Ultra-sensitive quantification of drugs and endogenous substances in human plasma using UPLC-MS/MS

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Mass spectrometry-based assays have become a powerful tool for the quantification of drugs and endogenous substances in biological samples. Especially, ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) achieves better sensitivity and selectivity with shorter run time and sharper peak. UPLC-MS/MS assays that allow ultra-sensitive quantification have been applied to innovative studies such as microdosing and quantification of endogenous substances in the lower picomolar range.

Chlorzoxazone (CZZ), a centrally acting muscle relaxant, is a well-established model compound for cytochrome P450 (CYP)2E1 phenotyping because it is mainly metabolized to 6-hydroxychlorzoxazone (6-OH-CZZ) by CYP2E1. We developed and validated an ultra-sensitive assay for the quantification of CZZ and 6-OH-CZZ in human plasma using UPLC-MS/MS, and applied this technique to a CZZ microdosing study. The assay fulfilled the requirements of the US Food and Drug Administration (FDA) guidelines for assay validation, with a lower limit of quantification of 2.5 pg/mL for both CZZ and 6-OH-CZZ. The assay was successfully applied to assess the pharmacokinetics of CZZ after administration of single oral doses (2.5–5000 μg) in two human volunteers. A minimum dose of 250 μg of CZZ allowed determination of plasma concentrations of CZZ and 6-OH-CZZ until 8 hours after administration.

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide and has been implicated in the development of vascular disease, pulmonary hypertension, heart failure, and renal disease. In patients with pulmonary arterial hypertension, plasma ET-1 is elevated and correlates with disease severity. Plasma ET-1 concentrations in humans are reported to be in the lower picomolar range. Thus, plasma ET-1 concentrations are typically quantified by immunoassays with high sensitivity, such as enzyme-linked immuno-sorbent assays (ELISA) and radioimmunoassays. ELISA is a popular method for quantification of endogenous substances but has a disadvantage of possible cross-reactivity. We developed and validated an ultra-sensitive and selective UPLC-MS/MS assay for the quantification of ET-1 in human plasma. The assay fulfilled the requirements of the FDA guidelines for assay validation, with a lower limit of quantification of 1.5 pg/mL for ET-1. The assay was successfully used to monitor the

time course of plasma ET-1 concentrations in two human volunteers after co-administration of bosentan and clarithromycin. In this trial, the concentrations measured by UPLC-MS/MS were slightly lower than those measured by ELISA, with a strong positive correlation between the two methods. The UPLC-MS/MS method can be applied clinically and may have better selectivity for ET-1 than ELISA.