

## SENSITIVE SINGLE QUADRUPOLE LC/MS METHOD FOR DETERMINATION OF LAPATINIB IN HUMAN PLASMA

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**Abstract:** A sensitive liquid chromatographic-single quadrupole mass spectrometric method was developed and validated for the determination of lapatinib in human plasma. Following a liquid-liquid extraction with methyl *t*-butyl ether, lapatinib and isotope labelled lapatinib, used as an internal standard (IS), were separated from the endogenous compounds on a Zorbax SB-C18 (150 × 3 mm, 3.5 μm) column. An isocratic elution with the mobile phase consisting of formic buffer and the mixture of acetonitrile, methanol and formic acid was used. Mass spectrometry with positive electrospray ionization in a single ion monitoring mode was applied. The proposed method provides the satisfactory recovery of lapatinib from human plasma and a sensitivity comparable to numerous tandem mass spectrometric methods, with a lower limit of quantification of 5 ng/mL. The validated method may be applied to pharmacokinetic studies in humans following a single 250 mg oral dose.

**Keywords:** lapatinib, liquid chromatography-mass spectrometry, bioanalytical method validation, liquid-liquid extraction, bioanalysis, pharmacokinetics

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). It is recognized that in 15–20% of patients breast cancer exhibits an over-expression of the epidermal growth factor receptor 2 (HER2), which is associated with a more aggressive course of the disease (2). Lapatinib acting as a tyrosine kinase inhibitor is able to inhibit the HER2 receptors and therefore remains a valuable choice of treatment of the HER2-positive carcinoma. The addition of lapatinib to capecitabine in the treatment of HER2-positive advanced breast cancer significantly improves the median time to progression by 8.5 weeks. However, the estimated cost of introducing this tyrosine kinase inhibitor to the combination therapy is nearly \$20,000 per patient (3). 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.

Lapatinib is available as 250 mg tablets. 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) (4). According to the European Medicines Agency guideline (5), 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,  $AUC_{(0-\infty)}$ . Taking into account the above recommendations and available pharmacokinetic data (4), the linearity range of 5.00–800.00 ng/mL seems to be suitable for bioequivalence studies.

Presently, several methods based on tandem mass spectrometry coupled with high-performance liquid chromatography (6–13), ultra-performance liquid chromatography (14) or without the separation technique (15) have been reported for lapatinib quantification in human plasma or serum samples. They were linear in the concentration ranges of 1–1000 ng/mL (6), 5–5000 ng/mL (8, 13), 10–5000 ng/mL (14), 20–10000 ng/mL (12), 25–10000 ng/mL (15), 50–3500 ng/mL (11), 50–5000 ng/mL (9), 100–5000 ng/mL (10) and 100–10000 ng/mL (7). However, a majority of the reported methods were designed for the measurement of lapatinib fol-

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lowing higher doses used in routine clinical practice. Hence, only three methods seem to be sensitive enough for pharmacokinetic studies following a 250 mg dose (6, 8, 13). The triple quadrupole detector was also used for the determination of intracellular levels (16). A high cost of the instrumentation is the main limitation of these assays, making them unattainable to many routine biomedical laboratories. The application of ultraviolet detection was presented during the American Association for Cancer Research 103rd Annual Meeting. However, the poster presentation did not provide details concerning this assay (17). To the best of our knowledge, no method using a single quadrupole mass spectrometric detector has been reported for lapatinib quantification in human plasma so far.

The most widely employed sample preparation methodology was the precipitation of proteins (PP) (8, 9, 11, 12, 15). In spite of the high throughput of PP, large amounts of residual endogenous compounds may significantly contaminate instrumentation, especially if thousands of samples are to be measured. A more effective sample cleanup can be achieved by a solid-phase (7, 14) and liquid-liquid extraction (13). Solid-phase extraction methods, though very effective, significantly increase the cost of sample analysis and with high sample load they may require automated solution handling in order to obtain suitable sample throughput. Another alternative is the application of special on-line extraction columns (6, 10). However, this technique is available only to more complex equipment setups. Therefore, the liquid-liquid extraction seemed to be most practical for the present study.

The aim of the present study was to develop and validate a sensitive liquid chromatographic-single quadrupole mass spectrometric method for the determination of lapatinib in human plasma suitable for pharmacokinetic studies following a single 250 mg oral dose.

## EXPERIMENTAL

### Chemicals and reagents

Reference standards: lapatinib, N-[3-chloro-4-[[3-(3-fluorophenyl)methoxy]phenyl]-6-[5-[[[2-(methylsulfonyl)ethyl]amino]methyl]-furan-2-yl]quinazolin-4-amine, as ditosylate monohydrate (CAS number: 388082-78-8) was synthesized at the Pharmaceutical Research Institute (Warszawa, Poland) (18), [<sup>13</sup>C,<sup>2</sup>H<sub>7</sub>]-lapatinib was supplied by Alsachim (Strasbourg, France).

Methanol (HPLC and LC/MS grade), sodium carbonate and formic acid 98–100% (both of analyt-

ical grade) were purchased from POCH (Gliwice, Poland). Methyl *t*-butyl ether (HPLC grade) was obtained from Chem-Lab (Zedelgem, Belgium). Acetonitrile (HPLC and LC-MS grade) and 25% ammonium hydroxide solution (analytical grade) were purchased from J.T.Baker (Deventer, Netherlands). Purified water from Milli-Q system, Millipore (Molsheim, France), was used throughout the study.

### Instrumentation

The Shimadzu LC/MS system (Duisburg, Germany), consisting of two LC-10ADVP pumps, an SIL-HTA autosampler, a CTO-10A column oven, a DGU-20A<sub>3</sub> degasser and an LCMS-2010 single quadrupole mass spectrometer, was used for lapatinib determination in human plasma. Data integration was performed with Shimadzu LCMS Solution software (version 2.05).

### Chromatographic conditions

The chromatographic separation from endogenous compounds was performed on a Zorbax SB-C18 column (150 × 3 mm, 3.5 μm) from Agilent Technologies (Santa Clara, CA, USA). The HPLC column was preceded by a SecurityGuard C-18 guard column (4 × 3 mm) purchased from Phenomenex (Torrance, CA, USA). The mobile phase, consisting of 10 mM formic buffer pH 4.0 (phase A) and acetonitrile/methanol/formic acid (25 : 75 : 0.1, v/v/v) (phase B) mixed in the ratio A : B 25 : 75, v/v, was delivered at the flow rate of 0.45 mL/min. The sample volume of 50 μL was injected onto the column. The column and the autosampler were maintained at 35 ± 2°C and 20 ± 5°C, respectively. The total chromatographic run time was 11.0 min with the retention time of lapatinib was approximately 3.5 min.

### Mass spectrometric conditions

The mass spectrometer was equipped with an electrospray ionization source operated in the positive mode, using single ion monitoring (SIM) as the data acquisition mode. Ions of lapatinib and the IS were monitored at *m/z* ratio of 581.0 and 589.1, respectively. The probe high voltage was 3.0 kV and nitrogen at 4.0 L/min was used as the nebulizer gas. The block temperature was 280°C, whereas the curved desolvation line temperature and voltage were 290°C and 75 V, respectively. The Q-Array lens voltages were 74 V, 55 V, 32 V, respectively. The detector voltage was 1.2 kV. Data were collected in 0.4 s intervals.

### Solution preparation

Stock solutions of lapatinib and the IS were prepared by dissolving the appropriate amounts of the substances in methanol. The stock solutions were further diluted with 75% methanol to the working solutions. The working solutions used to prepare the calibration standards and quality control samples were obtained from different stock solutions. The stock solutions were stored in a freezer at temperature =  $-6^{\circ}\text{C}$  and in a refrigerator at temperature =  $12^{\circ}\text{C}$  and the working solutions were stored in a refrigerator at temperature =  $12^{\circ}\text{C}$ .

### Preparation of the calibration standards and quality control samples

The calibration standards contained lapatinib at the following concentration levels: 5.00, 15.00, 50.00, 100.00, 200.00, 400.00, 600.00 and 800.00 ng/mL. The quality control samples contained lapatinib at the concentration levels: 15.00, 200.00 and 600.00 ng/mL. The calibration standards and quality control samples were prepared by spiking blank human plasma with the appropriate working solution of lapatinib. The calibration standards were prepared directly before the analysis and the quality control samples were stored in a freezer at temperature =  $-14^{\circ}\text{C}$ .

### Sample preparation

Twenty microliters of the IS solution and 250 mL of the 2M sodium carbonate solution were added to the 250 mL aliquot of human plasma and vortex mixed in a glass, screw cap extraction tube. Then, 2 mL of methyl *t*-butyl ether were added and shaken on a vibrax mixer for 10 min. After centrifugation, the aqueous phase was frozen and the organic layer was transferred to a glass tube and evaporated to dryness under the stream of nitrogen. The dry residue was reconstituted in 200 mL of the mobile phase and mixed. After centrifugation, the supernatant was transferred into an autosampler vial.

### Method validation

The validation parameters were defined according to the European Medicines Agency as well as the Food and Drug Administration guidelines (19, 20). The study was performed in compliance with the principles of Good Laboratory Practice. For the calculation of the precision, accuracy, calibration curve parameters and selected stability results, a normal distribution of measurements was assumed. The statistical analysis of the stability included the comparison of two sets of experimental data, assuming the log-normal distribution of mea-

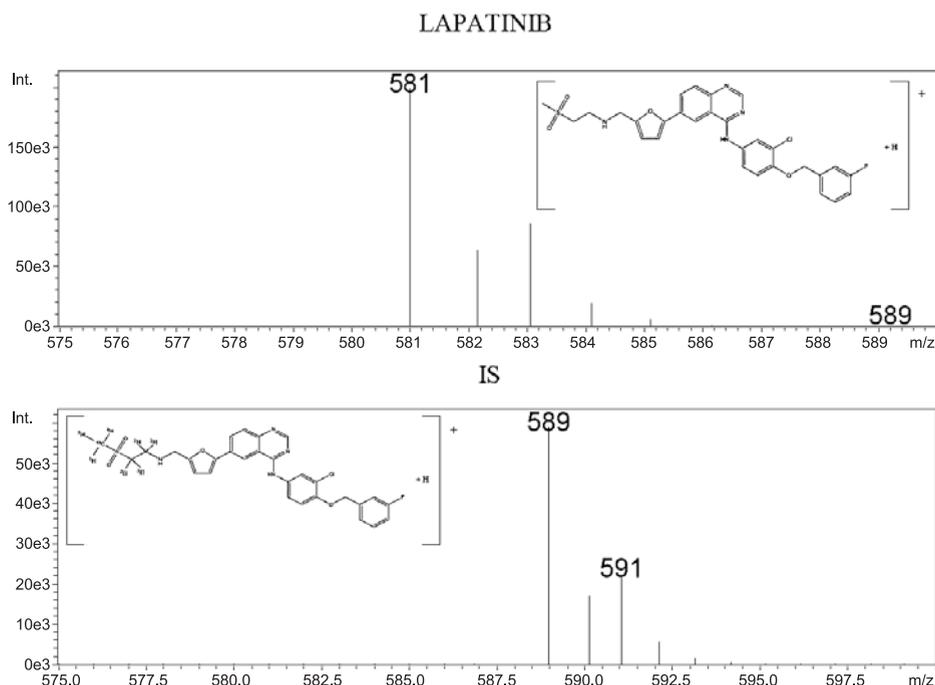


Figure 1. Positive ion electrospray mass scan spectra of lapatinib and IS. As reported previously (7, 9), the  $[M + H]^+$  peak of  $m/z$  581 was the most abundant one for lapatinib

surements' results and it was based on the application of confidence intervals (21, 22). The construction of confidence intervals depends on the variance equality, therefore the F-Snedecor test (significance level 0.01) was applied to test the hypothesis on the variance equality.

## RESULTS

### Method development

Manufacturing method of lapatinib of pharmaceutical purity was recently developed in Chemistry Department of the Pharmaceutical Research Institute (18). In this work, the chromatographic conditions, especially the chromatographic column and the composition of the mobile phase, were selected by subsequent iterations in order to achieve a suitable lapatinib retention and acceptable peak shape. To improve the peak shape a number of different mobile phases, sample solvents and reversed-phase columns including several brands of octadecyl and C6-phenyl phases were tested. Mobile phases consisted of acetonitrile and methanol mixed with 0.1% acetic acid or 10 mM formic buffer (pH 4.0) in various ratios changed to modify lapatinib retention or limit the back-pressure. Four sample solvents were used acetonitrile/0.1% formic acid and methanol/0.1% formic acid in two ratios 75 : 25 and 25 : 75 (v/v). It was found that the greatest influence on the peak shape had the composition of the sample solvent and no acceptable shape could be obtained unless the solvent was identical with the mobile phase. The selected mobile phase, which was used throughout the validation, consisted of a 10 mM formic buffer (pH 4.0) and acetonitrile/methanol/formic acid (25 : 75 : 0.1, v/v/v) in the ratio 25 : 75, v/v. Among tested reverse-phase columns, the best sensitivity with acceptable retention was obtained for a Zorbax SB-C18 column (150 × 3 mm,

3.5 μm, Agilent Technologies). Lapatinib and the IS were eluted at 3.5 min.

Positive ion electrospray mass scan spectra of lapatinib and the IS are shown in Figure 1. The  $[M + H]^+$  peak of  $m/z$  581.0 and  $[M + H]^+$  peak of  $m/z$  589.1 were the most abundant for lapatinib and the IS, respectively, and were selected for the analysis.

During the development of sample preparation, the extraction efficiencies using various extracting solvents were tested. Polar solvent like methyl *t*-butyl ether provided a much higher lapatinib recovery than the non-polar mixture of hexane and isopropanol and was selected for the sample cleanup. The influence of a modifier on extracting conditions was also studied. Saturated (2 M) sodium carbonate solution and 10 mM formic buffer pH 4.0 were tested as modifiers. The best results were achieved for the sodium carbonate. Finally, the optimization of the extraction time and extracting solvent volume led to the conclusion that shaking time of 10 min and the volume of 2 mL of methyl *t*-butyl ether are sufficient to obtain the high recovery (71–82%) of both lapatinib and the IS.

### Method validation

#### Linearity and lower limit of quantification

The calibration curve was linear within the range of 5.00–800.00 ng/mL regarding the peak area ratio of lapatinib to the IS *versus* the nominal concentration of lapatinib. The curve was obtained by a weighted linear regression analysis with the weighting factor of  $1/y^2$  selected according to the minimum sum of percentage relative errors (23). The values of regression parameters for the curve, described by the equation:  $y = ax + b$ , were:  $a = 2.727e^{-3}$ ,  $b = 2.016 e^{-3}$  and  $r = 0.9997$  ( $n = 6$ ), where  $x$  was the lapatinib nominal concentration,  $y$  was the lapatinib/IS instrument response ratio,  $a$  and  $b$  were slope and

Table 1. Validation summary.

Linear range [ng/mL]	5.00 – 800.00			
Lapatinib concentration [ng/mL]	5.00	15.00	200.00	600.00
Extraction recovery of lapatinib [%]	–	82.0	82.4	71.4
Matrix effect – RSD [%]	–	6.22	5.31	2.55
Within-run precision [%]*	4.75–11.37	4.33–10.38	2.19–5.26	1.80–4.32
Within-run accuracy [%]*	89.3–99.9	91.0–100.7	96.9–101.8	94.2–98.2
Between-run precision [%]*	2.37–11.37	3.02–10.38	2.19–14.69	1.11–5.34
Between-run accuracy [%]*	89.3–100.9	90.4–100.7	93.2–106.9	92.9–98.2

\*The accuracy and precision results are expressed as the 90% confidence intervals.

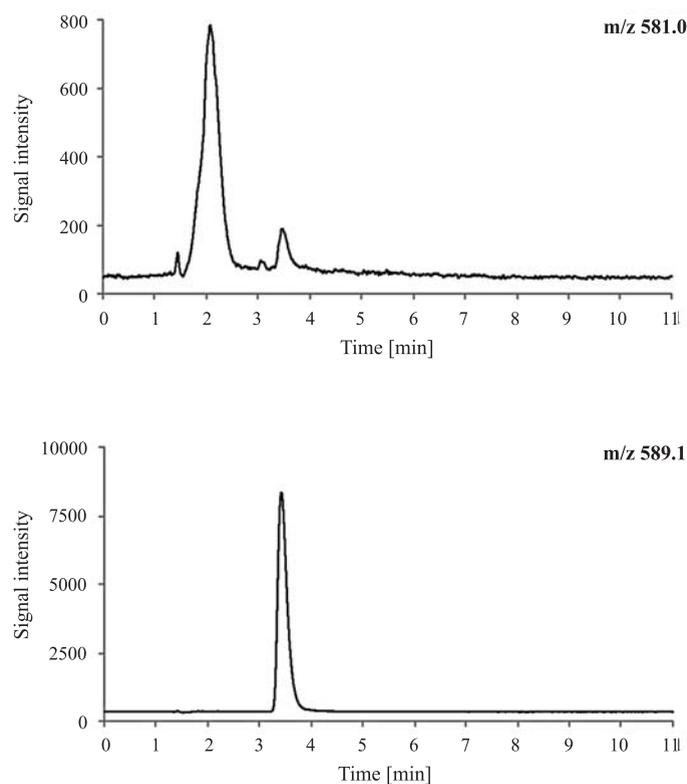


Figure 2. Chromatogram of the extracted plasma sample containing lapatinib at 5.00 ng/mL and the working concentration of the IS (the retention time of lapatinib and the IS – 3.5 min)

intercept, respectively (Table 1). All regression parameters were statistically significant (significance level 0.05,  $df = n - 2$ ).

The lower limit of quantification was determined at 5.00 ng/mL and met international acceptance criteria for the accuracy and precision (Table 1, Fig. 2).

#### **Selectivity and carry-over**

To confirm the method selectivity, blank human plasma from six different sources, including hemolyzed and lipophilic plasma, was analyzed. The chromatograms showed no peaks influencing the quantification near the retention times of lapatinib and the IS (Fig. 3).

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.

#### **Matrix effect and recovery**

To determine the extraction recovery of lapatinib for three quality control sample levels, blank

human plasma samples from six different sources, including hemolyzed and lipophilic plasma, were spiked with both lapatinib and the IS before the extraction (pre-extraction spiked plasma samples) and after the extraction of the plasma sample (post-extraction spiked plasma samples). The calculation of the lapatinib and IS recovery was based on the ratio of the peak areas determined in the pre- and post-extraction spiked plasma samples (24). The extraction recovery of lapatinib was stable across the studied concentration range and did not influence the recovery of the IS (Table 1).

The matrix factor was studied for lapatinib at three concentration levels and for the IS at the working concentration. It was calculated as the ratio of the instrument response for the substance in the presence of the matrix (post-extraction spiked plasma samples) to the instrument response in the absence of the matrix (standard solutions) (19). The matrix effect was evaluated from the RSD of the IS-normalized matrix factor (i.e., the ratio of the matrix factors calculated for lapatinib and the IS) calculated for six different sources of plasma, including hemolyzed and lipophilic plasma. The calculated

RSD did not exceed 15%, which confirmed the absence of matrix effects (Table 1).

#### Accuracy and precision

The results of the within-run (one sequence,  $n = 6$  for each concentration) and between-run (three sequences,  $n = 6$  for each concentration) accuracy and precision for the lower limit of quantification and three quality control sample levels are presented in Table 1. For the lower limit of quantification, the accuracy was within the acceptance criteria range of 80–120% and the precision was below the acceptance limit of 20%. For each quality control sample level, the accuracy was within the acceptance criteria range of 85–115% and the precision was below the acceptance limit of 15% (Table 1).

#### Stability

The results of the stability tests: autosampler, freeze and thaw, short-term and long-term at two

storage temperatures for 53 days, are presented in Table 2. For each concentration level, the 90% confidence interval for the mean stability met the acceptance criteria falling within the range of 85–115%. Moreover, respective stability tests confirmed the stability of lapatinib and the IS in the stock and working solutions (Table 3).

#### DISCUSSION AND CONCLUSION

To the best of our knowledge, the method developed is the first application of a single quadrupole mass spectrometry for the determination of lapatinib in human plasma. It offers an attractive lower limit of quantification of 5.00 ng/mL. Previously published tandem mass spectrometric methods presented wide linearity ranges, however, only one of them provided better sensitivity (6). Based on pharmacokinetic data (4), the upper limit of quantification proposed in this paper seems to be sufficient for the determination of lapatinib in plasma following a

Table 2. The stability of lapatinib in plasma expressed as the 90% confidence intervals ( $n = 6$ ).

Stability		Short-term	Freeze-thaw	Autosampler	Long-term	Long-term
Temperature		Ambient temperature	$\leq -14^{\circ}\text{C}$	$20 \pm 5^{\circ}\text{C}$	$\leq -14^{\circ}\text{C}$	$\leq -65^{\circ}\text{C}$
Storage period		4 h	3 cycles	18 h	53 days	53 days
Lapatinib concentration [ng/mL]	15.00	96.4–109.9	98.7–110.2	91.3–100.8	96.2–112.7	96.2–109.7
	600.00	101.3–105.4	90.3–100.2	99.1–100.3	91.8–98.8	92.1–99.5

Table 3. The stability of lapatinib and the IS in the solutions expressed as the 90% confidence intervals ( $n = 6$ ).

	Analyte	Concentration	Temperature	Storage period	Stability
Stock solutions	Lapatinib	1.00 mg/mL	$\leq -6^{\circ}\text{C}$	64 days	104.2–109.4
			$\leq 12^{\circ}\text{C}$	7 days	103.3–104.8
			Ambient temperature	24 h	101.6–103.9
	IS	0.10 mg/mL	$\leq -6^{\circ}\text{C}$	64 days	95.3–98.9
			$\leq 12^{\circ}\text{C}$	7 days	98.2–100.9
			Ambient temperature	24 h	98.7–102.3
Working solutions	Lapatinib	100.00 $\mu\text{g/mL}$	$\leq 12^{\circ}\text{C}$	57 days	99.4–102.5
			Ambient temperature	24 h	100.4–106.7
		62.50 ng/mL	$\leq 12^{\circ}\text{C}$	64 days	91.7–102.1
	Ambient temperature		24 h	94.2–105.4	
	IS	5.00 $\mu\text{g/mL}$	$\leq 12^{\circ}\text{C}$	57 days	97.7–103.7
			Ambient temperature	24 h	101.0–105.5

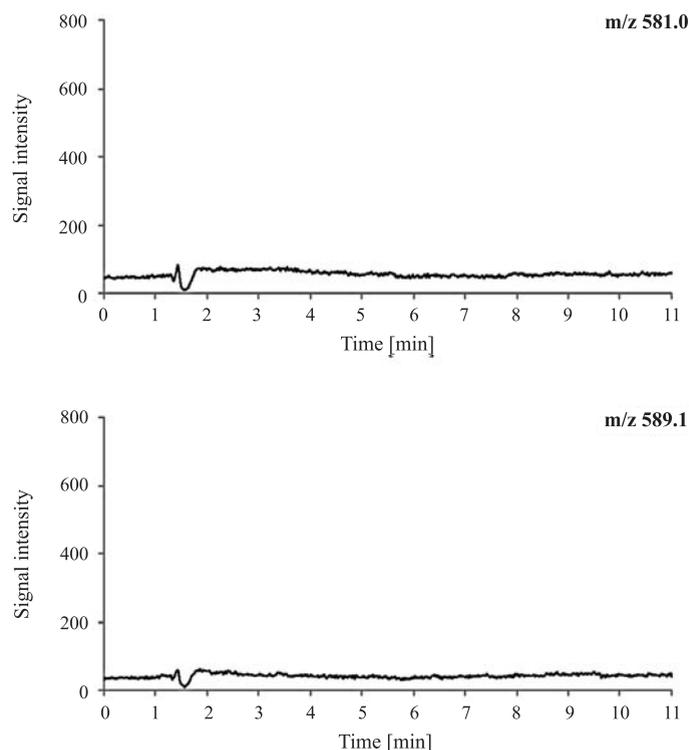


Figure 3. Chromatogram of the extracted blank plasma sample

250 mg oral dose. For economic reasons triple quadrupole mass spectrometers are not so widely available as the single quadrupole detectors. Therefore, the present method expands the list of instrumental setups applicable to the analysis of the drug and represents an attractive alternative approach for many biomedical laboratories.

Sample preparation is a critical part of the quantitative bioanalysis. Selecting a proper technique is always a good practice even with mass spectrometric detectors, as it limits matrix effects, improves the selectivity and sensitivity, extends column life as well as protects the liquid chromatographic system and the ion source from impurities. Protein precipitation is less effective in the removal of endogenous compounds compared to the extraction techniques and thus carries the highest risk of ion suppression (25–27). It also makes necessary a frequent ion source cleaning. Although a solid-phase extraction provides a selective sample cleanup, the use of expensive columns limits its availability. The liquid-liquid extraction procedure described in this paper allowed to obtain high sensitivity and good recovery (above 70%) of the method as well as avoid significant matrix effects.

The method was fully validated with respect to the European Medicines Agency and the Food and Drug Administration requirements, proving its reliability, and may be applied to pharmacokinetic studies in humans following a single 250 mg oral dose.

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## REFERENCES

1. Ferlay J., Soerjomataram I., Ervik M., Dikshit R., Eser S., Mathers C., Rebelo M. et al.: Lyon, France: International Agency for Research on Cancer (2013) (<http://globocan.iarc.fr>, accessed on 05/05/2014).
2. Baselga J.: *Ann. Oncol.* 21 (Suppl. 7), vii36 (2010).
3. Le Q.A., Hay J.W.: *Cancer* 115, 489 (2009).
4. Bence A.K., Anderson E.B., Halepota M.A., Doukas M.A., DeSimone P.A., Davis G.A., Smith D.A. et al.: *Invest. New Drugs* 23, 39 (2005).
5. European Medicines Agency. Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr \*\*). London, 20 January, 2010.
6. Hsieh S., Tobien T., Koch K., Dunn J.: *Rapid Commun. Mass Spectrom.* 18, 285 (2004).
7. Bai F., Freeman B.B. III, Fraga C.H., Fouladi M., Stewart C.F.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 831, 169 (2006).
8. Haouala A., Zanolari B., Rochat B., Montemurro M., Zaman K., Duchosal M.A., Ris H.B. et al.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 877, 1982 (2009).
9. Götze L., Hegele A., Metzelder S.K., Renz H., Nockher W.A.: *Clin. Chim. Acta* 413, 143 (2012).
10. Couchman L., Birch M., Ireland R., Corrigan A., Wickramasinghe S., Josephs D., Spicer J., Flanagan R.J.: *Anal. Bioanal. Chem.* 403, 1685 (2012).
11. Andriamanana I., Gana I., Duret B., Hulin A.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 926, 83 (2013).
12. Lankheet N.A.G., Hillebrand M.J.X., Rosing H., Schellens J.H.M., Beijnen J.H., Huitema A.D.R.: *Biomed. Chromatogr.* 27, 466 (2013).
13. Wu J., Wiegand R., LoRusso P., Li J.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 941, 100 (2013).
14. Bouchet S., Chauzit E., Ducint D., Castaing N., Canal-Raffin M., Moore N., Titier K., Molimard M.: *Clin. Chim. Acta* 412, 1060 (2011).
15. Mičová K., Friedecký D., Faber E., Adam T.: *Talanta* 93, 307 (2012).
16. Roche S., McMahon G., Clynes M., O'Connor R.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 877, 3982 (2009).
17. Escudero V., Valenzuela B., Rebollo J., Sureda M., Brugarolas A.: *Cancer Res.* 72 (Suppl. 1) abstract 757 (2012).
18. Łuniewski W., Krzeczyński P., Kłós K., Trzcińska K.: *Przem. Chem.* 91, 276 (2012) (Polish).
19. European Medicines Agency. Guideline on Bioanalytical Method Validation (EMA/CHMP/EWP/192217/2009). London, 21 July, 2011.
20. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Veterinary Medicine (CVM). Bioanalytical Method Validation. May, 2001.
21. Timm U., Wall M., Dell D.: *J. Pharm. Sci.* 74, 972 (1985).
22. Rudzki P.J., Leś A.: *Acta Pol. Pharm. Drug Res.* 65, 743 (2008).
23. Almeida A.M., Castel-Branco M.M., Falcão A.C.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 774, 215 (2002).
24. Matuszewski B.K., Constanzer M.L., Chavez-Eng C.M.: *Anal. Chem.* 75, 3019 (2003).
25. Bonfiglio R., King R.C., Olah T.V., Merkle K.: *Rapid Commun. Mass Spectrom.* 13, 1175 (1999).
26. Müller C., Schäfer P., Störtzel M., Vogt S., Weinmann W.: *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 773, 47 (2002).
27. Taylor P.J.: *Clin. Biochem.* 38, 328 (2005).



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