Tyrosine kinase inhibitors quantification in human plasma

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Introduction

- Tyrosine kinase inhibitors (TKIs) are a class of targeted drugs with antiangiogenic and antitumor activities. They are increasingly used in the treatment of malignant pathologies. Due to inter-individual metabolic variability, an accurate therapeutic drug monitoring is a key element for the patient treatment. [1-3]. Three TKIs tested in clinical trial (namely sunitinib, axitinib and pazopanib) are under investigation.

- The objective of this research study is to bring a proof of concept of the use of metabolomics and lipidomics in predicting toxicity and efficacy of TKIs in patients with metastatic clear cell renal cell carcinoma. The future generated data will be correlated with pharmacokinetic values. In this frame, a rapid and unique UPLC method coupled to UV detection for quantification and identification of each compound was developed. A pretreatment step by Solid Phase Extraction (SPE) of plasma samples was also optimized.

Extraction step

Solid phase Extraction (SPE) pretreatment protocols

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCX Oasis® 96 well plates SPE (µSPE)</th>
<th>MCX Oasis® cartridges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioning</td>
<td>1 x 100 µl H₂O ml Q 100%</td>
<td>2 x 1 ml CH₃OH 100%</td>
</tr>
<tr>
<td>Equilibration</td>
<td>2 x 200 µl CH₃OH 100%</td>
<td>1 x 1 ml H₂O ml Q 100%</td>
</tr>
<tr>
<td>Loading sample</td>
<td>1 x 0.25 ml sample (100 µl plasma + 5 µl standard solution analyte = 900 µl H₂O ml Q + 20 µl H₂PO₄)</td>
<td>1 x 205 µl or 430 µl sample (100 or 200 µl plasma + 5 or 10 µl standard solution analyte = 100 or 200 µl H₂O ml Q + 20 µl H₂PO₄)</td>
</tr>
<tr>
<td>Washing</td>
<td>1 x 1 ml HCOOH 2%</td>
<td>2 x 1 ml HCOOH 2%</td>
</tr>
<tr>
<td>Eluting</td>
<td>1 x 1 ml ACN/ CH₃OH NH₃ (57:38.5) Nₑ evaporation Resublimed either in 100% H₂O for sunitinib/axitinib or in 80% CH₃OH · 20% DMSO for pazopanib</td>
<td>1 x 50 µl ACN/CH₃OH NH₃ (57:38.5) 25 or 50 µl HCOOH 4% added in plates No Nₑ evaporation time saving and losses risk decreasing</td>
</tr>
</tbody>
</table>

UPLC method development & results

UPLC conditions for the three compounds

- Column: BEH C18 1.7µm 2,1 x 50mm Waters®
- Mobile phase: Ammonium formate – acetonitrile gradient
- UV detection: DAD (Diode Array Detection)
- Flow rate: 0.4 ml/min
- Run time: 5 minutes

Typical UPLC chromatogram obtained for sunitinib and its metabolite (n-desethyl sunitinib)

- Preliminary to the validation protocol for the three TKIs compounds, ranitidine HCl has been introduced as Internal Standard (IS).

- The graph of the ratio IS peak area/analyte peak area as a function of analyte concentration highlights a satisfying correlation coefficient (R² = 0.9941) leading us to suppose a linear range between 50 - 250 ng/ml.

Conclusion & outlooks

- A common extraction step from human plasma using µSPE technology has been performed with high recovery values for sunitinib, axitinib and pazopanib.
- The reverse-phase UPLC-UV method allows quantification in the targeted concentration ranges:
  - 20 - 50 ng/ml : axitinib and sunitinib
  - 5 - 250 µg/ml : pazopanib
- Prior to routinely use the developed method, a validation is required. To reach this goal an internal standard has been introduced and the response function studied.
- Once validated, this method will provide accurate plasma concentration and help physician to adapt the best posology of the relevant TKI for the patients.
- A complete validation protocol will be performed via the accuracy profile approach.