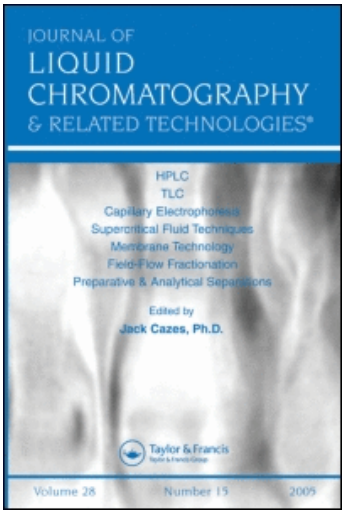

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HIGH SENSITIVITY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ELECTROSPRAY TANDEM MASS SPECTROMETRY DETERMINATION OF TERFENADINE IN HUMAN PLASMA

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ABSTRACT

A highly sensitive and selective HPLC-MS-MS method was developed for the determination of terfenadine in human plasma. The analytes, terfenadine and terfenadine related compound A (internal standard), were extracted from human plasma by methyl t-butyl ether/methylene chloride/n-butyl chloride (3:2:1). The organic solvent is evaporated to dryness and reconstituted in 100 µL of acetonitrile/0.02 M ammonium acetate buffer pH 3.5 (50:50). Chromatographic separation is achieved on a TSK gel ODS-80$_{TS}$ column with a mobile phase composed of acetonitrile/1% formic acid/0.01 M ammonium acetate pH 4.0 (85:13:2). The analytes were detected by HPLC in conjunction with electrospray tandem mass spectrometry. The assay was linear in the concentration ranges of 0.2 to 50 ng/mL. The analysis of pooled quality controls demonstrated excellent precision with relative standard deviations being less than 5.2%. The method is accurate with all intraday and over-all mean values being less than 5.3% from theoretical.

INTRODUCTION

Terfenadine (α-[4-(1,1-dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl) -1-piperidinebutanol) is an H_1 antihistaminic drug. This drug has been used in treatment of allergic rhinitis and a variety of skin allergies[1] without any anticholinergic, antiserotoninergic and anti-adrenergic effects.[1-3] Terfenadine undergoes extensive biotransformation on its first pass through the liver, to form mostly the acid metabolite. Other metabolites such as azacyclonol, the 'alcohol' metabolite and a 'ketone' metabolite[4] were also found in urine. Because of the high first pass effect, very low concentrations of terfenadine are found in the blood plasma.[5] Few analytical methods for the determination of terfenadine and its metabolites have been reported.[4,6-9] However, these methods lack the sensitivity (> 2 ng/mL) and the selectivity.

This paper presents a highly sensitive and selective high performance liquid chromatography (HPLC) electrospray (ESP) tandem mass spectrometry (HPLC- MS-MS) method for the quantitation of terfenadine in human plasma, which has an analysis time of less than 4 min. The short analysis time was due to the highly selective MS-MS detector.

EXPERIMENTAL

Materials

Terfenadine and internal standard (terfenadine related compound A) were obtained from USP (Rockville, MD, USA). Heparinized human plasma was purchased from Rockland (Gilbertsville, PA, USA). Acetonitrile, methylene chloride, methyl t-butyl ether and n-butyl chloride (HPLC-grade) were obtained from Burdick & Jackson (Muskegon, MI, USA). Acetic acid and hydrochloric acid (GR grade) were obtained from EM Science (Gibbstown, NJ, USA). Ammonium acetate (HPLC grade) was obtained from Fisher (Fairlawn, NJ, USA). Formic acid (ACS reagent, 98.7%) was obtained from Sigma (St. Louis, MO, USA). Deionized water was processed through a Milli-Q water purification system (Millipore, Bedford, MA, USA)

Chromatographic System

HPLC-MS-MS was performed on a VG Quattro I triple quadrupole (Fisons, Manchester, UK) mass spectrometer interfaced via a electrospray (ESP) probe to a Hewlett-Packard 1090 L HPLC system with a 25-μL sample loop. The source temperature was set at 200°C. The cone voltage and the collision energy were at 35 V and 30 eV, respectively. The bath gas (nitrogen) and ESP

nebulizing gas (nitrogen) were 450 l/hr and 15 l/hr, respectively. The dwell time was 0.2 s. The mass spectrometer was set to detect 472.2/436 and 470.2/203.2 parent/daughter ions for terfenadine and ISTD, respectively, in the positive ion mode. The analytical column was a TSK gel ODS-80_{TS} (150 mm x 2.0 mm I.D., 5-μm particle size, Tosho, Japan). The mobile phase was acetonitrile/1% formic acid/0.01 M ammonium acetate pH 4.0 (85:13:2) at a flow-rate of 0.2 mL/min. Data were collected and processed using IBM PC compatible computer and MassLynx (Version 2.1) software.

Preparation of Standard Solutions

A stock solution of terfenadine (100 μg/mL) was prepared by dissolving 2.5 μg of terfenadine in 25 mL of methanol. A stock solution of internal standard (100 mg/mL) was prepared by dissolving 2.50 mg of terfenadine related compound A (ISTD) in 25-mL of methanol. Standard solutions of terfenadine (2-500 ng/mL) and internal standard (250 ng/mL) were prepared by diluting the stock solutions with methanol/water (25:75).

Quality Control Samples

Pooled quality control samples (QC) (4, 20 and 40 ng/mL) were prepared to determine the precision and accuracy of the method, and to evaluate the stability of samples. A quality control pool at a concentration above the curve range (80 ng/mL, over-curve control) was also prepared to evaluate precision and accuracy when analyzed using partial volume.

Sample Preparation

Calibration standards were prepared by adding 50 μL of the appropriate terfenadine standard solutions (2-500 ng/mL) to 0.5 mL of blank human plasma. Calibration standards and quality controls were processed by adding 0.5 mL of plasma, 50 μL of internal standard solution (250 ng/mL) and 5 mL of methyl t-butyl ether/methylene chloride/n-butyl chloride (3:2:1) into labeled 16x125 screw-cap tubes. The tubes were capped and mixed on a vortex mixer for 1 min and centrifuged at 2500 rpm for 10 min. The aqueous layer was frozen in an acetone/dry ice bath and the organic layer was transferred to a clean tube. The plasma layer was extracted with 5 mL of methyl t-butyl ether/methylene chloride/n-butyl chloride (3:2:1) for a second time. The organic layers were pooled in a clean tube and evaporated to dryness under nitrogen at 40°C. Reconstituted in 100 μL of acetonitrile/0.02 M ammonium acetate pH 3.5 (50:50). Aliquots of 10 μL were injected onto the HPLC system.

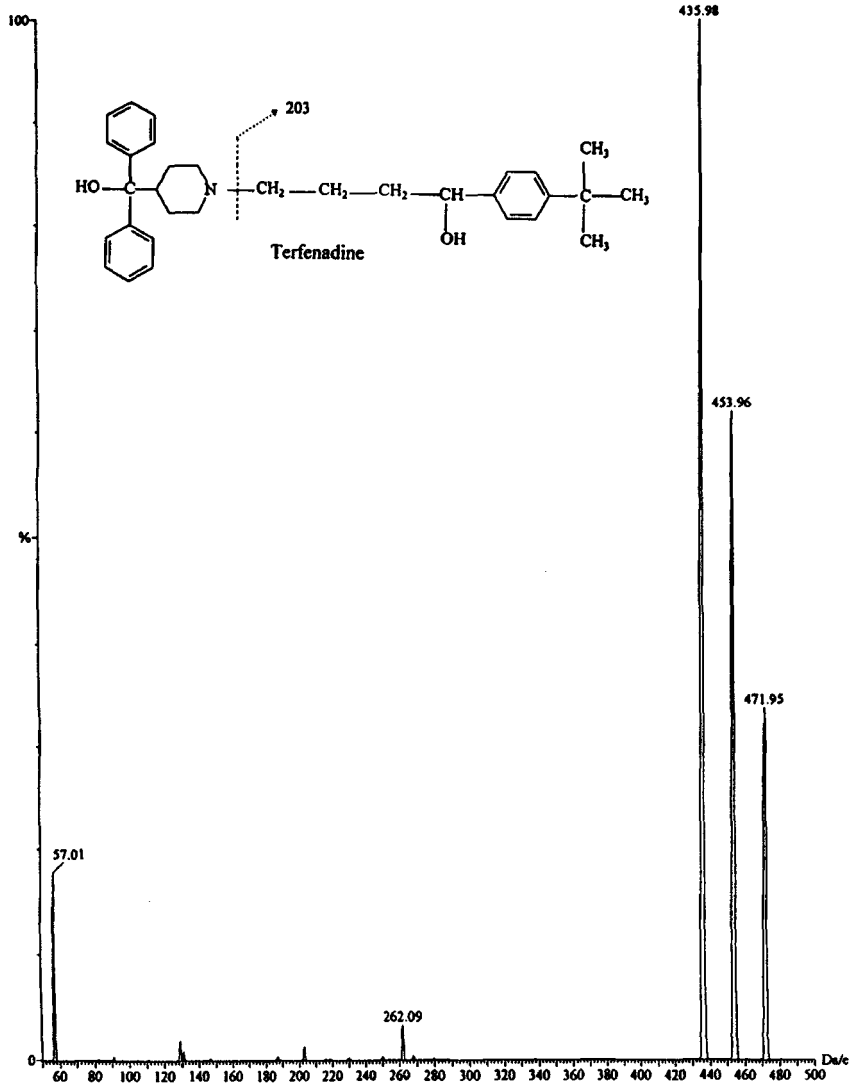

Figure 1. Molecular Structure and Mass Spectrum of Terfenadine.

Validation

Duplicate calibration curves (0.2, 0.5, 1.0, 5.0, 10, 25, and 50 ng/mL) were analyzed on each of three days. Triplicate quality control samples at each concentration (4.0, 20.0, and 40 ng/mL) were analyzed with each calibration

Table 1

Precision and Accuracy of Terfenadine Standards

Calibration Standard Concentration (ng/mL)	Calculated Concentration (Mean±S.D., n=6) (ng/mL)	R.S.D. (%)	Deviation (%)
0.2	0.2±0.011[a]	5.5	0.0
0.5	0.48±0.018[a]	3.8	-4.0
1.0	0.96±0.024	2.5	-4.0
5.0	4.59±0.166	3.6	-8.2
10	9.99±0.502	5.0	-0.1
25	26.9±1.06	3.9	7.6
50	53.1±1.77	3.3	6.2

[a] n = 5.

Table 2

Precision and Accuracy of Terfenadine Quality Controls

Control Concentration (ng/mL)	Calculated (Overall mean±S.D., n=18) (ng/mL)	R.S.D. (%)	Deviation (%)
4.00	4.20±0.220	5.2	5.0
20.0	20.2±0.99	4.9	1.0
40.0	40.0±1.64	4.1	0.0

curve. The calibration curves were obtained by weighted (1/concentration x concentration) least-squares linear regression analysis of the peak area ratios of terfenadine/ISTD vs. the concentration of terfenadine. The equations of the calibration curves were used to calculate the concentration of terfenadine in the controls from their peak area ratios

RESULTS AND DISCUSSION

The molecular structures and mass spectra of terfenadine and terfenadine related compound A (internal standard) are shown in Figures 1 and 2.

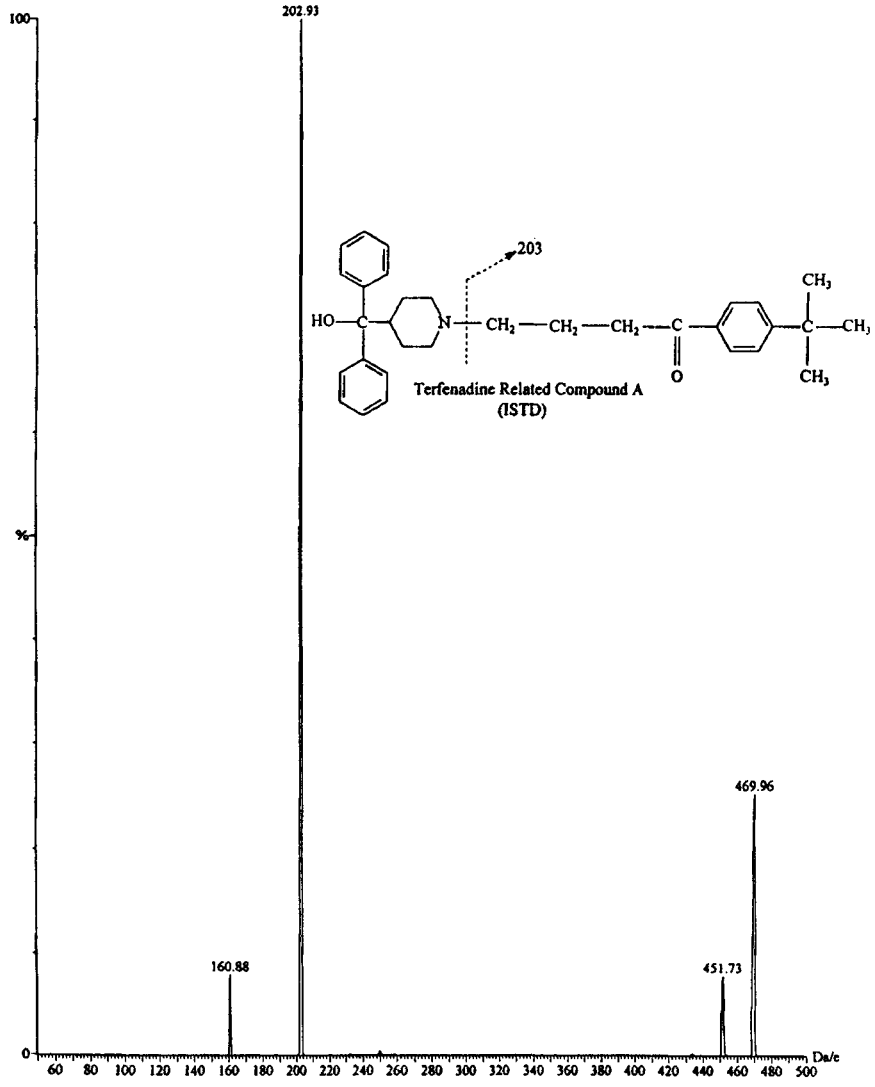

Figure 2. Molecular Structure and Mass Spectrum of Terfenadine Related Compound A (ISTD).

Representative chromatograms are shown in Figure 3 through 5. The mean retention times of terfenadine and the internal standard were 2.5 and 2.8 minutes, respectively. Blank human plasma from 17 pools was tested for endogenous interferences. There were no endogenous interferences found in the

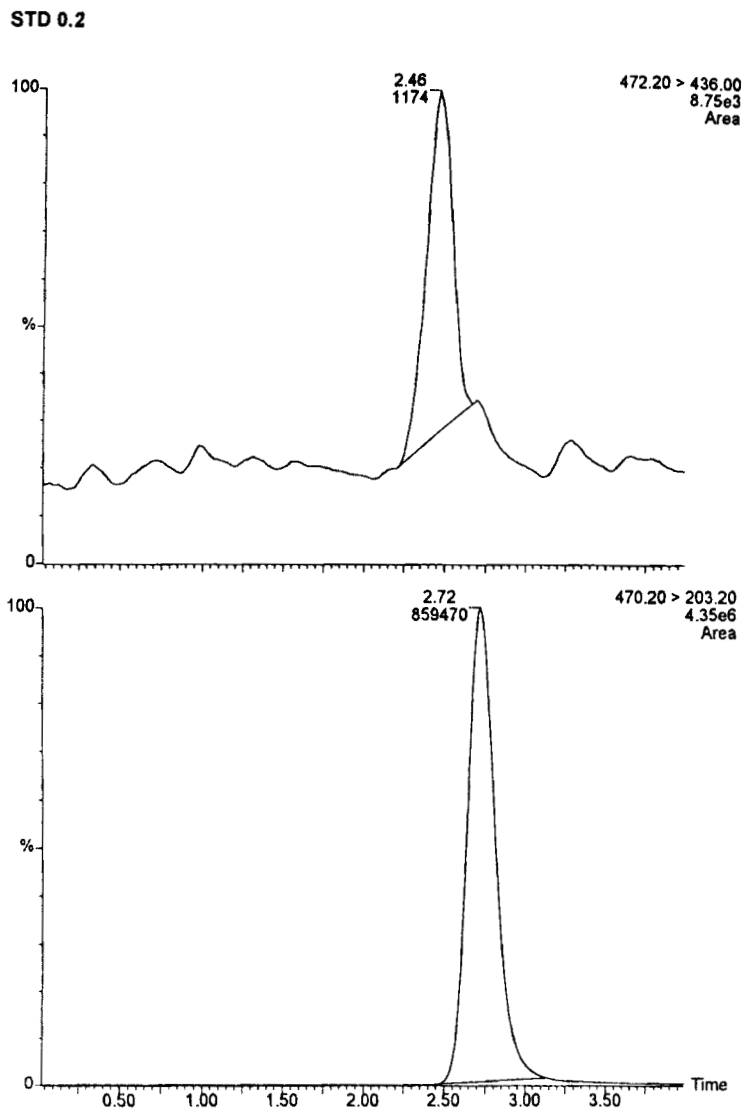

Figure 3. Chromatogram of a 0.20-ng/mL Calibration Standard.

terfenadine and ISTD regions for all the lots tested (Figure 5). Precision and accuracy of terfenadine standards are contained in Table 1. The standards show low values in deviation (<8.2%) and relative standard deviation (<5.5%). Calibration curves for terfenadine in human plasma were linear over the

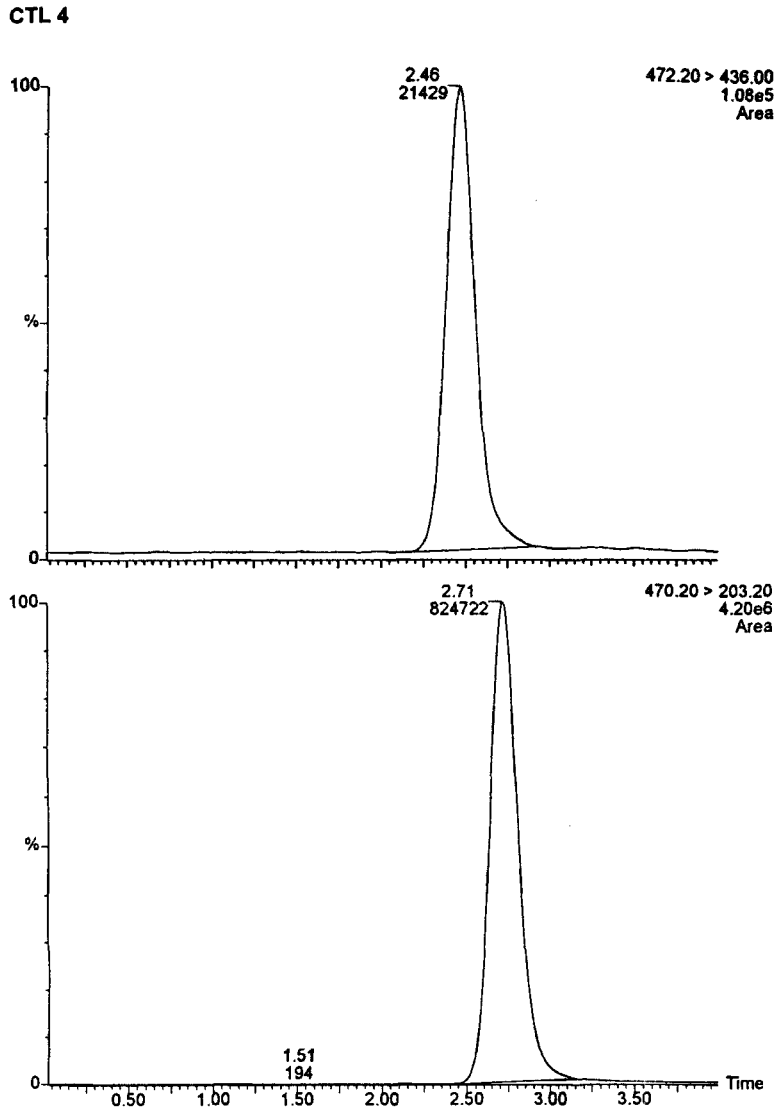

Figure 4. Chromatogram of an 4.00-ng/mL Quality Control Sample.

concentration range of 0.2 to 50 ng/mL. The correlation coefficients were greater than 0.9922 for all curves. Data from the spiked quality control samples are shown in Table 2. The within-day precision of the method, as measured by the relative standard deviation (RSD) of the daily mean (n=6), was less than

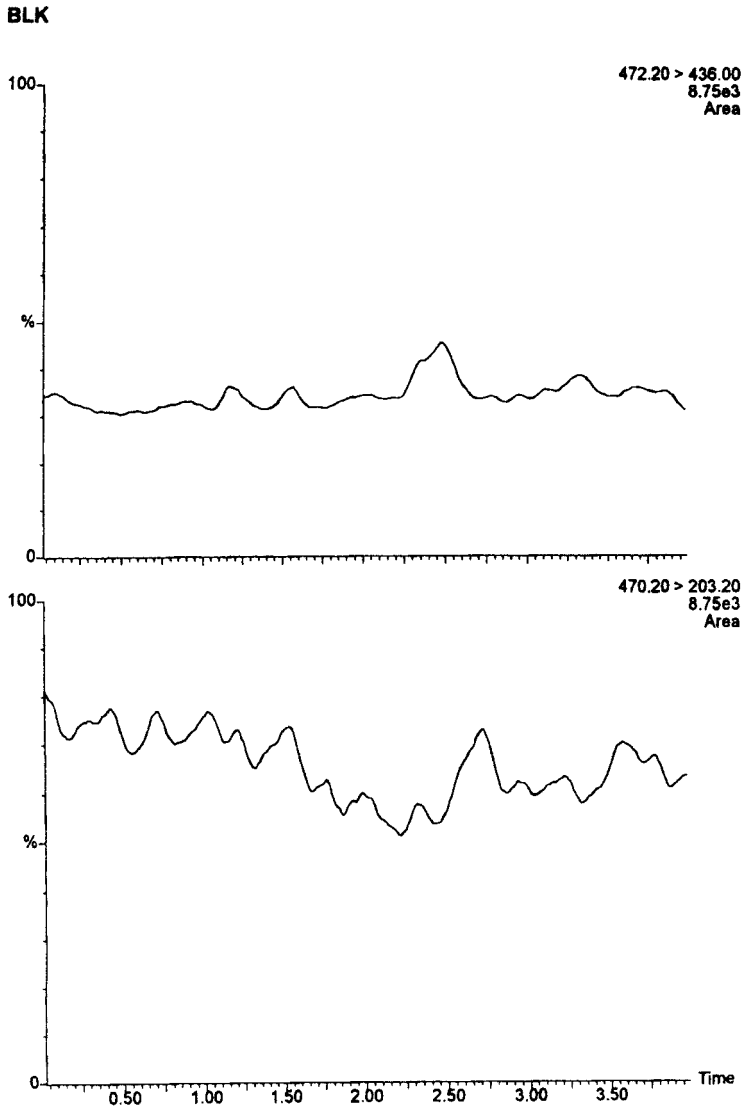

Figure 5. Chromatogram of Blank Human Plasma.

6.7% at the three control concentrations in human plasma. The overall precision was 5.2%, 4.9% and 4.1% (n = 18) for the 4.00-, 20.0- and 40.0-ng/mL terfenadine quality controls, respectively. The accuracy of the method was determined by comparing the means of the measured concentrations with the

nominal (theoretical) concentrations of terfenadine in plasma controls. All of the daily mean (n = 6) and overall mean (n = 18) values for the QC samples were within 5.3% of their expected values.

The lower limit of quantitation (LLOQ) was set at 0.20 ng/mL of terfenadine in human plasma. Five replicates of the lowest standard (0.2 ng/mL) were analyzed to evaluate the LLOQ. At the LLOQ, the RSD (n = 5) of the measured concentrations was 11.5%, and the deviation of the mean of the measured concentrations from their nominal value was 10.0%. Extraction recoveries were determined by comparing the peak areas of extracted QC samples with the peak areas of spiked extracted blanks at the same nominal concentrations. The mean recoveries for terfenadine and the internal standard were 125%, and 99.5%, respectively. Stock and working solutions of terfenadine in methanol and methanol/water (25:75) were stable for at least 2 months when stored at approximately 4°C. Stability was tested by subjecting the QC samples to three freeze/thaw cycles, and storage for 24 hours at room temperature. The thawing and refreezing of QC samples and the storage of QC samples at room temperature had little effect on the precision or accuracy of the results. The mean (n = 3) value was within 11.0% of the expected values.

Process stability was tested by storing the extracted samples in validation day 1 at room temperature overnight before analysis. The storage of extracted samples at room temperature had little effect on the accuracy and precision of the results.

The method presented here for the determination of terfenadine in human plasma shows acceptable linearity, precision, and accuracy down to a concentration of 0.2 ng/mL. The method is simple with relative short analysis time which can result in high sample analysis throughput.

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