Extremely Simple and Rapid HPLC Analysis of Tocilizumab in Human Serum with Selective Precipitation Using Alkylamine

Abstract

An assay using HPLC with fluorescence (FL) detection method for monitoring native FL of tocilizumab (TCZ) in human serum combined with extremely simple and rapid pretreatment without any antigenantibody reaction was developed. Good separation of TCZ was achieved within 13 min on a Presto FF-C18 column (100×4.6 mm i.d., 2 μm). Simple pretreatment with acetonitrile containing primary and secondary alkylamines having longer than C3 in the alkyl chain removed immunoglobulin G subclass 1 and TCZ could be recovered selectively. The spiked calibration curve of TCZ in human serum showed good linearity in the range of 40–1000 μg/mL ($r > 0.997$). The lower limit of quantitation (S/N=10) of the TCZ was 19.7 μg/mL. The accuracy was within 103.5–114.9%, and the intra- and inter-day precisions as relative standard deviations were less than 5.3 and 7.8% (n=5), respectively. The recovery of TCZ was 42.2±3.4% (n=3). The TCZ in pretreated sample was confirmed to be stable for 6 h (>95%) at room temperature and 24 h (>95%) at 4 ℃. The proposed method is considered extremely superior to the previous methods in terms of time requirement for analysis. Therefore, the developed method may be more useful than conventional methods in urgent situations, such as confirming therapeutic efficacy of cytokine-release syndrome by 2019 coronavirus disease.