INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Original Article

SENSITIVE AND RAPID ESTIMATION OF LAPATINIB, AN ANTICANCER DRUG IN SPIKED HUMAN PLASMA BY LC-MS/MS

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Received: 17 Oct 2015 Revised and Accepted: 18 Nov 2015

ABSTRACT

Objective: The work presents a sensitive, selective and rapid determination of lapatinib, a potent anticancer drug in human plasma by liquid chromatography-tandem mass spectrometry.

Methods: Liquid-liquid extraction of lapatinib and lapatinib-d4, added as an internal standard (IS) was carried out from 100 µl plasma sample. Chromatographic analysis was performed on ACE C18 (100 mm × 4.6 mm, 5 µm) column using 10 mmol ammonium formate buffer (pH 3.5) and acetonitrile (10:90, *v/v*) as the mobile phase. The precursor ion → product ion transitions for lapatinib (*m/z* 581.1 → 365.2) and IS (*m/z* 585.1 → 365.0) were monitored on a triple quadrupole mass spectrometer in the positive electrospray ionization mode. The method was validated in accordance with the US FDA guidelines.

Results: A linear concentration range was established from 2.50-2500 ng/ml for lapatinib. The intra-batch and inter-batch precision were ≤ 4.81 %. The recovery of lapatinib and IS from plasma samples ranged from 88.7 to 95.8 % and 85.9 to 96.5 % respectively. The accuracy and precision (% CV) for the stability of lapatinib under different storage conditions showed a variation from 95.2 to 102.2 % and 1.19 to 4.35 % respectively at low and high QC levels. Under optimized chromatographic conditions, the retention time for lapatinib was 1.406 min with a total run time of 2.5 min for each sample.

Conclusion: The validation results demonstrate that the method is simple, accurate, precise and reproducible. The developed method can be readily used for pharmacokinetics/bioequivalence studies in patients as well as healthy subjects.

Keywords: Lapatinib, Lapatinib-d4, Liquid chromatography-tandem mass spectrometry, Human plasma, Sensitive, High-throughput.

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INTRODUCTION

Lapatinib is an orally active and a dual tyrosine kinase (TK) reversible inhibitor of epidermal growth factor receptor (ErbB1/EGFR) and human epidermal growth factor receptor 2 (ErbB2/HER2). These ErbB receptors play a major role in tumor cell growth and survival and are therefore key therapeutic targets [1]. Lapatinib acts intracellularly by reversibly binding to the cytoplasmic ATP-binding site of the kinase and at the same time blocking receptor phosphorylation and activation. As a result, it prevents ensuing downstream signaling events.

The ability of lapatinib to specifically inhibit ErbB1 and ErbB2 receptors is unique compared to other small molecule TK inhibitors like erlotinib and gefitinib [2, 3]. Two effective combinations of lapatinib with capecitabine/letrozole have been approved by the US Food and Drug Administration for the treatment of metastatic HER2 positive breast cancer and for postmenopausal women with HER2 & estrogen receptor positive breast cancer respectively [4].

Currently, lapatinib is available as a 250 mg tablet formulation and is recommended as a single dose at least 1.0 h before or after a meal [1]. However, it has been demonstrated that food increases the bioavailability of the drug after oral administration [5]. Further, the peak plasma concentration reaches within 3-6 h post dosing. Lapatinib is mainly bound to plasma albumin (>99%) and has a high volume of distribution (>2200 L), suggesting good drug distribution [6]. Lapatinib exhibits large inter-individual variation in pharmacokinetics due to the contribution of several metabolizing enzymes and transporters involved in the process of absorption and disposition. Thus to support efficient lapatinib therapeutic drug monitoring and clinical pharmacokinetics it is essential to develop highly sensitive, rapid and reliable methods to monitor the plasma concentration of lapatinib in patient samples.

Several methods are described in the literature for the individual [7-10] as well as the simultaneous determination of lapatinib [11-18] with other TK inhibitors in human plasma [7-10, 12-18] or cellular samples [11]. These methods have mainly used liquid chromatography with mass spectrometry detection for the analysis of lapatinib except one report which utilizes UV as the detector [9]. Some of these developed methods are either less sensitive ≹ 50 ng/ml) [7, 15, 17, 18], have higher retention time for lapatinib (≥ 3.0 min) [8, 10, 12, 15-18] or require large plasma volume for processing (≥ 250 µl) [8, 10, 13]. Moreover, it has been emphasized that the use of isotope-labeled IS is essential to minimize variation in the recovery of anticancer drugs [8]. The comparative features of liquid chromatography-mass spectrometric (LC-MS/MS) method developed for lapatinib in human plasma is presented in table 1.

Thus, the primary objective of the work was to develop a simple extraction procedure with reduced sample volume for processing using a deuterated IS for precise and quantitative recovery. This was essential as lapatinib is an anticancer drug and is estimated clinically mainly in patients and therefore, it is important to minimize blood loss during subject sample analysis. The other aspect was to optimize chromatographic run time with adequate retention on a reversed phase column to maximize throughput. The developed method was fully validated as per current regulatory guidelines especially for matrix effect and stability of lapatinib under different conditions.

MATERIALS AND METHODS

Chemicals and reagents

Working standards of lapatinib (99.5%) and lapatinib-d4 (IS, 98.9%) were purchased from TLC Pharmachem (Toronto, Canada). HPLC grade acetonitrile and methanol were obtained from J. T. Baker (Mumbai, India). Guaranteed reagent grade formic acid, ammonium

formate, ammonia solution (30 %) and methyl *tert*-butyl ether were purchased from Merck Specialties Pvt. Ltd., (Mumbai, India). Water was purified using Milli-Q water purification system from Millipore (Bangalore, India). Blank human plasma was procured from Supratech Micropath Lab (Ahmedabad, India) and was stored at–70 °C until use.

Table 1: Comparative summary of liquid chromatography-mass spectrometry methods developed for lapatinib in human plasma

S. No.	Extraction procedure; internal standard; mean extraction recovery (%)	Sample volume (µl)	Linear range (ng/ml)	Retention time and run time (min)	Ref.
1	SPE on Strata X cartridge; lapatinib-d6; 75	100	100-10000	1.62 and 3.5	7
2	LLE with ethyl acetate; lapatinib-d3; 82.7	250	5.0-5000	4.4 and 10.0	8
3	LLE with MTBE; lapatinib-d7, ^{13}C; 78.6	250	5.0-800	3.5 and 11.0	10
4[a]	PP with ACN; imatinib-d8; 103.4	100	5.0-5000	8.77 and 15.0	12
5[a]	SPE on Oasis MCX cartridge; sorafenib-d4; 90.3	300	10.0-5000	1.84 and 3.0	13
6[a]	PP with MeOH; lapatinib-d4; --	20	4.55-2000	--	14
7[a]	PP with MeOH; imatinib-d8; 99.3	100	50-5000	6.6 and 12.0	15
8[a]	PP with ACN; lapatinib-d7, ^{13}C; 67.2	50	20-10000	4.6 and 10.0	16
9[a]	PP with ACN; imatinib-d8; 85-90	50	100-5000	3.2 and 5.5	17
10[a]	PP with ACN; imatinib-d8; 84-101.2	50	50-3500	3.28 and 6.0	18
11	LLE with methyl *tert*-butyl ether; lapatinib-d4; 91.9 %	100	2.5-2500	1.04 and 2.5	PM

[a]Together with other tyrosine kinase inhibitors; MTBE: methyl *tert*-butyl ether; ACN: acetonitrile; MeOH: methanol; SPE: solid phase extraction; LLE: liquid-liquid extraction; PP: protein precipitation; PM: present method

LC-MS Instrumentation and conditions

A Shimadzu HPLC system (Tokyo, Japan) consisting of binary solvent mixer, sample manager, and column manager was used for setting the reverse-phase liquid chromatographic conditions. The separation of lapatinib and IS was performed on ACE C18 (100 mm × 4.6 mm, 5 µm) column maintained at 40 °C in a column oven. The mobile phase consisted of 10 mmol ammonium formate buffer (pH 3.5, adjusted with formic acid): acetonitrile (10:90, *v/v*) and was delivered isocratically at a flow rate of 1.0 ml/min, with 90 % flow splitting. The flow directed to the ion spray interface was equivalent to 100 µl/min. The auto sampler temperature was maintained at 5 °C.

Ionization and detection of lapatinib and IS were performed on a triple quadrupole mass spectrometer, MDS SCIEX, API4000 (Applied Biosystems, Canada), equipped with electrospray ionization and operating in positive ion mode. Quantification was performed using multiple reaction monitoring (MRM) to monitor precursor → product ion transitions of *m/z* 581.1 → 365.2 and *m/z* 585.1 → 365.0 for lapatinib and IS respectively.

The source dependent parameters maintained for lapatinib and IS were curtain gas: 25.0 psig, ion spray voltage: 5500 V, temperature: 400.0 °C, nebulizer gas (GS1): 60.0 psig, heater gas (GS2): 40.0 psig and collision gas: 10.0 psig. Optimum values set for compound dependent parameters (MRM file parameters) were declustering potential: 42.0 V, entrance potential: 10.0 V, collision energy: 45.0 eV and collision cell exit potential: 13.0 V. The dwell time was set at 200 ms. Analyst software version 1.4.2 was used to control all parameters of HPLC and MS.

Preparation of standard stock, calibration standards, and quality control samples

The standard stock solution of lapatinib (1000 µg/ml) was prepared by dissolving the requisite amount in methanol. Further, an intermediate solution of lapatinib (10.0 µg/ml) was prepared in methanol: water (50:50, *v/v*). Calibration standards (CSs) and quality control (QC) samples were prepared by spiking blank plasma with working solution prepared from intermediate solution. CSs were made at the following concentrations 2.50, 5.00, 10.0, 20.0, 40.0, 100, 250, 625, 1250 and 2500 ng/ml while the QC samples were prepared at four concentration levels, 2000 ng/ml (HQC, high quality control), 1000 and 70.0 ng/ml (MQC-1 and 2, medium quality control) and 7.5 ng/ml (LQC, low quality control). Stock solution (1.0 mg/ml) of lapatinib-d4 was prepared by dissolving 10 mg in 10 ml methanol. An aliquot of 50 µl of this solution was further diluted to 50 ml in acetonitrile: water (50:50, *v/v*) to obtain a working solution of 1.0 µg/ml. The stock solutions were stored at 5 °C, while the CSs and QC samples were kept at–70 °C until use.

Sample preparation

Prior to analysis, all CSs and QC samples were thawed and allowed to equilibrate at room temperature. To an aliquot of 100 µl of spiked plasma sample, 50 µl working solution of IS was added and vortexed for 1.0 min. Further, 50 µl of ammonia solution (5.0 % in water) was added and vortexed again for 1.0 min. Samples were then extracted with 2.0 ml methyl *tert*-butyl ether at 50 rpm for 10 min, followed by centrifugation at 4000 rpm at 10 °C for 5 min. The supernatant was transferred in pre-labeled tubes, and the samples were evaporated to dryness under nitrogen at 40 °C. The dried samples were reconstituted with 500 µl of mobile phase and 5 µl was used for injection in the chromatographic system.

Method validation

The method was validated as per the current regulatory requirements to establish the accuracy and precision of the method [19]. The details of the parameters studied were the same as our previous study [20]. System suitability experiment was performed by injecting six consecutive injections using an aqueous standard mixture of lapatinib and IS at the start of each batch to evaluate the precision in the measurement of area response and retention time. Precision (% CV) of system suitability test was observed in the range of 0.64 to 1.05 % for the retention time and 0.32 to 2.16 % for the area response. System performance was checked by measuring the signal to noise (S/N) ratio for the lowest concentration of lapatinib in the calibration curve. The signal to noise ratio for system performance was≥ 26 for lapatinib. Carryover assessment was carried out through the following sequence of injections, extracted blank plasma, upper limit of the quantification (ULOQ) sample, two extracted blank plasma samples, lower limit of quantification (LLOQ) sample and extracted blank plasma at the beginning of the batch.

The selectivity of the method was investigated by analyzing eight individual blank plasma sources (5 normal K_3EDTA, 1 heparinized, 1 haemolysed and 1 lipemic). Each blank plasma sample was checked for interference by comparing with spiked plasma samples at LLOQ concentration. Method was also assessed for the interference of some commonly used medications by human volunteers. This included domperidone, paracetamol, ranitidine, diclofenac, caffeine, acetylsalicylic acid and ibuprofen. Their stock solutions (100 µg/ml) were prepared in methanol: water (50:50, *v/v*), spiked with the sample and analyzed to check for any interference in the quantification of lapatinib.

Five calibration curves with ten non-zero concentrations were prepared to determine the linearity of the method. The peak area ratio (lapatinib/IS) response obtained through multiple reaction monitoring vs. concentration plot was drawn using linear regression

with $1/x^2$ weighting. Intra-day accuracy and precision were evaluated by measuring the plasma concentration of lapatinib on the same day. The analytical run consisted of a calibration curve and six replicates of QC samples at five concentration levels. The inter-day accuracy and precision were assessed by analysis of five separate precision and accuracy batches on three consecutive days. The reinjection reproducibility for retention time was also checked by reinjection of one entire analytical batch of 80 samples.

The extraction recovery of lapatinib and IS from human plasma was evaluated in six replicates by comparing the mean peak area response of pre-extraction spiked samples to those of post-extraction spiked samples. Matrix effect expressed as matrix factors (MFs) was assessed by comparing the mean area response of post-extraction spiked samples with a mean area of solutions prepared in mobile phase solutions (neat standards) with identical concentration. The relative matrix effect was determined by assessment of precision (% CV) values for slopes of calibration lines from eight different plasma lots [21]. For qualitative assessment of matrix effect, post-column infusion experiment was performed by infusing a standard solution of lapatinib (2500 ng/ml) and IS (1000 ng/ml) post column at a constant flow rate of 10 µl/min. An aliquot of 5 µl of extracted control blank plasma sample/mobile phase was then injected into the column by the autosampler and the chromatograms were acquired.

The stability of analytes in plasma was examined at LQC and HQC levels under different study conditions: bench top at 25 °C, freeze-thaw at-20 °C and-70 °C, wet extract (autosampler) at 5 °C and 25 °C, dry extract at 5 °C, and long-term stability at-20 °C and-70 °C respectively. The area ratio response (labatinib/IS) of stability samples was compared against freshly prepared comparison standards with identical concentration. The procedure also included an evaluation of short-term (25 °C) and long term (5 °C) stability of lapatinib and IS in stock and working solutions.

The ruggedness of the method was tested on two different columns of the same make but different batch numbers and also by employing different analysts using two precision and accuracy batches. Further, dilution reliability of the method was also studied by analyzing six replicates samples prepared as spiked standard at 2 times ULOQ concentration (5000 ng/ml) after five and ten-fold dilution respectively. The precision and accuracy for dilution reliability were determined by comparing the samples against freshly prepared calibration curve standards.

RESULTS AND DISCUSSION

LC-MS/MS method optimization

Mass spectrometry settings were carried out in the positive electrospray ionization mode due to the presence of secondary amino groups which can be readily protonated under acidic conditions in the mobile phase. Initially, the Q1 MS spectra obtained by infusing 500 ng/ml solutions of lapatinib and IS contained abundant protonated precursor ions at *m/z* 581.1 and 585.1 respectively (fig. 1 and fig. 2). The most consistent and stable product ions in Q3 MS spectra for lapatinib and IS were observed at *m/z* 365.2 and 365.0 by applying 45 eV collision energy.

These identical product ions were formed as a result of the elimination of methyl sulfonyl ethyl groups from their respective precursor ions as shown in fig. 1 and fig. 2. The source dependent and compound dependent parameters were suitably optimized to obtain a consistent and sufficient response for lapatinib. A dwell time of 200 ms afforded a sufficient number of data points for the quantitation of lapatinib, avoiding cross talk between lapatinib and IS with identical product ions. Under the optimized conditions, the cross talk experiment showed no detectable change in the peak area of lapatinib and IS at their respective MRMs.

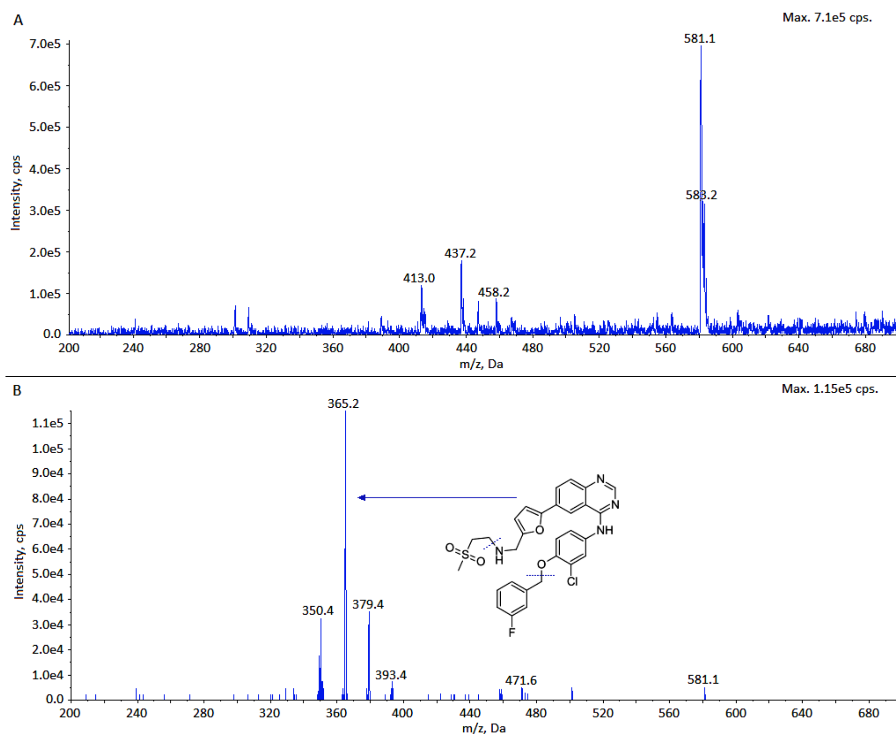

Fig. 1: Mass spectra of (A) Q1 precursor ion and (B) Q3 product ion of lapatinib in the positive electrospray ionization mode

Chromatographic conditions were optimized by careful selection of mobile phase composition (aqueous and organic ratio), pH and flow rate to obtain proper retention, symmetric peak shape, and adequate response. Three reversed phase columns having identical dimensions were studied during initial trials namely ACE C18 (100 mm × 4.6 mm, 5 µm), Hypersil C18 (100 mm × 4.6 mm, 5 µm) and Gemini C18 (100 mm × 4.6 mm, 5 µm). The concentration of ammonium formate (pH 3.0-5.5, adjusted with formic acid) in the range of 2.0-10 mmol was critically examined to get symmetric peak shape and better analyte response. Additionally, the flow rate was varied from 0.6-1.0 ml/min to minimize any possible interference at the retention time of lapatinib. The results obtained on all three

columns was comparable in terms of chromatographic run time and response but the best peak shape was found on ACE C18 column at a flow rate of 1.0 ml/min using 10 mmol ammonium formate buffer (pH 3.5): acetonitrile (10:90, *v/v*) as the mobile phase. The reproducibility of retention time for lapatinib expressed as % CV was ≤ 0.82 % for the entire batch of 80 samples on the same column. The selectivity of the method is evident from fig. 3 which shows the chromatograms of double blank plasma, blank plasma spiked with IS, lapatinib and IS at LLOQ and ULOQ concentration. There was no interference of endogenous components at the retention time of the lapatinib and IS. Similarly, none of the commonly used medications by human volunteers interfered at the retention of lapatinib and IS.

Wu *et al.* [8] have critically examined the impact of isotope labeled and non-isotope labeled IS and found that only isotope labeled IS could correct for variability in the recovery of lapatinib from patient samples. Thus, we employed a deuterated analog, lapatinib-d4 as IS in the present work, which gave acceptable accuracy and precision in the determination of lapatinib from plasma samples.

Ion suppression effect from the LLE extracted blank human plasma and mobile phase solution after post-column infusion of lapatinib and IS is depicted in fig. 4. The results demonstrate the absence of ion suppression or enhancement at the retention time of lapatinib and IS.

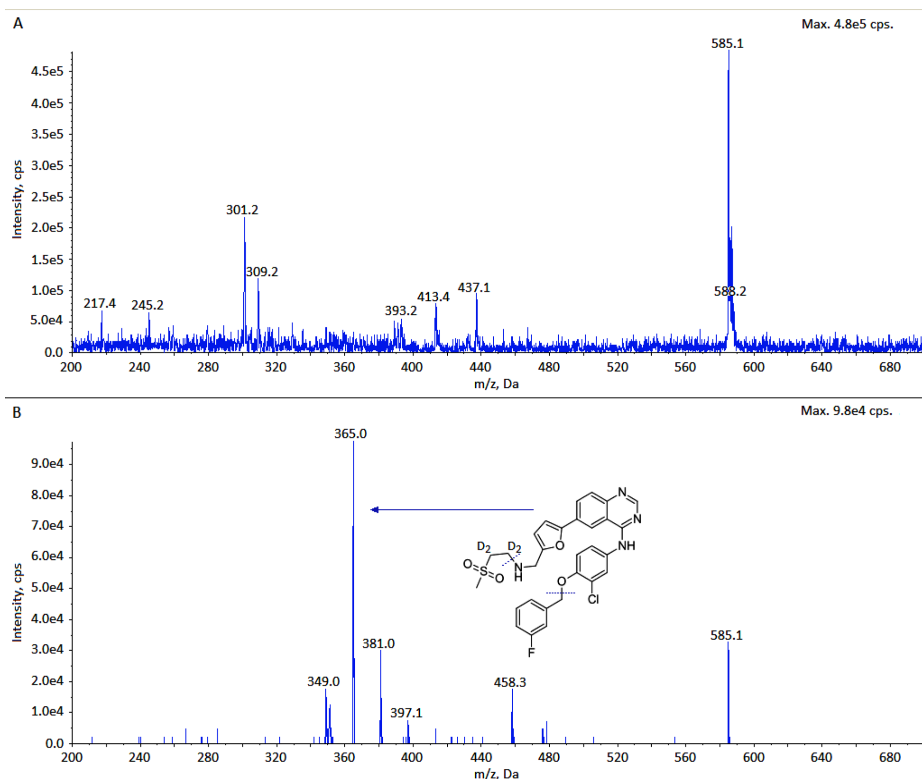

Fig. 2: Mass spectra of (A) Q1 precursor ion and (B) Q3 product ion of lapatinib-d4, internal standard in the positive electrospray ionization mode

Lapatinib is a non polar hydrophobic drug having log *P*>4.6 [7] and is slightly basic in nature (pK_a ~ 7.2) [11]. Thus, in principle either, liquid-liquid extraction (LLE) or solid-phase extraction (SPE) on non-polar reversed phase C18 cartridges can be used as reported previously [7, 8, 10, 11]. We therefore, tried both these techniques using *n*-hexane, ethyl acetate methyl *tert*-butyl ether and dichloromethane as extraction solvents for LLE and Waters Oasis HLB and Phenomenex Strata-X cartridges for SPE. On SPE, trials were performed after pre-treatment of the plasma samples with an acid (acetic acid/formic acid), base (30 % ammonia) and also under neutral conditions. Elution conditions were also optimized using acetonitrile/methanol and their mixture together with an acidic buffer (formic acid-ammonium formate).

The results showed that base treated samples afforded more consistent recovery (45-54 %) as compared to acid pretreated (41-67 %) or untreated samples (38-56 %). However, to have a quantitative and precise recovery of lapatinib we switched over to LLE under alkaline conditions (30 % ammonia solution) to render the drug more hydrophobic for better extraction. Indeed, the results thus obtained with methyl *tert*-butyl ether were much superior compared to SPE. The mean recovery across three QC levels was 91.9 % (varying from 88.7 to 95.8 %), which is higher and more consistent than two previous studies using ethyl acetate [8] and methyl *tert*-butyl ether [10] as extraction solvents.

Validation results

Selectivity and carryover

Selectivity of the assay was assessed in eight different batches of blank plasma to establish that quantitation of lapatinib is unaffected by the presence of biological matrix and other interfering components. There was no significant interference at the retention time of the lapatinib in any of the batches studied. The carry-over evaluation was performed before and after each analytical run to ensure that it does not influence the accuracy and the precision of the proposed method. The column and autosampler carry-over evaluation showed negligible carry over in blank plasma (≤ 0.24 % of LLOQ area response) after subsequent injection of ULOQ sample.

Linearity, accuracy and precision

Regression analysis was carried out to assess the linearity between the peak area ratio (lapatinib/IS) and lapatinib concentration. All calibration curves were linear (r^2 ≥ 0.9985) through the studied concentration range of 2.50-2500 ng/ml of lapatinib. The mean linear equation for calibration curve concentrations was $y = (0.0034 \pm 0.0001)x + (0.0033 \pm 0.0012)$. The accuracy and precision (% CV) observed for the calibration curve standards ranged from 96.40 to 102.75 % and 1.37 to 4.12 % respectively for lapatinib. The limit of detection (LOD) and LLOQ of the method was 1.0 ng/ml and

2.50ng/ml (% CV<20) at S/N ratio of 12 and 26 respectively. The intra-batch and inter-batch precision and accuracy results across five QC levels are shown in table 2. The intra-batch precision (% CV) ranged from 1.43-2.56 % and the accuracy was within 97.8-105.5 % for lapatinib. Similarly for inter-batch experiments, the precision varied from 1.39-4.81 % and the accuracy was within 97.7-105.0 %.

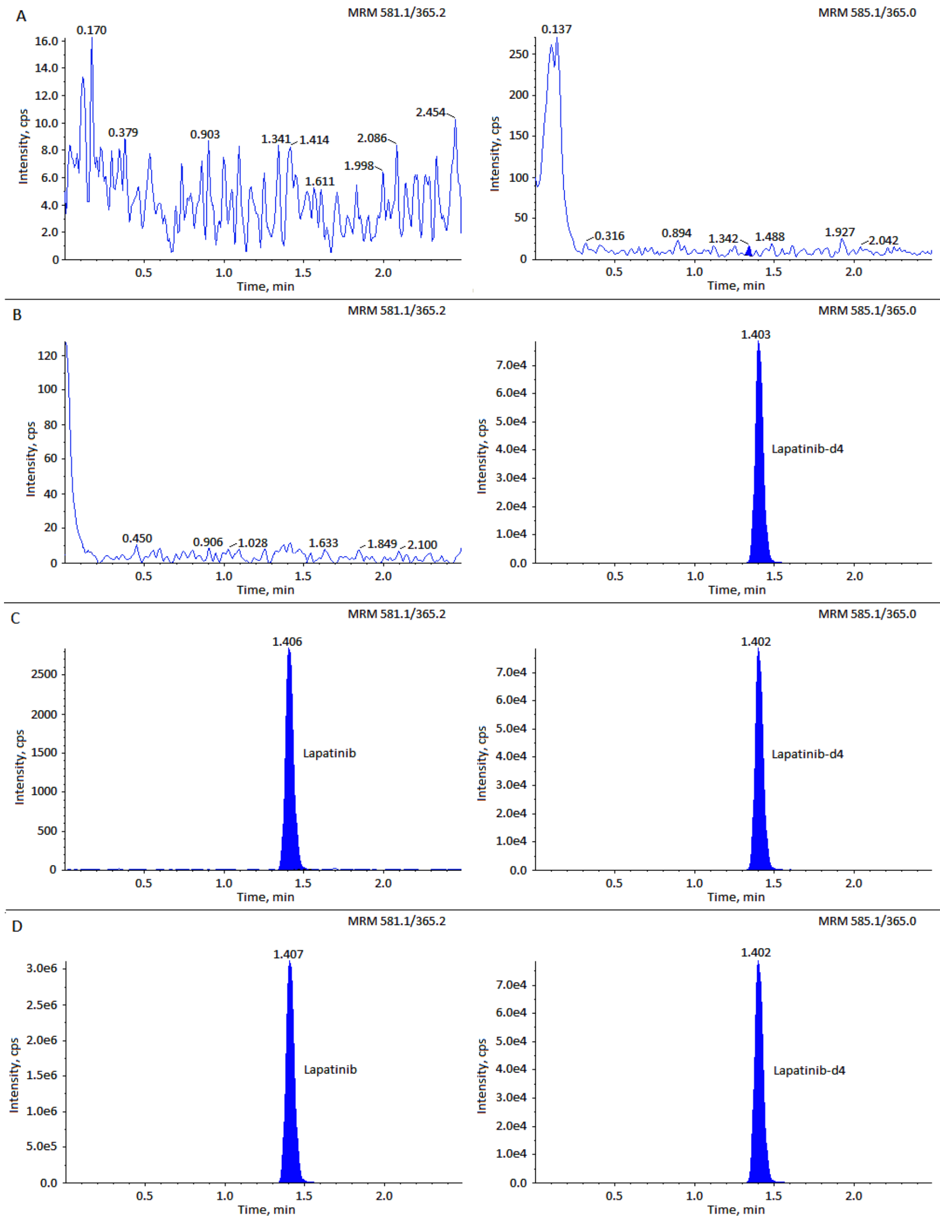

Fig. 3: MRM ion-chromatograms of (A) double blank plasma (without lapatinib and lapatinib-d4), (B) blank plasma and lapatinib-d4 (*m/z* 585.1 → 365.0), (C) lapatinib (*m/z* 581.1 → 365.2) at LLOQ and lapatinib-d4, (D) lapatinib at ULOQ and lapatinib-d4

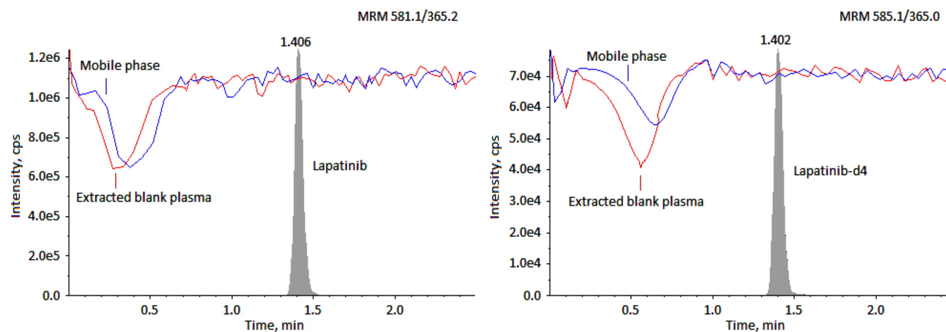

Fig. 4: Matrix effect profiles after post-column infusion of lapatinib (250 ng/ml) and lapatinib-d4 (1000 ng/ml) while injecting extracted blank plasma and mobile phase solution

Table 2: Intra-batch and inter-batch precision and accuracy for lapatinib

Nominal conc. (ng/ml)	Intra-batch (*n* = 6; single batch)			Inter-batch (*n*=30, 6 from each batch)		
	Mean conc. found (ng/ml)	Precision (% CV)	Accuracy (%)	Mean conc. found (ng/ml)	Precision (% CV)	Accuracy (%)
2000	2020	1.43	101.0	2046	2.05	102.3
1000	1055	1.87	105.5	1036	1.39	103.6
75.0	77.8	1.79	103.7	78.6	3.63	105.0
7.50	7.33	2.36	97.8	7.40	3.13	98.7
2.50	2.55	2.56	102.0	2.44	4.81	97.7

n: number of replicates; CV: coefficient of variation

Extraction recovery and matrix effect

The extraction recovery and matrix effect results for lapatinib and IS are presented in table 3 and 4 respectively. The extraction recovery for lapatinib and IS varied from 88.7 to 95.8 % and 85.9 to 96.5 % respectively across QC levels. The potential impact of matrix effect on the quantification of lapatinib was expressed as matrix factor (MF). MFs can be determined from the peak area response of lapatinib and IS separate while the ratio of the two factors gives the IS-normalized MF.

The IS-normalized MFs using stable-isotope-labeled IS should be close to unity because of the similarities in the chemical properties and elution times. The IS-normalized MFs ranged from 0.974-1.044.

Table 3: Extraction recovery for lapatinib

QC level (nominal conc., ng/ml)	Mean area response (*n* = 6)		Extraction recovery, % (B/A)
	A (spiking after extraction)	B (spiking before extraction)	
LQC (7.50)	30876	27387	88.7 (85.9)[a]
MQC-2 (75.0)	303155	274052	90.4 (92.1)[a]
MQC-1 (1000)	3942066	3776499	95.8 (94.6)[a]
HQC (2000)	7829487	7250105	92.6 (96.5)[a]

[a]Mean recovery of lapatinib-d4; *n*: number of replicates

Table 4: Matrix effect results for lapatinib

QC level (nominal conc., ng/ml)	Mean area response (*n* = 6)		Matrix factor		
	A (spiking after extraction)	B (spiking in mobile phase)	Analyte (A/B)	IS	IS-normalized
LQC (7.50)	30876	31094	0.993	1.002	0.991
MQC-2 (75.0)	303155	306837	0.988	1.014	0.974
MQC-1 (1000)	3942066	3812443	1.034	0.990	1.044
HQC (2000)	7829487	7900592	0.991	0.987	1.004

IS: internal standard, lapatinib-d4; *n*: number of replicates

Further, matrix effect needs to be checked in lipemic and haemolysed plasma samples in addition to normal K_3EDTA plasma. The coefficient of variation (% CV) of the slopes of calibration lines for relative matrix effect in eight different plasma lots was 2.07 %, which is within the acceptance criteria of 3.0 % [21].

Table 5: Stability of lapatinib under different conditions (*n* = 6)

Storage conditions	Nominal conc. (ng/ml)	Mean stability Sample (ng/ml)±SD	Accuracy (%)	Precision (% CV)
Bench top stability	2000	2043±59	102.2	2.92
at 25 °C, 12 h	7.50	7.36±0.32	98.1	4.35
Freeze & thaw stability	2000	2035±45	101.7	2.21
at-20 °C, 5 cycles	7.50	7.42±0.27	98.9	3.64
Freeze & thaw stability	2000	2014±24	100.7	1.19
at-70 °C, 5 cycles	7.50	7.31±0.19	97.5	2.60
Auto sampler stability	2000	1976±38	98.8	1.92
at 5 °C, 24 h	7.50	7.45±0.22	99.3	2.95
Dry extract stability	2000	1947±64	97.4	3.28
at 5 °C, 12 h	7.50	7.21±0.15	96.1	2.11
Wet extract stability	2000	1968±27	98.4	1.37
at 25 °C, 12 h	7.50	7.33±0.17	97.7	2.32
Long term stability	2000	2029±35	101.5	1.72
at-20 °C, 92 d	7.50	7.39±0.12	98.5	1.62
Long term stability	2000	1989±56	99.5	2.81
at-70 °C, 92 d	7.50	7.14±0.28	95.2	3.92

SD: standard deviation; *n*: number of replicates

Stability, dilution reliability and method ruggedness

Samples kept for short term and long term stock and working solution stability remained unaffected up to 20 h and 66 d respectively for lapatinib and IS. Bench top stability of lapatinib in plasma was established up to 12 h and for a minimum of five freeze and thaw cycles at–20 °C and–70 °C. Autosampler stability (wet extract) of the spiked quality control samples maintained at 5 °C was determined up to 24 h without significant loss of lapatinib. Spiked plasma samples stored at–20 °C and–70 °C, for long term stability experiment were found stable for a minimum period of 92 d.

For method ruggedness, the precision (% CV) and accuracy values for different columns and analysts ranged from 0.88 to 1.68 % and 99.3 to 104.0 % respectively for lapatinib at five QC levels. The precision (% CV) for dilution reliability of $1/5^{th}$ (1000 ng/ml) and $1/10^{th}$ (500 ng/ml) of 5000 ng/ml solution was between 1.68 and 2.48 %, while the accuracy results were within 98.4 and 103.1 % respectively, which is within the acceptance limit of 15 % for precision (% CV) and 85 to 115 % for accuracy.

CONCLUSION

The present work successfully demonstrates a sensitive, rapid and precise method for determination of lapatinib from endogenous plasma matrix. The selectively of this bioanalytical method enhances its utility for clinical pharmacokinetic studies. The salient features include the use of small plasma sample for analysis, quantitative and precise recovery, high sensitivity (2.50 ng/ml) and high throughput (2.5 min). Use of deuterated IS minimizes inter-individual variability in the recovery of lapatinib following efficient LLE approach under alkaline conditions. Finally, the method shows good accuracy and precision as evident from the results of all validation parameters.

ACKNOWLEDGEMENT

The authors are thankful to the Director of Veeda Clinical Research, Ahmedabad for providing technical support and infrastructure facility to carry out this work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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