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

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**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  $\mu$ L of saline and 2.0 mL of ethyl acetate were added to 20  $\mu$ L of plasma. The mixture was centrifuged and the upper organic layer was aspirated and dried up. The residue was dissolved in 200  $\mu$ L of 50% methanol, and 25  $\mu$ L of the solution was injected to HPLC. The mobile phase consisted of the mixture of 25 mM Na<sub>2</sub>PO<sub>3</sub> / acetonitrile (57/43, v/v) with a flow rate of 1.0 mL/min. The column was YMC-Pack Pro C<sub>18</sub> 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  $\mu$ 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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