ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1675–1680

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

# Development and validation of an LC–MS–MS method for the determination of terfenadine in human plasma[1]

A. Xu*, K. Linderholm, L. Peng, J. Hulse

*Harris Laboratories, 624 Peach Street, P.O. Box 80837, Lincoln, NE, 68501, USA*

Received for review 1 December 1995; revised manuscript received 1 February 1996

**Abstract**

A sensitive LC–MS–MS method capable of quantifying terfenadine at levels down to 100 pg ml$^{-1}$ in human plasma is reported. The method was validated over a linear range from 0.1 to 5.0 ng ml$^{-1}$ using a liquid–liquid extraction with a deuterium-labelled internal standard. The between-run precision and accuracy of the calibration standards were 2.6–6.0% RSD and −2.0 to +2.2% relative error (RE). The between-run and within-run precision and accuracy of quality control samples (0.3, 1.5 and 3.5 ng ml$^{-1}$) were 1.0–5.9% RSD and +1.7 to +6.3% RE. This method was applied to the analysis of human plasma samples.

*Keywords:* LC–MS–MS; Terfenadine; Antihistamine

## 1. Introduction

Terfenadine {α-[4-(1,1-dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl) - 1 - piperidinebutanol} (T; Fig. 1) is a non-sedating antagonist of histamine H1-receptor developed by Marion Merrell Dow under the trade-name Seldane. It is the first antihistaminic drug in this group devoid of sedative effects on the central nervous system [1].

T undergoes extensive (99.5%) and rapid biotransformation by hepatic cytochrome P4503A4 (CYT3A4) to pharmacologically active metabolites, primarily tert-butylcarboxyterfenadine (MDL 16,455), and a pharmacologically inactive metabolite of azacyclonol (MDL 4829) [2,3]. The first-pass metabolism results in very low concentrations of terfenadine in plasma. In addition to the two major metabolites, three other metabolites, an alcohol metabolite (MDL 17,523), an aldehyde metabolite and a "ketone-acid" metabolite, have also been detected in urine [4].

The therapeutic dose of T is 60 mg twice daily; $C_{max}$ is 1.5 ng ml$^{-1}$ for a 60 mg dose [2]. Dose

* Corresponding author. Tel.: +402-476-2811; fax: +402-476-7598.

[1] Presented at the Analysis and Pharmaceutical Quality Section of the Tenth Annual American Association of Pharmaceutical Scientists Meeting, November, 1995, Miami, Florida, USA.


*PII* S0731-7085(96)01804-3

proportionality has been studied and confirmed [2]. For the pharmacokinetic study of T, the active metabolite of tert-butylcarboxyterfenadine can be easily detected by HPLC with fluroescence detection because of its relatively high plasma level [5]. However, to measure the plasma level of T is still a major challenge. Previously, radioimmunoassay (RIA) [6] was the only method for the quantitation of T in human plasma, but the test kit is not commercially available. An HPLC method with fluorescence detection had a limit of quantitation (LOQ) of 10 ng $ml^{-1}$ for T [5]. This could be applied to an interaction study or a unique clinical situation, but in normal subjects the T levels are usually below the LOQ of this method. A GC-MS method has also been used for metabolite identification [7], but hydrolysis and multiple-step derivatizations were involved, resulting in a relatively long sample preparation time. Therefore, the GC–MS method might not be suitable for pharmacokinetic studies with large numbers of plasma samples. This paper describes a simple and rapid LC–MS–MS procedure for the quantitative determination of T in human plasma.

## 2. Experimental

### 2.1. Materials and reagents

Terfenadine was purchased from USP (Washington, DC) and terfenadine-$d_{10}$ the internal standard (I.S.), was custom synthesized. Ammonium acetate and ammonia solution were obtained from Mallinckrodt (Paris, KY). Acetonitrile, methanol, hexane and HPLC-grade water from Fisher (Fair Lawn, NJ) and heparinized control plasma from Worldwide Biologicals (Cincinnati, OH).

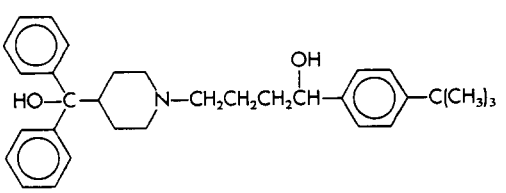


Fig. 1. Terfenadine.

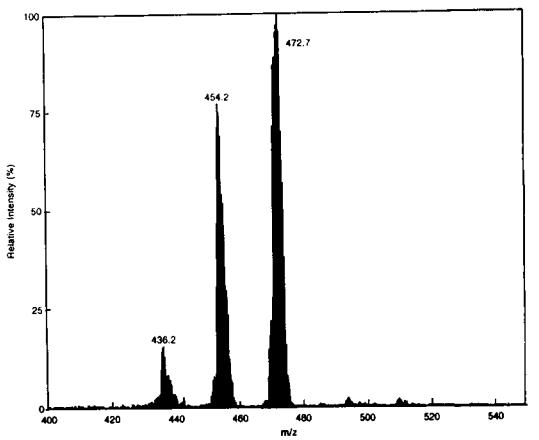


Fig. 2. Full-scan Q1 mass spectrum of terfenadine. The protonated molecular ion ($MH^+$) at $m/z$ 472.7 was used for the product ion scan.

### 2.2. Sample preparation

Three primary stock solutions of T were prepared from separate weighings. The primary stock solutions were verified using a UV spectrophotometer, scanning from 200 to 400 nm. Two of these three stock solutions with the closest abundance were chosen to prepare standards and quality control samples (QCs). The primary and subsequent working stock solutions were prepared in methanol and stored at − 20°C during the validation.

Working standards were prepared fresh daily. The addition of 100 $\mu$l of working stock solution

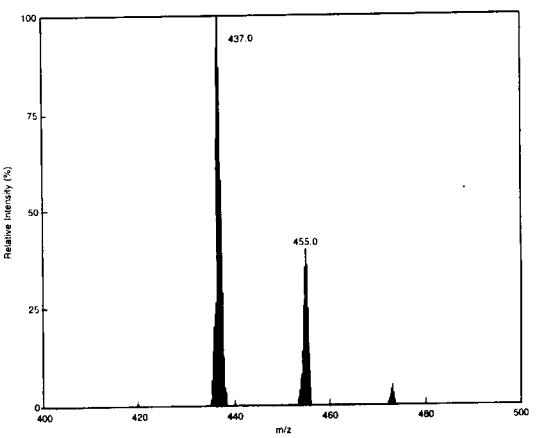


Fig. 3. Daughter ion mass spectrum of terfenadine.

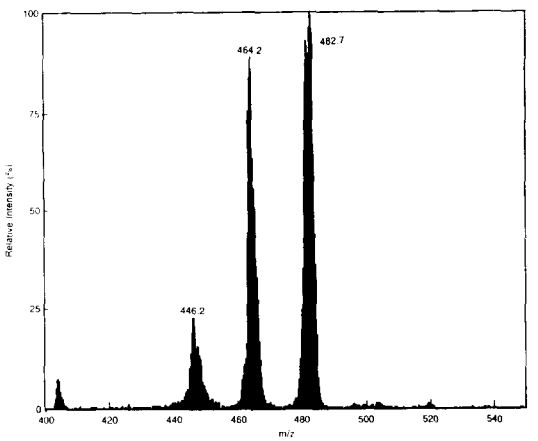


Fig. 4. Full-scan Q1 mass spectrum of terfenadine-$d_{10}$. The protonated molecular ion ($MH^+$) at $m/z$ 482.7 was used for the product ion scan.

to 1.0 ml of blank control plasma yields the plasma concentration. The plasma concentrations of calibration standards were 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ng $ml^{-1}$. Three levels of QCs, at 0.3, 1.5 and 3.5 ng $ml^{-1}$, were prepared at the beginning of the study and were stored at −20°C with the clinical samples.

### 2.3. Instrumentation

The HPLC system consisted of LDC 3500 pump (River Beach, FL) and a Waters Model 717 autosampler (Milford, MA). A BDS Hypersil $C_{18}$ guard column (10 × 2 mm i.d., 5 μm) and a BDS Hypersil $C_{18}$ analytical column (50 × 3 mm i.d., 3μm) were purchased from Keystone (Bellefone, PA). The HPLC system was operated isocratically at 0.8 ml $min^{-1}$ and at room temperature. The mobile phase consisted of acetonitrile–methanol–10 mM ammonium acetate (625:375:150, v/v/v).

The mass spectrometer was a Perkin Elmer Sciex API-III Plus triple-quadrupole mass spectrometer (Thornhill, Canada) equipped with an atmospheric pressure chemical ionization (APCI) interface. The heated nebulizer temperature was 480°C, the auxiliary nitrogen gas flow rate was 1.2 l $min^{-1}$ and the interface heater temperature was 55°C. Ions monitored [multiple reaction monitoring (MRM)] for T were $m/z$ 472.7 (parent) and $m/z$ 437 (product) and for terfenadine-$d_{10}$ (I.S.) were $m/z$ 482.7 (parent) and $m/z$ 447 (product). Argon was used as the collision gas and the electron multiplier was set at 4 kV.

### 2.4. Data treatment

A weighted $1/y$ linear regression was used to determine slopes, intercepts and correlation coefficients, where $y$ is the ratio of the compound peak area to the I.S. peak area. The resulting ratios were used to calculate T concentration from the equation concentration = ($y$ − intercept)/slope.

### 2.5. Extraction procedure

To 1.0 ml of heparinized plasma sample, 100 μl of I.S. in methanol (20 ng $ml^{-1}$) were added. After vortex mixing briefly, 50 μl of 1 N ammonia solution were added, followed by 4 ml of hexane and shaking for 5 min on a horizontal shaker to extract T and I.S. from the plasma sample. The organic phase was then separated from the aqueous phase by centrifugation at 2500 rpm for 5 min. After freezing the aqueous layer in a dry ice–acetone bath, the organic phase was transferred into a clean conical tube and evaporated to dryness under a stream of nitrogen gas in a 40°C water-bath. The residue was reconstituted in 100 μl of mobile phase and 30 μl were injected into the LC–MS–MS system.

## 3. Results and discussion

The LC–MS–MS method for the detection of T in human plasma was investigated. Fig. 2 shows the full-scan Q1 mass spectrum of T where the molecular ion ($MH^+$) was $m/z$ 472.7. The daughter ion spectrum of T (Fig. 3) illustrates two major peaks $m/z$ 455.0 and 437.0 which are due to loss of water from the protonated molecular ion ($MH^+$). The full-scan Q1 mass spectrum and the daughter ion spectrum of I.S. (Figs. 4 and 5) displayed a pattern similar to T, in that the protonated molecular ion ($MH^+$) was at $m/z$ 482.7 and the base peak of daughter ion spectrum was at $m/z$ 447.0.

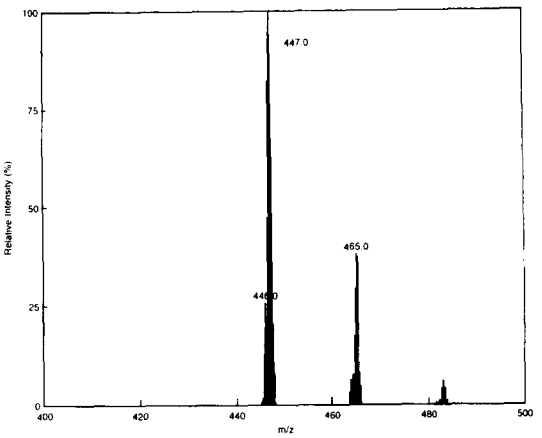


Fig. 5. Daughter ion spectrum of the I.S.

MRM ion chromatograms were used to determine T and I.S. levels in plasma. The ions monitored for T were $m/z$ 472.7 and 437.0, those for the I.S. were $m/z$ 482.7 and 447.0. The dwell time was 100 ms for each ion with a 10 ms pulse time between scans. Both T and I.S. eluted at 1.1 min. A relatively short run time, 2 min, was achieved with the short analytical column (50 × 3 mm i.d.) and a high percentage of organics in the mobile phase.

At the initial stage of method development, both an ionspray interface and a heated nebulizer probe with corona discharge chemical ionization (APCI interface) were evaluated. The APCI interface was chosen owing to its consistent response to the analytes in the MRM mode. By adjusting the resolutions (RE1 and RE3) and peak width (delta mass), the instrument selectivity and sensitivity were greatly enhanced.

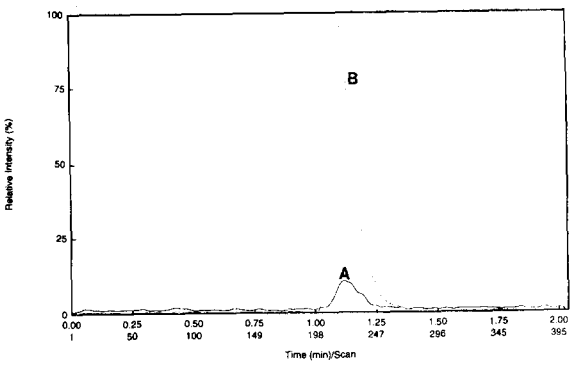


Fig. 7. Chromatogram of extracted 0.1 ng ml$^{-1}$ standard: (A) terfenadine; (B) I.S.

Fig. 6 is a chromatogram of blank control plasma fortified with I.S. Thirty-six lots of commercial blank control plasma were screened during the method validation, and no interference was observed. Figs. 7 and 8 show, the extracted 0.1 ng ml$^{-1}$ standard and the extracted 0.3 ng ml$^{-1}$ QC, respectively. In each chromatogram, the I.S. concentration was 2.0 ng ml$^{-1}$. The chromatograms are clean and with baseline resolution.

The calibration curves were plotted as the peak-area ratio (T/I.S.) vs. T concentration, with eight points ranging from 0.1 to 5.0 ng ml$^{-1}$. The resulting calibration curves were linear and gave correlation coefficients greater than 0.999.

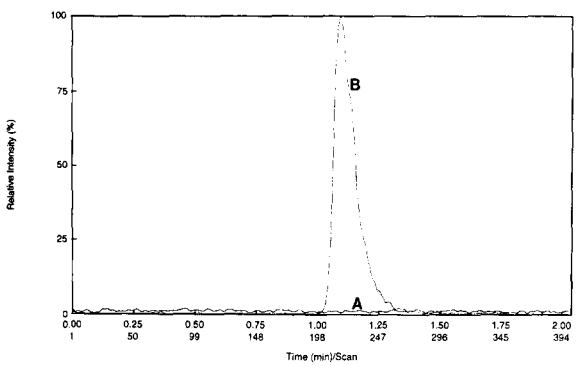


Fig. 6. Chromatogram of blank control plasma with I.S. added: (A) terfenadine; (B) I.S. No terfenadine was observed.

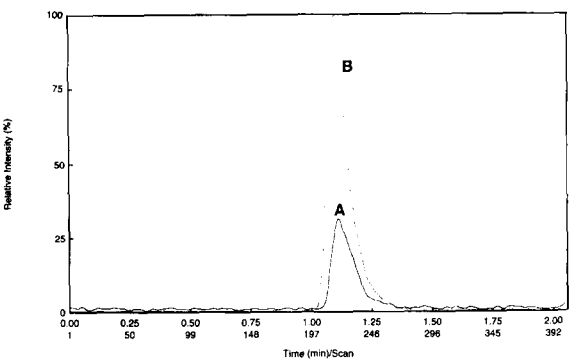


Fig. 8. Chromatogram of extracted 0.3 ng ml$^{-1}$ quality control sample: (A) terfenadine; (B) I.S.

Table 1
Inter-day precision and accuracy for terfenadine standard concentrations

| | Terfenadine (ng ml$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.10 | 0.20 | 0.50 | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 |
| Mean (ng ml$^{-1}$) | 0.100 | 0.196 | 0.51 | 1.019 | 2.009 | 2.963 | 3.965 | 5.052 |
| RSD (%) | 6.00 | 5.10 | 3.72 | 4.32 | 3.14 | 2.73 | 3.86 | 2.63 |
| RE (%) | 0.00 | −2.00 | +2.20 | +1.90 | +0.45 | −1.23 | −0.88 | +1.04 |
| $n$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

### 3.1. Recovery

A simple liquid–liquid extraction procedure was introduced to extract T and I.S. from plasma. The recoveries were calculated by direct comparison of the peak areas of extracted standards with those of unextracted test solutions prepared in an interference-free matrix at the same concentrations. The recoveries of T were 54.2–60.2% over the concentration range 0.1–5.0 ng ml$^{-1}$ ($n = 6$). The I.S. (terfenadine-$d_{10}$) recovery was 58.8% ($n = 6$), which was comparable to that for T.

### 3.2. Performance characteristics

Five validation curves were run on three separate days. Consistently good correlation coefficients ($r > 0.999$) were obtained throughout the validation process. Table 1 shows the inter-day precision and accuracy data for each standard concentration.

Table 2
Precision and accuracy for quality control samples

| | Terfenadine (ng ml$^{-1}$) | | |
|---|---|---|---|
| | 0.3 | 1.5 | 3.5 |
| Inter-day | | | |
| Mean (ng ml$^{-1}$) | 0.311 | 1.560 | 3.588 |
| RSD (%) | 5.88 | 4.84 | 5.03 |
| RE (%) | +3.67 | +4.00 | +2.51 |
| $n$ | 30 | 30 | 30 |
| Intra-day | | | |
| Mean (ng ml$^{-1}$) | 0.305 | 1.594 | 3.621 |
| RSD (%) | 2.95 | 2.13 | 0.99 |
| RE (%) | +1.67 | +6.27 | +3.46 |
| $n$ | 6 | 6 | 6 |

The LC–MS–MS method, with an LOQ of 100 pg ml$^{-1}$, was substantially more sensitive than the RIA method described by Cook et al. [6], which had an LOQ of 200 pg ml$^{-1}$. The signal-to-noise ratio obtained at the LOQ was 20.

Table 2 displays the inter-day and intra-day precision and accuracy for three quality control levels. The data shows that this LC–MS–MS method is very consistent and reliable with low RE and RSD for both the standards and QCs over the entire concentration range.

Table 3
Stability of quality control samples

| | Terfenadine (ng ml$^{-1}$) | | |
|---|---|---|---|
| | 0.30 | 1.5 | 3.5 |
| Processed sample stability | | | |
| Refrigeration ($n = 6$) | | | |
| Initial (ng ml$^{-1}$) | 0.298 | 1.52 | 3.56 |
| 66 h (ng ml$^{-1}$) | 0.311 | 1.52 | 3.45 |
| As % of initial | 104 | 100 | 96.9 |
| Stability of samples in biological matrix | | | |
| Benchtop ($n = 6$) | | | |
| Control (ng ml$^{-1}$) | 0.302 | 1.47 | 3.46 |
| 4 h (ng ml$^{-1}$) | 0.292 | 1.58 | 3.69 |
| As % of control | 96.7 | 107 | 107 |
| Freeze–thaw ($n = 6$) | | | |
| 1st cycle (ng ml$^{-1}$) | 0.295 | 1.52 | 3.56 |
| 3rd cycle (ng ml$^{-1}$) | 0.311 | 1.48 | 3.53 |
| As % of 1st cycle | 105 | 97.4 | 99.2 |
| Sample storage ($n = 6$, −20°C) | | | |
| Initial (ng ml$^{-1}$) | 0.319 | 1.60 | 3.54 |
| 10 weeks (ng ml$^{-1}$) | 0.288 | 1.40 | 3.32 |
| As % of initial | 90.3 | 88.0 | 93.8 |

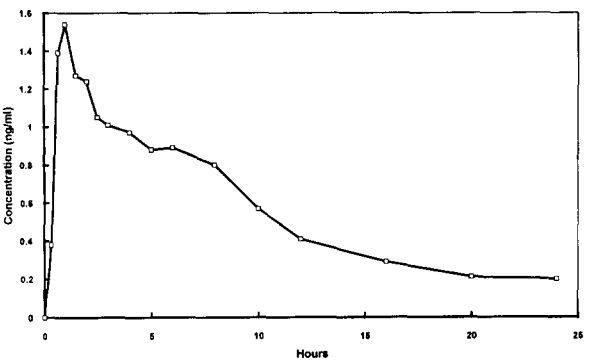


Fig. 9. Pharmacokinetic profile of a subject dosed with 120 mg of terfenadine.

### 3.3. Stability results

The stabilities of the processed samples and the samples in a biological matrix were studied during the method validation. The results are given in Table 3. No significant degradation of T was observed during the stability test. The samples were stable and the results were consistent.

### 3.4. Clinical application

This LC–MS–MS method was used to provide pharmacokinetic data for T in human plasma following oral administration. Volunteers were given a single 120 mg dose of T and subsequently sampled at specific times for the determination of T in the plasma. A total of 1250 clinical samples from 33 subjects were analyzed in 4 days. A plot of T concentrations in plasma versus time after treatment from a normal volunteer is shown in Fig. 9.

## 4. Conclusion

A simple, rugged and sensitive LC–MS–MS method was developed using a liquid–liquid extraction procedure. The method was validated to meet the requirements of the pharmacokinetic investigation of terfenadine. The LOQ was 0.10 ng $ml^{-1}$. More than 1000 clinical samples were analyzed in 4 days. The calibration curve is suitable for generating reliable pharmacokinetic parameters.

## References

[1] E. Sorkin and R. Heel, Drugs, 29 (1985) 34–56.
[2] R. Okerholm, D. Weiner, R. Hook, B. Walker, G. Leeson, S. Biedenbach, M. Cawein, T. Dusebout, G. Wright, M. Myers, V. Schindler and C. Cook, Biopharm. Drug Dispos., 2 (1981) 185–190.
[3] A. Rodrigues, D. Mulford, R. Lee, B. Surber, M. Kukulka, J. Ferrero, S. Thomas, M. Shet and R. Estabrook, Drug Metab. Dispos., 23 (1995) 765–775.
[4] T. Chen, K. Chan, J. Coutant and R. Okerholm, J. Pharm. Biomed. Anal., 9 (1992) 929–933.
[5] J. Coutant, P. Westmark, P. Nardella, S. Walter and R. Okerholm, J. Chromatogr., 570 (1991) 139–148.
[6] C. Cook, D. Williams, M. Meyers, C. Tallent, G. Leeson, R. Okerholm and G. Wright, J. Pharm. Sci., 69 (1980) 1419–1423.
[7] D. Garteiz, R. Hook, B. Walker and R. Okerholm, Arzneim.-Forsch./Drug Res., 32 (1982) 1185–1190.