Wenzhou Medical University, Wenzhou, China

An UPLC-MS/MS method for the determination of EAI045 in plasma and tissues and its application to pharmacokinetic and distribution studies in rats

Handeng Lv#, Enzhao Shen#, Lu Luo, Yong Xu, Yaqian Pan, Kaiqi Wu, Zhicheng Xing, Faqing Ye*, Yuan Zhang*

Received June 26, 2018, accepted August 17, 2018

**Corresponding authors: Faqing Ye, Yuqan Zhang, Wenzhou Medical University, Wenzhou 325035, China zy8428@126.com*
#These authors contributed equally to this work.

Pharmazie 73: 630–634 (2018) *doi: 10.1691/ph.2018.8123*

EAI045 represents a fourth generation allosteric EGFR TKI compound which targets *T790M* and *C797S* EGFR mutants. The study reported herein describes a method to explore the distribution of EAI045 in rat tissues as well as to quantify it in plasma. The method used here is an ultra-performance liquid chromatography-tandem mass spectrometry with high sensitivity and selectivity. An ACQUITY UPLC BEN HILIC column with dimensions of 2.1 × 100 mm, 1.7 μm was used to separate the analytes and IS. As mobile phase acetonitrile as well as 0.1 % of formic acid/water was used combined with an elution gradient and 0.40 mL/min flow rate. This eluent was also used for electrospray ionization in positive ion mode. A mode on multiple reactions monitoring (MRM) was also employed in the quantification. This quantification included the use of targeted segment ions with m/z 384.1→100.8 for EAI045, and m/z 285.1→193.3 for IS, respectively. It was found that the linearity of this method was appropriate and the concentration range could be kept within a range of 2-2000 ng/mL for EAI045 in rat plasma and tissues. The level of EAI045 was found to be highest in the liver, followed by kidneys, lungs and heart. Furthermore, the results provided evidence that EAI045 could be absorbed quickly and distributed widely in different tissue types.

1. Introduction

The presence of activating mutations generally reduces the sensitivity of non-small cell lung cancer (NSCLC) cells to first, second and third generation epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) (Sharma et al. 2007; Shan et al. 2012; Brewer et al. 2013; Wang et al. 2016). Three generations of EGFR inhibitors include gefitinib, erlotinib, afatinib, and AZD9291 (Mok et al. 2009; Cataldo et al. 2011; Yu and Pao 2013; Finlay et al. 2014). EAI045 represents a fourth generation allosteric EGFR TKI that targets *T790M* and *C797S* EGFR mutants (Thress et al. 2015; Wang et al. 2016, 2017). Different from the first, second and third generation EGFR TKIs, EAI045 binds to an allosteric pocket, a conformation of the kinase called 'C-helix' (Jia et al. 2016; Russo et al. 2017). Because of the high selectivity of EAI045, exploring the pharmacokinetic and pharmacological characteristics of this EGFR TKIs remains a critical, albeit unmet, scientific goal. To achieve this goal, it is crucial to find an appropriate analytical method to determinate EAI045 in biological samples.

The key properties of EAI045 as fourth generation EGFR inhibitor have just been reported (Patel et al. 2017). Therefore, full methodological details in terms of pharmacokinetics properties *in vivo* have not yet been studied. Compared with current chromatographic methods, UPLC-MS/MS was employed as an analytical tool with both high accuracy and efficiency (de Villiers et al. 2006; Goodwin et al. 2007; Du et al. 2014). In the study reported herein, UPLC-MS/MS was selected and used to detect EAI045 in rat plasma and tissues. Using this method, crucial information on the pharmacokinetics and tissue distribution characteristics of EAI045 could be obtained.

2. Investigations and results

2.1. Method development

The MS detector parameters were calculated via a direct infusion of a standard solution to the ESI source. Upon optimization of the MS-MS conditions, the collision energy together with the capillary voltage were increased to obtain records of the daughter ion spectrum of the $[M+H]^+$ ions. The most common segment was examined at m/z 384.1, together with 1500 V capillary voltage and a 20 V collision energy. Hence, a m/z 384.1→100.8 transform was selected in the UPLC-MS/MS analysis (MRM mode, Fig. 1).

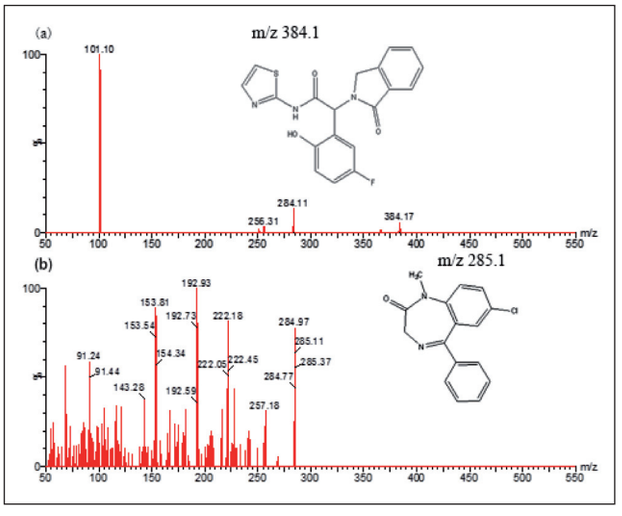

Fig. 1: Mass spectrum of EAI045 (a) and diazepam (IS, b).

In general, to obtain suitable ionization features and satisfying chromatographic characterizations, an appropriated mobile phase is required. The effects of various mobile phase systems with different annexing agents were investigated. Acetonitrile was selected as the organic solvent of choice. Unlike methanol, the use of acetonitrile resulted in a sharper peak shape as well as a

lower pump pressure. The concentration of formic acid added to the water phase was evaluated from 0.01 to 0.2 %. Eventually, a formic acid concentration of 0.1 % was selected and resulted in an appropriate peak shape and retention time.

The removal of proteins as well as other potential interferences from the biological samples is crucial since this feature determines whether a suitable LC-MS analysis can be developed (Ma et al. 2014a,b; Zhao et al. 2015). Different organic solvent mixtures such as acetonitrile, methanol, methanol/water, acetonitrile/water and acetonitrile/methanol were evaluated in an effort to extract EAI045 and IS from plasma and tissues. The results showed that the analyte recovery yields (> 80 %) using acetonitrile proved to be the most acceptable and the process was also both fast and simple.

2.2. *Specificity*

Figure 2 shows the corresponding chromatograms of blank plasma, blank plasma spiked with EAI045 as well as IS, as well as a plasma sample. We could not observe an endogenous substance in the holding time of EAI045 and IS.

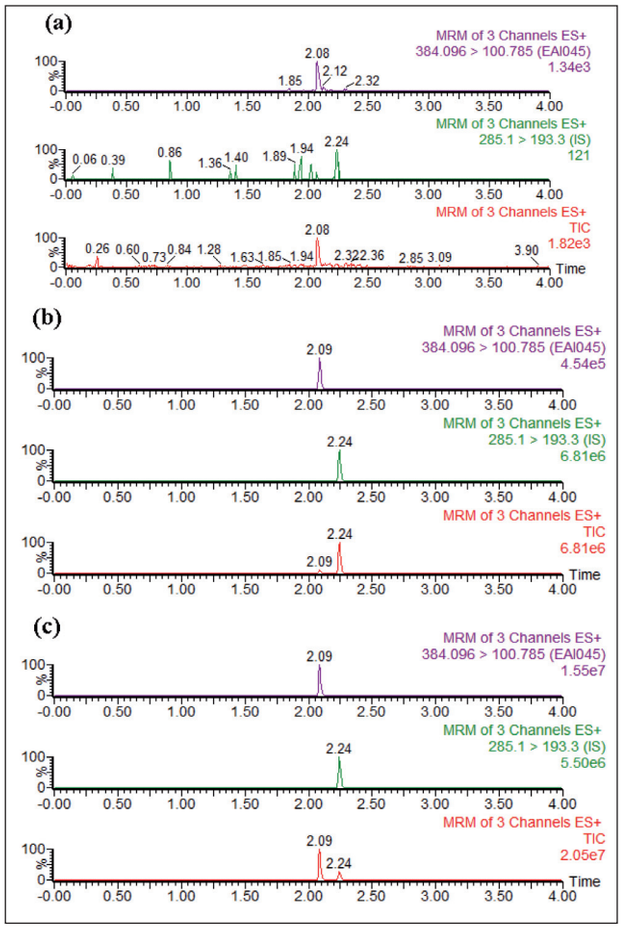

Fig. 2: Representative UPLC-MS/MS chromatograms of EAI045 and Diazepam (IS), (a) blank plasma; (b) blank plasma spiked with EAI045 (2 ng/mL) and IS (50 ng/mL); (c) rat plasma sample after 2 h of oral administration of a single dose of EAI045 (30 mg/kg).

2.3. *Linearity and lower limits of quantification (LLOQ)*

In rat plasma, the linear regressions based on the peak ratio area against the concentrations were fitted within the concentrated-range from 2 to 2000 ng/mL for EAI045 (Table 1). The equation of Y = 0.00846384 × X - 0.01661, r = 0.998044 was obtained from the calibration curve. Here, Y describes the rate of the EAI045 peak area to the ratios of the peak area of IS while X stands for the plasma concentration. The LLOQ of EAI045 within the plasma was examined to be 2 ng/mL. The accuracy and precision of LLOQ were 90.2% and 10.8%, respectively.

In the rat tissue, to fit the linear regressions of the rates of peak area against the concentrations, EAI045 was set with the concentration ranging from 2 to 2000 ng/mL. The calibration curve was determined by the following set of equations: Y = 0.00817237 × X + 0.121214, r = 0.994898 for hearts; Y=0.0095502 × X - 0.144366, r = 0.994591 for livers; Y = 0.0108422 × X + 0.041733, r = 0.993584 for spleens; Y = 0.00985224 × X + 0.187033, r = 0.990023 for lungs; Y = 0.00955502 × X + 0.140849, r = 0.997750 for kidneys; Y = 0.00883214 × X + 0.155031, r = 0.991523 for brains. The LLOQ of EAI045 in the tissues was demonstrated to be 2 ng/mL. The obtained precision (n = 6) for LLOQs ranged from 8.5% to 16.9% and the obtained accuracy ranged from 84.5% to 110.2%, respectively.

Table 1: Precision and accuracy of calibrator standards in the calibration curve of EAI045 (n = 6)

Conc. (ng/mL)	Measured conc. (ng/mL)	Accuracy (%)	RSD (%)
2	1.8	90.2	10.8
5	4.8	96.8	10.7
10	10.7	107	8.0
20	21.9	109.5	5.9
50	55.3	110.6	5.1
100	108.9	108.9	6.4
200	205.5	102.7	2.7
500	500.6	100.1	6.7
1000	994.9	99.5	8.5
2000	2013.7	100.6	8.8

2.4. *Precision and accuracy*

The RSD for QCs at three levels of concentration was determined in a three-day validation tests in order to investigate the precision and accuracy of the method. The intra-day precision was 7% or less. For each QC level, the precision of the inter-day precision proved to be 9% or less. Within every QC level, the method accuracy ratio was shown to be extended from 97.5% to 105.2%. Table 2 illustrates the data of assay performance.

Table 2: Precision and accuracy of EAI045 for QC samples in rat plasma (n = 6)

Conc. (ng/mL)	RSD (%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
3	2.5	5.9	97.5	103.5
600	6.2	8.4	100.4	105.2
1200	3.6	4.6	99.6	98.2

2.5. *Carry-over*

None of the analytes presented any significant peaks (³ 20% LLOQ and 5% IS) in the blank sample injected after the upper limit of quantitation (ULOQ) sample. However, an additional flow time of 0.4 min at the end of the gradient elution was shown to efficiently clean the system and eliminate any residuals between the samples.

2.6. *Extraction recovery and matrix effect*

The matrix effect at concentrations of 3, 600 and 1200 ng/mL EAI045 was separately determined at 99.0, 98.2, and 103.2 %, with the RSD of 9.5, 8.4 and 8.3 % (n = 6). In addition, the matrix effect

for 0.05 μg/mL IS was determined to be 97.8 %, with the RSD of 7.1 % (n = 6). Therefore, the plasma matrix effects were believed to be negligible. The extraction recoveries of EAI045 in rat plasma at three QC levels were 80.2-87.2 %, the mean extraction recovery of IS was 85.6 % (Table 3).

Table 3: Extraction recovery and matrix effect for EAI045 in rat plasma (n = 6)

Conc. (ng/mL)	Extraction recovery		Matrix effect	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)
3	85.4±5.2	7.5	99.0±6.8	9.5
600	87.2±8.4	9.3	98.2±8.1	8.4
1200	80.2±6.5	5.6	103.2±8.9	8.3
IS	85.6±7.3	8.2	97.8±7.4	7.1

2.7. *Stability*

Several different conditions were evaluated to analyze the stability. Those conditions included room temperature, freezing and thawing as well as a long-term period that lasted 20 days. The findings obtained indicated that the stability of EAI045 was acceptable for studies of both pharmacokinetics and tissue distribution since the bias in concentrations was within the range 93.3-106.7% according to the nominal values (Table 4).

Table 4: Stability summary of EAI045 and IS under various storage conditions (n = 3)

Condition	Conc. (ng/mL)		RSD (%)	Accuracy (%)
	Added	Measured		
Ambient, 4 h	3	2.9	4.3	96.7
	600	605.4	5.7	100.9
	1200	1192.8	3.2	99.4
	IS (50)	49.2	3.6	98.4
-20 °C, 20 days	3	3.2	10.8	106.7
	600	599.5	7.2	99.9
	1200	1207.0	8.9	100.5
	IS(50)	51.3	7.8	102.6
3 freeze-thaw cycles	3	2.8	6.4	93.3
	600	598.2	6.6	99.7
	1200	1194.6	5.2	99.5
	IS(50)	52.2	4.7	104.4
Autosampler ambient for 24 h	3	3.1	3.0	103.4
	600	603.2	3.5	100.5
	1200	1205.2	2.4	100.4
	IS(50)	50.6	4.8	101.2

2.8. *Pharmacokinetic study*

After oral (30 mg/kg) and intravenous (2 mg/kg) administration of EAI045, a mean EAI045 concentration-time curve in rats was obtained as shown in Fig. 3. The calculation of primary pharmacokinetics parameters was conducted in a non-compartment model analysis as illustrated in Table 5. The absolute bioavailability of berberrubine was determined to be 15.9 % (F = AUCpo × Div/AUCiv × Dpo × 100%).

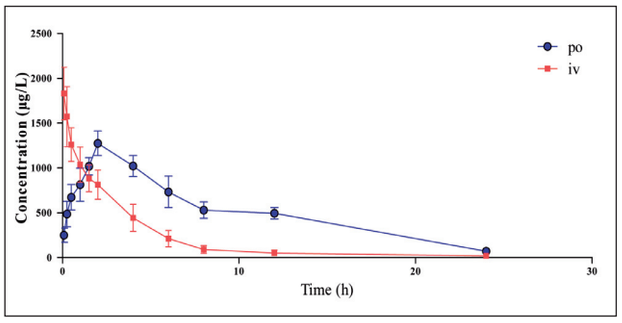

Fig. 3: Mean plasma concentration profile of EAI045 after oral administration (30 mg/kg) and intravenous (2 mg/kg) administration of EAI045 in rats.

Table 5: Major pharmacokinetic parameters after oral administration of EAI045 in rats (n = 6)

Parameters	Oral administration 30 mg/kg	Intravenous administration 2 mg/kg
$AUC_{(0\text{-}t)}$ (ng/mL h)	12379.529±655.334	5204.978±1218.775
$AUC_{(0\text{-}\infty)}$ (ng/mL h)	12929.394±639.154	5253.931±1255.675
$t_{1/2}$ (h)	5.297±0.606	3.58±1.661
CL (L/h/kg)	2.325±0.114	0.199±0.044
T_{max} (h)	2.214±0.035	0.083±0.002
V (L/kg)	17.782±2.314	0.997±0.424
C_{max}/C_{5min} (ng/mL)	1275.802±137.146	1831.146±292.158
Absolute bioavailability, F	15.9%	

AUC describes the area under the plasma concentration-time curve; $t_{1/2}$ stands for half-life; CL represents clearance; T_{max} stands for maximum plasma concentration; V describes the volume of distribution; C_{max} is the maximum plasma concentration (oral administration); C_{5min} represents the plasma concentration at 5 min (intravenous administration).

2.9. *Tissue distribution*

After oral administration of EAI045 at a dose of 30 mg/kg, the mean concentration-time curves of EAI045 in plasma, hearts, livers, spleens, lungs, kidneys and brains were obtained as shown in Fig. 4. The results indicated that EAI045 is quickly and extensively distributed to various tissue types. However, the level of EAI045 was found to be highest in the liver, followed by kidneys, lungs and heart. Importantly, the levels of EAI045 found in the lungs maintained a high concentration for about four hours. However, in the brain, the EAI045 concentration was lower than that in other

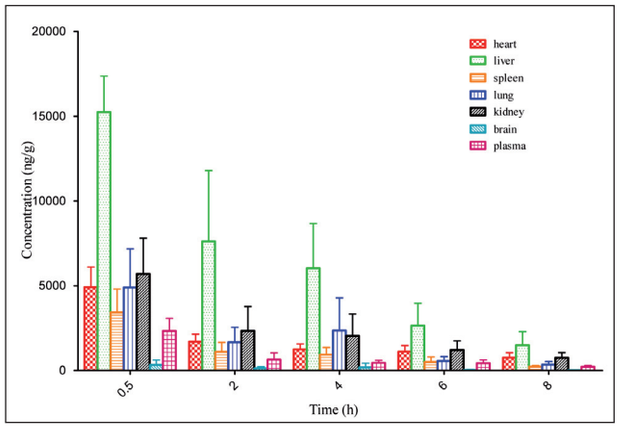

Fig. 4: Mean concentration of EAI045 in different tissues (heart, liver, spleen, lungs, kidneys, brain, and plasma) at different time points (0.5, 2, 4, 6 and 8 h) after oral administration of EAI045 (30 mg/kg) in rats (n = 6, mean±SD).

tissues, a finding that may indicate that EAI045 could accumulate in the liver and lungs, but does not cross the blood brain barrier. This phenomenon also suggests that EAI045 isprimarily metabolized in the liver.

2.10. Conclusions

In this study, the accuracy, recovery, selectivity, precision, stability and linearity of a UPLC-MS/MS approach for EAI045 was determined. This UPLC-MS/MS approach exhibited a LLOQ of 2 ng/mL and an overall run time of 4 min. The method could be effectively employed to the study of pharmacokinetics and distribution of tissue characteristics of EAI045. The levels of EAI045 were found to be highest in the liver, following by kidney, lung and heart tissues. Overall, it may be concluded that EAI045 is rapidly absorbed and widely distributed into different tissue types.

3. Experimental

3.1. Chemicals and reagents

EAI045 (purity > 99%) was acquired from Selleck Chemicals LLC. (Shanghai, China) and diazepam (IS, purity > 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and LC-grade acetonitrile were obtained from Merck & Co. (Darmstadt, Germany). To produce ultra-pure water, a Millipore Milli-Q purification system (Bedford, MA, USA) was employed. Drug-free rats provided blank rat plasma (Laboratory Animal Center of Wenzhou Medical University).

3.2. Instrumentation and experimental conditions

The UPLC-MS/MS system consisted of a XEVO TQS triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA), an electrospray ionization interface and an ACQUITY I-Class UPLC system. The UPLC system used throughout this study consisted of two managing systems, one of which was a binary solvent manager and another was a sample manager with a flow-through needle. Masslynx 4.1 software was applied to conduct both data collection and instrument control (Waters Corp., Milford, MA, USA).

At a constant column temperature of 40 °C, a UPLC BEH HILIC column with dimensions of 2.1 mm × 100 mm, 1.7 µm was used for the effective separation of EAI045 and diazepam (IS). Formic acid at a concentration of 0.1 % and acetonitrile were used as mobile phase and the gradient elution was kept at a flow rate of 0.4 mL/min, with an injection volume of 2 µL. A linear gradient was employed and the acetonitrile amount was increased from 10 to 70 % within 2.5 min. The gradient was then maintained at 70 % acetonitrile for 0.5 min, and finally, the acetonitrile ratio was decreased from 70 to 10 % within 0.5 min. A gradient of 10 % acetonitrile was then kept constant until the end of the program, with the entire process lasting 4 min. The following step included rinsing with acetonitrile-water, 90/10, *v/v*, as well as rinsing with acetonitrile-water, 10/90, *v/v*. Then, the sample managing system was cleaned by a needle rinse process after each injection.

Both the 1000 L/h desolvation gas and the 50 L/h cone gas was nitrogen. The relevant conditions of ion monitoring were set at follows: 2.5 kV capillary voltage, and 150 °C source temperature, plus 500 °C desolvation temperature. MRM modes of ions with m/z 384.1→100.8 for EAI045 and m/z 285.1→193.3 for IS were employed for quantitative analysis (Fig. 1).

3.3. Calibration standards and quality control samples

Stock solutions of EAI045 with 1.0 mg/mL and diazepam (IS) at a concentration of 1.0 mg/mL were prepared in methanol. A solution of 0.5 µg/mL diazepam (IS) as working standard solution was prepared by diluting the IS stock solution with methanol. Similarly, the working solutions were used for calibration and the controls were prepared using stock solutions. All solutions were stored at 4 °C before use.

According to calibration standards, EAI045 at different concentrations was used to spike blank plasma or tissue with suitable amounts of the work solutions. At a concentration range of 2-2000 ng/mL, the calibration plots for EAI045 in the plasma of rats (2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL) and tissues (2, 5, 10, 20, 50, 100 500, 1000 and 2000 ng/mL) were generated. For every 100 µL of the blank plasma or tissues, 10 µL of the IS working solution (0.5 µg/mL) was added and the resulting solutions were mixed by vortex mixing. The prepared samples of quality-control (QC) were produced in a similar way with three different plasma concentrations (3, 600, 1200 ng/mL). All of the samples, including QC samples and the analytical standards, were stored at -20 °C before use. Before conducting the UPLC-MS/MS study, the calibration standards as well as the QC samples were pretreated via precipitation with acetonitrile.

3.4. Sample preparation

The samples of plasma were defrosted at room temperature prior to analysis. Thereafter, an aliquot of 10 µL of the IS working solution (0.5 µg/mL) was added to 100 µL of the plasma samples, followed by the addition of 200 µL of acetonitrile. The samples were prepared in 1.5 mL centrifuge tubes. Then, vortex mixing in the tubes was carried out for 1.0 min. After centrifugation at 13,000 *g* for 10 min, the supernatants (2 µL) were used for injection into the UPLC–MS/MS system.

The tissue samples were defrosted at room temperature prior to analysis. Then, 100 mg of heart, liver, spleen, lung, kidney and brain tissues were weighed and placed in 1.5 mL centrifuge tubes. Next, 10 µL of the IS working solution (0.5 µg/mL) and 200 µL of acetonitrile were added. The corresponding mixtures were stored at -80 °C for 20 min, and then grinded for 2 min using a SCIENTZ-48 Tissue Grinder (1800 r/s, 64 Hz). Then, the samples were vortex-mixed for 1.0 min. After centrifugation at 13,000 *g* for 10 min, the supernatants (2 µL) were injected into the UPLC-MS/MS system.

3.5. Method validation

According to the United States Food and Drug Administration (FDA) (Brodie & Hill 2002) and the European Medicines Agency (EMA)(Smith 2010), our proposed method proves to be validated for specificity, linearity, precision, accuracy, recovery and stability.

3.5.1. Specificity

The method specificity was assessed by analyzing six sample types obtained from blank rat plasma, blank plasma spiked with EAI045 and IS, as well as a sample of rat plasma.

3.5.2. Linearity and lower limits of quantification (LLOQ)

Calibration curves were obtained by analyzing spiked calibration samples on three separate days. The peak area rates for EAI045-to-IS were drawn against the concentration of EAI045. Considering regression with a weighting element of the reciprocal concentration value ($1/x$) at 2-2000 ng/mL, the standard curves, according to the regression results, were fitted to the equations. The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curves.

3.5.3. Precision and accuracy

In order to evaluate the degree of precision and accuracy of the determination of the QC samples at three concentrations, i.e. 3, 600, and 1200 ng/mL, the samples were analyzed in six replicates over three validations. Relative standard deviation (RSD) expressed the precision.

3.5.4. Carry-over

For the purpose of assessing the carry-over, an injection into a blank plasma sample was carried out as soon as the three repeats for the upper limit of quantification (ULOQ) were completed. The response was examined in terms of precision (Williams et al. 2012).

3.5.5. Extraction recovery and matrix effect

The matrix effect and extraction recovery were evaluated by six replicates in parallel for each concentration. The extraction recovery of EAI045 from rat plasma at three different QC levels was investigated by comparing the peak areas from the analytes spiked in post-extraction plasma samples with those originally added to blank plasma. The matrix effects were measured by the peak areas gained from plasma samples spiked with standard solutions with pure standard solutions and methanol at QC levels using the same extraction method.

3.5.6. Stability

To determine the steadiness of EAI045 for rat plasma, three replicates of the plasma samples at concentrations of 3, 600 and 1200 ng/mL were analyzed separately and the samples were examined under various conditions. These results were compared with those obtained from freshly-prepared plasma samples and an assessment regarding the short-term steadiness was conducted. The spiked samples were exposed to room temperature for a period of 4 h. Simultaneously, other samples were exposed to room temperature for a period of 24 h after which the samples were injected in the UPLC autosampler. Protein precipitation was carried out at room temperature. Furthermore, an evaluation of freeze/thaw processes was carried out. This stability assessment was conducted at complete freeze/thaw cycles (-20 to 25 °C) covering a period of three continuous days. The samples of standard-spiked plasma were then stored at a temperature of -20 °C, covering a 20-day period after which the long-term stability was evaluated.

3.6. Pharmacokinetic study

Twelve male Sprague-Dawley (SD) rats (200-220 g) were obtained from the Laboratory Animal Center of Wenzhou Medical University. These male rats served for the analysis of the pharmacokinetics of EAI045. The Animal Care and Use Committee of Wenzhou Medical University reviewed and approved all animal protocols and procedures. Before the experimental processes were carried out, the sample animals were fasted for 12 h, however, water was freely provided. Blood samples were taken from the tail vein and the samples were placed in heparinized 1.5 mL centrifuge tubes at time points of 0.0833, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h after oral (30 mg/kg) or intravenous (2 mg/kg) administration of EAI045. The samples were centrifuged at 3,000 *g* for 10 min immediately afterwards. The plasma obtained by this method was then stored at -20 °C until UPLC-MS/MS analysis. Analysis of EAI045 plasma concentration at different time points for each rat was carried out via DAS, a Drug and Statistics software package (version 2.0, Wenzhou Medical University).

3.7. Tissue distribution model

Thirty-six rats were randomly selected and divided into six separate groups (n=6). The rats received 30 mg/kg of EAI045 via oral administration. Afterwards, the rats

were euthanized by decapitation at time points of 0 (blank group), 0.5, 2, 4, 6 and 8 h after administration of EAI045. Saline was used to dissect and wash the tissues from the rat hearts, livers, spleens, lungs, kidneys and brains. Then, filter paper was used to blot the tissues. Afterwards, the tissues were stored at -20 °C before UPLC-MS/MS analysis.

Conflicts of interest: None declared.

Acknowledgements: This work was supported by Xinmiao Talent Project of Zhejiang Province [grant number 2016R413079], and the National Training Program of Innovation and Entrepreneurship for Undergraduates [grant number 201610343012].

References

Brewer MR, Yun CH, Lai D, Lemmon MA, Eck MJ (2013) Mechanism for activation of mutated epidermal growth factor receptors in lung cancer. Proc Natl Acad Sci USA 110: 20344-20344.

Brodie RR, Hill HM (2002) Validation issues arising from the new FDA guidance for industry on bioanalytical method validation. Chromatographia 55: S91-S94.

Cataldo VD, Gibbons DL, Perez-Soler R, Quintas-Cardama A (2011) Treatment of non-small-cell lung cancer with erlotinib or gefitinib. New Engl J Med 364: 947-955.

de Villiers A, Lestremau F, Szucs R, Gelebart S, David F, Sandra P (2006) Evaluation of ultra performance liquid chromatography. Part I. Possibilities and limitations. J Chromatogr A 1127: 60-69.

Du J, Zhang Y, Chen Y, Liu D, Chen X, Zhong D (2014) Enantioselective HPLC determination and pharmacokinetic study of secnidazole enantiomers in rats. J Chromatogr B 965: 224-230.

Finlay MRV, Anderton M, Ashton S, Ballard P, Bethel PA, Box MR, Bradbury RH, Brown SJ, Butterworth S, Campbell A, Chorley C, Colclough N, Cross DAE, Currie GS, Grist M, Hassall L, Hill GB, James D, James M, Kemmitt P, Klinowska T, Lamont G, Lamont SG, Martin N, McFarland HL, Mellor MJ, Orme JP, Perkins D, Perkins P, Richmond G, Smith P, Ward RA, Waring MJ, Whittaker D, Wells S, Wrigley GL (2014): Discovery of a potent and selective EGFR inhibitor (AZD9291) of both sensitizing and T790M resistance mutations that spares the wild type form of the receptor. J Med Chem 57: 8249-8267.

Goodwin L, White SA, Spooner N (2007) Evaluation of ultra-performance liquid chromatography in the bioanalysis of small molecule drug candidates in plasma. J Chromatogr Sci 45: 298-304.

Jia Y, Yun CH, Park E, Rcan DE, Manuia M, Juarez J, Xu CX, Rhee K, Chen T, Zhang HK, Palakurthi S, Jang JB, Lelais G, DiDonato M, Bursulaya B, Michellys PY, Epple R, Marsilje TH, McNeill M, Lu WS, Harris J, Bender S, Wong KK, Janne PA, Eck MJ (2016) Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature 534: 129-132.

Ma J, Ding X, Sun C, Lin C, An X, Lin G, Yang X, Wang X (2014a) Development and validation a liquid chromatography mass spectrometry for determination of solasodine in rat plasma and its application to a pharmacokinetic study. J Chromatogr B 963: 24-28.

Ma J, Lin C, Wen C, Xiang Z, Yang X, Wang X (2014b) Determination of bicuculline in rat plasma by liquid chromatography mass spectrometry and its application in a pharmacokinetic study. J Chromatogr B 953-954: 143-146.

Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. New Engl J Med 361: 947-957.

Patel H, Pawara R, Ansari A, Surana S (2017) Recent updates on third generation EGFR inhibitors and emergence of fourth generation EGFR inhibitors to combat C797S resistance. Eur J Med Chem 142: 32-47.

Russo A, Franchinaa T, Ricciardi GRR, Smiroldo V, Picciotto M, Zanghi M, Rolfo C, Adamo V (2017) Third generation EGFR TKIs in EGFR-mutated NSCLC: Where are we now and where are we going. Crit Rev Oncol Hemat 117: 38-47.

Shan YB, Eastwood MP, Zhang XW, Kim ET, Arkhipov A, Dror RO, Jumper J, Kuriyan J, Shaw DE (2012) Oncogenic mutations counteract intrinsic disorder in the EGFR kinase and promote receptor simerization. Cell 149: 860-870.

Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. Nature Rev Cancer 7: 169-181.

Smith G (2010) Bioanalytical method validation: notable points in the 2009 draft EMA Guideline and differences with the 2001 FDA Guidance. Bioanalysis 2: 929-935.

Thress KS, Paweletz CP, Felip E, Cho BC, Stetsonl C, Dougherty B, Lai ZW, Markovets A, Vivancos A, Kuang Y, Ercan D, Matthews SE, Cantarini M, Barrett JC, Janne PA, Oxnard GR (2015) Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nature Med 21: 560-562.

Wang SH, Song YP, Liu DL (2017) EAI045: The fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. Cancer Lett 385: 51-54.

Wang SH, Song YP, Yan FF, Liu DL (2016) Mechanisms of resistance to third-generation EGFR tyrosine kinase inhibitors. Front Med 10: 383-388.

Wang SH, Tsui ST, Liu C, Song YP, Liu DL (2016) EGFR C797S mutation mediates resistance to third-generation inhibitors in T790M-positive non-small cell lung cancer. J Hematol Oncol 9: 59.

Williams JS, Donahue SH, Gao H, Brummel CL (2012) Universal LC-MS method for minimized carryover in a discovery bioanalytical setting. Bioanalysis 4: 1025-1037.

Yu HA, Pao W (2013) Targeted therapied. Afatinib-new therapy option for EGFR-mutant lung cancer. Nat Rev Clin Oncol 10: 551-552.

Zhao LS, Sun XH, Xiong ZL, Wang SJ (2015) Simultaneous determination of mosapride and its active des-p-fluorobenzyl and 4'-N-oxide metabolites in rat plasma using UPLC-MS/MS: An application for a pharmacokinetic study. Talanta 137: 130-135.