Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 705–711
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Rapid and sensitive LC/MS/MS analysis of the novel tyrosine kinase inhibitor ZD6474 in mouse plasma and tissues

Joseph A. Zirrolli, Erica L. Bradshaw, Michael E. Long, Daniel L. Gustafson *

School of Pharmacy, Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, 4200 East 9th Ave., Denver, CO 80262, USA

Received 10 November 2004; received in revised form 29 March 2005; accepted 6 April 2005
Available online 2 June 2005

Abstract

ZD6474 (*N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy] quinazolin-4-amine) is a tyrosine kinase inhibitor with anti-angiogenic and anti-tumor activity that is currently undergoing human trials for cancer treatment. Pharmacokinetic studies in animal models are an important component in clinical development of this agent to relate pre-clinical models to patient treatment. A liquid chromatography tandem mass spectrometry method was developed for the determination of ZD6474 levels in mouse plasma and tissues. Plasma (0.05 mL) and tissue homogenates (0.1 mL of 10 mg/mL) were extracted under alkaline conditions with ethyl acetate:pentane (1:1, v/v) after addition of the internal standard (trazodone, 2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-1,2,4-triazolo[4,3-a]pyridine-3(2H)-one). Separation was achieved on a C18, 50 mm × 2 mm column with quantitation by internal standard reference and multiple reaction monitoring of the ion transitions *m*/*z* 475 → 112 (ZD6474) and *m*/*z* 372 → 176 (trazodone). The calibration curve was linear from a range spanning 20–20,000 ng/mL in plasma and 10–320 ng/mg in tissue homogenates. Mean recoveries from plasma and tissue homogenates were 88 and 90%, respectively. The accuracy in plasma was 88% at the lower limit of quantitation (20 ng/mL with a 50 μL plasma sample) with high precision (R.S.D.% < 10%). Assay performance in liver and other tissue homogenates is also reported. The assay was applied to a pharmacokinetic study in mice to determine dosing schedules that would approximate therapeutic ZD6474 levels determined in humans.

Keywords: Reversed-phase HPLC; Tandem mass spectrometry; ZD6474; Tyrosine kinase inhibitors; VEGFR inhibitors; Pharmacokinetics; Quantitative analysis

1. Introduction

Cancer progression: tumor growth, invasion, angiogenesis and metastasis, is largely regulated by autocrine and paracrine signaling through growth factors and their binding to receptor tyrosine kinases (RTKs). Upon binding of growth factors to respective RTKs, dimerization and subsequent phosphorylation of the intracellular kinase domain occurs. Resulting phosphotyrosines serve as docking sites that recruit signal transduction intermediates, ultimately leading to cell proliferation [1]. Approaches to therapies designed to target the signal transduction pathway to inhibit tumor growth, angiogenesis or both include: anti-growth factor antibodies, receptor antagonism, anti-receptor monoclonal antibodies, anti-sense and small molecule tyrosine kinase inhibitors (TKIs) [2,3].

4-Anilinoquinazolines are a class of orally available synthetic small molecules designed to bind to the intracellular kinase domain of RTKs, preventing phosphorylation and disrupting the necessary signal transduction for cell proliferation [4,5]. ZD6474 (*N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy] quinazolin-4-amine) has demonstrated potent and selective activity against the vascular endothelial cell growth factor receptor (VEGFR2, Flk1/KDR) [4,5], which plays a key role in tumor angiogenesis [6] and the epidermal growth-factor receptor (EGFR) [4,5], which is overexpressed in several cancer types [7]. The dual activity of ZD6474 allows a single molecularly targeted agent to be effective against the dividing tumor vasculature

* Corresponding author. Tel.: +1 303 315 0755; fax: +1 303 315 6281.
E-mail address: Daniel.Gustafson@UCHSC.edu (D.L. Gustafson).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.04.024

as well as the tumor cell population. ZD6474, administered orally as a once daily treatment, has demonstrated anti-tumor and anti-angiogenic capability in vitro and in vivo [8,9] and is currently in Phase II clinical trials.

Pharmacokinetic studies conducted in pre-clinical models provide useful information about absorption, distribution, elimination and efficacy that can be translated to the clinical setting. Presently, there are no available or published methods for the measurement of ZD6474. The 4-anilinoquinazolines with basic side chains demonstrate good tandem mass spectrometric performance. Therefore, we developed a simple, rapid and sensitive LC/MS/MS assay to measure ZD6474 from mouse plasma and tissue.

2. Experimental

2.1. Chemicals and reagents

ZD6474 was a generous gift from AstraZeneca (Macclesfield, UK) and trazodone was obtained from Sigma (St. Louis, MO). All other chemicals and solvents were of reagent or higher grade and obtained from Fisher Scientific (Pittsburgh, PA).

2.2. Standards preparation

Standard dilutions of ZD6474 were prepared in acetonitrile. For analysis in plasma and tissue, ZD6474 was added to 10 mg/mL control tissue homogenate (10–320 ng/mg tissue) and plasma (20–20,000 ng/mL) and them extracted as described in Section 2.3. Each dilution contained 50 ng of trazodone, the internal standard, in a final volume of 1 mL acetonitrile. Trazodone was prepared at 1 μg/mL in acetonitrile.

2.3. Sample preparation

Tissue samples were homogenized at 10 mg/mL in 10 mM ammonium acetate, pH 9.0 (adjusted with ammonium hydroxide) and 100 μL of tissue homogenate was used for the extraction. For the analysis of blood samples 50 μL of plasma was used. To each sample, 50 ng (50 μL of 1 μg/mL) of trazodone was added followed by 1 mL of pentane/ethyl acetate (1/1, v/v) containing 0.1% (v/v) ammonium hydroxide (30%, w/v) and extraction by vortexing for 10 min. Organic and aqueous layers were separated by centrifugation (10 min, 12,000 RCF) and the organic layer removed, evaporated by vacuum centrifugation at room temperature and reconstituted in 1 mL of acetonitrile for LC/MS/MS analysis.

2.4. Mass spectrometry

Positive ion electrospray ionization (ESI) mass spectra were obtained with a PE Sciex API-3000 triple quadrupole mass spectrometer (Foster City, CA) with a turbo ionspray source interfaced to a PE Sciex 200 HPLC system. Samples were chromatographed with a Prodigy ODS, 5 μm, 100 Å, 50 mm × 2 mm column (Phenomenex, Torrance, CA). The LC elution was isocratic with 80% acetonitrile containing 10 mM ammonium acetate and 0.1% acetic acid at a flow rate of 200 μL/min and sample injection volume of 20 μL. The analysis time was 5 min.

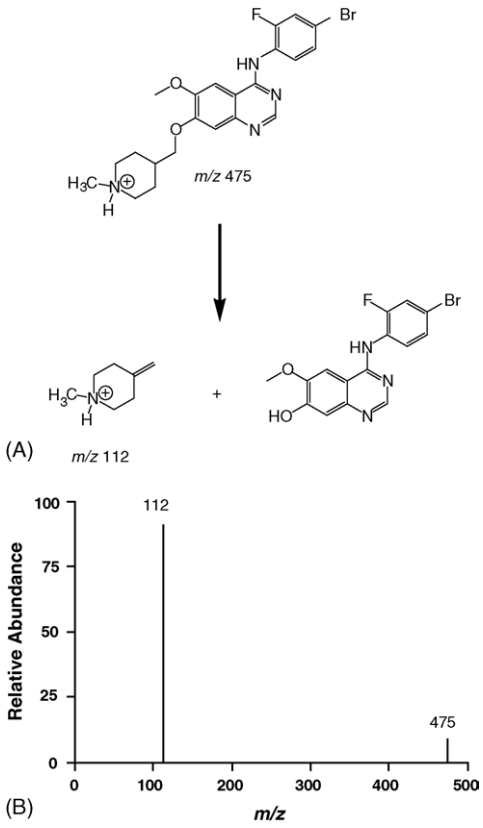

Fig. 1. Structure of ZD6474 $[MH]^+$ ion and product ion, m/z 112 (A) and the product ion spectrum (B) for ZD6474 under the described mass spectrometric conditions.

The mass spectrometer settings were: turbo ionspray temperature, 250 °C; spray needle, 4500 V; declustering potential (DP), 15 V; focus plate (FP), 75 V; collision energy (CE), 40 V; collision gas, N_2 (CAD), 10 units. Samples were quantified by the internal standard reference method in the MRM mode by monitoring the transition m/z 475 → 112 for the analyte ZD6474 (loss of *N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-hydroxy-quinazolin-4-amine [MW 363]) as shown in Fig. 1 and m/z 372 → 176 for the internal standard trazodone (loss of 1-(3-chlorophenyl)-piperazine [MW 196]) as shown in Fig. 2. Each ion transition was integrated for 500 ms. Q_1 was operated in the low resolution mode (4 amu window) to pass both halogenated isotopic species of ZD6474 (m/z 475–478) and trazodone and Q_3 remained at unit resolution to retain mass specificity for the non-halogenated product ions.

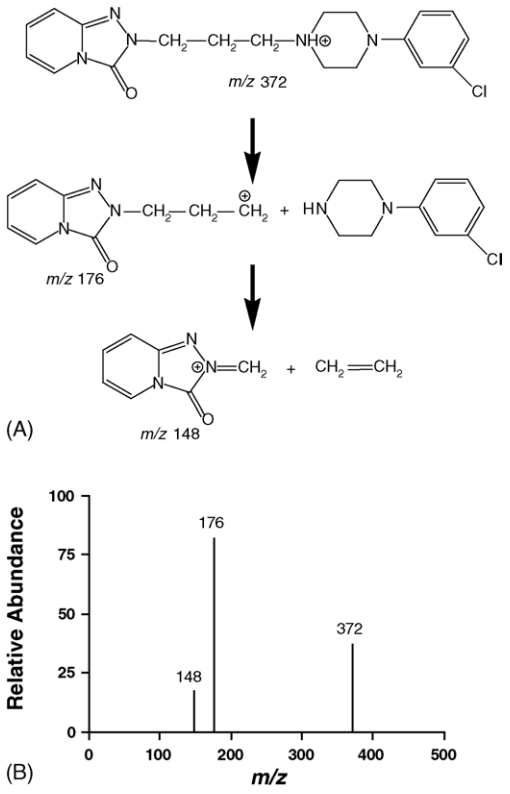

Fig. 2. Structure of trazodone (internal standard) $[MH]^+$ ion and product ions, *m/z* 176 and 148 (A) and the product ion spectrum (B) for trazodone under the described mass spectrometric conditions.

2.5. Pharmacokinetic study in mice

Female balb/c mice (8–10 weeks old) were purchased from Charles River Laboratories (Wilmington, MA) and allowed to acclimate for 7 days. Animals were housed (three per cage) in polycarbonate cages and kept on a 12-h light:12-h dark cycle. Food and water were given ad libitum. All studies were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, and animals were housed in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Mice were treated with ZD6474 daily at a dose of 30 mg/kg by *intraperitoneal* injection. ZD6474 was made up as a suspension in sterile filtered 1% Tween 80 and 50 μL injections were used. Mice were dosed daily for 10 days, and following dosing on day 10 animals were killed by exsanguination under isoflurane anesthesia at 30 min, 1, 4, 8 and 24 h post-dosing. Two animals were included at each time point and plasma and tissues (liver, intestine, kidney and heart) were collected, frozen in liquid nitrogen and stored at −80 °C prior to extraction and analysis.

2.6. Data analysis

Quantitation of ZD6474 was based on standard curves in spiked matrix using the ratio of ZD6474 peak area to trazodone peak area using $1/x$ weighting. Parameters for the assessment of assay performance were calculated as:

$$\text{Accuracy}\,(\%) = \left(1 - \left|\frac{\text{Theoretical} - \text{Measured}}{\text{Theoretical}}\right|\right) \times 100$$

$$\text{Precision}\,(\text{R.S.D.}\%) = \frac{\text{Standard deviation calculated values}}{\text{Mean calculated value}} \times 100$$

Pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin 4.1 (Pharsight, Mountain View, CA) on a PC.

3. Results and discussion

3.1. Chromatography

ZD6474 and the internal standard trazodone were eluted at 2.3 and 2.4 min, respectively (Figs. 3 and 4). Peaks were detected by monitoring the transition from *m/z* 475 → 112 for ZD6474 and from *m/z* 372 → 176 for trazodone. No interfering peaks were detected at either monitored ion transition in extracted matrix (plasma or tissue homogenates). Chromatographic conditions were optimized for peak shape.

3.2. Inclusion of trazodone as an internal standard

Trazodone (2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-1,2,4-triazolo[4,3-a]pyridine-3(2H)-one) was chosen as an internal standard for the analysis of ZD6474 due to the lack of available structural homologs and isotopomers. Trazodone has many physical and chemical characteristics similar to ZD6474, including similar pK_a's (piperdine, pK_a = 11.1 and piperazine, pK_a = 9.7), both compounds have halogenated phenyl groups and poly nitrogen containing aromatic groups (quinazoline and triazolopyridinone groups) and similar solubility and extractability (Table 4). Importantly, both compounds have very similar ESI-MS performance and CID behavior. Because of the similar and high pK_a's, both compounds form very abundant $[M+H]^+$ ions and the collision induced dissociation is directed by the protonated basic nitrogens of the piperadine and piperazine groups to form few, but abundant, product ions through similar fragmentation mechanisms (Figs. 1A and 2A). The inclusion of trazodone as an internal standard enhanced the accuracy and precision of ZD6474 analysis, when compared to calculations using just the analyte alone, in spiked plasma samples (Table 1). Similar results were obtained when comparing analysis of ZD6474 in tissues, with the calculations including the ZD6474/IS ratio increasing both the accuracy and the precision of the assay (data not shown).

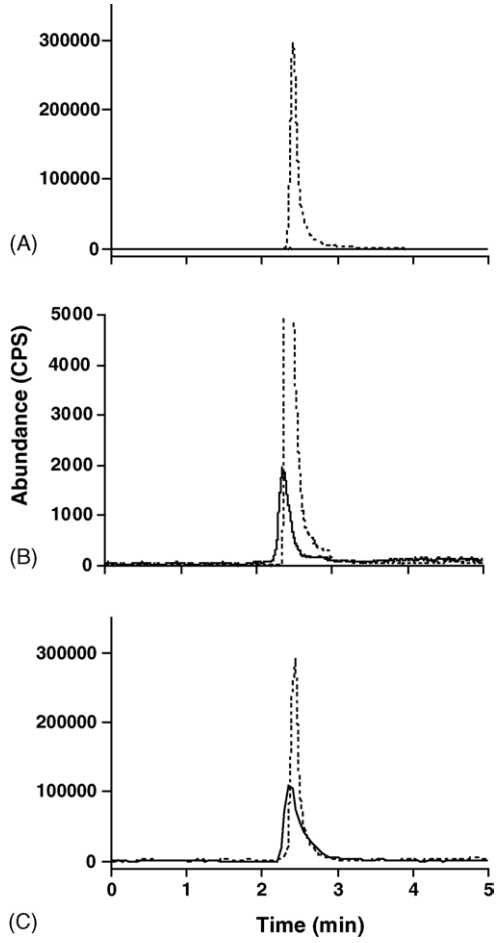

Fig. 3. MRM chromatogram of blank mouse plasma sample spiked with 50 ng internal standard (trazodone) (A), a ZD6474 plasma standard at 20 ng/mL (LLOQ) spiked with 50 ng trazodone (B) and a plasma sample from a mouse treated with 30 mg/kg ZD6474 for 10 consecutive days 24 h after the last dose (C). The dashed line represents *m*/*z* transition from 372 to 176 (trazodone, R_t = 2.4 min) and the solid line represents *m*/*z* transition from 475 to 112 (ZD6474, R_t = 2.3 min).

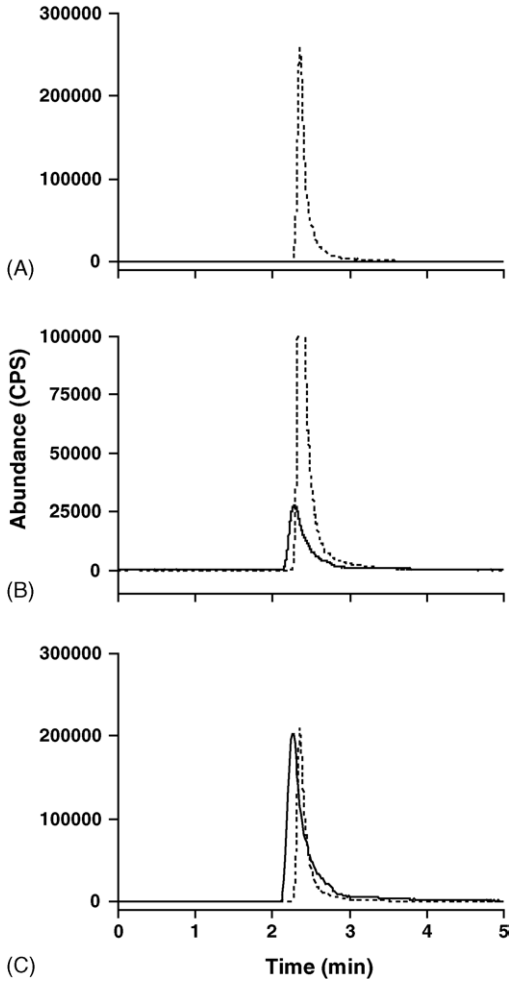

Fig. 4. MRM chromatogram of blank mouse liver homogenate sample spiked with 50 ng internal standard (trazodone) (A), a ZD6474 liver homogenate standard at 10 ng/mg (LLOQ) spiked with 50 ng trazodone (B) and a liver tissue sample from a mouse treated with 30 mg/kg ZD6474 for 10 consecutive days 24 h after the last dose (C). The dashed line represents *m*/*z* transition from 372 to 176 (trazodone, R_t = 2.4 min) and the solid line represents *m*/*z* transition from 475 to 112 (ZD6474, R_t = 2.3 min).

3.3. Assessment of ion suppression and matrix effects

Ion suppression and matrix effects were assessed with this method by comparison of the results obtained with standard dilutions of ZD6474 in 1 mL of acetontrile (solvent standards) and standards added to dried extracts of plasma and tissue homogenates and then diluted to 1 mL of acetontrile (post-extraction standards). Both sets of solvent and post-extraction standards also contained 50 ng of trazodone (IS). No ion suppression or matrix effects were observed. The final dilutions are very clean as this method uses small samples volumes (50 μL of plasma and 100 μL of tissue homogenate). The volumes are 1/10–1/20 of the typical sample

Table 1
Effect of the internal standard trazodone on the accuracy and precision of ZD6474 analysis in plasma[a]

Concentration (ng/mL)	Analyte only (n = 7)			Internal standard normalized (n = 7)		
	Observed	Accuracy	Precision (R.S.D.%)	Observed	Accuracy	Precision (R.S.D.%)
200	258 ± 15	71.1	5.6	223 ± 19	88.4	8.5
2000	2305 ± 402	79.5	17.4	1901 ± 222	90.1	11.7
10000	10098 ± 447	96.4	8.8	9421 ± 237	94.2	5.0

[a] Values were obtained from seven independent determinations at each concentration in spiked plasma samples and represent mean values ± S.D. for the observed concentrations. Calculations for the analyte only were done using the area of the ZD6474 peak whereas the internal standard normalized data were calculated using ZD6474 peak area divided by the peak area of the internal standard (trazodone).

Table 2
Precision and accuracy for ZD6474 assay in mouse plasma within and between runs

Concentration[a] (ng/mL)	Within runs ($n = 3$)			Between runs ($n = 6$)		
	Observed	Accuracy	Precision (R.S.D.%)	Observed	Accuracy	Precision (R.S.D.%)
20	17.6 ± 1.6	88.3	8.9	17.4 ± 1.2	87.2	6.4
100	90.7 ± 4.8	90.7	5.3	87.8 ± 5.6	87.9	6.3
200	184.8 ± 6.8	92.4	3.6	182.0 ± 10.6	91.0	5.8
1000	1008 ± 102	99.2	10.0	978 ± 126	97.9	12.8
2000	1938 ± 274	96.9	14.2	1880 ± 194	94.0	10.4
10000	8980 ± 220	89.8	2.4	8880 ± 520	88.9	5.9
20000	21080 ± 3340	94.6	15.9	21400 ± 2620	93.0	12.2

[a] Values are concentration in the plasma (50 μL) that was extracted for analysis.

volumes used in many drug assays (0.5–1 mL of plasma). Additionally the extracted analytes are diluted 20× instead of concentrated by 2–4× as described in many methods. The method results in a 1/400–1/1600 dilution of the final sample as compared to typical drug assays. The use of small sample volumes and subsequent final dilution is possible because of the high instrumental sensitivity of the LC/MS/MS assay. As measured in plasma extracts the LOD is 0.25 ng/mL and the LLOQ is 1.0 ng/mL in acetontrile. ZD6474 and trazodone have non-polar and hydrophobic characteristics and sample extraction with ethyl acetate:pentane, which is non-polar, is more selective than other solvents. Full mass spectral scans (100–1000 amu) did not detect any extraneous compounds extracted from plasma and tissue samples.

3.4. Linearity and LLOQ

Standard curves in spiked matrix were linear from a range of 20–20,000 ng/mL in mouse plasma and from 10 to 320 ng/mg in tissue homogenates. The linearity of the ratio of ZD6474 peak area to trazodone (IS) peak area in plasma (Fig. 5) and tissue homogenates were greater than $r^2 = 0.98$ using $1/x$ weighting. Both uniform and $1/x$ weighting gave linear regression analysis with r^2 approaching 1, however, the use of $1/x$ weighting for the standard curve resulted in better prediction of standard samples across the concentration range. Most notably, the $1/x$ weighting yielded greater accuracy at lower standard values.

The lower limit of quantitation (LLOQ), based on standard analytical method validation guidelines [10], was determined to be 20 ng/mL in plasma and 10 ng/mg in tissue homogenate. The LLOQ values translate to an instrument sensitivity of 1 ng/mL ZD6474 following extraction based on a volume of 50 μL plasma and 100 μL of liver homogenate (10 mg/mL) per extraction diluted into a final volume of 1 mL. With an injection volume of 20 μL, we can quantify 20 pg (~40 fmol) per sample introduction using the extraction and analysis conditions described.

3.5. Precision, accuracy and recovery

The precision and accuracy of ZD6474 measurement were assessed in spiked plasma and liver homogenates and the results are shown in Tables 2 and 3. The accuracy of within-run drug measurements in plasma at concentrations from 20 to 20,000 ng/mL ranged from 88.3 to 99.2% with the precision (as measured by R.S.D.%) ranging from 2.4 to 15.9%. The between-run accuracy in plasma ranged from 87.2 to 97.9% and the precision from 5.8 to 12.8%. The accuracy of the

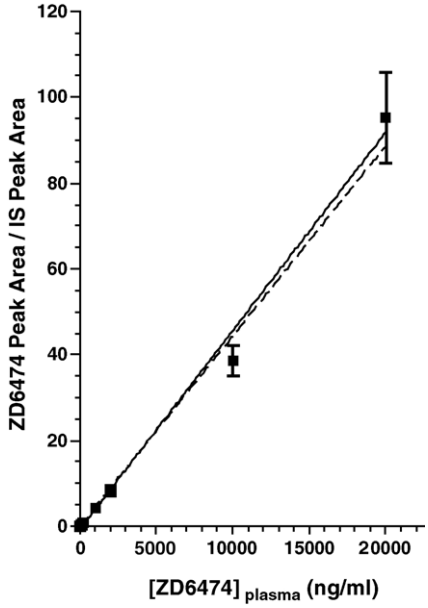

Fig. 5. Standard curve of ZD6474 in mouse plasma with 50 ng trazodone as an internal standard. The solid line ($y = 0.0046x - 0.7163$) represents uniform weighting for least squares calculation and the dashed line ($y = 0.0044x - 0.0024$) $1/x$ weighting. Both regression algorithms gave calculated adjusted r^2 values >0.98.

Table 3
Precision and accuracy of ZD6474 analysis in mouse liver tissue ($n = 3$)

Concentrations[a] (ng/mg)	Observed	Accuracy	Precision (R.S.D.%)
10	7.73 ± 0.67	77.3	8.6
20	15.5 ± 1.4	77.7	8.8
40	39.7 ± 1.8	99.2	4.6
80	73.7 ± 3.8	92.1	5.1
160	166 ± 11	96.3	6.8
320	328 ± 15	97.4	4.4

[a] Values are amounts per mg wet weight of liver tissue in the spiked homogenate.

Table 4
Summary of ZD6474 assay performance in mouse plasma and tissues

	Accuracy[a]	Precision[a] (R.S.D.%)	ZD6474 extraction[b] (%)	IS extraction[b] (%)
Plasma	90.9 ± 7.0	7.6 ± 4.7	88 ± 3	96 ± 10
Liver	90.0 ± 10.0	6.4 ± 2.0	84 ± 12	82 ± 10
Kidney	87.0 ± 2.9	8.4 ± 3.5	91 ± 6	92 ± 8
Gut	89.9 ± 4.4	6.1 ± 1.9	84 ± 6	74 ± 7
Heart	94.4 ± 3.4	4.2 ± 1.8	94 ± 5	93 ± 8

[a] Accuracy and precision were calculated as the mean ± S.D. for individual concentrations contained within the range of the assay for each tissue.
[b] Extraction values represent the mean ± S.D. of at least three independent determinations at various concentrations for ZD6474 and at 50 ng per sample for the IS (trazodone).

assay in liver homogenates at concentrations ranging from 10 to 320 ng/mg was between 77.3 and 99.2%. The precision of the assay in liver homogenates was between 4.4 and 8.8%. A summary of the accuracy and precision of ZD6474 analysis in plasma and liver, kidney, intestine and heart tissues across the tested range of the assay is presented in Table 4. All accuracy and precision measurements were within the minimum accuracy (80%) and precision (±20%) ranges for acceptable analytical method validation [10]. Recovery of ZD6474 from spiked plasma samples at 100, 1000 and 10,000 ng/mL was between 84.9 and 89.6% (Table 5). Recovery of ZD6474 and trazodone from tissues is shown in Table 4. Recoveries were determined by comparing the abundance of ZD6474 in spiked matrix samples with solvent dilutions, as no ion suppression or matrix effects were observed in any of the samples.

3.6. Stability of ZD6474 samples

ZD6474 in plasma and tissue samples were stable for over 6 months when stored at −80 °C. Sample stability was determined by analyzing sample aliquots within a week of collection and analyzing another aliquot of the same sample after 6 months storage at −80 °C. The sample analysis gave similar results within the accuracy and precision of the assay thus showing no detectable loss of ZD6474 in the stored samples. Extracted samples in acetonitrile were repeatedly measured and proved to be stable at room temperature for up to 1 month.

3.7. Pharmacokinetic study of ZD6474 in mouse plasma and tissues

The LC/MS/MS assay developed was applied to a pharmacokinetic study of ZD6474 in mice. Following 10 successive daily intraperitoneal (i.p.) treatments (30 mg/(kg day)),

Table 5
Recovery of ZD6474 from mouse plasma[a]

Plasma concentration (ng/mL)	Recovery (%)	R.S.D.%
5	88.5	4.4
50	89.6	4.2
500	84.9	2.7

[a] Values were calculated from three independent determinations.

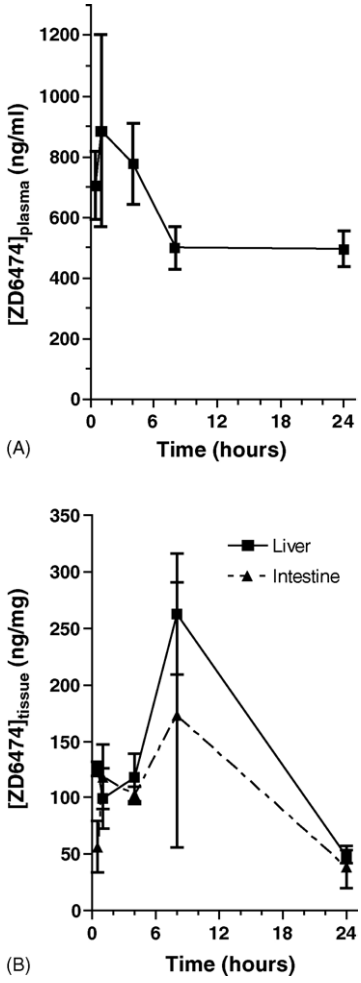

Fig. 6. Plasma (A) and liver and intestine (B) concentrations of ZD6474 in mice following treatment at 30 mg/(kg day) for 10 days. Drug levels were determined post-dosing on day 10. Values represent the mean ± S.D. of three independent determinations.

ZD6474 accumulates relatively slowly in the liver and intestine with a T_{max} of 8 h post-final dose and a long plasma terminal half-life is observed (Fig. 6; Table 6). The long half-life in plasma is consistent with Phase I data in humans that

Table 6
Pharmacokinetic parameters of ZD6474 in balb/c mice treated at 30 mg/(kg day) on day 10 of treatment[a]

	C_{max}[b]	C_{min}[b]	T_{max}[c]	$AUC_{0\rightarrow t}$[d]	$t_{1/2}\lambda$[c]
Plasma	1.8	1.0	1	27.2	31.0
Liver	525	96	8	7320	6.5
Intestine	173	19	8	2847	7.4

[a] Female balb/c mice were treated with ZD6474 by i.p. dosing at 30 mg/(kg day). Drug levels were determined in plasma and tissues following drug treatment on day 10. Parameters were calculated by non-compartmental analysis.
[b] C_{max} and C_{min} values are expressed as μM for plasma and nmol/g for liver and intestine.
[c] T_{max} and $t_{1/2}\lambda$ values are expressed in hours.
[d] AUC values are expressed as μM h for plasma and (nmol/g) h for liver and intestine.

has shown a terminal half-life approaching 100 h for ZD6474 [11]. Table 6 lists pharmacokinetic parameters for the plasma, liver and intestine as determined by non-compartmental analysis.

4. Conclusions

Quantitation of ZD6474 is necessary for the investigation of pharmacokinetics in animal models, which is a fundamental component for clinical development. The LC/MS/MS assay developed provides clean chromatography with a low limit of quantitation. The hydrophobicity of ZD6474 allows for simple and rapid extraction in alkaline ethyl acetate:pentane. The use of trazodone as the internal standard is advantageous since trazodone demonstrates similar physicochemical analytical properties as well as similar extractability (recovery) as ZD6474, in addition to being commercially available and inexpensive. The developed LC/MS/MS assay for ZD6474 is robust, capable of measuring drug in plasma and tissues and can be used for pre-clinical and clinical quantitation of ZD6474 in biological samples.

Acknowledgements

The authors would like to thank AstraZeneca for supplying ZD6474 and for financial support. We would also like to thank Andrea L. Merz for help in carrying out the animal studies and for animal dosing.

References

[1] C.H. Heldin, Cancer Surv 27 (1996) 7–24.
[2] A. Levitzki, A. Gazit, Science 267 (1995) 1782–1788.
[3] F. Ciardiello, G. Tortora, Clin. Cancer Res. 7 (2001) 2958–2970.
[4] L.F. Hennequin, A.P. Thomas, C. Johnstone, E.S.E. Stokes, P.A. Plé, J.-J.M. Lohmann, D.J. Ogilvie, M. Dukes, S.R. Wedge, J.O. Curwen, J. Kendrew, C. Lambert-van der Brempt, J. Med. Chem. 42 (1999) 5369–5389.
[5] L.F. Hennequin, E.S.E. Stokes, A.P. Thomas, C. Johnstone, P.A. Plé, D.J. Ogilvie, M. Dukes, S.R. Wedge, J. Kedrew, J.O. Curwen, J. Med. Chem. 45 (2002) 1300–1312.
[6] N. Ferrara, Eur. J. Cancer 32 (1996) 2413–2422.
[7] R.I. Nicholson, J.M.W. Gee, M.E. Harper, Eur. J. Cancer 37 (2001) 9–15.
[8] F. Ciardiello, R. Bianco, R. Caputo, R. Caputo, V. Damiano, T. Troiani, D. Melisi, F. De Vita, S. De Placido, A.R. Bianco, G. Tortora, Clin. Cancer Res. 10 (2004) 784–793.
[9] F. Ciardiello, R. Caputo, V. Damiano, R. Caputo, T. Troiani, D. Vitagliano, F. Carlomagno, B.M. Veneziani, G. Fontanini, A.R. Bianco, G. Tortora, Clin. Cancer Res. 9 (2003) 1546–1556.
[10] V.P. Shah, K.K. Midha, J.W.A. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551–1557.
[11] H. Hurwitz, S.N. Holden, S.G. Eckhardt, M. Rosenthal, R. de Boer, D. Rischin, M. Green, R. Basser, Proc. Am. Assoc. Clin. Oncol. 22 (2002) 325.