ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 1319–1329

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of bioequivalence of lomefloxacin tablets using urinary excretion data

Shailesh A. Shah *, Ishwarsinh S. Rathod, Shrinivas S. Savale, Dharmesh B. Patel

Department of Quality Assurance, L.M. College of Pharmacy, P.O. Box #4011, Navrangpura, Ahmedabad 380 009, India

Received 8 May 2002; received in revised form 23 July 2002; accepted 30 July 2002

Abstract

The present study describes development of a sensitive and simple HPTLC method for estimation of lomefloxacin (LMF) in human urine. The drug was extracted using chloroform after adjusting the pH of urine to 7.0. Chloroform extract was spotted on silica gel 60 F_{254} TLC plate and was developed in a mixture of *n*-butanol–methanol–ethyl acetate-6 M ammonia (4:2:3:2, v/v/v/v) as the mobile phase and scanned at 290 nm. The peak for LMF resolved at R_F of 0.40 ± 0.02. The method was validated in terms of linearity (50–600 μg/ml), precision, specificity and accuracy. The limit of detection and limit of quantification for LMF in urine were found to be 20 and 50 μg/ml, respectively. The average recovery of LMF from urine was 91.93%.

The proposed method was applied to generate urinary excretion data for LMF after administration of two market LMF tablet formulations (400 mg, Formulation *R* and Formulation *T*) to six healthy human volunteers in a two-treatment, open, crossover design. Various pharmacokinetic parameters like peak excretion rate ($(\mathrm{dAU}/\mathrm{d}t)_{max}$), time for peak excretion rate (t_{max}), AUC_{0-48}, $AUC_{0-\infty}$, cumulative amount and % cumulative amount of LMF excreted, elimination half-life ($t_{1/2}$), terminal elimination rate constant (k_{el}) and overall elimination rate constant (K), were calculated for both the formulations.

The average cumulative amounts of LMF excreted in urine after administration of Formulation *R* and Formulation *T* were found to be 321.60 mg (80.40% of dose) and 296.51 mg (74.13% of dose), respectively. The urinary excretion profiles of LMF upto 48 h for both the formulations were found to be similar. Statistical comparison (90% confidence intervals of ratio) of various pharmacokinetic parameters of Formulation *T* with that of Formulation *R* revealed that Formulation *T* is bioequivalent with Formulation *R*.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Lomefloxacin; HPTLC; Urinary excretion; Tablet formulations; Bioequivalence

1. Introduction

Lomefloxacin (LMF) is a member of the fluoroquinolone class of antibacterial agents. It is active against a wide range of gram-negative and gram-

* Corresponding author. Tel.: +91-79-6302746; fax: +91-79-6304865

E-mail address: shrini_s@hotmail.com (S.A. Shah).

0731-7085/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.

PII: S0731-7085(02)00453-3

positive bacteria. It is used in treatment of infections of respiratory tract, urinary tract, joints, skin, mouth, ear, nose, throat, eye and in obstetric and gynaecological infections [1].

On oral administration, more than 95% of the total dose of LMF is rapidly absorbed, about 10% is bound to plasma proteins and about 60–78% gets excreted unchanged in urine. The elimination half-life of LMF ranges from 6 to 8 h in healthy subjects. Its glucuronide appears as the major metabolite with highest concentration of about 9% of the dose [2].

It is well documented that in a typical bioavailability or bioequivalence study, samples of an assessible biological fluids, such as blood or urine, are analyzed for drug and/or its metabolite concentrations [3]. Urine provides a non-invasive sample collection method and determination of drug levels in urine is comparatively less complex than plasma and other body fluids [4,5]. Several reports indicate that urinary excretion data can be used to arrive at bioequivalence decision of different drug formulations [6–9]. HPLC methods have been reported for estimation of LMF in biological fluids (plasma, urine) [10–14]. Different HPTLC methods for determination of LMF in pharmaceutical formulations have been reported in literature [15,16]. Due to its speed and versatility, it was thought of interest to develop HPTLC method for estimation of LMF in urine.

The present study describes development, validation and application of a simple and specific HPTLC method for estimation of LMF in urine. The urinary excretion data, thus obtained, was successfully utilized to compare bioavailability of LMF after administration of two market LMF tablet formulations in six healthy volunteers for assessment of bioequivalence.

2. Experimental

2.1. Instruments

A HPTLC system consisting of Camag Linomat IV semiautomatic spotting device, Camag glass twin-trough chamber (20×10 cm^2), Camag TLC Scanner 3, Camag CATS 4 software (Camag Sonnenmattstr., Muttenz, Switzerland) and a 100 µl HPTLC syringe (Hamilton Company, Reno, NV) were used for chromatographic analysis.

2.2. Reagents

Analytically pure lomefloxacin hydrochloride (LMF.HCl) was received as a gift sample from Cadila Pharmaceuticals Ltd, Ahmedabad, India. Two market tablet formulations—Formulation *R* (Lomaday, Dr Reddy's Laboratories Ltd, India) and Formulation *T* (Lomef 400, Torrent Pharmaceuticals Ltd, India)—containing LMF.HCl equivalent to 400 mg of LMF were studied for bioequivalence. Chloroform, methanol, ethyl acetate (AR, Ranbaxy Laboratories, SAS Nagar, India), *n*-butanol, strong ammonia, anhydrous sodium sulphate (LR, JC's Chemicals, Vadodara, India), disodium hydrogen phosphate and potassium dihydrogen phosphate (LR, S.D. Fine-Chem. Ltd, Mumbai, India) were used. TLC aluminum sheets precoated with silica gel 60 F_{254} (20×10 cm^2; layer thickness, 0.2 mm) (E. Merck, Darmstadt, Germany) were used as stationary phase.

2.3. Preparation of standard solutions

A 500 µg/ml stock solution of LMF was prepared in methanol (Solution S1). An appropriate volume of stock solution was further diluted with methanol to obtain a standard solution of LMF having a final concentration of 10 µg/ml (Solution S2).

2.4. Preparation of phosphate buffer (pH 7.0)

A phosphate buffer of pH 7.0 was prepared by mixing 41.3 ml of 1/15 M solution of potassium dihydrogen phosphate with 58.7 ml of 1/15 M solution of disodium hydrogen phosphate.

2.5. Chromatographic conditions

TLC plates (20×10 cm^2) were activated by prewashing with methanol followed by drying in oven for 5 min (50 ± 1 °C) and bringing down to room temperature. Chromatographic estimations were

performed using activated TLC plates under following conditions-

Mobile phase:-*n*-butanol–methanol–ethyl acetate-6 M ammonia (4:2:3:2, v/v/v/v)
Volume of mobile phase: 11 ml
Chamber saturation time: 45 min
Temperature: 25 ± 1 °C, Relative humidity: 35–40%
Migration distance: 60 mm
Wavelength of detection: 290 nm
Band width: 4 mm
Space between two bands: 4 mm
Spraying rate: 10 s/μl

2.6. Extraction of LMF from urine

One millilitre of urine (drug-free or drug-spiked or volunteer urine sample) was transferred into 10-ml volumetric flask. Volume was adjusted to 10 ml with phosphate buffer (pH 7.0). One millilitre of this solution was extracted with chloroform (2×1 ml) by vortexing for 45 s at high speed, followed by centrifugation (5 min, 1200–1300 rpm). 0.8 ml of chloroform layer was collected on each extraction and combined (total of 1.6 ml).

2.7. Chromatographic separation

Appropriate volumes of the combined extract or standard solution of LMF (Solution S2) were spotted on the TLC plate 10 mm from bottom edge using Camag Linomat IV semiautomatic spotting device. TLC plate was developed in ascending mode in twin-trough chamber previously saturated for 45 min with mobile phase, *n*-butanol–methanol–ethyl acetate-6 M ammonia (4:2:3:2, v/v/v/v). The plate was removed from chamber, dried in air and scanned in absorbance/reflectance mode using Camag TLC Scanner 3 at 290 nm. Data of peak area was recorded using Camag CATS 4 software.

2.8. Preparation of calibration curve

2.8.1. Calibration curve for standard LMF

Aliquots of 5, 10, 20, 30, 40, 50 and 60 μl of Solution S2 were spotted on TLC plate. The plate was developed, dried and scanned as described in Section 2.7. A plot of peak area versus corresponding LMF concentration was constructed.

2.8.2. Calibration curve of LMF spiked in urine

One millilitre of drug-free urine was transferred in seven different 10-ml volumetric flasks. Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml of S1 were added separately in the volumetric flasks. The solutions were diluted upto the mark with phosphate buffer (pH 7.0). One millilitre of the solution was extracted and analyzed as described under Section 2.6 and Section 2.7.

Quantitative determination was performed by fitting areas of the peaks corresponding to LMF from the chromatograms into corresponding calibration curve equation.

2.9. Validation of the method

2.9.1. Linearity

The linearity of response for LMF was assessed in the range of 50–600 ng/spot for standard LMF and LMF spiked in urine after extraction.

2.9.2. Limit of quantitation and limit of detection

Limit of quantitation of LMF was taken as the lowest concentration of LMF in the calibration range. For limit of detection, concentrations of LMF lower than the limit of quantitation were spotted and the minimum concentration detected under given chromatographic conditions was considered as limit of detection.

2.9.3. Precision

Precision of the proposed method in terms of intra-day variation (RSD) was determined by analyzing urine samples spiked with LMF at different concentrations (50–600 ng/spot) for 3 times on the same day and inter-day precision (RSD) was assessed by analyzing urine samples spiked with different concentrations of LMF (50–600 ng/spot) on 7 different days over a period of 1 week.

2.9.3.1. Repeatability of measurement of peak area. Ten microlitres of S2 (10 μg/ml) were spotted on TLC plate, developed, dried and the spot was

scanned for seven times without changing the plate position and RSD for measurement of peak area was determined.

2.9.3.2. Repeatability of sample application. Ten microlitres of S2 (10 μg/ml) were applied seven times on TLC plate by semiautomatic spotting device. The plate was developed and analyzed as described under Section 2.7 and RSD for peak area for different peaks was calculated.

2.9.4. Accuracy

The accuracy was determined by standard addition method at different concentration levels of LMF. Different volumes of LMF were added to urine samples spiked with LMF (100 μg/ml). The samples were extracted with chloroform and analyzed as described under Section 2.7. Amount of total LMF in urine was determined by fitting the corresponding peak area into the calibration curve equation for LMF spiked in urine. Accuracy of determination of LMF in urine was computed using the formula:

$$(\text{Total amount of LMF found in urine}/\text{Total amount of LMF spiked in urine})\times 100.$$

2.9.5. Specificity

The specificity of the method was ascertained by analyzing standard LMF, drug-free urine and urine spiked with LMF. The spot for LMF spiked in urine was confirmed by comparing its R_F and absorbance/reflectance spectrum with that of standard LMF. The peak purity of LMF spiked in urine was assessed by comparing the spectra at peak start, peak apex and peak end positions of the LMF spot.

2.9.6. Recovery studies

Recovery of LMF from urine was calculated as the ratio of area of LMF peak after extraction from urine to the area of standard LMF at respective concentrations followed by application of the correction factor.

2.10. Bioavailability study

2.10.1. Study protocol

Two LMF tablet formulations Formulation *R* (Reference) and Formulation *T* (Test), containing LMF.HCl equivalent to 400 mg of LMF, were studied for bioequivalence. A pilot bioequivalence study was performed on six healthy male volunteers (20–23 yr, 55–70 kg), employing a single dose, two-treatment, two-period, open randomized crossover design with a wash-out period of minimum 7 days between the treatments. The written informed consent was obtained from all the volunteers. Healthy status of the volunteers was assessed by history, physical examination and laboratory investigations including serum chemistries, total and differential blood count. Volunteers with a history of major kidney, liver or heart diseases were excluded from the study. Renal functionality of the volunteers was assessed on the basis of creatinine clearance test. Individuals with known gastrointestinal disease that might affect absorption of the drug, history of adverse reactions and hypersensitivity to fluoroquinolones were excluded. Only non-smoking and non-alcoholic individuals with no clinically significant abnormal findings during medical history, physical examination and laboratory evaluations were allowed to participate in the study. The study protocol was approved by the local Ethical Committee. None of the volunteers received any other drug at least 2 weeks prior to day 1 of the study and during the study. The volunteers were abstained from consumption of xanthine containing foods and beverages (chocolates, tea, coffee or coke) for 24 h before administration of the dose and were fasted overnight (at least 10 h). A standard breakfast and standard lunch were provided after 4 and 6 h of sampling, respectively. Each volunteer received a formulation (*R* or *T*) along with 200 ml of potable water. Water consumption was restricted upto 4 h after administration and was allowed ad libitum thereafter.

Urine samples were collected before administration and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h after administration of the formulation. The volume of urine collected during sampling time from each volunteer was measured. Representative

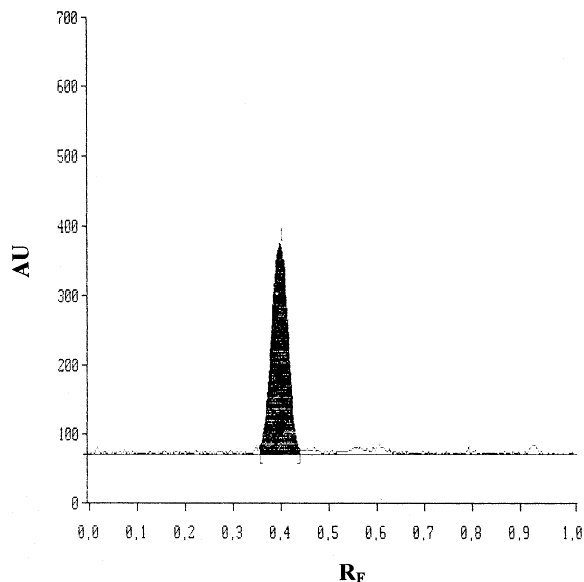


Fig. 1. Chromatogram showing peak of LMF (peak 1, $R_F = 0.40 \pm 0.02$) extracted from urine.

samples of urine (10 ml) were stored, in glass test tubes sealed with aluminum foil, at −20 °C until analysis.

The urine samples, after bringing to room temperature, were analyzed for LMF content by the proposed HPTLC method and urinary excretion profiles were used to determine various pharmacokinetic parameters.

2.10.2. Pharmacokinetic analysis

The peak excretion rate ($(dAU/dt)_{max}$) and peak excretion time (t_{max}) values were obtained from the urinary excretion rate (dAU/dt) versus time curves obtained for each volunteer after administration of Formulation R and Formulation T. Various other pharmacokinetic parameters such as overall elimination rate constant (K), terminal elimination rate constant (k_{el}), elimination half-life ($t_{1/2}$) were obtained from log-transformed urinary excretion rate ($\log(dAU/dt)$, mg/h) versus mid-point of time (h) curves. Both, AUC_{0-48} and $AUC_{0-\infty}$, were calculated using untransformed (dAU/dt) data. AUC_{0-48} was calculated using linear trapezoidal rule and was extrapolated to infinite time, $AUC_{0-\infty}$. k_{el} was calculated from the slope of terminal linear portion of $\log(dAU/dt)$ versus mid-point of time curve. K was obtained as quotient of intercept of terminal linear line extrapolated to Y-axis and dose (mg). The elimination half life ($t_{1/2}$) was calculated using the formula, $t_{1/2} = 0.693/k_{el}$. Cumulative amount of LMF excreted in 48 h (C_{total}, mg) and % dose of LMF excreted were also estimated.

2.10.3. Statistical analysis

The relative bioavailability was determined in terms of AUC_{0-48}, $AUC_{0-\infty}$ and cumulative amount of LMF excreted (C_{total}) from Formulation T relative to Formulation R. Classical 90% confidence intervals were estimated for AUC_{0-48}, $AUC_{0-\infty}$, $(dAU/dt)_{max}$ and C_{total}. Two one-sided t-test was also performed for these parameters.

3. Results and discussion

3.1. HPTLC method development and validation

Due to its versatility and speed of analysis, HPTLC technique was found suitable for estimation of LMF levels in urine.

Literature review reveals that LMF is less soluble at pH 7.14 [17]. Therefore, at this pH, it is easily extracted from urine by means of organic solvents. Various solvents viz. chloroform, dichloromethane, ethyl acetate were tried for quantitative extraction of LMF from urine. Use of chloroform could provide better clean-up and recovery of LMF. Adjustment of pH of urine to 7.0, followed by two times extraction with chloroform could improve extraction efficiency to more than 90%, which was satisfactory. It was observed that about 5 times volume of phosphate buffer (pH 7.0), as compared to that of the volume of urine sample, was required to make the pH of urine sample to 7.0.

Different compositions of n-butanol, methanol and ethyl acetate were tried to obtain optimum R_F and separation of LMF from urine components on the TLC plate. Various modifiers like triethylamine, diethylamine, ammonia solution were tried to achieve sharp band of LMF. A mixture of n-butanol–methanol–ethyl acetate-6 M ammonia (4:2:3:2, v/v/v/v), could provide sharp peak of

Table 1
Precision of proposed HPTLC method for estimation of LMF spiked in human urine

Concentration of LMF (ng/spot)	Intra-day ($n=3$) RSD	Inter-day ($n=7$) RSD
50	8.92	9.43
100	3.54	3.78
200	7.05	8.06
300	4.05	4.23
400	3.04	2.79
500	3.13	3.15
600	2.50	2.99

LMF well resolved from other urine components at R_F of 0.40 ± 0.02 (Fig. 1).

It was observed that activation of TLC plates (pre-washing with methanol followed by drying at 50 °C) and pre-saturation of TLC chamber with mobile phase for 45 min ensures good reproducibility and peak shape of LMF.

Densitometric evaluation was performed at 290 nm, the wavelength of maximum absorbance of LMF (λ_{max}), in absorbance/reflectance mode.

3.1.1. Validation

Using the optimized extraction method and chromatographic conditions, developed HPTLC method was validated in terms of linearity, limit of detection, limit of quantitation, precision, accuracy and specificity.

3.1.1.1. Linearity. Peak areas of standard LMF were found to be linear in the range of 50–600 ng/spot (i.e. 50–600 μg/ml, $n=3$) with correlation coefficient of 0.9936. Peak areas of LMF spiked in urine were found to be linear in the range of 50–600 ng/spot (i.e. 50–600 μg/ml, $n=7$). The average linear regressed equations for the corresponding curves were $y=26.59x+2814.02$ (standard error, slope = 0.92, intercept = 116.04) and $y=26.45x+2139.75$ (standard error, slope = 0.38, intercept = 119.04), where 'y' is the concentration of LMF in ng/spot and 'x' is the corresponding peak area.

3.1.1.2. Limit of quantitation and limit of detection. The limit of quantitation was 50 ng/spot for LMF spiked in urine, while minimum detectable quantity of LMF was found to be 20 ng/spot.

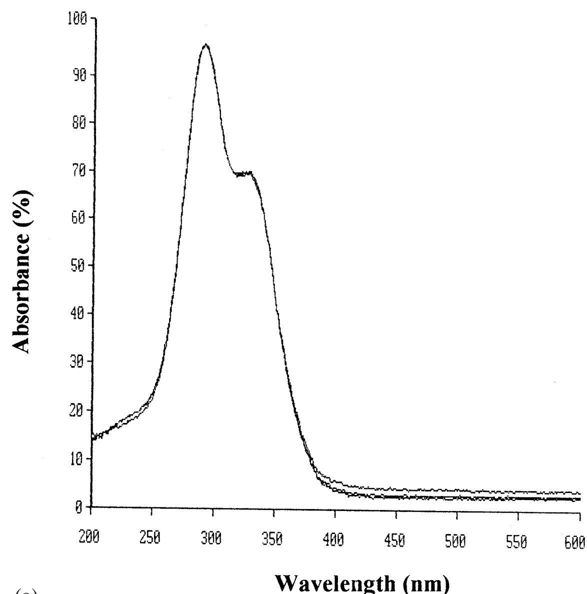
3.1.1.3. Precision. The intra-day variation for determination of LMF in urine was in the range of 2.50–8.92%, while inter-day variation was ranging from 2.79 to 9.43% (Table 1). Incase of repeatability of sample application, peak area values showed a RSD of 2.84 while for repeatability of measurement of peak area, the corresponding RSD value was 0.64 (Table 3). Both these values were found to be satisfactory.

3.1.1.4. Accuracy. The percentage accuracy for estimation of LMF in urine, determined using standard addition method, was found to be

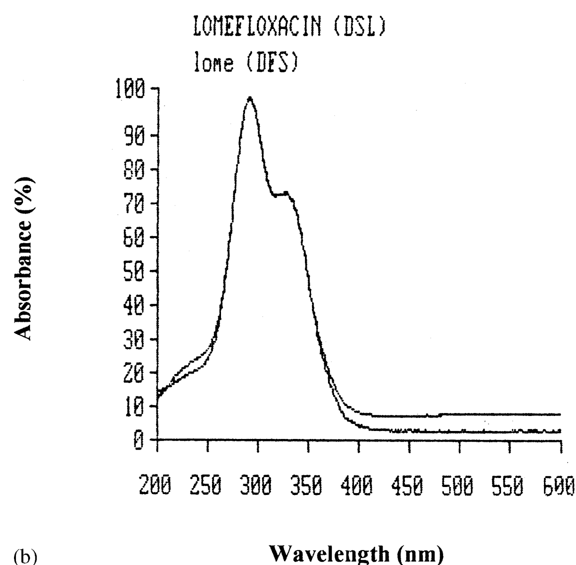
Table 2
Accuracy data for proposed HPTLC method for LMF in urine

Concentration of LMF (ng/spot)			Total quantity of LMF found[a] (ng/spot)	% accuracy
Initial quantity spiked (a)	Quantity of Std. added (b)	Total quantity spiked ($a+b$)	(Mean ± S.D.) ($n=3$)	(Mean ± S.D.) ($n=3$)
100	0	100	93.73 ± 4.33	93.73 ± 4.33
100	50	150	149.00 ± 2.09	99.33 ± 1.39
100	150	250	261.28 ± 4.27	104.51 ± 1.71
100	250	350	344.40 ± 17.19	99.35 ± 3.27
100	350	450	450.25 ± 23.35	100.05 ± 5.19
100	450	550	529.98 ± 17.79	96.35 ± 3.23

[a] Total quantity found was determined by utilizing calibration curve equation for LMF spiked in urine for three replicate samples.



(a)



(b)

Fig. 2. (a) Peak purity spectra for LMF, extracted from urine sample, scanned at the peak start, peak apex and peak end positions of the spot (correlation, $r_{start,apex} = 0.9999$, $r_{apex,end} = 0.9996$); (b) Comparison of spectra of LMF extracted form urine with that of standard LMF (correlation = 0.9985).

between 93.73 and 104.51% over the concentration range studied (Table 2).

Table 3
Summary of validation parameters for the proposed HPTLC method for estimation of LMF in urine

No.	Parameter	Result for LMF
1	Linearity range	
	(a) Standard lomefloxacin	50–600 ng/spot ($y = 26.59x + 2814.02$, $r = 0.9936$)
	(b) Lomefloxacin spiked in urine	50–600 ng/spot (50–600 μg/ml) ($y = 26.45x + 2139.75$, $r = 0.9960$)
2	Limit of detection	20 ng/spot
3	Limit of quantitation	50 ng/spot
4	Precision (RSD)	
	(a) Repeatability of sample application	2.84
	(b) Repeatability of measurement	0.64
	(c) Intra-day	2.50–8.92
	(d) Inter-day	2.79–9.43
5	Accuracy (%)	93.73–104.51
6	Specificity	Specific

3.1.1.5. Specificity. Comparison of chromatograms of urine spiked with LMF and blank (drug-free) urine, showed no interference from the urine components in the separation of LMF. Peak purity check showed high degree of correlation between spectra scanned at peak start, peak apex and peak end positions ($r_{start,apex} = 0.9999$ and $r_{apex,end} = 0.9996$) of LMF peak which confirmed that the peak represents a pure single component i.e. LMF (Fig. 2a). This was further supported by equally good correlation ($r = 0.9985$) between spectrum of standard LMF and the spectrum of LMF spiked in urine (Fig. 2b).

Average recovery of LMF, from urine, over the range of spiked concentration of 50–600 ng/spot, was found to be 91.93%. LMF in urine was found to be stable over a period of 7 days at −20 °C.

Different validation parameters for the proposed HPTLC method are summarized in Table 3. Thus, the proposed method is simple, sensitive, specific, precise and accurate and can be utilized for estimation of LMF excreted in human urine.

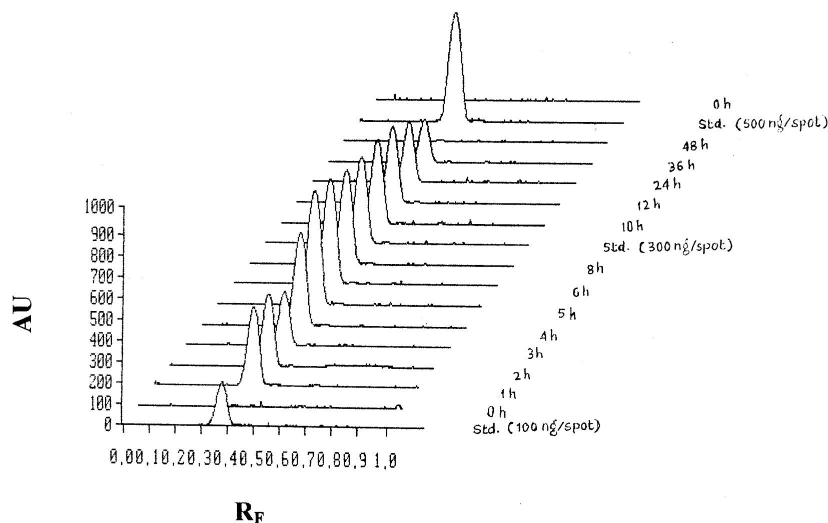


Fig. 3. Typical chromatogram showing levels of LMF in urine samples of a volunteer (volunteer 1) collected over a period of 48 h after administration of LMF tablet formulation (Formulation *R*).

Table 4
Average % cumulative LMF excreted, rate of LMF excretion and log-transformed rate of LMF excretion data after administration of Formulation *R* and Formulation *T*

Time (mid-point) (h)	Percentage cumulative excreted		Rate of excretion (dAU/d*t*)(mg/h)		log(dAU/d*t*) (mg/h)	
	Formulation *R*	Formulation *T*	Formulation *R*	Formulation *T*	Formulation *R*	Formulation *T*
0.5	3.82±2.00	3.49±1.64	15.26±8.01	13.95±6.56	1.11±0.31	1.09±0.30
1.5	10.19±2.89	8.79±2.70	25.50±4.33	21.22±4.64	1.40±0.09	1.32±0.10
2.5	16.40±3.39	14.81±2.55	24.84±3.33	24.08±2.61	1.39±0.06	1.38±0.05
3.5	21.82±3.70	19.82±2.57	21.60±2.80	20.03±1.57	1.33±0.06	1.30±0.03
4.5	26.67±4.00	24.58±2.59	19.81±2.66	19.01±1.12	1.29±0.06	1.28±0.03
5.5	31.28±4.28	28.928±2.41	18.07±2.21	17.38±1.03	1.25±0.05	1.24±0.03
7.0	39.58±4.70	36.96±1.94	16.60±2.55	16.09±1.20	1.22±0.06	1.21±0.03
9.0	47.17±4.91	44.26±1.45	15.19±1.93	14.60±2.35	1.18±0.05	1.16±0.07
11.0	52.61±5.15	49.64±1.09	10.88±2.53	10.64±1.76	1.03±0.11	1.03±0.06
18.0	72.68±4.96	66.76±3.20	6.69±0.85	5.71±1.33	0.82±0.06	0.75±0.10
30.0	79.52±4.27	72.64±3.74	2.28±1.21	1.96±0.66	0.36±0.19	0.27±0.15
42.0	80.40±3.07	74.13±5.21	0.29±0.45	0.50±0.64	−0.02±0.03	−0.02±0.14

Note: All the values indicate mean±S.D. for the data from six volunteers. Formulation *R*, Lomaday, Dr Reddy's Laboratories Ltd, India; Formulation *T*, Lomef 400, Torrent Pharmaceuticals Ltd, India.

3.2. Bioavailability study

Urinary excretion levels of LMF after administration of Formulation *R* and Formulation *T* (both containing LMF.HCl equivalent to 400 mg of LMF) in six volunteers was estimated using proposed HPTLC method. Typical chromatogram showing LMF excreted in urine during different time intervals is shown in Fig. 3.

The average values (±S.D.) for % cumulative amount of LMF excreted, rate of excretion (dAU/d*t*) and log-transformed rate of excretion (log(dAU/d*t*)) for both the formulations with respect to mid-point of time are given in Table 4.

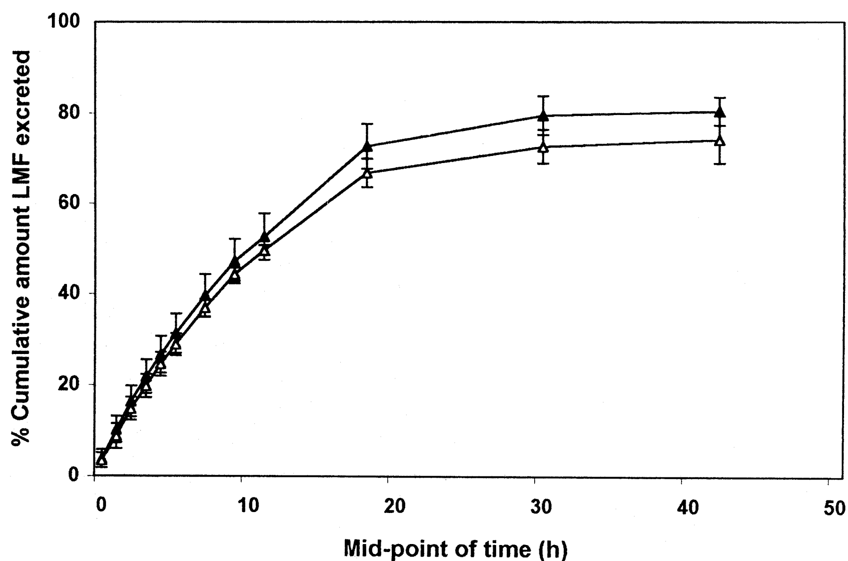


Fig. 4. Average % cumulative LMF excreted, with respect to LMF dose, versus mid-point of time plots after administration of LMF tablet formulations (—▲—, Formulation *R*; —△—, Formulation *T*) to six healthy male volunteers (*Note:* The vertical lines indicate S.D. in % cumulative excreted at corresponding mid-point of time).

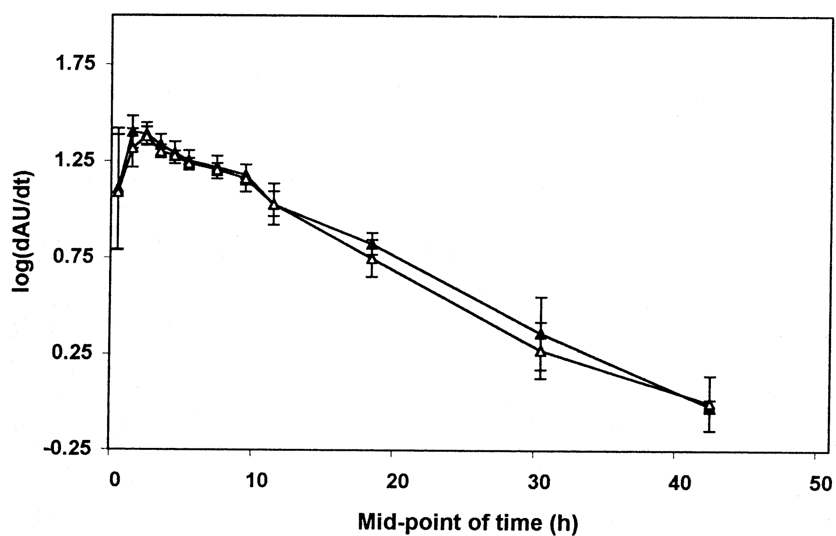


Fig. 5. Average log excretion rate (log(dAU/d*t*)) versus mid-point of time plots for LMF after administration of LMF tablet formulations (—▲—, Formulation *R*; —△—, Formulation *T*) to six healthy male volunteers (*Note:* The vertical lines indicate S.D. in log(dAU/d*t*) at corresponding mid-point of time).

The plots of average % cumulative of LMF excreted (±S.D.) over a period of 48 h versus mid-point of time and average (log(dAU/d*t*)) (± S.D.) versus mid-point of time, are shown in Fig. 4 and Fig. 5, respectively. From these figures it is evident that both the formulations show similar excretion behavior, which in turn, indicates similarity in their bioavailability. About 321.60 ± 12.27 mg (80.40 ± 3.07% of dose) and 296.51 ± 20.85 mg (74.13 ± 5.21% of dose) of LMF is excreted in 48 h

Table 5
Summary of pharmacokinetic parameters of LMF after administration of LMF tablet formulations

Pharmacokinetic parameter	Formulation *R*	Formulation *T*
t_{max} (h)	2.00±0.55	2.00±0.55
$(dAU/dt)_{max}$ (mg/h)	26.50±2.98	25.21±1.46
AUC_{0-48} (mg)	326.86±16.28	302.99±16.42
$AUC_{0-\infty}$ (mg)	359.57±33.48	315.87±19.60
$t_{1/2}$ (h)	8.57±1.41	7.48±1.36
Cumulative amount of LMF excreted (C_{total}, mg)	321.60±12.27	296.51±20.85
% cumulative amount of LMF excreted[a]	80.40±3.07	74.13±5.21
K (1/h)	0.0037±0.0003	0.0038±0.0001
k_{el} (1/h)	0.0830±0.0164	0.0950±0.0154

Note: Each value indicates mean±S.D. for data from six volunteers. Formulation *R*, Lomaday, Dr Reddy's Laboratories Ltd, India; Formulation *T*, Lomef 400, Torrent Pharmaceuticals Ltd, India.
[a] % cumulative amount of LMF excreted with respect to the administered dose (400 mg).

(C_{total}) after oral administration of Formulation *R* and Formulation *T*, respectively. It was observed that both the formulations showed maximum excretion rates in the interval of 1.5–2.5 h (t_{max}) in terms of mid-point of time (Fig. 5).

Average values (±S.D.) of various pharmacokinetic parameters are reported in Table 5. AUC_{0-48} value for Formulation *R* was found to be 326.86±16.28 mg and that for Formulation *T* was 302.99±16.42 mg, which are comparable. Maximum amounts excreted in corresponding time intervals ($(dAU/dt)_{max}$) from Formulation *R* and Formulation *T* were 26.50±2.98 and 25.21±1.46 mg/h, respectively (Table 5). Values of other pharmacokinetic parameters of Formulation *T* were also comparable with that of reference formulation (Formulation *R*). Comparison of all these parameters indicated similar bioavailability of LMF from Formulation *T* as compared to Formulation *R*. The relative bioavailability of LMF from Formulation *T*, in terms of AUC_{0-48}, $AUC_{0-\infty}$ and C_{total} was found to be 92.69, 87.84 and 92.20%, respectively (Table 6).

Table 6
Statistical analysis of different pharmacokinetic parameters of LMF obtained after administration of LMF tablet formulations

Parameter	90% confidence interval	
	Lower limit	Upper limit
AUC_{0-48}	100.2	102.4
$AUC_{0-\infty}$	100.5	103.9
C_{total}	100.2	102.7
$(dAU/dt)_{max}$	97.90	105.0
Relative bioavailability (%)[a]		
AUC_{0-48}	92.69	
$AUC_{0-\infty}$	87.84	
C_{total}	92.20	

Note: The pharmacokinetic parameters of Formulation *T* (Test formulation) are compared with that of Formulation *R* (Reference formulation).
[a] Calculated as (ratio of pharmacokinetic parameter of Formulation *T* to corresponding parameter of Formulation *R*) × 100.

For bioequivalence decision, AUC_{0-48}, $AUC_{0-\infty}$, C_{total} and peak excretion rate ($(dAU/dt)_{max}$) values were subjected to statistical analysis like 90% confidence interval. The estimates of 90% confidence interval ($P = 0.05$) for the ratio of these four parameters were found to be within the specified limits of 80–125% for log-transformed data as per US FDA requirement for bioequivalence (Table 6). Thus, it is concluded that Formulation *T* is bioequivalent to Formulation *R*.

4. Conclusions

A HPTLC method was developed for estimation of LMF excreted in urine. The method was validated and found to be simple, sensitive, specific, accurate and precise. The proposed method was successfully used to obtain urinary excretion data for LMF after administration of LMF tablet formulations in six healthy human volunteers. Statistical analysis of various pharmacokinetic parameters calculated using urinary excretion data of LMF revealed that Formulation *T* is bioequivalent with Formulation *R*.

Acknowledgements

Authors are grateful to Cadila Pharmaceuticals Ltd, Ahmedabad, India, for generous gift of analytically pure sample of LMF.HCl. The manuscript was prepared during the tenure of Research Associateship of one of the authors (S.S.S.), awarded by Council for Scientific and Industrial Research (CSIR), New Delhi, India, and he is thankful to CSIR.

References

[1] Y. Sanzgiri, S. Knaub, C. Riley, in: K. Florey (Ed.), Analytical Profiles of Drug Substances and Excipients, vol. 23, Academic Press, California, 1994, pp. 321–370.
[2] A. Wadworth, K. Goa, Drugs 42 (1991) 1018–1060.
[3] USP24, NF19, Mack Printing Company, Easton, PA, 2000, 2056–2061.
[4] L. Shargel, B. Andrew, Applied Biopharmaceutics and Pharmacokinetics, fourth ed., Prentice-Hall International, Inc, USA, 1999, pp. 247–279.
[5] S. Shah, I. Rathod, S. Savale, B. Patel, J. Chromatogr. -B 767 (2002) 83–91.
[6] A. Straughan, A. Melikan, M. Meyer, J. Pharm. Sci. 68 (1979) 1099–1102.
[7] B. Martin, M. Uihlein, R. Ings, L. Stevens, J. McEwen, J. Pharm. Sci. 73 (1984) 437–441.
[8] C. Shishoo, S. Shah, I. Rathod, S. Savale, M. Vora, Int. J. Pharm. 228 (2001) 53–67.
[9] D. Adriana, M. Raul, H. Marcela, Drug Dev. Ind. Pharm. 26 (2000) 821–828.
[10] A. Shibl, A. Tawfik, S. El-Houfy, F. Al-Shammary, J. Clin. Pharm. Ther. 16 (1991) 335–339.
[11] C. Chan, A. Lam, G. French, J. Antimicrob. Chemother. 23 (1989) 597–604.
[12] D. Griggs, R. Wise, J. Antimicrob. Chemother. 24 (1989) 437–445.
[13] T. Hunt, M. Adams, Diagn. Microbiol. Infect. Dis. 12 (1991) 181–187.
[14] I. Morse, Biopharm. Drug Dispos. 11 (1990) 543–551.
[15] A. Argekar, S. Kapadia, S. Raj, Indian Drugs 33 (1996) 107–111.
[16] J. Chawla, R. Sodhi, R. Sane, Indian Drugs 34 (1997) 512–519.
[17] K. Takacs-Novak, B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi, G. Szasz, J. Pharm. Sci. 79 (1990) 1023–1028.