



Simultaneous Determination of Cortisone and Cortisol in Serum by HPLC-DAD and Application for Pharmacokinetics

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SUMMARY. To develop a high performance liquid chromatography method for the simultaneous determination of cortisone and cortisol in rat serum and apply it for pharmacokinetics. After addition of pirfenidone as internal standard (IS), a liquid-liquid extraction with ethylacetate was employed for the sample preparation. Samples were separated on Zorbax SB-C18 column at 25 °C using mobile phase consisting of acetonitrile–water–0.1 % trifluoroacetic acid with flow rate of 0.9 mL/min, utilizing DAD detection at 246 nm. Excellent liner relationships of the cortisone and cortisol concentrations were obtained from 50 to 6000 ng/mL, with $r = 0.9997, 0.9999$ respectively, and the lower limit of quantitation (LLOQ) were both 50 ng/mL. The developed method was successfully applied to pharmacokinetic studies of cortisone and cortisol in rats following single dose of 20 mg/kg via intraperitoneal injection.

KEY WORDS: Cortisone, Cortisol, HPLC, Pharmacokinetics, Serum.

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