ملتقى البحث العلمي

مركز بحوث الدراسات العلمية والطبية
Comparative pharmacokinetic profiles of selected irreversible tyrosine kinase inhibitors, neratinib and pelitinib, with apigenin in rat plasma by UPLC-MS/MS

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Blocking EGFR as a targeted anticancer therapy

Monoclonal antibodies

Small molecules

- **Tyrosine kinase inhibitors**
- Serine/threonine kinase inhibitors
- Small molecule drug conjugates

Phosphorylation of tyrosine residues on the epidermal growth factor receptor (EGFR) is an important early event in signal transduction, leading to cell replication for major human carcinomas. This receptor is widely expressed in advanced cancers.

Li et al, Target Oncol, 2012
**Reversible and irreversible TKIs**

- **ATP-competitive inhibitors** inhibit protein catalytic activity in a reversible manner. Examples of which are gefitinib (GEF), and erlotinib (ERL).
- In the past decade, much progress has been made in the development of a new class of potent and selective TKIs that irreversibly inhibit their target protein.

Several theoretical advantages of the second-generation irreversible EGFR TKIs over the first-generation reversible EGFR TKIs are that some have a **higher affinity** and **selectivity** for the EGFR kinase domain, and may allow a more complete blockade of the EGFR signaling pathway. **The number of irreversible TKIs entering clinical trials studies is steadily increasing.** Examples of which are neratinib (NER) and pelitinib (PEL).
Intra/Inter-individual pharmacokinetic (PK) variability

- However, **complexity in the pharmacokinetics (PK)** of these drugs results from their **oral administration**.

**Intra/Inter-individual PK variability**

- Genetic heterogeneity of drug targets
- **Patient characteristics**
  - *environmental factors* (drug–drug interactions)
  - *adherence to treatment.*
- Pharmacogenetic background of the patient
  - (e.g. cytochrome P450 and ABC transporter polymorphisms)
  - plays an important role in the inter-individual variability.

*Widmer, Eur. J. Cancer, 2014*
Drug-drug interactions (DDIs) with TKIs

Potential sites of drug interactions with orally-administered kinase inhibitors.

1. Oral administration
2. A change in stomach pH
3. Activity of drug transporters and drug metabolising enzymes within the intestine, liver and kidney
   - Formation of insoluble complexes interfering with absorption
   - Inhibition or induction of intestinal P-glycoprotein
4. Substrates of intestinal CYP3A4

Protein binding of the agent within the systemic circulation (distribution)
TKIs are mainly metabolized by CYP3A4. Thus they are susceptible to drug-drug interactions with any co-administered drug which has been known to be an inhibitor or inducer of CYP450 enzymes. Inhibitors block the activity of a particular CYP450 enzyme with a slow clearance of the TKI with increased frequency of side effects while inducers increase the enzyme activity resulting in enhancing chemotherapy clearance with a potential decrease in the effectiveness and increasing risk of relapse.
Drugs altering the activity of drug transporters (P-gp) within the intestine

Impact of transporter-based drug-drug interactions on pharmacokinetics in intestine and liver

Co-administration of natural products contained in food, which have potential to inhibit P-gp, may cause unexpected drug-induced toxicity due to their enhanced systemic exposure.

Nakanishi et al, Current Drug Metabolism, 2015.
It is noteworthy to mention that cancerous patients mostly require multiple medications besides the anticancer agents. They include

- **Supportive care medications** used to alleviate the side effects of the particular anticancer drugs. Since many of these supportive drugs are either inhibitors or inducers of the CYP450, they could potentially affect the metabolism of co-administered TKIs
- **Complementary & alternative medicine (CAM)**
- **Anticancer medications or drugs used to treat underlying diseases.**
Administration of herbs with TKIs in some cancer cases

Herb-drug interactions with TKIs
Flavonoids are widely used as remedies because of their spasmolytic, antiphlogistic, antiallergic, and diuretic properties. **Flavonols and flavones are flavonoids of particular importance as they were found to contain antioxidant and free radical scavenging activity in foods.**

These molecules have been shown to possess numerous anti-inflammatory, antiangiogenic, and anti-carcinogenic effects in cell culture and in various animal models.
• Apigenin, to some extent, is a potent inhibitor of the cytochrome P450 (CYP) enzyme system which is responsible for the metabolism of considerable pharmaceutical drugs.

• Given the widespread availability of apigenin, it is important to understand what effects its concomitant use may have on the disposition of medications.

Concurrent administration of other drugs or herbal products that modulate cytochrome P450 enzymes activity may alter TKIs exposure. Therefore, a combination of flavonoids and selected TKIs is expected TO HAVE POTENT DRUG INTERACTIONS.

Review of the literature revealed that, to our knowledge, no method has been found so far dealing with the study of the effect of food on the PK of NER/PEL.

Thus, this work aims at the development and validation of a rapid and highly selective UPLC–MS/MS method for the determination of NER/PEL in rat plasma samples.

The validated method was successfully applied to PK interaction studies as a result of possible co-administration of API, along with NER/PEL, in the oncology practice.
UPLC–MS/MS analysis was performed using a Waters Model Xevo TQ-S separation system with a triple-quadrupole mass spectrometric detector. The instrument was controlled by the Masslynx™ Version 4.1 software, Micromass.

Chromatographic conditions

- **Acquity UPLC BEH™ C 18 column** (100 ×1.0 mm, i.d., 1.7 µm particle size) (Waters, Ireland).

- **The mobile phase** was composed of two solvent systems namely, the aqueous phase, A (0.1 % formic acid in water) and the organic modifier, B (0.1 % formic acid in acetonitrile). Isocratic elution was applied using a mobile phase of A: B (30: 70). **Column temperature** was maintained at 45 °C while the **auto-sampler temperature** was kept at 10 °C throughout the runtime (2 min.). The **flow rate** was 0.2 mL/min and the **injection volume** was 5 µL using partial loop mode.
**Mass spectrometric conditions**

- Electrospray ionization (EI) was operated in the positive ionization mode.
- Quantitation was performed using multiple reaction monitoring (MRM). Nitrogen was used as the desolvating gas at a flow rate of 800 L/h. Cone gas flow was adjusted at 150 L/h.

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### LC–MS/MS optimized parameters

<table>
<thead>
<tr>
<th>Target compound</th>
<th>Precursor ion [M+H]+</th>
<th>Daughter ion</th>
<th>Cone voltage (V)</th>
<th>Capillary voltage (KV)</th>
<th>Collision energy (eV)</th>
<th>Desolvation Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NER</td>
<td>557.30</td>
<td>112.05</td>
<td>90</td>
<td>4</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td>PEL</td>
<td>468.21</td>
<td>395.22</td>
<td>3</td>
<td>3</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td>DOM (IS)</td>
<td>426.27</td>
<td>175.18</td>
<td>30</td>
<td>3.5</td>
<td>30</td>
<td>200</td>
</tr>
</tbody>
</table>
Product ion spectra of the studied compounds

- **NER**
  - $m/z$ 557.30 $>$ 112.05

- **PEL**
  - $m/z$ 468.21 $>$ 395.22

- **DOM**
  - $m/z$ 426.27 $>$ 175.18
Optimization of sample clean-up and extraction procedure

Methanol was used for protein precipitation. The supernatant was purified by passing through a C18 Bond Elut cartridge used for solid phase extraction. The retained drugs were eluted with methanol and the eluate was evaporated to dryness. The residue was further reconstituted in acetonitrile then injected into the UPLC-MS/MS.
Multiple reaction monitoring (MRM) of a blank plasma, and a plasma sample spiked with a standard mixture of NER and PEL at their LLOQ levels.

Matrix-based calibration.....
- Linearity range (0.5-200 ng/mL)
- LLOQ 0.5 ng/mL
- LLOD 0.3 ng/mL

High method detectability, sensitivity
Concentration spiked (ng/ml) | Mean recovery (%) | $\pm$ RSD$^a$ | $E_r$(%)$^b$
--- | --- | --- | ---
**Extraction recovery** | | | |
NER | 0.5 | 93.01±5.85 | -6.99 |
 | 5 | 92.42±3.44 | -7.58 |
 | 50 | 93.25±6.75 | -6.75 |
 | 150 | 94.84±0.86 | -5.16 |
PELL | 0.5 | 90.99±8.87 | -9.01 |
 | 5 | 89.73±7.01 | -10.27 |
 | 50 | 92.89±4.88 | -7.11 |
 | 150 | 94.56±9.44 | -5.44 |
**Matrix effect** | | | |
NER | 0.5 | 94.21±3.63 | -5.79 |
 | 5 | 98.81±1.73 | -1.19 |
 | 50 | 97.32±1.56 | -2.68 |
 | 150 | 100.23±1.39 | 0.23 |
PELL | 0.5 | 98.50±0.64 | -1.50 |
 | 5 | 98.44±3.21 | -1.56 |
 | 50 | 99.69±2.86 | -0.31 |
 | 150 | 96.56±1.77 | -3.44 |

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<table>
<thead>
<tr>
<th>Stability</th>
<th>Concentration added (ng/mL)</th>
<th>Mean recovery (%)</th>
<th>$\pm$RSD$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auto-sampler stability (10°C, 56 h)</td>
<td>5</td>
<td>98.88±1.00</td>
<td>97.85±2.67</td>
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<tr>
<td>150</td>
<td>100.43±1.15</td>
<td>98.78±1.58</td>
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<tr>
<td>Short-term stability (25°C, 6 h)</td>
<td>5</td>
<td>98.83±3.92</td>
<td>99.04±2.24</td>
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<tr>
<td>150</td>
<td>97.75±1.16</td>
<td>99.47±1.16</td>
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<tr>
<td>Long-term stability (-30°C, 30 days)</td>
<td>5</td>
<td>95.42±3.13</td>
<td>97.68±7.26</td>
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<tr>
<td>150</td>
<td>96.30±0.93</td>
<td>97.17±0.80</td>
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<tr>
<td>Freeze-thaw stability (-30°C, 3 cycles)</td>
<td>5</td>
<td>98.95±0.63</td>
<td>100.06±3.15</td>
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<tr>
<td>150</td>
<td>97.22±1.75</td>
<td>101.25±2.24</td>
<td></td>
</tr>
<tr>
<td>Refrigerator (4°C, 3 months)</td>
<td>5</td>
<td>95.04±1.81</td>
<td>97.73±2.88</td>
</tr>
<tr>
<td>150</td>
<td>100.83±1.93</td>
<td>98.26±3.35</td>
<td></td>
</tr>
</tbody>
</table>

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$^a$ Mean recovery (%) $\pm$ RSD of six determinations

$^b$ Percentage relative error.
## Accuracy and Precision

<table>
<thead>
<tr>
<th>Concentration added (ng/mL)</th>
<th>Intra-day (n=6)</th>
<th>Inter-day (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean recovery (%) ± RSD</td>
<td>E, (%)</td>
</tr>
<tr>
<td>NER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>98.07±2.85</td>
<td>-1.93</td>
</tr>
<tr>
<td>5</td>
<td>98.26±4.99</td>
<td>-1.74</td>
</tr>
<tr>
<td>50</td>
<td>92.18±6.33</td>
<td>-7.82</td>
</tr>
<tr>
<td>150</td>
<td>100.72±1.56</td>
<td>0.72</td>
</tr>
</tbody>
</table>

| PEL                        |                |    |                        |      |
| 0.5                        | 97.44±4.84     | -2.56 | 101.34±2.70          | 1.34  |
| 5                          | 90.08±2.19     | -9.92 | 99.09±2.70           | -0.91 |
| 50                         | 91.08±5.60     | -8.92 | 94.18±6.10           | -5.82 |
| 150                        | 99.67±1.52     | -0.33 | 91.38±10.02          | -8.62 |

### Dilution integrity (n=6)

<table>
<thead>
<tr>
<th>Concentration added (ng/mL)</th>
<th>1:2</th>
<th>1:5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NER</td>
<td>300</td>
<td>98.38±2.67</td>
</tr>
<tr>
<td>PEL</td>
<td>300</td>
<td>99.49±1.62</td>
</tr>
</tbody>
</table>

QC samples: Error, RSD within **15%** for conc. other than LLOQ (**20%**)

Error, RSD within **15%** for conc. other than LLOQ (**20%**)

Method Validation
Blood samples (0.3 mL) from control and treated groups were collected from orbital plexus in EDTA-K2 tubes. Blood samples were collected at different time intervals (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 10.0, and 24.0 h) following drug administration, spiked with a constant volume of 0.1 mL of DOM, IS (5 ng/mL). Plasma samples were then treated by SPE then analyzed by UPLC-MS/MS.
Multiple reaction monitoring (MRM) of plasma sample of treated rats collected 3 h after the oral administration of a combination of **NER (30 mg/kg)** and **API (100 mg/kg)** and plasma sample of treated rats collected 4 h after the oral administration of a combination of **PEL (10 mg/kg)** and **API (100 mg/kg)**.
The concentration–time profile of the studied drugs in rats after oral administration of NER (30 mg/kg) or PEL (10 mg/kg), and API (100 mg/kg), compared with single administration of each drug alone at the same dose levels.

Thus API-induced increased bioavailability of NER/PEL could be attributed to the inhibitory effect of API on both CYP3A4 enzymes as well as P-gp efflux proteins.
In the present study, a **UPLC-MS/MS method** has been developed and validated for the quantification of the two irreversible TKIs, NER and PEL, in plasma samples. The proposed method has many advantages over the previously reported methods for the determination of either NER or PEL including higher sensitivity, smaller sample volume, and shorter analysis time.

The applicability of the developed SPE-UPLC-MS/MS method was extended to studying the **possibility of PK interaction between NER/PEL and the widely used flavonoid, API**. The results showed that API could affect the CYP-mediated metabolism of NER/PEL with increased drug plasma levels and enhanced drug-induced toxicity. **Thus TDM of these drugs**, when co-administered with API, is very important for the sake of public health.
Particular care should be paid with the intake of CAM medication with TKIs

Thus TDM of these drugs, when co-administered with API, is very important for the sake of public health.
Although this study was conducted in rats and not on humans and that differences in their PK pattern could exist, the possibility of occurrence of PK interactions between NER/PEL and API could exist when shifting to clinical studies.
Acknowledgements
This research project was supported by a grant from the “Research Center of the Center for Female Scientific and Medical Colleges,” Deanship of Scientific Research, King Saud University.


Thank you