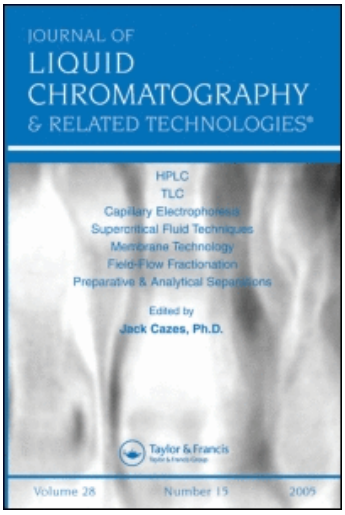

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A Sensitive HPLC Method for the Determination of Terfenadine and Its Metabolite in Human Plasma

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A SENSITIVE HPLC METHOD FOR THE DETERMINATION OF TERFENADINE AND ITS METABOLITE IN HUMAN PLASMA

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ABSTRACT

A sensitive and selective HPLC assay with fluorescence detection was developed for the analysis of terfenadine and its acid metabolite in human plasma. The compounds were isolated from plasma by liquid extraction with methyl-t-butyl ether:isopropyl alcohol (95:5 % v/v). The chromatographic separation was carried on cyanopropylsilane column (15 cm X 4,6 mm) with a mobile phase consisting of 0.001 M acetate buffer, pH 4.0 : acetonitrile (25:75 % v/v). The eluent was monitored at 230 nm excitation and 300 nm emission wavelengths with a 270 nm cut-off filter. The range of quantification was 2 to 1000 ng/ml for terfenadine and 5 to 1000 ng/ml for acid metabolite, respectively. The assay showed linearity over the range of quantification ($r^2 > 0.998$). This method has been applied to the analysis of human plasma samples.

INTRODUCTION

Terfenadine (TRF) is a non-sedating H1-receptor antagonist used in the treatment of allergic rhinitis[1]. Terfenadine undergoes extensive first-pass metabolism (over 99% of absorbed dose) resulting in two metabolites[2,3]. The major metabolite, a carboxylic acid analogue of terfenadine, has approximately one third of the antihistaminic activity of terfenadine. The peak plasma levels of terfenadine are below 10 ng/ml after administration of single or twice daily doses of 60 mg tablet[4]. However, the active metabolite levels are much higher and readily detectable for approximately 16 hours. Few analytical methods for the determination of terfenadine and its acid metabolite have been reported[5-7]. However, these methods lack good sensitivity or specificity[7]. The present paper describes a simple, sensitive, and selective HPLC method for determination of terfenadine and its acid metabolite in human plasma.

MATERIALS AND METHODS

Chromatographic System

Chromatographic determination was performed using a Hewlett Packard model 1050 liquid chromatograph equipped with a model 1046 A HP fluorescence detector, an autosampler, and a 3390 a integrator. The chromatography was carried out using a Microsorb 5 μm cyanopropylsilane (15 cm X 4.5 mm ID) column (Rainin, CA, USA) maintained at ambient temperature. The eluent was monitored at 230 nm excitation and 300 nm emission wavelengths with a cut-off filter at 270 nm.

Reagents

Terfenadine and acid metabolite were obtained from KV Pharmaceuticals and Teva Pharmaceuticals, respectively. Propranolol was obtained from Sigma (St.Louis, MO, USA). Methyl-t-butyl ether, isopropyl alcohol, and acetonitrile (all HPLC grade) were obtained from EM Science. All other chemicals were of analytical grade.

Drug Solutions

A 200 μg/ml stock solutions of terfenadine and its acid metabolite were prepared in methanol. Working solutions were prepared from the stock solution.

Internal Standard (IS) and Extraction Solutions

A 100 μg/ml stock solution of propranolol was prepared in methanol. The final extraction solution consisted of 50 ng of propranolol per ml of methyl-t-butyl ether:isopropyl alcohol (95:5 % v/v).

Mobile Phase

Sodium acetate buffer 0.001 M was prepared in deionized distilled water, and the pH was adjusted to 4.0 with acetic acid. The mobile phase consisted of acetonitrile and buffer (75:25 % v/v).

Preparation of Plasma Standards

To 1 ml of plasma in a 15 ml screw capped centrifuge tube was added an aliquot of drug solution containing 2 to 1000 ng of terfenadine and acid metabolite, 0.5 ml of 0.1 N HCl, and 5 ml of extraction solution containing internal standard. The tubes were vortexed for 20 seconds and centrifuged for 15 min at 3000g. The organic layer was transferred to clean evaporating tube. The aqueous phase was re-extracted with another 5 ml of extraction solution and the pooled organic fraction was evaporated to dryness under gentle stream of nitrogen at 40° C. The residue was dissolved in 100 μl methanol and 40 μl was injected onto the column.

Quantification

Standard curves for plasma were constructed using four replicates at each concentration (2 - 1000 ng/ml). The peak height ratios of standard to the IS were plotted against concentration (ng/ml).

TABLE I
Absolute Recovery of Terfenadine and Acid Metabolite from Plasma

Concentration (ng/ml)	% Recovery	
	Terfenadine	Acid metabolite
50	100.9	85.33
100	92.64	92.55
250	95.06	85.1
500	94.20	81.12

Absolute Recovery

The absolute recovery of terfenadine and acid metabolite from plasma was determined at final concentrations of 50 - 500 ng/ml plasma. The recoveries were calculated by comparing the peak heights of extracted spiked sample to the peak heights of methanolic standard solutions of the same concentration.

RESULTS AND DISCUSSION

The chromatographic column and conditions were chosen to provide complete resolution of terfenadine, acid metabolite, and internal standard. Figure 1 shows the chromatograms obtained from blank plasma and plasma spiked with 100 ng/ml of terfenadine and its acid metabolite. The peaks are sharp and well resolved from each other without any interference from endogenous plasma constituents. Retention time for acid metabolite, propranolol(IS), and terfenadine were 9.5, 12.2, and 15.1 minutes, respectively.

The absolute recovery results are presented in Table I. The present extraction procedure resulted in absolute recoveries ranging from 81.12 to 92.55 % for acid metabolite and from 92.24 to 100.90 % for terfenadine. This method produced superior absolute recoveries for terfenadine and comparable or better recoveries for acid metabolite than those reported in the previous method[5]. Propranolol was chosen as the internal standard since it extracts with the same extraction solution as the drug and metabolite. Propranolol also separates well from the drug and metabolite, and can be detected by fluorescence under the same conditions.

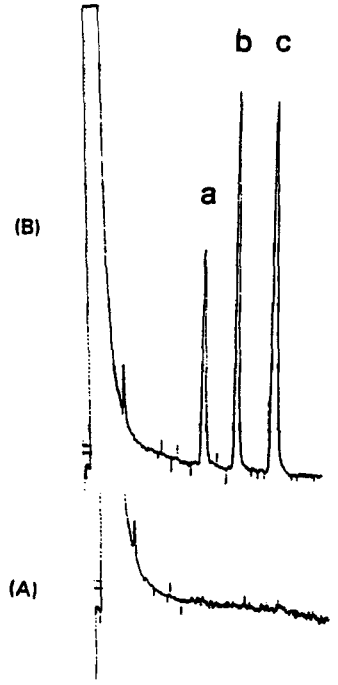

FIGURE 1. Chromatograms of (A) blank plasma (B) extract of plasma containing 100 ng/ml of tefenadine and acid metabolite.
Peaks: a - acid metabolite(9.5 min) , b - propranolol (12.2 min) c - terfenadine(15.1 min).

The ratio of the peak height of terfenadine or its metabolite to the peak height of internal standard was plotted against drug concentration spiked plasma. Standard curves were linear over the concentration range 2 to 1000 ng/ml for terfenadine and 5 to 1000 ng/ml for acid metabolite, with correlation coefficients (r^2) consistently greater than 0.998. The sensitivity of the assay was 2 ng/ml for terfenadine and 5 ng/ml for the acid metabolite. Intra-day and inter-day accuracy and precision are presented in Table II and Table III,

TABLE II

Intra-day Validation of the Assay for Terfenadine and its Acid Metabolite (n = 4).

TERFENADINE

Amount added	Amount found	Accuracy	CV
2	1.95 ± 0.11	97.50	5.60
5	4.17 ± 0.10	83.41	8.50
10	8.66 ± 0.30	86.67	3.40
25	24.7 ± 1.40	98.80	5.20
50	48.03 ± 1.31	96.06	2.71
100	87.34 ± 1.52	87.34	1.38
250	267.08 ± 9.06	106.80	3.40
500	536.08 ± 8.08	107.20	1.50
1000	980.5 ± 23.22	98.10	2.40

TERFENADINE ACID METABOLITE

Amount added	Amount found	Accuracy	CV
5	5.69 ± 0.1	113.7	2.0
10	9.33 ± 1.0	93.3	10.7
25	25.95 ± 1.0	103.8	3.9
50	49.15 ± 7.8	98.30	15.9
100	91.33 ± 0.5	91.33	1.0
250	256.34 ± 7.9	102.50	3.1
500	522.25 ± 7.9	102.50	3.1
1000	989.3 ± 12.5	98.93	1.3

respectively. The intra-day precision showed a CV range of 1.38 to 8.5 % for terfenadine and 1.0 to 15.9 % for acid metabolite. The Inter-day precision CVs varied from 1.8 to 14.3 % for terfenadine and 1.0 to 8.7% for acid metabolite.

A variety of drugs such as pseudoephedrine, ibuprofen, aspirin, acetaminophen, and tricyclic antidepressants which might be coadministered were tested for interference. The results indicated no

TABLE III

Inter-day Validation of the Assay for Terfenadine and its Acid Metabolite (n = 4).

TERFENADINE

Amount added (ng/ml)	Amount found (ng/ml)	Accuracy (%)	CV (%)
2	1.71 ± 0.14	85.5	7.8
5	5.712 ± 0.4	114.2	6.5
10	9.36 ± 0.4	93.6	4.2
25	26.3 ± 0.9	105.0	3.2
50	54.04 ± 7.7	108.08	14.3
100	89.22 ± 1.67	89.20	1.8
250	267.63 ± 6.1	107.10	2.2
500	483.79 ± 17.9	96.80	3.7
1000	1004.5 ± 13.9	100.50	8.7

TERFENADINE ACID METABOLITE

Amount added (ng/ml)	Amount found (ng/ml)	Accuracy (%)	CV (%)
5	5.59 ± 0.4	111.4	6.7
10	9.24 ± 0.5	92.4	4.3
25	26.14 ± 1.0	104.6	3.2
50	53.26 ± 4.6	106.50	8.7
100	94.19 ± 7.5	94.19	7.9
250	247.77 ± 14.9	99.19	7.9
500	510.87 ± 4.6	102.00	1.0
1000	995.83 ± 4.8	99.50	1.0

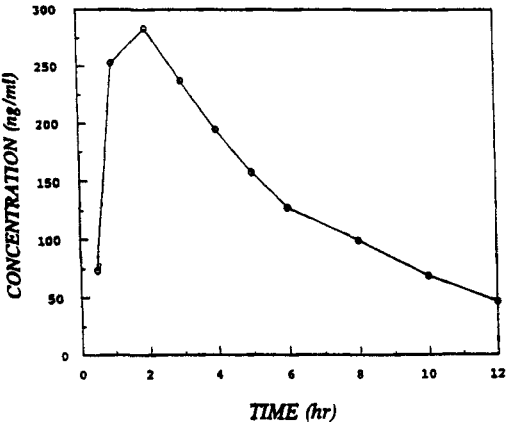

FIGURE 2. Mean plasma concentration profile of acid metabolite

interference from these compounds with the peaks of the drug, metabolite, or internal standard.

This method has been successfully applied to the analysis of plasma samples of five subjects. A typical mean plasma concentration-time profile for acid metabolite following oral administration of 60 mg of terfenadine is shown in Figure 2.

CONCLUSION

The HPLC method described in this paper offers excellent separation of terfenadine, its acid metabolite, and internal standard under isocratic conditions. The method provides excellent recoveries, linearity, and reproducibility. The sensitivity, selectivity, and linearity of this method makes it applicable for bioavailability or pharmacokinetic studies.

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