

DP-028

CLINICAL APPLICATION FOR DETERMINATION OF DARUNAVIR USING VERY LIMITED VOLUME OF PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

Daisuke Nagano^{1,2}, Takuya Araki¹, Tomonori Nakamura^{1,2} and Koujiro Yamamoto^{1,2}

¹Department of Clinical Pharmacology, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi 371-8511, Japan

²Department of Pharmacy, Gunma University Hospital, 3-39-15 Showa-machi, Maebashi 371-8511, Japan

E-mail: dnagano@gunma-u.ac.jp

ABSTRACT

【Purpose】 Darunavir (DRV) is a new protease inhibitor and used for the treatment of human immunodeficiency virus (HIV) type-1. Therapeutic drug monitoring of DRV is an important tool to obtain a reliable efficacy of DRV and to avoid its adverse effects such as hepatic disorder. To develop a less-invasive monitoring method, we established a high sensitive analysis of DRV, in very limited volume of plasma by HPLC with fluorescence detection. 【Methods】 Twenty microliter of IS (voriconazole) dissolved by 50% methanol, 140 μ L of saline and 2.0 mL of ethyl acetate were added to 20 μ L of plasma. The mixture was centrifuged and the upper organic layer was aspirated and dried up. The residue was dissolved in 200 μ L of 50% methanol, and 25 μ L of the solution was injected to HPLC. The mobile phase consisted of the mixture of 25 mM Na₂PO₃ / acetonitrile (57/43, v/v) with a flow rate of 1.0 mL/min. The column was YMC-Pack Pro C₁₈ column. The peaks of DRV and IS were detected by fluorescence detector at 235 nm (excitation) and 337 nm (emission). 【Results and Discussion】 Good linearity ($R^2 = 0.999$) was obtained over the range from 0.5 to 10 μ g/mL. The intra-assay precision and accuracy varied between 1.9–4.3% and -14.5–1.5%, respectively. We believe that this method reduces the damage of patients invasion in blood sampling and is clinically useful for the treat of HIV infection.

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