Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 848 (2007) 379–383
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

The quantification of erlotinib (OSI-774) and OSI-420 in human plasma by liquid chromatography–tandem mass spectrometry

Andrea R. Masters, Christopher J. Sweeney, David R. Jones *

Indiana University School of Medicine, Indiana University Cancer Center, Department of Medicine, Clinical Pharmacology Analytical Core Laboratory, Wishard Memorial Hospital, Myers Building W7123, 1001 West 10th Street, Indianapolis, IN 46202, United States

Received 5 December 2005; accepted 24 October 2006
Available online 13 November 2006

Abstract

An accurate and precise method was developed using HPLC-MS/MS to quantify erlotinib (OSI-774) and its O-desmethyl metabolite, OSI-420, in plasma. The advantages of this method include the use of a small sample volume, liquid–liquid extraction with high extraction efficiency and short chromatographic run times. The analytes were extracted from 100 μL plasma volume using hexane:ethyl acetate after midazolam was added to the sample for internal standardization. The compounds were separated on a Phenomenex C-18 Luna analytical column with acetonitrile:5 mM ammonium acetate as the mobile phase. All compounds were monitored by tandem mass spectrometry with electrospray positive ionization. The intra-day accuracy and precision (% coefficient of variation, % CV) estimates for erlotinib at 10 ng/mL were 90% and 9%, respectively. The intra-day accuracy and precision estimates for OSI-420 at 5 ng/mL were 80% and 4%, respectively. This method was used to quantify erlotinib and OSI-420 in plasma of patients ($n = 21$) administered 150 mg erlotinib per day for non-small cell lung cancer.

Keywords: Erlotinib; OSI-774; HPLC-MS/MS

1. Introduction

Erlotinib (OSI-774, Tarceva®) is a quinazolinamine analogue that is approved for use in the treatment of patients with locally advanced or metastatic non-small cell lung cancer [1]. Erlotinib is an epidermal growth factor receptor tyrosine kinase inhibitor with well documented anti-cancer properties [2]. The plasma concentrations of erlotinib have been highly variable in phases I and II trials with dosages as high as 200 mg/day [3]. To date, a variety of methods have been developed to quantify erlotinib and OSI-420, the O-desmethyl metabolite (Fig. 1), in plasma samples of subjects. All of the methods are HPLC with UV or MS/MS detection, each with its own advantages and disadvantages [3–6]. In this study, a HPLC-MS/MS method is described that is accurate and precise and requires only 0.1 mL of plasma. The advantages of this method include the use of a small sample volume, liquid–liquid extraction with high extraction efficiency and short chromatographic run times. This method was applied to a study to quantify erlotinib and OSI-420 in plasma from subjects enrolled on an IUPUI IRB approved protocol. The phase I dose escalation protocol enrolled patients with a variety of metastatic solid tumors. Each patient was administered 150 mg erlotinib once per day in combination with escalating doses of docetaxel.

2. Method

2.1. Chemicals and reagents

Erlotinib and OSI-420 were provided by OSI Pharmaceuticals (Melville, NY). The internal standard, midazolam and formic acid (reagent grade 95%) were purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile (HPLC grade), hexane (HPLC grade), ethyl acetate (HPLC grade), glycine, sodium chloride, ammonium acetate and sodium hydroxide (98% pellets) were purchased from Fisher Scientific (Fairlawn, NJ). Deionized water was purified using a Barnstead Nanopure Infinity ultrapure water system (Boston, MA).

* Corresponding author. Tel.: +1 317 630 8795; fax: +1 317 630 8185.
E-mail address: drjones1@iupui.edu (D.R. Jones).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.10.046

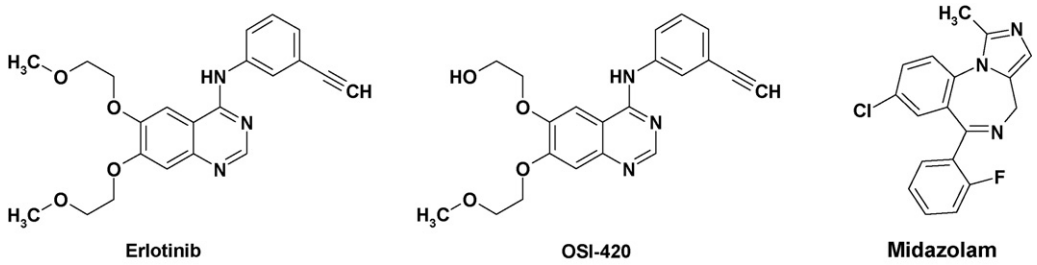

Fig. 1. Chemical structures of erlotinib, OSI-420 and midazolam.

2.2. Standard curve and quality control samples

Solutions of OSI-774, OSI-420 and midazolam were prepared separately in polypropylene tubes by adding methanol to create a 1 mg/mL solution. Erlotinib and OSI-420 were prepared in duplicate such that one solution of 1 mg/mL was used for quality control (QC) samples and the other solution was used for standard curve samples. These solutions were stored at −20 °C. The standard curve and QC samples were prepared daily from methanolic dilutions of the 1 mg/mL stock solutions. These dilutions were mixed with non-supplemented plasma (total volume of 100 μL) to prepare the standard curve and QC samples. The standard curve for erlotinib was: 0, 10, 30, 100, 300, 1000, 3000 and 5000 ng/mL in plasma. The standard curve for OSI-420 was: 0, 5, 15, 50, 150, 500, 1500 and 5000 ng/mL. Quality control samples were prepared daily, in duplicate. The QC samples had the following concentrations: OSI-774: 20 ng/mL (low QC), 300 ng/mL (medium QC) and 4000 ng/mL (high QC); OSI-420: 10 ng/mL (low QC), 150 ng/mL (medium QC) and 4000 ng/mL (high QC).

2.3. Sample preparation

Frozen plasma samples (stored in a −80 °C freezer) were thawed to ambient temperature. A 0.1 mL aliquot of the sample was placed in a 1.5 mL polypropylene microcentrifuge tube. A volume of 40 μL of 0.0001 ng/mL of internal standard, midazolam, was added to the tube followed by 100 μL of 100 mM NaOH/glycine pH 12 buffer (preparation of buffer: solution A, 0.1 M glycine and 0.1 M NaCl; solution B, 0.1 M NaOH; equal volumes of solutions A and B were mixed then adjusted to pH 12 by adding solution B). The tube was then mixed for 10 s on a vortex mixer. Next, a volume of 1 mL of hexane:ethyl acetate (50:50; v/v) was added to the tube and mixed vigorously for 20 s on a vortex mixer. The sample was centrifuged at high speed, 7450 rcf, at ambient temperature for 3 min to assure phase separation. Then, the organic layer was transferred to a 1.5 mL polypropylene microcentrifuge tube and evaporated to dryness in a rotary evaporator. Mobile phase (150 μL) was added to the tube and mixed for 10 s on the vortex mixer. Next, 75 μL was transferred to a 1.5 mL polypropylene microcentrifuge tube and diluted with 500 μL of mobile phase. If the known concentration of the sample was greater than 100 ng/mL, then 10 μL of the 575 μL sample was injected into the HPLC. Otherwise, an aliquot volume of 10 μL from the original 75 μL was injected into the HPLC.

2.4. Conditions for HPLC-MS/MS

Chromatographic analysis was performed using an Agilent 1100 series HPLC. Reverse phase chromatography was achieved at ambient temperature using a Phenomenex C18 Luna 5 μm 4.6 mm × 150 mm column. The mobile phase consisted of acetonitrile:5 mM ammonium acetate (45:55, v/v), and was delivered isocratically at a flow rate of 600 μL/min. The column effluent was monitored using an Applied Biosystems API 4000 triple-quadrupole mass spectrometer (Foster City, CA) equipped with an electrospray probe in positive ionization mode. It was controlled by Analyst software Version 1.4.1. in conjunction with Windows 2000. A flow injection analysis was performed, after the standard infusions, on each analyte to maximize sensitivity. The analytes were optimized at a source temperature of 600 °C, under unit resolution for quadrupoles 1 and 3, and were given a dwell time of 100 ms and a settling time of 75 ms. Optimal gas pressures for all three analytes were: collision gas 10 psi, curtain gas 10 psi, ion source gas (1) 40 psi, ion source gas (2) 45 psi. The *m/z* of the parent and daughter ions for the analytes were as follows: erlotinib, 394.1/278 and 394.1/336; OSI-420, 380.2/278 and 380.2/336; midazolam, 326.2/291. Mass spectrometry settings for the voltages are listed in Table 1. Multiple daughter ions were acquired for erlotinib and OSI-420 to increase selectivity for quantification. There was detectable OSI-420 in the erlotinib standard. Therefore, the OSI-420 standards, QC samples and method validation samples were run separately from the erlotinib samples.

2.5. Method validation

2.5.1. Intra-day variability

Standards (in singular) and QC samples (in duplicate) were prepared with additional samples at the following concentrations ($n = 6$ for each): the limit of quantification (LOQ) at 10 ng/mL for erlotinib and 5 ng/mL for OSI-420, low QC, medium QC and high QC concentrations. The acceptable accuracy was: 20% for the LOQ, 20% for the low QC, 15% for the medium QC and 10% for the high QC samples for erlotinib and OSI-420.

2.5.2. Inter-day variability

Standards (in singular) and two sets of QC samples (in duplicate) were prepared each day for 4 days. Accuracy and precision were assessed from one set of QC samples at each of the following concentrations: LOQ, low QC, medium QC and high QC

Table 1
Mass spectrometry settings for erlotinib, OSI-420 and midazolam

Compound	Q1 (amu)	Q3 (amu)	Declustering potential (V)	Entrance potential (V)	Collision energy (V)	Exit potential (V)
Erlotinib	394.1	278	80	10	43	6
Erlotinib	394.1	336	80	10	33	20
OSI-420	380.2	278	51	7	45	16
OSI-420	380.2	336	51	7	31	4
Midazolam	326.2	291	80	7	33	14

concentrations. Acceptable precision and accuracy for the QC samples were the same percentages that were stated previously for the intra-day samples.

2.5.3. Extraction efficiency

The efficiency of the extraction procedure was estimated for QC samples (from the intra-day variability assessments) by comparing the response of extracted analyte to the response of non-extracted analyte.

2.5.4. Stability

Stability was assessed in triplicate at each QC concentration for erlotinib and OSI-420. The stability compared non-extracted samples to samples prepared three ways: non-extracted samples prepared in mobile phase and allowed to set overnight at room temperature prior to injection into HPLC; plasma samples that were extracted then reconstituted with mobile phase and allowed to set overnight at room temperature in mobile phase prior to injection; plasma samples that were prepared then allowed to set overnight at room temperature prior to extraction and injection into HPLC.

2.5.5. Specificity and matrix effects

Samples (with internal standard only) were prepared with six different non-supplemented plasma samples following the sample preparation previously stated. Acceptable specificity occurred when all six samples exhibited an undetectable (below the LOQ) concentration of the analyte. In addition to the six samples stated above, one batch of plasma was randomly selected to use for addition of standards, pre-extraction and post-extraction, to assess any matrix effects. The slopes of these standard curves were compared to determine the matrix effects.

3. Results and discussion

A chromatogram of blank and supplemented samples (at the limit of quantification) for erlotinib, OSI-420 and midazolam is shown in Fig. 2 with retention times of 4.34, 2.11 and 4.47 min, respectively. Intra-day accuracy and precision, inter-day accuracy and precision, and extraction efficiency for erlotinib are listed in Table 2. The intra-day precision ranged from 2% to 11% and the intra-day accuracy ranged from 80% to 100%. The inter-day precision ranged from 3% to 17% and the inter-day

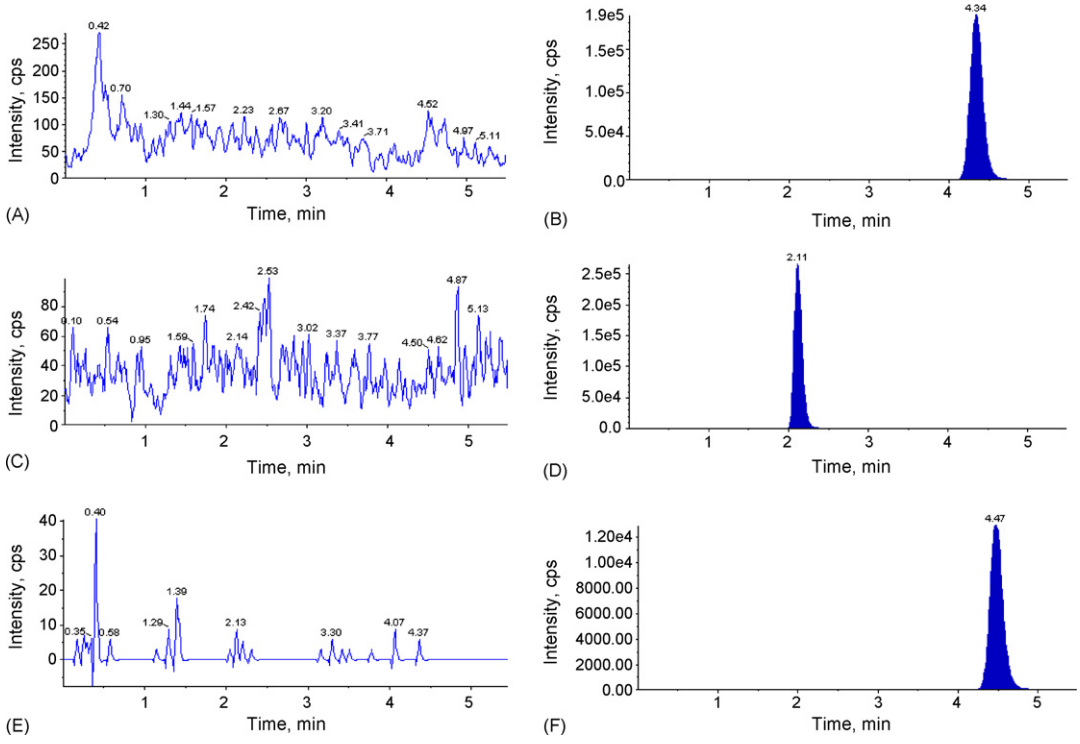

Fig. 2. Chromatograms of non-supplemented plasma for erlotinib (A), non-extracted erlotinib (B), non-supplemented plasma for OSI-420 (C), non-extracted OSI-420 (D), non-supplemented plasma for midazolam (E) and non-extracted midazolam (F).

Table 2
Intra-day and inter-day mean, standard deviation, accuracy, precision and extraction efficiency for erlotinib and OSI-420

	Intra-day				Inter-day				Extraction efficiency (%)
	Mean (ng/mL)	Standard deviation (ng/mL)	Accuracy (%)	Precision (%CV)	Mean (ng/mL)	Standard deviation (ng/mL)	Accuracy (%)	Precision (%CV)	
Erlotinib concentration (ng/mL)									
10	11	1	90	9	11	0.5	90	4	79
20	24	3	80	11	23	4	85	17	88
300	305	7	98	2	314	9	95	3	87
4000	3992	147	100	4	4024	345	99	9	85
OSI-420 concentration (ng/mL)									
5	6	0.3	80	4	6	1	80	17	89
10	11	1	90	13	11	2	90	13	92
150	159	23	94	14	153	22	98	15	74
4000	3896	347	97	9	3845	163	96	4	69

accuracy ranged from 85% to 99%. By comparing peak area ratios of non-extracted erlotinib to extracted erlotinib, the extraction efficiency was 79% or greater.

Intra-day accuracy and precision, inter-day accuracy and precision, and extraction efficiency for OSI-420 are listed in Table 2. OSI-420 intra-day precision ranged from 4% to 14% and the intra-day accuracy ranged from 80% to 97%. The inter-day precision and accuracy ranges were 4–17% and 80–98%, respectively. The extraction efficiency for OSI-420 was 69% or greater.

The stability of erlotinib and OSI-420 was tested with three different treatments: non-extracted samples, extracted plasma samples and plasma samples. The stability of erlotinib at each QC concentration and treatment was greater than 90% (Table 3).

Table 3
Stability data for erlotinib and OSI-420

	Treatment	Mean estimated concentration (ng/mL)
Erlotinib concentration (ng/mL)		
20	A	20
20	B	20
20	C	20
300	A	314
300	B	310
300	C	300
4000	A	4069
4000	B	3918
4000	C	3914
OSI-420 concentration (ng/mL)		
10	A	10
10	B	11
10	C	10
150	A	177
150	B	170
150	C	164
4000	A	4278
4000	B	3848
4000	C	3876

Treatment A: non-extracted compound in mobile phase that set overnight at room temperature; Treatment B: plasma that was extracted then reconstituted with mobile phase that set overnight at room temperature; Treatment C: plasma that was prepared and allowed to set overnight at room temperature. The next morning the sample was extracted and run on the HPLC.

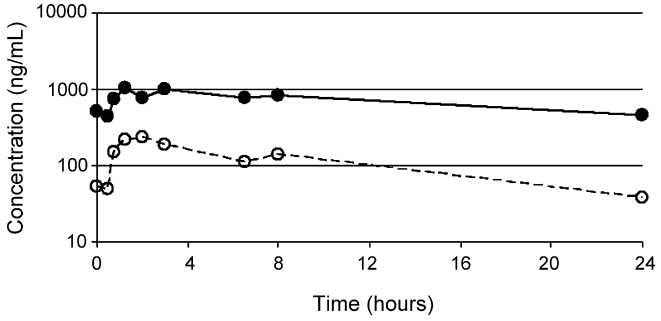

Fig. 3. Plasma concentration vs. time plot of erlotinib (●) and OSI-420 (○) from one representative subject who was administered 150 mg erlotinib per day.

The stability of OSI-420 under the same conditions exhibited acceptable stability (only one QC concentration, of nine, exhibited greater than 15% change from the non-extracted sample; Table 3).

The method exhibited acceptable specificity because all six plasma samples exhibited an undetectable (below the LOQ) concentration of erlotinib or OSI-420. In addition, the slope of the standard curve line for the pre- and post-extraction addition of standards was 0.46 versus 0.40 for erlotinib and 0.36 versus 0.34 for OSI-420, respectively. Thus, the plasma matrix had little to no effect on erlotinib and its metabolite.

The current method was used to quantify erlotinib and OSI-420 in plasma of patients ($n = 21$) administered 150 mg erlotinib per day in combination with docetaxel. Fig. 3 illustrates plasma concentrations of erlotinib and OSI-420 versus time plots of one subject administered erlotinib.

This manuscript describes an accurate and precise method to quantify erlotinib and its O-demethyl metabolite, OSI-420. Advantages of this method include the use of a small sample volume, liquid–liquid extraction that exhibits high extraction efficiency and allows concentration of the plasma sample, and short chromatographic run times.

References

[1] M.H. Cohen, J.R. Johnson, Y.-F. Chen, R. Sridhara, R. Pazdur, Oncologist 10 (2005) 461.
[2] J. Dowell, J.D. Minna, P. Kirkpatrick, Nat. Rev. 4 (2005) 13.

[3] M. Hidalgo, L.L. Siu, J. Nemunaitis, J. Rizzo, L.A. Hammond, C. Takimoto, S.G. Eckhardt, A. Tolcher, C.D. Britten, L. Denis, K. Ferrante, D.D. Von Hoff, S. Silberman, E.K. Rowinsky, J. Clin. Oncol. 19 (2001) 3267.
[4] M. Zhao, P. He, M.A. Rudek, M. Hidalgo, S.D. Baker, J. Chromatogr. B 793 (2003) 413.
[5] E.R. Lepper, S.M. Swain, A.R. Tan, W.D. Figg, A. Sparreboom, J. Chromatogr. B 796 (2003) 181.
[6] W. Zhang, L.L. Siu, M.J. Moore, E.X. Chen, J. Chromatogr. B 814 (2005) 143.