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Simultaneous determination of gefitinib and its major metabolites in mouse plasma by HPLC–MS/MS and its application to a pharmacokinetics study

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ABSTRACT

Gefitinib (Iressa) is the first oral EGFR tyrosine kinase inhibitor and it brings benefits to non-small cell lung cancer patients with EGFR mutation. In this study, a simple, rapid and credible high performance liquid chromatography–tandem mass spectrometry method was established and validated for the simultaneous quantification of gefitinib and its main metabolites M523595, M537194, M387783 and M608236 in NSCLC tumor-bearing mouse plasma. Sample extraction was done by protein precipitation using acetonitrile containing dasatinib as the internal standard. The chromatography run time was 6 min using an Agilent RRHD SB-C18 column with a gradient of acetonitrile and water (0.1% formic acid, v/v). The mass analysis was performed by a triple quadrupole mass spectrometry in positive multiple reaction monitoring mode. The calibration range was 0.5–100 ng/mL for M608236 and 1–200 ng/mL for other analytes with the correlation coefficients (r^2) ≥ 0.99. For quality control samples, inter- and intra-assay precision was less than 15% and accuracies ranged from 92.6% to 107.58% for all analytes. The extraction recoveries were in the range of 86–105% and no significant matrix effect was observed. This simple and reproducible high-throughput method was successfully applied to the pharmacokinetic study of gefitinib and its major metabolites in mouse.

1. Introduction

Gefitinib (Iressa®) is an anilinoquinazoline compound with the chemical name 4-quinazolinamine, *N*-(3-chloro-4-flurophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy]. It is an orally active and a selective small-molecule epidermal growth factor receptor (EGFR) TKI indicated for the treatment of locally advanced or metastatic NSCLC with sensitive mutations of EGFR [1,2]. Many clinical studies have confirmed that NSCLC patients with EGFR-active gene mutations could get benefits from gefitinib treatment [3,4].

Gefitinib is extensively metabolized by cytochrome P-450 (CYP) 3A4 and 2D6 [5]. Oxidation of the morpholine ring, oxidative defluorination and *O*-demethylation of the methoxy-substituent on quinazoline nucleus represent the main routes of gefitinib metabolism [5,6]. CYP3A4 catalyzed gefitinib to produce metabolites of M537194, M608236 and M387783, while CYP2D6 exerted rapid and extensive metabolism of gefitinib to M523595 [6]. Monitoring gefitinib and its metabolites may help to minimize the risk of drug–drug interactions and dose-related toxicity due to its extensive hepatic metabolism. Therefore, an analytical method is required to determine the concentrations of gefitinib and its major metabolites as well as their pharmacokinetic (PK) profiles.

To our knowledge, there are some validated methods for quantification of gefitinib and M523595 (Table 1), but other metabolites

Abbreviations: TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; LC–MS/MS, liquid chromatography–tandem mass spectrometry; EGFR, epidermal growth factor receptor; MRM, multiple reaction monitoring; CYP, cytochrome P-450; PK, pharmacokinetic; ESI, electrospray ionization; QC, quality control; IS, internal standard; LLOQ, lower limit of quantification; CE, collision energy; S/N-ratio, signal to noise ratio; RSD, relative standard deviation; RE, relative errors; ULOQ, upper limit of quantification; UHQC, ultra-high quality control; $t_{1/2}$, half-time; T_{max}, peak time; AUC, area under concentration–time curve; C_{max}, the peak concentration.

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Table 1
Published methods for quantification of gefitinib using liquid chromatography–tandem mass spectrometry.

Compound	Matrix	Sensitivity (LOQ)	Sample extraction	Run time	Mobile phase (v/v)
Gefitinib [7]	Human plasma	0.50 ng/mL	LLE	4 min	70% acetonitrile:30% formic acid (1%)
Gefitinib [8]	Human plasma; mouse plamsa and tissues	1 ng/mL (human); 5 ng/mL (mouse)	PP	3 min	70% acetonitrile:30% water containing 0.1% formic acid
Gefitinib [9]	Human plasma	0.1 ng/mL	LLE	10 min	80% acetonitrile:20% aqueous ammonium acetate (1% w/v)
Gefitinib [10]	Human plasma	0.5	LLE	5 min	80% acetonitrile:20% aqueous ammonium acetate (1% w/v)
Gefitinib, M523595 [11]	Human plasma	5 ng/mL	LLE	3 min	30% acetonitrile:70% water containing 0.1% formic acid
Gefitinib & metabolites [16]	Rat plasma; dog plamsa	5 ng/mL (M523595, M537194); 2 ng/mL for others	PP	–	80% acetonitrile:20% aqueous ammonium acetate (1% w/v)
Three TKIs including gefitinib [12]	Human plasma	5 ng/mL	LLE	–	65% water:35% methanol containing 0.01% formic acid and 2 mM ammonium acetate
Nine TKIs including gefitinib [13]	Human plasma	0.40 ng/mL	SPE	4 min	90% acetonitrile:10% 4 mM ammonium formate
Nine TKIs including gefitinib [14]	Human plasma	20 ng/mL	PP	10 min	20% ammonium hydroxide in water 10 mM:80% ammonium hydroxide in methanol 1 mM
Four TKIs including gefitinib [15]	Human plasma and cell culture	11.2 nM	PP	5 min	66.6% acetonitrile:25% 20 mM ammonium acetate:8.3% methanol

LOQ: limit of quantification; LLE: liquid–liquid extraction; PP: protein precipitation; SPE: solid phase extraction.

Table 2
Summary of the MRM transitions for gefitinib, its metabolites and internal standard used in the LC–MS/MS analysis.

Compound	Transitions (Da)	Dwell time (ms)	Fragment-or (V)	Collision energy (V)	Mean retention time (min)
Quantifier ions					
Gefitinib	447 → 128	40	150	28	1.86
M523595	433 → 128	40	150	23	1.82
M537194	421 → 320	40	150	22	1.80
M387783	445 → 128	40	150	24	1.46
M608236	449 →n130	40	150	24	2.28
Dasatinib(IS)	488 → 401	40	190	31	2.32
Qualifier ions					
Gefitinib	447 → 100.1	40	150	28	1.86
M523595	433 → 100.1	40	150	25	1.82
M537194	421 → 102.1	40	150	18	1.80
M387783	445 → 100.1	40	150	35	1.46
M608236	449 → 320	40	150	28	2.28

of gefitinib were not involved [7–15]. McKillop et al. reported a method for analyzing gefitinib and its metabolites, but the methodology and validation was not studied, and the analytes were with unsatisfactory purity (ranging from 44.7% to 89.8%) [16]. As mentioned above, there are a number of researches involving the PK behavior of gefitinib, but lack of assays for PK profiles of its metabolites.

In this study, we developed a simple, rapid and accurate LC–MS/MS method to simultaneously determine gefitinib, M523595, M537194, M387783 and M608236 in mouse plasma. We applied this established method to study the PK profiles of gefitinib and its major metabolites in tumor-bearing mouse after a single oral administration of gefitinib.

2. Materials and methods

2.1. Chemicals and materials

Gefitinib was purchased from AstraZeneca (Cheshire, UK), and dasatinib was produced from Bristol-Myers Squibb (NY, USA). M537194, M523595, M387783, M608236 were synthesized in Yaosu Technology Ltd. (Purity ≥ 98%, Beijng, China). HPLC grade methanol and acetonitrile were from Merck (Darmstadt, Germany). Analytical standard formic acid was from Sigma–Aldrich (98% purity, mass spectrometry grade, St. Louis, Missouri, USA). Chromatographic pure water was produced by Milli-Q® Water Purification Systems (Merck Millipore, MA, USA). The other chemicals used in this study were all of chromatographic grade.

2.2. Instrumentations and chromatographic conditions

The LC–MS/MS method was performed on an Agilent 1290 Infinity LC system (Agilent Technologies, USA), which mainly consisted of a G4220A binary pump, a G4226A infinity auto-sampler, a G1316C column heater and a G6460A triple-quadrupole mass spectrometer with an electrospray ionization (ESI) source.

The separation was achieved on an Agilent RRHD SB-C18 column (2.1 mm × 50 mm, 1.8 μm, Agilent Technologies, USA). An aliquot of 2.0 μL of the sample was injected on the column. Mobile phase was a mixture of 0.1% (v/v) formic acid in water (A) and acetonitrile (B). The gradient grogram of mobile phase was as follows: 10% B at 0–0.5 min; 10–50% B at 0.5–2.5 min; 50–95% B at 2.5–3 min; held 95% B at 3–4.5 min; 95–10% B at 4.5–4.6 min, 10% B for equilibration of the column. The total analytical run time was 6.0 min per sample, including equilibration time. The flow rate was 0.4 mL/min and the column was maintained at 40 °C. The quantifier- and qualifier-ions for gefitinib and its metabolites are also tabulated in Table 2.