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Investigation of metabolic degradation of new ALK inhibitor: Entrectinib by LC-MS/MS

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ABSTRACT

Entrectinib (ENC) is a potent orally available anaplastic lymphoma kinase (ALK) inhibitor. In 10 July 2017, biotechnology company (Ignyta) announced that granted orphan drug designation approval was given by the FDA to ENC for "treatment of NTRK fusion-positive solid tumors". A validated LC-MS/MS methodology was developed for ENC quantification in human plasma matrix. The supposed method characterized by high speed, specificity and sensitivity. This established method was applied for metabolic degradation assessment of ENC. Reversed stationary phase (C_{18} column) and elution mobile phase (48% 10 mM ammonium formate in H_2O (pH: 4.2 adjusted by adding few drops of formic acid): 52% ACN) were utilized for chromatographic resolution of ENC and lapatinib as internal standard (IS). Total elution time, flow rate and injection volume were 4 min., 0.25 mL/min., and 5 µL, respectively. Electrospray ionization source was used for ions generation, while positive multiple reactions monitoring (MRM) mode was used for ion analysis. The data of calibration curve of ENC in human plasma was linear in the range of 5–500 ng/mL with correlation coefficient (r^2) > 0.999. *LOQ* and *LOD* for ENC were 2.17 ng/mL and 0.71 ng/mL, respectively. Inter-day and intra-day precision and accuracy were 97.52 to 101.83%, and 0.38 to 1.32%, respectively. Intrinsic clearance (Clint) and *in vitro* half-life ($t_{1/2}$) were equal to 15.67 mL/min/kg and 9.1 min, respectively. To our knowledge, this is considered the first method for ENC quantification in human plasma and its metabolic degradation assessment.

1. Introduction

ALK is considered a receptor tyrosine kinase that is responsible for different tumor types development [1,2]. Even though crizotinib showed in the beginning remarkable clinical activity, the resistance mutations emergence and brain metastases frequently causes patients relapse [3,4].

ENC (Fig. 1) overcomes the crizotinib drawbacks as it penetrates the blood–brain barrier (BBB) in various animal species. It is very efficient in *in vivo* xenograft models [5]. Furthermore, ENC showed very potent effect on tyrosine kinases ROS1 and TRKs that were activated in several tumor types. ENC is undergoing phase I/II clinical trial for patients treatment that suffered from ALK-, ROS1-, and TRK-positive tumors [6]. ENC is considered the only TRK inhibitor that demonstrated clinical activity against primary and metastatic CNS disease without exhibiting unwanted off-target activity [7]. In 10 July 2017, biotechnology company (Ignyta) announced that granted orphan drug designation approval was given by the U.S. Food and Drug Administration (FDA) to ENC for "treatment of NTRK fusion-positive solid tumors" [8].

Our aim is to study ENC metabolic degradation and clearance through establishing a new LC-MS/MS method. The metabolic degradation assessment of ENC in RLMs was applied using the proposed method by measuring the rate of ENC disappearance in RLMs incubations. Clint and *in vitro* $t_{1/2}$ were used *in vitro* for metabolic degradation assessment. Hepatic clearance, bioavailability and *in vivo* $t_{1/2}$ can be computed. *In vivo* bioavailability of a certain compound may be low if it exhibited a fast metabolic degradation [9,10].

2. Experimental

2.1. Chemicals and reagents

ENC and lapatinib (IS) were purchased from Med Chem. Express (USA). Acetonitrile (ACN), ammonium formate, formic acid and poly

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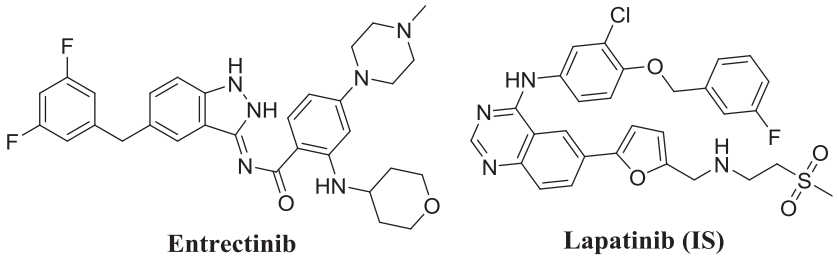

Fig. 1. Chemical structure of (A) Entrectinib (ENC) and (B) Lapatinib (IS).

ethylene glycol 300 (PEG 300) were purchased from Sigma-Aldrich (USA). Tween 80 was purchased from Eurostar Scientific Ltd. (UK). Ultrapure water was generated by Milli-Q plus filtration system (USA). Human plasma was supplied by King Khaled University Hospital (Saudi Arabia), after informed consent was obtained from donners. The protocol was approved by College of Pharmacy ethics committee and the Institutional Review Board of King Khalid University Hospital at King Saud University (Saudi Arabia). Human blood was obtained from volunteer by clean venous puncture from into 50-mL polypropylene heparinized tubes (10 IU heparin/mL of blood) from Sastedt Company (Germany). Immediately, plasma was separated by centrifugation at 5500*g* (2 °C for 20 min.) and stored at −70 °C until use. RLMs were prepared in-house following a published method using Sprague Dawley rats which were brought from the King Saud University (KSU) experimental animal care center (Saudi Arabia) [11–14].The Ethics Review Committee at KSU approved the animal experimental design.

2.2. Chromatographic conditions

All LC-MS/MS chromatographic parameters were tuned to achieve high resolution in a fast run time (Table 1). A pH of 10 mM Ammonium formate buffer was optimized at 4.2 using few drops of HCOOH as increasing pH more 4.2 resulted in longer run time (> 6 min) and broad tailed eluted peaks. While, decreasing pH < 4.2 resulted in overlapped peaks. The perfect results were attained by adjusting the ratio of ACN to buffer system to be 52%: 48%. Increasing ACN percent resulted in worsen separation and overlapped peaks, while decreasing it led to longer elution time (> 7 min). Different types of columns were tested (example: normal columns) and analytes were not retained. The best separation and resolution were attained using C_{18} column with isocratic binary mobile phase (48% 10 mM Ammonium formate in H_2O: 52% ACN).

Upon literature review, it was found that ENC wasn't used in combination with lapatinib for the same patient for treatment of any type of cancer. So, the patient who is under ENC treatment will not use lapatinib so no interference from it during chromatographic analysis. Also, lapatinib exhibited the same behavior in extraction as ENC with high recovery using the same extraction procedure. Furthermore, elution time of lapatinib is close to that of ENC using an isocratic mobile phase, so no need for longer elution time and analytes are well separated from one another.

2.3. Preparation of ENC and IS working solutions

ENC was solubilized in appropriate volume of dimethyl sulfoxide (DMSO) to make stock solution (2 mg/mL) followed by ten folds dilution by mobile phase to prepare ENC first working solution (200 µg/mL) then further twenty folds dilution with mobile phase to make ENC second working solution (10 µg/mL). Lapatinib (IS) was solubilized in appropriate volume of DMSO then further diluted by mobile phase to prepare the IS working solution (1.5 µg/mL).

2.4. Preparation of ENC calibration curves

Proper volumes of second ENC second working standard solution were mixed with human plasma to make twelve concentrations levels of

Table 1
Optimized LC-MS/MS parameters.

LC			MS/MS	
LC	Agilent 1200		MS spectrometer	Agilent 6410 QQQ
Isocratic mobile phase	52% ACN 48% 10 mM Ammonium Formate in H_2O (pH: 4.2 adjusted by adding few drops of Formic acid Flow rate: 0.25 mL/min. Run time: 4 min.		ESI	Positive mode Drying gas: N_2 gas Flow rate (12 L/min) Pressure (60 psi)
Agilent Zorbax eclipse plus C_{18} Column	Length	50 mm		T: 350 °C Capillary voltage: 4000 V
	Internal diameter	2.1 mm		
	Pore size	95 Å		
	Particle size	1.8 µm	Collision gas	High purity N_2
	Temperature:	22 ± 1 °C	Mode	Multiple Reaction monitoring (MRM)
Analyte	ENC		ENC transitions	*m/z* 561 → *m/z* 302, FV[a]: 135 V CE[b]: of 15 eV *m/z* 561 → *m/z* 284, FV: 135 V CE: of 14 eV
Internal standard (IS)	LAP		LAP (IS) transitions	*m/z* 581 → *m/z* 367 FV: 135 V, CE: 30 eV *m/z* 581 → *m/z* 352 FV: 140 V, CE: 32 eV

[a] Fragmentor voltage.
[b] Collison energy.