The present work describes a rapid and sensitive advance liquid chromatographic technique, ultra high-pressure liquid chromatography (UHPLC) method with UV detection to quantify antiviral drug ganciclovir (GCV) in rabbit aqueous humor. After deproteinisation with acetonitrile, gradient separation of GCV was achieved on a Waters Acquity BEH C18 (50 mm x 2.1 mm, 1.7 µm) column at 50°C. The mobile phase consisted of 0.1% trifluoroacetic acid in water (pH 3.5) and acetonitrile (95:5, v/v) at a flow rate of 0.45 mL/min. GCV analysis was performed at a wavelength of 254 nm with total run time of 3 min. Method was found to be selective, linear ($r^2 = 0.999$), accurate (recovery, 97.0–100.2%) and precise (CV, ≤ 3.1%) in the selected concentration range of 0.1–1.0 µg/mL. Detection and quantitation limit of GCV in aqueous humor were 3.0 and 10.0 ng/mL, respectively. The method was applied to compare aqueous humor levels of GCV after single topical instillation of GCV solution, GCV nanoparticles, GCV nanocomplexes and GCV niosomal dispersions. Topical instillation of GCV-NCs (AUC$_{0\rightarrow t}$, 3440.7±26.2 ng.hr/mL) and GCV-NDs (AUC$_{0\rightarrow t}$, 3380.5±29.3 ng.hr/mL) provided approximately 5 fold increase in the relative ocular bioavailability compared with GCV solution (AUC$_{0\rightarrow t}$, 650.8±14.9 ng.hr/mL) and nearly 2.5 fold higher than the GCV-NPs (AUC$_{0\rightarrow t}$, 1350.2±18.5 ng.hr/mL). The results indicate that the nanocomplexes and niosomal dispersions increases ocular bioavailability of GCV and prolong its residence time in the eye.