Comparing the present method to other reported assays Recently, Alvarez et al. developed an HPLC–MS/MS method and applied it to assess a patient who was intravenously administered remdesivir.This reliable method will provide sufficient refer– ence and mirror for pharmacokinetics and clinical efficacy in patients with COVID–19A comparison of the method reveals several advantages of the present HPLC–MS/MS method, such as simple plasma preparation, satisfactory matrix effect and recovery, detailed pharmacokinetic application, and ro– bustness owing to measuring the uncertainty and incurred sample reanalysis (Table 5).Worsening of the COVID–19 pan– demic has necessitated the development of an HPLC–MS/ MS method that can accurately quantitate Nuc to provide plasma exposure information, because obtaining this informa– tion has become a bottleneck in the clinical treatment of COVID–19.The optimized HPLC–MS/MS method reported here offers significant advantages including cost–effective, high–throughput sample preparation (protein precipitation ex– traction), and requiring only small volume of biological ma– trix (20 uL).Conclusions A high–throughput, robust, sensitive, and reproducible HPLC–MS/MS method was developed and fully validated for the quantitation of Nuc, the active metabolite of remdesivir, in plasma.Moreover, detailed recovery, uncertainty of measurement, and incurred sample reanalysis were not determined.Furthermore, this method meets the requirements for extraction recovery and matrix effects.This quantitative method was also successfully applied in a rat pharmacokinet– ics study.However, the re– quired plasma volume was 50 uL and matrix effects were not reported.