Clinical pharmacokinetic studies of ciprofloxacin require accurate and precise measurement of plasma drug concentrations. We describe a rapid, selective and sensitive HPLC method coupled with fluorescence detection for determination of ciprofloxacin in human plasma. Internal standard (IS; sarafloxacin) was added to plasma aliquots (200 ε L) prior to protein precipitation with acetonitrile. Ciprofloxacin and IS were eluted on a Synergi Max-RP analytical column (150 mm × 4.6 mm i.d., 5 ε m particle size) maintained at 40 °C. The mobile phase comprised a mixture of aqueous orthophosphoric acid (0.025 M)/methanol/acetonitrile (75/13/12%, v/v/v); the pH was adjusted to 3.0 with triethylamine. A fluorescence detector (excitation/emission wavelength of 278/450 nm) was used. Retention times for ciprofloxacin and IS were approximately 3.6 and 7.0 min, respectively. Calibration curves of ciprofloxacin were linear over the concentration range of 0.02ï 4 ε g/mL, with correlation coefficients (r2) Ó0.998. Intra- and inter-assay relative standard deviations (SD) were <8.0% and accuracy values ranged from 93% to 105% for quality control samples (0.2, 1.8 and 3.6 ε g/mL). The mean (SD) extraction recoveries for ciprofloxacin from spiked plasma at 0.08, 1.8 and 3.6 ε g/mL were 72.8 ± 12.5% (n = 5), 83.5 ± 5.2% and 77.7 ± 2.0%, respectively (n = 8 in both cases). The recovery for IS was 94.5 ± 7.9% (n = 15). The limits of detection and quantification were 10 ng/mL and 20 ng/mL, respectively. Ciprofloxacin was stable in plasma for at least one month when stored at ī 15 °C to ī 25 °C and ī 70 °C to ī 90 °C. This method was successfully applied to measure plasma ciprofloxacin concentrations in a population pharmacokinetics study of ciprofloxacin in malnourished children.