

# **Simultaneous Determination of Caffeic Acid, Ferulic Acid and Isoferulic Acid in Rabbit Plasma by High Performance Liquid Chromatography**

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## **Abstract**

A simple and sensitive high performance liquid chromatographic method with reverse-phase column for the simultaneous quantification of caffeic acid, ferulic acid, and isoferulic acid in rabbit plasma was developed. An ODS column (150 mm × 4.6 mm I.D., 5 µm) was used as the stationary phase and the mobile phase consisted of acetonitrile/glacial acetic acid/water (15:0.5:85, v/v, pH adjusted to 4.5). Flow-rate was 1.0 mL/min and UV absorbance was set at 321 nm. One hundred microliter of plasma was used to simultaneously measure the concentrations of caffeic acid, ferulic acid, and isoferulic acid. After a direct clean-up procedure with 10% of trifluoroacetic acid, the lower limits of quantification were 0.1 µg/mL and the standard curves were found to be linear over the concentration ranges of 0.1-100 µg/mL for caffeic acid, ferulic acid, and isoferulic acid. The average recoveries for caffeic acid, ferulic acid, and isoferulic acid were 96.2%, 98.0%, and 98.3%, respectively. The results for all compounds also showed good stability after three freeze/thaw cycles and storage at ambient temperature and 4°C for 24 hr. The assay method was successfully applied to the pharmacokinetic study of caffeic acid, ferulic acid, and isoferulic acid after an intravenous administration of caffeic acid to rabbits.