Sensitive quantification of free lenvatinib using ultra-high performance liquid chromatography coupled to tandem mass spectrometry and assessment for clinical application. Sueshige Y[1], Shiraiwa K[1], Tanaka R[1], Saito T[2], Iwao M[2], Arakawa M[2], Endo M[2], Tatsuta R[1], Murakami K[2], Itoh H[1]. [1]Department of Clinical Pharmacy, Oita University Hospital, Yufu, Oita, Japan; [2]Department of Gastroenterology, Oita University Faculty of Medicine, Yufu, Oita, Japan

Background: The therapeutic effects and adverse effects of drugs are associated with the distribution of the free drug (unbound to plasma proteins) to tissues and organs. For drugs with high protein binding rate, the pharmacokinetics of the free drugs may vary depending on the patient's clinical conditions and drug-drug interactions. Although several reports demonstrated the usefulness of measuring plasma lenvatinib concentrations, these studies measured only total lenvatinib concentrations. For drugs with high protein binding rates, such as lenvatinib, measuring free rather than total drug concentration may be more clinically useful. In this study, we aimed to develop a highly sensitive quantification method for free lenvatinib concentration in human plasma using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) and to evaluate the developed method for clinical applicability.

Methods: Plasma sample was ultrafiltrated using Centrifree® ultrafiltration device. Ultrafiltrated plasma containing free lenvatinib was loaded into a conditioned and equilibrated Oasis® MCX μElution plate for solid phase extraction. Free lenvatinib was analyzed with a UHPLC-MS/MS system using lenvatinib-d_4 as internal standard. UHPLC separation used an Acquity UPLC® BEH C18 column (2.1 × 50 mm, 1.7 μm) and a mobile phase gradient composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, with a run time of 6 minutes. MS analysis was done in positive electrospray ionization mode. Full validation was performed according to FDA guidance.

Results: The calibration curve for free lenvatinib was linear over the 20-4000 pg/mL concentration range. The lower limit of quantification for free lenvatinib was 20 pg/mL. The average recovery rate was 102.4%. Average precision was below 8.9% CV (coefficient of variation), and accuracy was within 12.0% for all the quality control levels. Matrix effect was higher than 31.0%. This assay was successfully applied to measure free lenvatinib concentrations in patients with hepatocellular carcinoma after administration of lenvatinib.

Conclusions: We have developed a high throughput UHPLC-MS/MS method for quantification of free lenvatinib in human plasma. This method can be applied to measure free lenvatinib concentrations in patients receiving lenvatinib in the clinical setting.