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Research Article

LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY/MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF LAPATINIB IN RAT PLASMA: APPLICATION TO PHARMACOKINETIC STUDIES IN WISTAR RATS

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ABSTRACT

Objective: The objective of the study was to develop and validate a simple, accurate, and sensitive liquid chromatography–mass spectrometry (LC–MS)/MS method for the determination of lapatinib a dual tyrosine kinase inhibitor in rat plasma using gefitinib as internal standard.

Methods: An Inertsil ODS column (50 mm×4.6 mm×5 µm) was used for separation with isocratic elution of 10 mM ammonium formate-acetonitrile (5:95 v/v). Analyte and internal standard were extracted from 50 µl of plasma using tertiary butyl methyl ether followed by subsequent reconstitution in a mixture of water-acetonitrile.

Results: The extraction recoveries were 95% and 98% for lapatinib and gefitinib, respectively. The lower limit of quantification was 5 ng/ml with a precision of 6.2% and accuracy of 108%. The response was found to be linear over the range of 5–1000 ng/ml with a correlation coefficient of 0.999. The intraday and interday precision expressed as relative standard deviation was <15%.

Conclusion: This validated method was applied to the pharmacokinetic study in Wistar rats. The proposed bioanalytical LC–MS/MS method for lapatinib is a simple, sensitive, and accurate to quantify the concentrations in rat plasma.

Keywords: Lapatinib, Liquid chromatography–mass spectrometry/mass spectrometry, Bioanalytical method validation, Pharmacokinetic study, Gefitinib, Rat plasma.

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INTRODUCTION

Lapatinib was developed as a targeted therapy against breast cancer [1] and acts specifically by inhibiting tyrosine kinase associated with epidermal growth factor (ECF) receptor or ErbB1 and HER-2/*neu* (erbB2) receptors of the EGF family [2,3]. Lapatinib tablets contain monohydrate of the ditosylate salt, with chemical name N-(3-chloro-4-{{(3-flurophenyl) methyl] oxy} phenyl)-6-{5-({[2-methylsulfonyl) ethyl] amino} methyl)-2-furanyl}-4-quinazolinaminebis (4-methyl benzene sulfonate) monohydrate. The structures of lapatinib and gefitinib are shown in Fig. 1.

The literature survey revealed liquid chromatography–mass spectrometry (LC–MS)/MS methods published for the quantification of lapatinib in human plasma of a Phase I study [4], using solid-phase extraction with a recovery of only 75% while another LC–MS/MS method for the determination of lapatinib in tissue samples [5]. There are also other methods for lapatinib alone and simultaneous determination of lapatinib and other tyrosine kinase inhibitors in human plasma using LC–MS/MS, LC–MS, and LC-UV [6-14]. These methods are comparatively less sensitive and also require large volumes of plasma. In this study, a bioanalytical LC–MS/MS method for the estimation of plasma levels was developed and validated using liquid-liquid extraction. The method is sensitive requires very less volumes of plasma and cheaper with higher extraction efficiency compared to reported methods. Furthermore, this method was successfully applied to study pharmacokinetics of lapatinib in Wistar rats.

METHODS

Chemicals and reagents

The reference standards of lapatinib and gefitinib were obtained from Chemical Division, Natco Pharma Limited, India. Analytical grade acetonitrile and tertiary butyl methyl ether were purchased from JT Bakers, ammonium formate was purchased from Fluka, and water used was of Milli-Q.

Instrumentation and operating conditions

The LC–MS/MS analysis was performed using a Waters Quattro Micro API MS equipped with 2695 LC separation module connected to a triple quadrupole analyzer. The separation was carried out using Inertsil ODS (50 mm×4.6 mm and 5 µm) column equipped with an ODS guard column. An isocratic elution of 10 mM ammonium formate-acetonitrile (5:95 v/v) was adopted with a run time of 3.0 min and an injection volume of 5 µl. A constant flow rate of 1.0 ml/min with a splitter ratio of 6:4 was used. The mass spectral analysis was carried out by electrospray ionization in positive ion detection mode with multiple reaction monitoring using parent-daughter transition of 581.36 >362.2 for lapatinib and 447.59 >128.2 for gefitinib. The voltages, temperatures, desolvation gas, and collision gas are optimized to produce maximum intensity of the ions. The source parameters of the MS are summarized in Table 1.

Primary stock solutions and working standard solutions

The primary stock solution of lapatinib and gefitinib (internal standard) was prepared by dissolving appropriate amounts in methanol. The working standard solutions for calibration curve and quality control and internal standard were prepared by dilution of the primary stock solutions. All the stock solutions were stored at 4°C.

Spiking of calibration curve and quality control samples

Spiking was done by adding appropriate amounts of working standard solutions to drug-free rat plasma. The calibration curve samples at concentrations of 5, 10, 50, 100, 250, 500, and 1000 ng/ml and quality

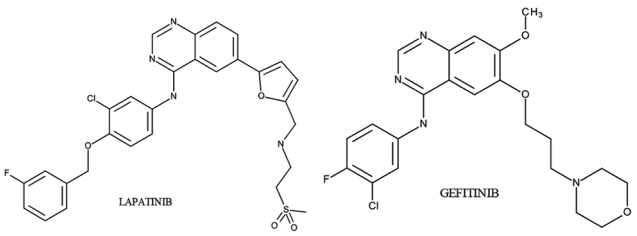

Fig. 1: Chemical structures of lapatinib and gefitinib

Table 1: LC–MS/MS source parameters

Source parameters	Value
Capillary voltage (KV)	3
Cone voltage (V)	55
Extractor voltage (V)	2
RF voltage(V)	0.2
Source temperature (°C)	120
Desolvation temperature (°C)	350
Desolvation gas flow (L/h)	650
Cone gas flow (L/h)	50
Resolution	14
Collision energy (eV)	37

control samples at concentrations of 5, 15, 350, and 900 ng/ml were spiked. After spiking aliquots, each of 50 µl was transferred into Eppendorf tubes and was stored at −70°C until further analysis. Three successive validation batches were done, and on each validation, the aliquots were thawed, 20 µl of internal standard was added and extracted by liquid-liquid extraction method.

Plasma sample extraction procedure

To 50 µl plasma, 20 µl of internal standard (2.5 µg/ml) was added and vortexed for about 30 s. To this, 4 ml of tertiary butyl methyl ether was added, vortexed for 5 min using Multi-Pulse Vortexer (Glas-Col) then 2 ml of the supernatant clear organic layer is transferred to a 7.5 ml test tube and evaporated to dryness using SpeedoVap at 40°C under a stream of nitrogen. Then, the dried extract is reconstituted with 100 µl of diluent (Water:acetonitrile – 1:1) and a 20 µl aliquot is injected into chromatographic system. During each run, a plasma blank sample and a zero standard sample (IS) were also analyzed.

Application of the method

To show the applicability of the developed and validated bioanalytical method, a pharmacokinetic study in Wistar rats was performed. The animal study was approved (1236/PO/08/c/CPCSEA) by the Institutional Ethics Committee (IEC) of Natco Research Centre constituted for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was performed on male Wistar rats (weight range=180–200 g). The drug was administered orally with a single dose of 20 mg/kg. Blood samples (0.5 ml) were collected with a capillary into heparinized tubes from retro-orbital sinus at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 8.0 h. The blood samples were immediately centrifuged at 3000 rpm for 10 min; the plasma was separated and stored at −70°C until analysis. The pharmacokinetic parameters were calculated by Win Nolin software.

Assay validation

The linearity was tested for the concentration range of 5–1000 ng/ml. Seven different concentrations were used for constructing the calibration curve by spiking in blank rat plasma. A least square linear regression analysis was performed to determine the intercept, slope, and the correlation coefficient.

The selectivity was established by checking blank plasma samples obtained from rats. Blank plasma and blank plasma spiked with lapatinib and internal standard were analyzed to check for potential interferences.

The lower limit of quantification was set at 5 ng/ml with an accuracy of 80–120% and precision ±20%.

The accuracy of the assay is defined as the ratio of the mean of the assay values to the actual values expressed in percentage. The accuracy and precision were checked by analyzing six replicates of all the three quality control samples (15, 350, and 900 ng/ml) against a single linearity curve on 3 different days.

The extraction efficiency expressed as the percentage recoveries was determined by measuring the peak area of the extracted plasma quality control and compared to the peak area of extracted blank plasma spiked with standards containing the same concentrations.

Autosampler stability/post preparative stability was determined for ~24 h to cover the anticipated run time for analytical batch and also to allow for delayed injection due to unforeseen circumstances like instrument malfunction. The extracted six replicate QC samples at three different concentrations were kept at autosampler temperature of 5°C for ~24 h and analyzed against fresh standards. The concentrations of the stability samples and fresh samples were determined from a calibration curve prepared on the same day.

Bench-top stability/short-term stability was measured to cover the duration of the time taken to extract the samples. Bench-top stability was checked by analyzing six replicate QC samples at three different concentrations. The spiked QC samples were kept for 6 h at ambient temperature and processed thereafter. The concentration of the stability samples was compared against freshly spiked and processed standards.

Freeze-thaw stability of the spiked quality control samples was determined during three freeze-thaw cycles. Low, medium, and high QC samples were analyzed in six sets. The percentage degradation was determined by comparing the concentration of lapatinib from the freshly prepared plasma validation samples at the same concentrations.

RESULTS AND DISCUSSION

Selectivity

The plasma samples analyzed showed that there is no interference detected due to any endogenous components. A representative chromatogram of blank plasma and blank plasma spiked with IS and analyte is shown in Figs. 2-4, respectively.

Linearity

Five different calibration curves were analyzed and the correlation coefficients are >0.99. This confirms that the calibration curves are linear over the range of 5–1000 ng/ml. The curve parameter summary of five calibration curves is given in Table 2.

Lower limit of quantification

The lower limit of quantification was determined as 5 ng/ml. The response is >5 times compared to the response of blank plasma sample. The accuracy obtained was 108% and precision 6.2%.

Accuracy and precision

The accuracy and precision were checked by analyzing six replicates of all the three quality control samples (15, 350, and 900 ng/ml) against a single linearity curve on 3 different days. The intraday/within run accuracy ranged from 99.5% to 103.0% and the intraday/within run precision ranged from 1.5% to 6.7%. The interday/between run accuracy ranged from 93% to 101% and the interday/between run precision ranged from 7.0% to 8.2%. The intraday and interday accuracy and precision are given in Table 3.

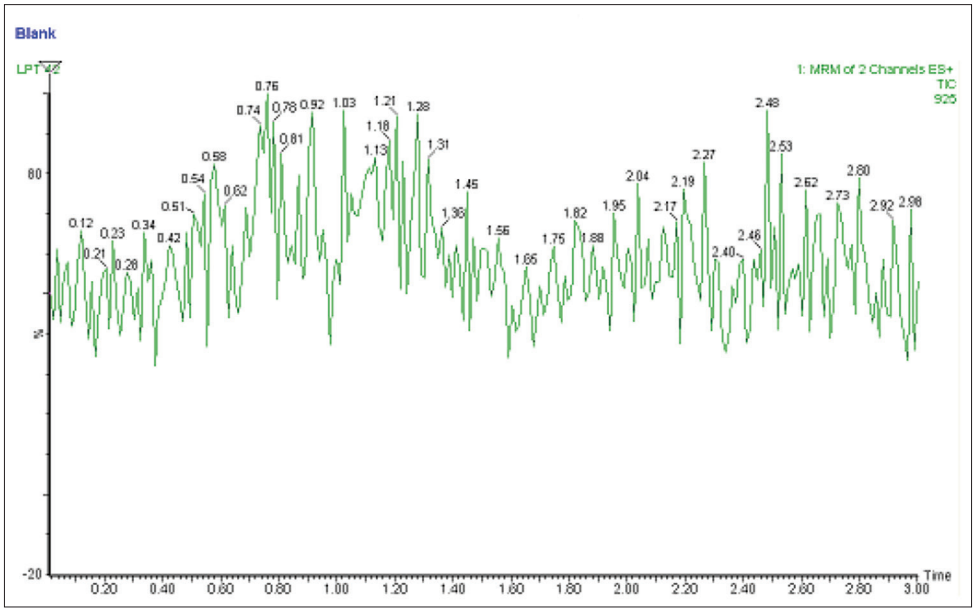

Fig. 2: Blank plasma

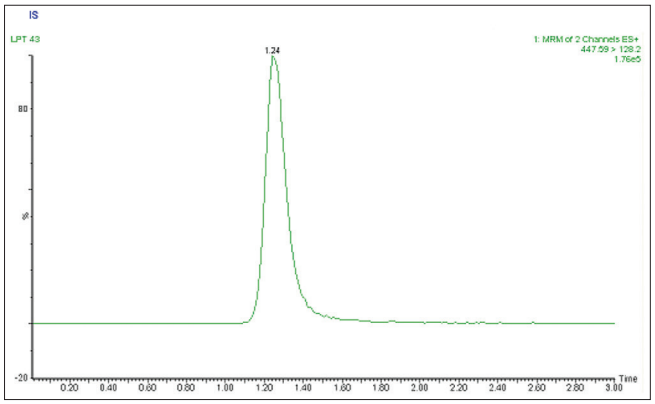

Fig. 3: Blank plasma spiked with internal standard

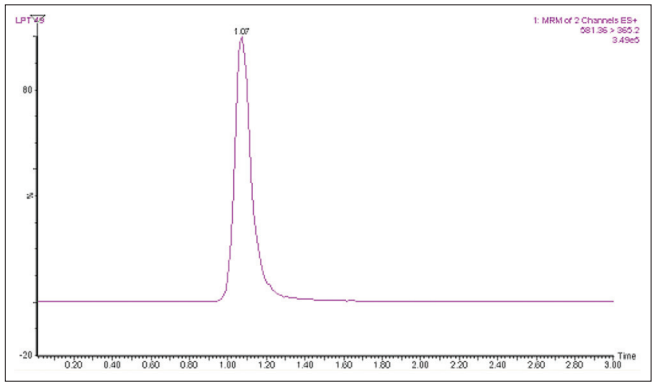

Fig. 4: Blank plasma spiked with analyte

Recovery (extraction efficiency)

The extraction efficiency or the recovery of the analyte and the internal standard are >90%. The recovery of lapatinib and internal standard were 95% and 98%, respectively.

Stability

The concentrations of the stability samples kept at autosampler temperature of 5°C for ~24 h and the concentrations of the freshly prepared samples were determined from a calibration curve prepared on the same day. The accuracy and precision of the stability samples are very much within the limits.

The concentration of the bench-top stability samples was compared with freshly spiked and processed standards. Lapatinib was found to be stable even after 6 h. It was found that even after three freeze-thaw cycles, the concentrations of lapatinib are nearly the same with the original concentrations and the percentage remaining in between 88% and 94% at the end of the three cycles.

Table 2: Curve parameter summary of five calibration curves

Curve code	Slope (a)	y-intercept (b)	Correlation coefficient (r^2)
1	0.002855	0.004234	0.999
2	0.002875	0.002945	0.992
3	0.003138	0.002974	0.998
4	0.002918	0.002555	0.996
5	0.002847	0.008440	0.997

Table 3: Precision of lapatinib in rat plasma

Spiked concentration (ng/ml)	Intraday precision (n=6)		Interday precision (n=6)	
	Mean±SD	RSD (%)	Mean±SD	RSD (%)
15	14.94±0.23	1.53	15.63 ±1.205	7.708
350	360.64±24.24	6.72	345.66±24.29	7.028
900	989.9±69.02	6.97	969.8 ±79.78	8.227

Pharmacokinetic study

To determine the plasma concentration of lapatinib followed by oral administration of the drug to Wistar rats, blood was collected by puncturing the retro-orbital sinus; the plasma was separated and stored at −70°C until analysis. In each analytical run, the concentrations of the quality control samples run were found to be within ±15% of the nominal values. There was no interference peak found during the analysis of the pharmacokinetic study samples. The plasma concentration versus the time curves of lapatinib in rats is represented in Fig. 5 and the pharmacokinetic data are listed in Table 4. The C_{max} and T_{max} values are found to be 951.503 ng/ml and 4 h, respectively.

Table 4: Pharmacokinetic data in rats after oral administration of 20 mg/kg

C_{max} (ng/ml)	T_{max} (h)	AUC_{last} (ng/ml*h)	AUC_{extra} (ng/ml*h)	AUC_{tot} (ng/ml*h)	$T_{1/2}$ (h)	MRT (h)
951.503	4	4682.06	579.136	5261.19	1.671	4.676

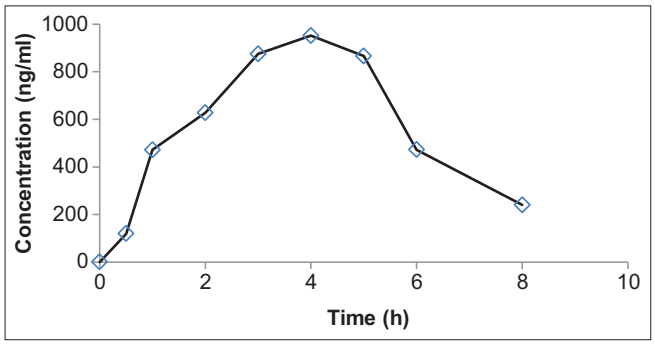

Fig. 5: Mean plasma concentration-time profile of lapatinib after oral administration of 20 mg/kg in rats

CONCLUSION

The proposed bioanalytical LC–MS/MS method for lapatinib is a simple, sensitive, and accurate to quantify the concentrations in rat plasma. A simple liquid-liquid extraction procedure which is cheaper compared to costly solid-phase extraction procedure and a run time of only 3.0 min is very useful to analyze large number of samples in a short-time period. The method was found to be suitable for the quantification of lapatinib in rat plasma samples for pharmacokinetic studies.

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AUTHORS' CONTRIBUTIONS

Nalini Kotapati has performed the work. Literature search, data interpretation, and drafting the manuscript were done by Narmada Palnati. Critical review of the manuscript was provided by Gopal Vaidyanathan.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

The authors declare that no funding was received for the conduct of this study.

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