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BIO ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LAPATINIB IN HUMAN PLASMA BY LC - MS/MS.

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ABSTRACT
A highly sensitive and simple high-performance liquid chromatographic tandem mass spectrometric (LC–MS/MS) assay is developed and validated for the quantification of Lapatinib in human plasma. Lapatinib is extracted from human plasma by Liquid Liquid Extraction by 50µl 1mM NaOH and 2.5mL of Ethyl Acetate and analyzed using a reversed phase isocratic elution on a Kromacil 100 C18 (4.6 X 50mM, 5µm) column. A acetonitrile: 5mM ammonium formate (80:20%v/v) pH adjusted to 3.80 using formic acid is used as mobile phase and detection was performed by Ultra triple quadrupole mass spectrometry LC-MS/MS using electrospray ionization in positive mode. Pioglitazone is used as the internal standard. The lower limit of quantification is 15.004ng/mL for lapatinib. The calibration curves are consistently accurate and precise over the concentration range of 15.004 to 2000.540 ng/mL in plasma for Lapatinib. This novel LC–MS/MS method shows satisfactory accuracy and precision and is sufficiently sensitive for the performance of pharmacokinetic, bioavailability and bioequivalence studies in humans.

KEYWORDS: Lapatinib, LC-MS/MS, Bioanalytical Method Validation, Human Plasma, Liquid Liquid Extraction, Breast Cancer.

1. INTRODUCTION
Breast cancer is the second most frequent cause of cancer related death in women in developed countries with over 1.67 million cases diagnosed worldwide in 2012.[1] The incidence of breast cancer is increasing in the developing world due to increase life expectancy, increase urbanization and adoption of western lifestyles. It is estimated that worldwide over 508 000 women died in 2011 due to breast cancer (Global Health Estimates, WHO 2013)[2] and the same also accounting for 571 000 deaths in 2015 as per WHO Cancer Fact sheet Updated February 2017.[3] Although breast cancer is thought to be a disease of the developed world, almost 50% of breast cancer cases and 58% of deaths occur in less developed countries (GLOBOCAN 2008).[2] Approximately 15%–22% of breast cancers exhibit over expression of gene amplification of human epidermal growth factor receptor 2 (HER2)[4,5], which is known to be associated with aggressive disease and a greater risk of disease progression and death.[5,6]

Lapatinib is a small molecule and member of the 4-anilinoquinazoline. It is present as the monohydrate of the ditosylate salt, with chemical name N-(3-chloro-4-{[(3fluorophenyl) methyl]oxy}phenyl)-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furanyl]-4quinazolinamine bis(4-methylbenzenesulfonate) monohydrate. It has the molecular formula C29H26ClFN4O4S (C7H8O3S) 2 H2O and a molecular weight of 943.5.[7]

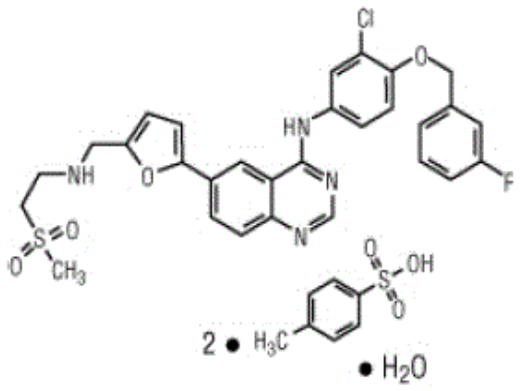

Figure 1: Chemical Structure of Lapatinib ditosylate monohydrate

Lapatinib is an orally active drug for breast cancer (approved by U.S. FDA in 2007) and for some solid tumours.[8] It is a dual tyrosine kinase inhibitor which

interrupts the HER2/neu and epidermal growth factor receptor (EGFR) pathways.[9,10] It is used in combination therapy for HER2 positive breast cancer and it is used for the treatment of patients with advanced or metastatic breast cancer whose tumors over express HER2 (ErbB2)[11] and is also used as an adjuvant therapy when patients have progressed on Herceptin.[12] Glaxo Smith Kline (GSK) announced the approval of lapatinib in first line therapy in triple positive (hormone receptor, EGFR, HER2) breast cancer patients.[13]

Considering the need for pharmaco economic cancer treatments, it is essential to develop reliable and sensitive bioanalytical method for lapatinib determination in human plasma in order to conduct bioequivalence studies of new generic drug products. After the oral administration of a single 250 mg dose, maximum lapatinib plasma concentrations occur within 3 to 6 h (mean 4 h) and range from 192 ng/mL to 524 ng/mL (mean 317 ng/mL).[14] According to the European Medicines Agency guideline[15], a method designed for the application in bioequivalence studies should allow to assess maximum drug concentrations and analyze the area under the plasma concentration curve from the administration to the last observed concentration at a time t, AUC (0-t), which covers at least 80% of the area extrapolated to the infinity time, AUC (0-∞). Taking into account the above recommendations and available pharmacokinetic data[14], the linearity range of 15.000 to 2000.000 ng/mL seems to be suitable for bioequivalence studies.

Several chromatographic methods including liquid chromatography UV (LC–UV)[16], HPLC tandem mass spectrometry (LC-MS/MS)[17,18] have been developed to measure Lapatinib in biological fluids. All these reported methods are inadequate because of insufficient sensitivity, along high chromatographic runtime, more plasma volume required for sample processing and a high solvent usage. Yet not much sensitive and effective LC–MS/MS method has been developed and validated in human plasma. The objectives of this research study were to develop and validate a simple, rapid, sensitive and accurate method of the separation with short run time and quantification of Lapatinib in human plasma.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Lapatinib ditosylate monohydrate working standard was supplied by Natco pharma limited and was certified to contain 99.80% for lapatinib ditosylate monohydrate. Pioglitazone hydrochloride standard was supplied by Cadila pharmaceuticals limited and was certified to contain 99.40% w/w for Pioglitazone hydrochloride. Both standard and internal standard were used without further purification. The organic solvent methanol of HPLC grade used and was obtained from Merck for standard preparation and further dilution was made by Acetonitrile: Water (90:10 v/v). Acetonitrile used was of HPLC grade, Sodium Hydroxide and Ethyl Acetate was of Analytical Grade were obtained from Merck. Water was obtained from a Milli-Q Gradient water purification system (Millipore, Barnstead). Ammonium formate and formic acid used in mobile phase preparation was of Guaranteed Reagent (GR) grade obtained from Sigma Aldrich.

2.2. Chromatographic conditions

A Thermo Surveyor Liquid Chromatography (LC) instrument was used in this study. Separation was carried out on a Kromacil 100 C18 column (4.6 x 50mm, 5µm) maintained at 35°C. The LC mobile phase consisted of Acetonitrile: 5mM Ammonium formate (80:20% v/v) pH adjusted to 3.80 with formic acid. The flow rate was 0.650 mL/min. The injection volume was 5.0 µL and the runtime was 3.0 minutes (mins).

2.3. Mass spectrometry conditions

Detection was carried out by a Thermo TSQ Quantum Ultra triple quadrupole MS/MS fitted with heated electrospray ionization (HESI) probe and operated in the positive ion mode. Detection was carried out in multiple reactions monitoring (MRM) mode. Argon 99.995% was used as the collision gas. The optimal parameters are shown in Table 1.

2.4. Preparation of standard and sample solutions

Standard stock solutions of Lapatinib 1mg/mL (w/v) and the IS Pioglitazone 1mg/mL (w/v) were separately prepared in 10mL volumetric flasks with methanol. Working solutions for calibration and controls were prepared from the stock solution by adequate dilution using diluents Acetonitrile: Water (90:10v/v). The Internal Standard (IS) working solution (500.000 ng/mL) was prepared by diluting the stock solution with diluent. 20 µL of working solutions were added to 980 µL drug free human plasma to obtain Lapatinib concentration levels of 15.004, 30.008, 60.016, 150.040, 300.081, 600.162, 1200.324, 1600.432 and 2000.540 ng/mL respectively. Quality control (QC) samples were prepared as a bulk based on an independent weighing of standard drug, at concentrations of 15.010ng/mL (LLOQ), 41.464ng/mL (LQC), 829.280ng/mL (MQC), 1594.770ng/mL (HQC) as a single batch at each concentration. These samples were divided into aliquots in micro centrifuge tubes (Tarson, 0.3mL) and stored in the freezer at below -80^0 C until analysis.

2.5. Sample preparation

Sample preparation involved a liquid liquid extraction with Ethyl Acetate with 1mM NaoH Buffer. A spiked plasma stability sample of Lapatinib was removed from the deep freezer maintained at -80^0C and left at room temperature to thaw. The samples were vortexed, mixed adequately and centrifuged before pipetting. As soon as the stability samples were thawed, these samples were aliquoted (0.3 ml) and freshly prepared. Calibration Standards(CS) and Quality Control (QC) samples were spiked with 50 µL IS (500.000ng/mL) into pre labeled RIA vials to each tube except blank and mixed for 30

seconds on vortexer. Add 50 µL 1mM NaoH, vortex the sample for 10 Seconds. To this 2.5 ml of solvent Ethyl acetate was added to each tube and vortexed in Vibramax for 10 minutes at 2500 rpm. Then samples were centrifuged at 4500 rpm for 5 min at 4^0C. 1.8mL of supernatant layer was transferred, separated and evaporated under gentle steam of nitrogen gas pressure at 40°C up to dryness, reconstituted with 500.00 µL mobile phase and vortexed for 1 min then transferred to a HPLC vials for analysis.[19]

2.6. Method validation

2.6.1. Specificity and carry over

To verify the absence of interfering substances around the retention time of analyte, the specificity of the method was investigated by chromatograms obtained from eight different sources of blank plasma one heamolysed and one lipeamic source spiked at LLOQ, ULOQ levels.

The carry over experiment, in which blank human plasma samples were analyzed immediately after the highest concentration calibration standards (procedure repeated six times), showed no peaks influencing the quantification.

2.6.2. Linearity

Calibration curves were constructed using matrix matched calibration standard solutions by plotting the peak area of the quantitative ion of each analyte versus concentrations. Concentration range of Lapatinib was found to be accurate and precise from 15.004 to 2000.540ng/mL respectively. Correlation coefficient was greater than 0.99 for Lapatinib.

2.6.3. Limit of detection and quantitation

The limits of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, the limit of detection (LOD) was also defined as the concentration that produced a signal three times above the noise level of a blank preparation. LOD were estimated for those concentrations that provide a signal-to-noise ratio of 3:1 was found. The LOQs were determined in Back calculated values of the lowest calibration samples with a bias and CV% below ±20% enables to determine the LOQ values, in accordance with the results mentioned. The LLOQ concentrations were finally selected as the lowest levels of the calibration curves established during the analytical method validation.

2.6.4. Accuracy and precision

Intra assay precision and accuracy of Lapatinib were calculated at lower limit of quantification (LLOQ) (15.010ng/mL), low quality control (LQC) (41.464ng/mL), Middle quality control (MQC) (829.280ng/mL) and high quality control (HQC) (1594.770ng/mL) levels for the six replicates, each of the same analytical run. Inter assay precision and accuracy were calculated after the replicates in three different analytical runs.

2.6.5. Recovery

The recovery of Lapatinib was calculated by comparing the peak area of the analyte from the extracted plasma standard with that obtained from an unextracted standard at the same concentration for the QC samples containing 41.464, 829.280, 1594.770ng/mL for Lapatinib. Internal Standard recovery was tested at 500.000ng/mL by comparing six extracted and unextracted samples at each concentration.

2.6 6. Matrix effect

The matrix effects were investigated for eight different lots including one heamolysed and one lipeamic lots of blank K3EDTA human plasma were processed in duplicate and reconstituted with aqueous HQC and LQC samples. Extracted and aqueous samples were then compared to determine the matrix factor for analyte and Internal Standard (IS). IS normalized matrix factor for individual lot is also determined. The calculated matrix factor of all LQC and HQC samples must be within 0.85-1.15 of their nominal concentration. At least 67% of QC samples must fall within the above mentioned criteria at each LQC and HQC levels.

2.6 7. Dilution Integrity

The dilution integrity was calculated by spiking the matrix with an analyte concentration above the ULOQ and diluting this sample with blank matrix. Accuracy and precision should be within ±15%. Dilution integrity should cover the dilution applied to the study samples.

2.6 8. Stability

Exhaustive experiments were performed to assess the stability of Lapatinib in stock solution and in plasma samples under different conditions, simulating the conditions occurring during the analysis of study samples. Room temperature stability, extracted sample stability (process stability), Auto sampler stability, freeze thaw stability, long term stability of plasma samples, dry extract stability and stock solution stability were performed.

3. RESULTS AND DISCUSSION

3.1. Method development and optimization

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analyte and the IS, as well as short run time. Modifiers such as ammonium formate alone or in combination in different concentrations were added. It was found that a mixture of Acetonitrile: 5mM Ammonium formate (80:20% v/v) pH adjusted to 3.80 with formic acid could achieve this purpose and was finally adopted as the mobile phase. Formic acid was found to be necessary in order to lower the pH and act as a peak modifier for Lapatinib and thus deliver a good peak shape. The percentage of formic acid was optimized

to maintain a good peak shape, while being consistent with good ionization and fragmentation in the mass spectrometer.

The tandem mass spectrometer allows the selective detection of substances with varying masses or fragments without chromatographic separation. The development of the chromatographic system was focused on short retention times in order to assure high throughput, paying attention to matrix effects as well as good peak shapes. The high proportion of organic solvent Acetonitrile: 5mM Ammonium formate (80:20% v/v) pH adjusted to 3.80 with formic acid eluted the analyte at retention times 1.74 min and the IS at retention times of 1.40 min, respectively. A flow rate of 0.650 mL/min produced good peak shapes and permitted a run time to 3.0 min.

Internal standard (IS) is necessary for the determination of analyte and metabolite in biological samples. In the initial stages of this work, several compounds were investigated to find a suitable internal standard and finally Pioglitazone structurally not related to Lapatinib, but was found to be the best for the present purpose. Clean chromatograms were obtained and no significant direct interferences in the MRM channels at the relevant retention times were observed. However in ESI signal suppression or enhancement may occur due to co eluting endogenous components of the sample matrix. Liquid liquid extraction was necessary and important because this technique not only purifies but also concentrates the samples.

Ethyl acetate was used because of its high extraction efficiency and low interference. These potential matrix effects were evaluated by spiking blank plasma extracts at the low and high QC levels. The resulting chromatograms were compared with those obtained for clean standard solutions at the same concentrations. Three independent plasma lots were used with three samples from each lot. The results showed that there was no significant difference between peak responses for spiked plasma extracts and clean solutions.

3.2. Method validation

The developed method was validated in terms of specificity, linearity, precision and accuracy, recovery, matrix effect, dilution integrity, stability, Carry over Test and Ruggedness. The validation was carried out with respect to FDA, Guidance for Industry: Bioanalytical Method Validation.[20, 21, 22, 23, 24,25]

3.2.1. Specificity

The Specificity was assessed by analyzing extracted samples of analyte at LLOQ and ULOQ without internal standard, internal standard sample at working concentration without analyte and blank sample without analyte and internal standard. The area observed at the retention time of Lapatinib was found to be less than 20% of the LLOQ area (15.010ng/mL). It was found that internal standard is not interfering with analyte and vice versa. Representative chromatograms attached in Figure 2 and Figure 3.

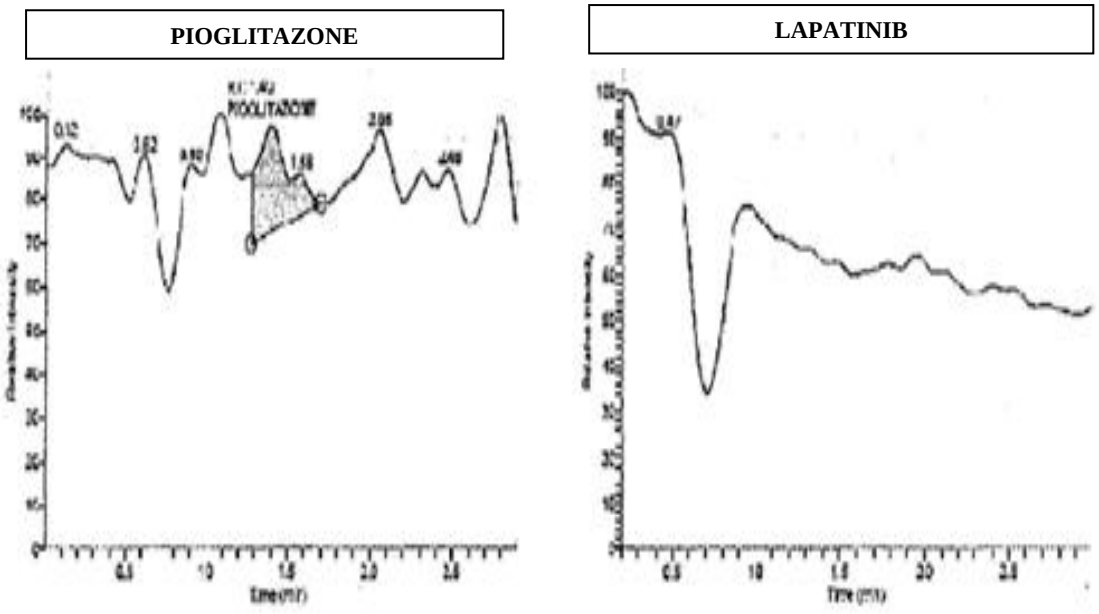

Figure 2: Representative ion Chromatogram for Standard Blank

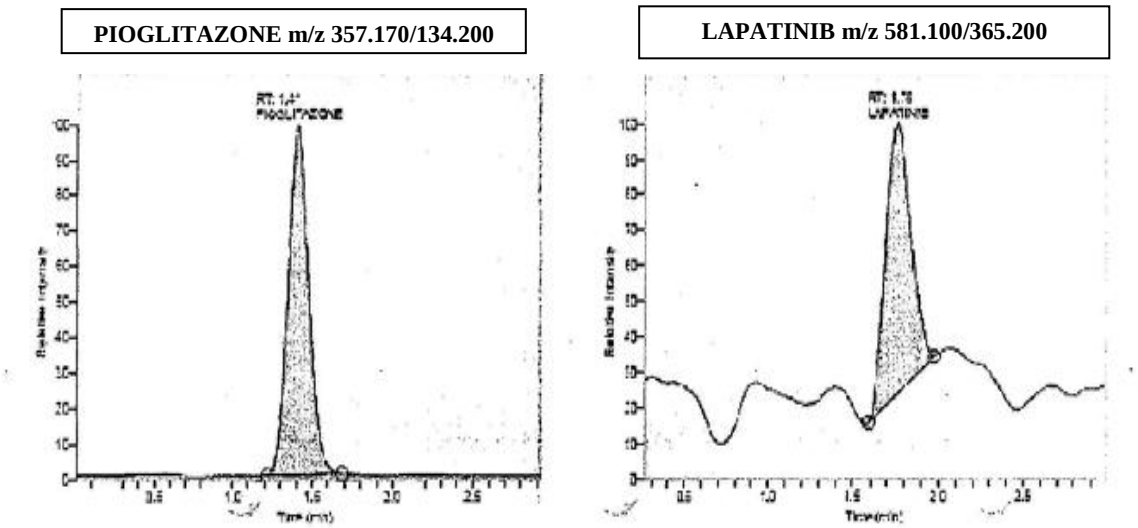

Figure 3 Representative ion Chromatogram for LLOQ QC

3.2.2. Linearity

The linearity of the method was determined by a weighted quadric regression analysis of standard plots associated with nine point standard calibration curves. Best fit calibration curves of peak area ratio against the concentration were drawn. The concentration of Lapatinib was calculated from the simple quadric equation using a regression analysis of the spiked plasma Calibration Standards with a reciprocal of the drug concentration 1/x as a weighting factor. The calibration plots of Lapatinib were quadric and calibration curves are found to be consistently accurate and precise over a range of 15.004 to 2000.540 ng/mL respectively with r2 greater than 0.99.

3.2.3. Accuracy and precision

The Intra batch coefficients of variation ranged from 3.24 to 10.89% and percentage accuracy ranged from 88.64 to 118.65% for Lapatinib. The results are within ±15% and for LLOQ the results are within ±20% and shown in Table 2. The Inter batch coefficients of variation ranged from 10.40 to 15.51% and percentage accuracy ranged from 99.42 to 102.11% for lapatinib respectively. Results are presented in Table 3.

3.2.4. Recovery

Recovery of Lapatinib and the internal standard Pioglitazone were evaluated by comparing mean peak response of six extracted low, middle and high level quality control samples to those of six appropriately diluted aqueous solutions. Mean recovery values of Lapatinib are 41.83, 53.63 and 51.64. Mean recovery values of internal standard Pioglitazone are 85.89, 108.61 and 104.92 for low, middle and high quality control levels respectively. Total mean recovery of analyte Lapatinib and the internal standard Pioglitazone are 49.03% and 99.81% respectively. Results shown in Table 4.

3.2.5. Matrix effect

Processed and analyzed calibration standards in the same matrix which is to be used during validation experiment and duplicates from six different lots of plasma at LQC and HQC levels as per the procedure are described in the sample preparation section. The calculated matrix factor of all LQC and HQC samples must be within 0.85-1.15 of their nominal concentration. At least 67% of QC samples must fall within the above-mentioned criteria at each LQC and HQC levels and results shown in Table 5.

3.2.6. Dilution integrity

Dilution integrity standard having double the concentration of HQC was diluted 2 and 4 times with screened pooled blank plasma. 6 replicates of each dilution are extracted and analysed. The extracted samples were injected along with calibration curve standards. Percentage accuracy for 1/2 dilution of Lapatinib was 92.00% and for 1/4 dilution are 96.60% respectively and within ±15% of the nominal concentration, the coefficient of variation for 1/2 dilution are 4.11% and for 1/4 dilution are 4.53% respectively. Results are presented in Table 6.

3.2.7. Stability

The stability of the analyte and IS in human plasma under different temperature and time conditions were evaluated, as well as the stability in stock solution, was also evaluated and follows. All the stability Figure of the studies were carried out at two concentration levels of Lapatinib (41.464 ng/mL and 1594.770 ng/mL) as low and high values with six determinations for each stability test along with a calibration curve standards. The results are shown in Table 7.

For short term stability determination, stored plasma aliquots were thawed and kept at room temperature for a period of time exceeding that was expected to be encountered during the routine sample preparation (around 6 h). These results indicate reliable stability behavior under the experimental conditions of the regular analytical procedure.

The stability of QC samples kept in the auto sampler for 24 hours 16 mins was also assessed. The results indicate

that solutions of Lapatinib were found to be stable for 24 hours 16 mins in auto sampler with percentage stability 96.34 to 103.36%, percentage accuracy 93.90 to 101.30% respectively. Coefficient of variation was 10.57 to 11.80% respectively. Lapatinib and Internal Standard Pioglitazone can remain in the auto sampler for at least 24 hours 16 mins hours, without showing a significant loss in the quantified values, indicating that samples should be processed within this Period.

The data representing the stability of Lapatinib in plasma at two QC levels over four freeze and thaw cycles are given in result Table 7. These tests indicate that the analyte is stable in human plasma for four freeze and thaw cycles, when stored at below -80^{0}C and thawed to room temperature. The stability study of Lapatinib in human plasma showed reliable stability behavior, as the mean of the results of the tested samples was within the acceptance criteria of ±15% of the initial values of the controls. These findings indicate that storage of Lapatinib in plasma samples at below -80^{0}C is adequate, and no stability related problems would be expected during routine analysis for pharmacokinetic, bioavailability or bioequivalence studies.

The Analyte were found to be stable as dry extract for 24 hours, found to be stable as wet extract 10 hours 30 mins and found to be stable as bench top for 08 hours 12 mins. The results revealed optimum stability in Plasma samples.

Lapatinib were found to be stable in human plasma stored at -20°C for 02 days 06 hours 30 mins. Results of Intermediate stability are shown in Table 7.

The stability of stock solutions was tested and established at room temperature for 09 hours 42 minutes and under refrigeration (2^{0}C–8^{0}C) for 04 days 01 hour 8 mins. The results revealed optimum stability for the prepared stock solutions throughout the period intended for their daily use.

Table 1: Mass Spectrometer Parameters

Parameters	**Values**	
Compound	**Laptinib**	**Pioglitazone**
Parent mass (m/z)	581.100	357.170
Product mass (m/z)	365.200	134.200
Collision Energy	35	27
Tube lens off set	240	141
Spray voltage	5000	5000
Sheath gas pressure	65	65
Aux Gas pressure	35	35
Ion Sweep gas	10	10
Skimmer off set	-24	-24
Capillary Temperature	270	270

Table 2: Lapatinib Intra batch accuracy and precision

Quality Control Samples	**Lapatinib**			
	Nominal conc.(ng/mL)	**Conc. found (mean ±S.D.) (ng/mL)**	**Precision (%) CV**	**Accuracy (%)**
LLOQC	15.010	17.8090±0.57656	3.24	118.65
LQC	41.464	39.5130±3.22108	8.15	95.29
MQC	829.280	762.1657±83.02761	10.89	91.91
HQC	1594.770	1413.6305±142.12525	10.05	88.64

Table 3: Lapatinib Inter batch accuracy and precision

Quality Control Samples	**Lapatinib**			
	Nominal conc.(ng/mL)	**Conc. found (mean ±S.D.) (ng/mL)**	**Precision (%) CV**	**Accuracy (%)**
LLOQC	15.010	15.3272±2.37723	15.51	102.11
LQC	41.464	41.4067±4.30631	10.40	99.86
MQC	829.280	845.0924±105.31057	12.46	101.91
HQC	1594.770	1585.5268±192.82330	12.16	99.42

Table 4: Recovery Results

Percentage Recovery		
Description	**Lapatinib**	**Pioglitazone (IS)**
LQC	41.83	85.89
MQC	53.63	108.61
HQC	51.64	104.92
Global Recovery	49.03	99.81
SD	6.3171	12.193
% CV	12.88	12.22

Table 5: Internal Standard Normalised Matrix Factor

IS normalized Matrix Factor			
S.NO	**Lot ID**	**Lapatinib**	
		HQC	**LQC**
1	LS_01	0.89	0.97
2	LS_02	0.93	0.98
3	LS_03	1.03	1.14
4	LS_04	0.96	1.05
5	LS_05	0.95	1.04
6	LS_06	0.86	0.96
7	LS_07(Lipimic)	0.99	0.95
8	LS_08(Hemophilic)	0.92	0.98
Mean		**0.9413**	**1.0088**
SD		**0.05410**	**0.06424**
% CV		**5.75**	**6.37**
Acceptance		**% CV<15%**	

Table 6: Dilution Integrity Results

	DI-2 TIMES	**DI-4 TIMES**
Nominal values (ng/ml)	**1600.432**	**800.216**
QC ID	**Actual concentrations (ng/ml)**	
1	1391.831	840.159
2	1450.611	794.311
3	1507.254	813.107
4	1427.759	786.878
5	1497.971	816.868
6	1558.945	725.036
Mean	**1472.3951**	**796.6537**
SD	**60.52704**	**36.08990**
%CV	**4.11**	**4.53**
%Nominal	**92.00**	**99.60**

Table 7: Stability Data Results of Lapatinib.

S.No.	Stability Experiments	Lapatinib				
		Stability Duration And Temperature	Nominal sample Conc. (ng/mL) (n = 6) LQC, HQC	Conc. Found (ng/mL) (mean ± S.D.)	% Mean change at QC level Acceptance Limit (85-115%)	%Stability
1A	Four freeze thaw cycles (FT)-LQC	After 4th FT cycle at (-80°C)	41.464	39.7093± 4.45123	95.77	98.26
1B	Four freeze thaw cycles (FT)-HQC	After 4th FT cycle at(-80°C)	1594.770	1461.3272± 75.11508	91.63	93.50
2A	Auto sampler Stability-LQC	24 h 16m at 10°C	41.464	38.9337 ±4.59400	93.90	96.34
2B	Auto sampler Stability-HQC	24 h 16m at 10°C	1594.770	1615.4480 ±170.74400	101.30	103.36
3A	Bench top Stability-LQC	08h12m at 20°C	41.464	38.4897 ±3.56000	92.80	95.24
3B	Bench top Stability-HQC	08h12m at 20°C	1594.770	1598.6272± 77.53300	100.20	102.28
4A	Dry extract Stability-LQC	24h at 20°C	41.464	37.9207± 2.516	91.50	93.83
4B	Dry extract Stability-HQC	24h at 20°C	1594.770	1418.6413± 24.894	89.00	90.76
5A	Wet extract Stability-LQC	10h30m at 2-8°C	41.464	40.8852± 3.90379	98.60	101.17
5B	Wet extract Stability-HQC	10h30m at 2-8°C	1594.770	1555.2248± 100.37183	97.52	99.50
6A	Intermediate stability-LQC	02days 06h30m at-20°C	41.464	39.5280± 2.62755	95.33	97.81
6B	Intermediate stability-HQC	02days 06h30m at-20°C	1594.770	1670.7478± 61.37337	104.76	106.89

4. CONCLUSION

In summary, the LC–MS/MS method for the quantitation of Lapatinib in human plasma was developed and fully validated as per FDA guidelines. This method offers significant advantages over those previously reported in terms of improved sensitivity and selectivity, faster run time (3.0 min) and shorter sample preparation time, lower sample requirements and less solvent consumptions, Thus the volume of samples to be collected per time point from an individual's during trial is reduced significantly, allowing inclusion of additional points. With dilution integrity up to 4 fold, we have established that the upper limit of quantification is extendable up to 2000.540 ng/mL. Hence, this method is useful for single and multiple ascending dose studies in human subjects. The current method has shown acceptable precision and adequate sensitivity for the quantification of Lapatinib in human plasma samples obtained for pharmacokinetic, bioavailability and bioequivalence studies.

The desired sensitivity of Lapatinib was achieved with an LLOQ of 15.010 ng/mL, which has a within and between-batch CV of 3.24 to 10.89% respectively. The sensitivity could be further improved by sample concentration. The simplicity, liquid liquid extraction and sample turnover rate of 3.0 min per sample, make it an attractive procedure in high throughput bioanalysis of Lapatinib. The validated method allows quantification of Lapatinib in the range 15.004 to 2000.540 ng/mL.

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