washed, test tubes. They were spiked to contain different amounts of theophylline and 100 µL of the internal reference standard of acetaminophen (1 mg/mL stock solution) was added. Plasma samples were deproteinized with 0.2 mL of 1 M hydrochloric acid and centrifuged at 3000 rpm and 5°C for 15 min. The supernatant was extracted with 5 mL mixture of chloroform-isopropanol (1:1). The organic phase was transferred to another acid-washed test tube and evaporated at 40°C (water bath) under a stream of nitrogen. The dry residue was dissolved in 120 µL of methanol. This methanolic solution was used for the HPTLC procedure.

Thin layer chromatography

HPTLC was performed on 10×20 cm silica gel 60 F 254 pre-coated plates (Art. 5642, Merck). The plates were previously developed in chloroform-methanol (1:1) and stored in a 60 °C oven for 15 min to remove elutable components and produce a clean layer background for visual evaluation, and a straighter baseline and high signal-to-noise ratio when scanning.[11] Ascending chromatography was performed in a twin-trough TLC chamber (Camag, Muttenz, Switzerland), using toluene-isopropanol-acetic acid (80:10:5) as the mobile phase, without chamber saturation. The migration distance was 50 mm and migration time about 25 min. Bands of samples and standards (4mm) were applied with a Camag Linomat IV using the data-pair technique.[12] The chromatoplates were dried by nitrogen flow. The spots were detected under UV light at 254 nm. Scanning was performed at 278 nm with a Camag TLC Scanner II in the reflectance/absorbance mode.

RESULTS AND DISCUSSION

Selectivity

Baseline separation was obtained between theophylline, caffeine (as the most probable interfering compound in human plasma), and acetaminophen, which is used as internal standard (Fig. 1). This baseline separation was also obtained between theophylline, acetaminophen, and matrix components (Fig. 2).

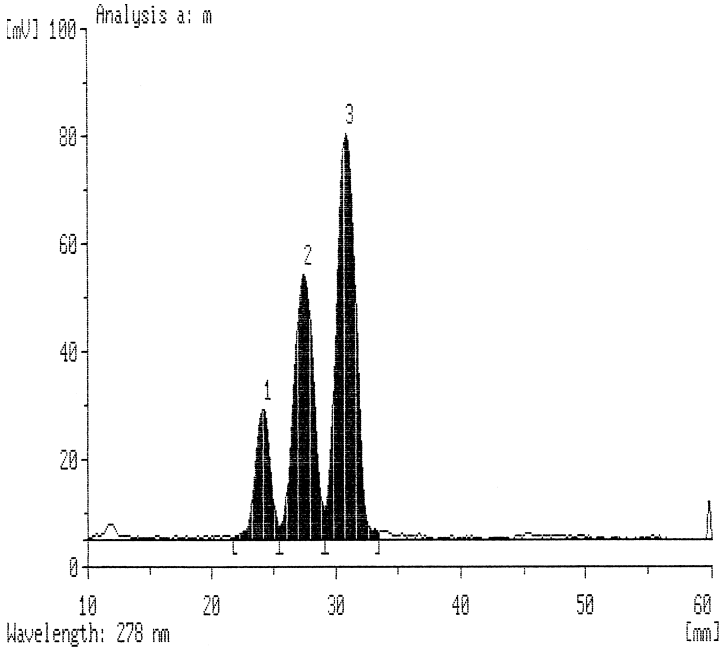

Figure 1. Baseline separation between caffeine, 1, theophylline, 2, and acetaminophen, 3.

Table 1

Calibration Data

Concentration (ng/spot)	Peak Area Ratio (x 1000)
80.2	708.7
90.2	745.5
100.2	778.8
110.2	880.1
120.2	919.2
130.3	999.9
140.3	1086.1
150.3	1062.4
160.3	1114.2

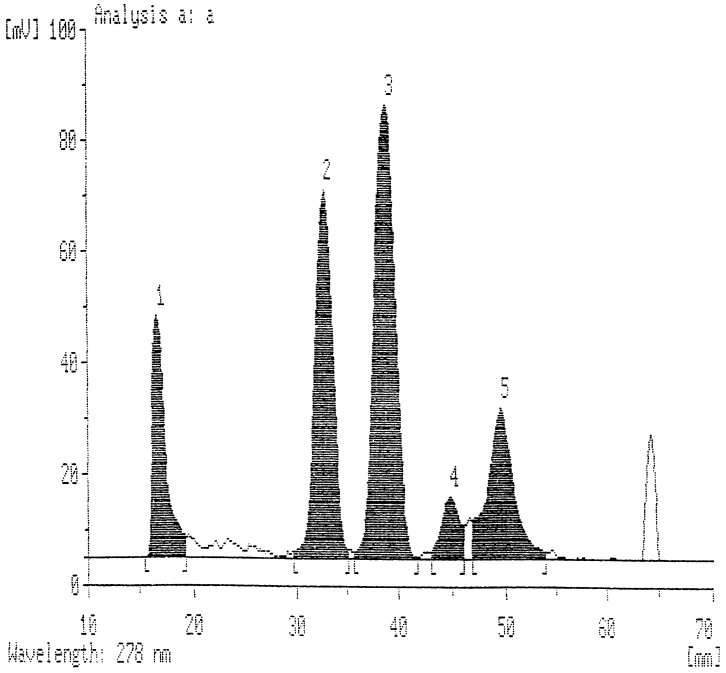

Figure 2. Baseline separation between theophylline, 2, acetaminophen, 3, and other matrix components.

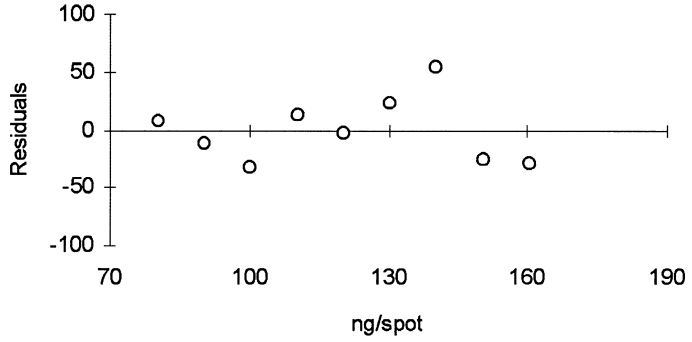

Figure 3. Calibration function for theophylline in the linear working range.

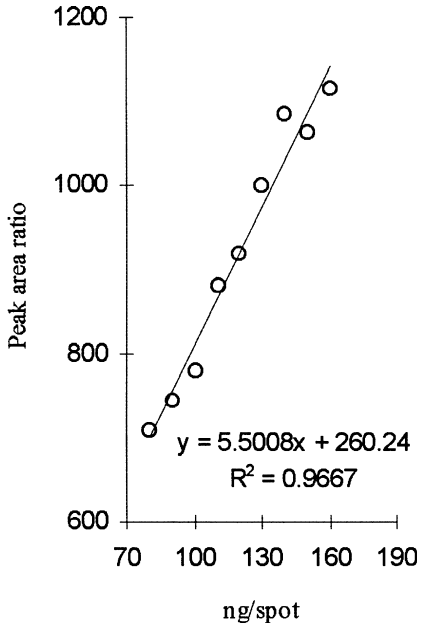

Figure 4. Residual plot.

Calibration and Working Range

In quantitative TLC, the results are highly influenced by the quality of the calibration.[13] Lack of linearity can easily be overcome by specifying working ranges with linear response.[14] The linearity of peak area ratio to different concentrations of theophylline was determined over a concentration range of 80 to 160 ng/spot (Table 1, Fig. 3). Residuals were plotted as an illustrative tool to check the adequacy of the regression model.[15,16] The residuals were distributed at random around the regression zero line (Fig. 4) and the calibration graph can be assumed to be linear.

Accuracy

Accuracy of the method gives information about the recovery of the drug from the sample, and about matrix effects.[16] It was tested by determination of efficiency of recovery of analyte used for spiking. Three laboratory-made

Table 2

Accuracy of Theophylline Determination

Expected (ng/spot):	85.0	85.0	120.3	120.3	155.8	155.8
Found (ng/spot):*	84.1	85.3	118.9	121.6	156.2	156.2
Recovery (%):	98.9	100.3	98.8	101.1	100.2	100.2

* Average of four different experiments.

Table 3

Repeatability Data

Measurement	**Theophylline Concentration (ng/spot)***		
	I	**II**	**III**
1	833.3	118.9	156.2
2	83.3	118.9	156.2
3	84.1	118.8	156.0
4	84.4	118.9	156.0
5	83.2	118.8	156.3
6	83.3	118.8	156.2
7	84.4	118.8	156.2
Mean (ng/spot)	83.7	118.8	156.1
SD	0.56	0.05	0.11
RSD	0.69	0.04	0.07

* I: Lower region of the calibration graph; II: Middle region of the calibration graph; III: Upper region of the calibration graph.

samples were prepared in duplicate, spiked with theophylline in different amounts covering the lower, middle, and upper region of the calibration graph. The analyte concentration were determined in duplicate, using in-system calibration. The results are shown in Table 2.

Repeatability

Repeatability of the method was determined by multiple analysis using the same method of measurement, analyst, instruments, and location over a short

period of time.[14,17,18] Three laboratory-made samples in the lower, middle, and upper region of the calibration graph were examined. The results are shown in Table 3.

CONCLUSION

An accurate and precise HPTLC method for determination of theophylline in human plasma has been developed. The analyte is well separated from matrix components and assayed for theophylline. High sample throughput and ease of operation are significant advantages of the method.

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