

**Biodegradable Polymeric Nano formulation of Acyclovir and Acyclovir prodrug for Enhanced Therapeutic Efficacy Against Herpes Simplex Virus Infections**

**Sushruta S Hakkimane<sup>1#</sup>, Divakarareddy Vemanna Paladugulu<sup>2#</sup>, Santosh L Gaonkar<sup>3</sup>, Kavitha Karunakaran<sup>4</sup>, Piya Paul Mudgal<sup>4</sup>, Yogeesha N Nayak<sup>3</sup>, Bharath Raja Guru<sup>2\*</sup>**

Department of Biotechnology, Manipal Institute of Technology Bengaluru, Manipal Academy of Higher Education Manipal, India

Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education Manipal, India

Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education Manipal, India

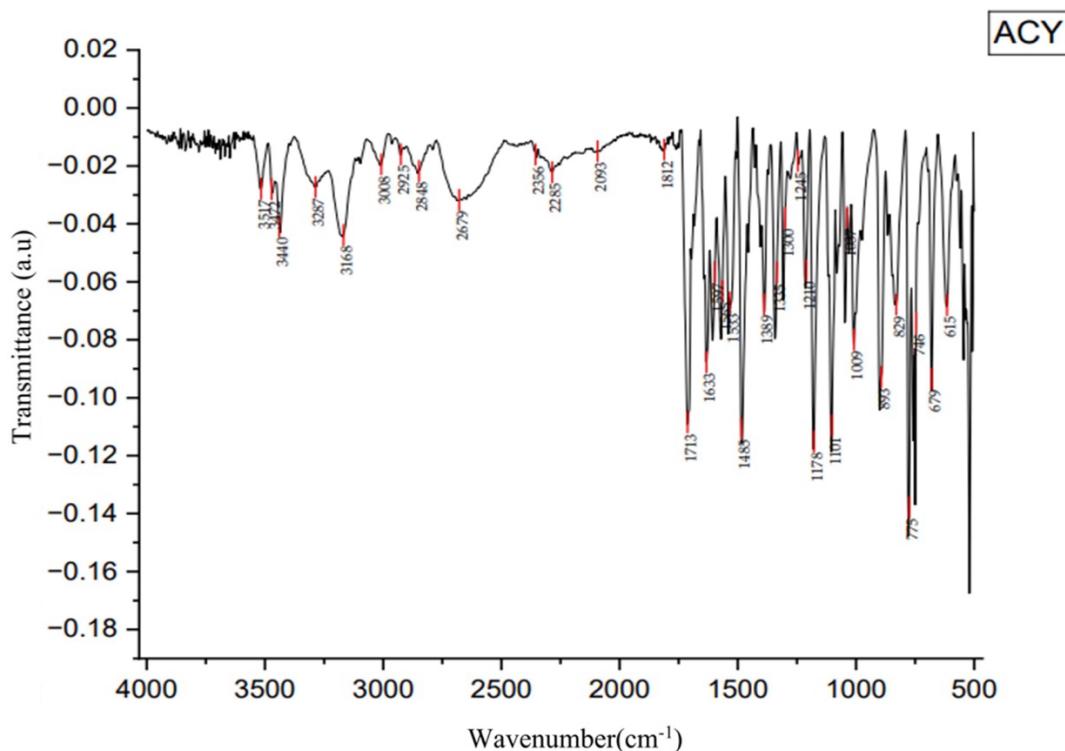
Manipal Institute of Virology, Manipal Academy of Higher Education Manipal, India

# Authors contributed equally

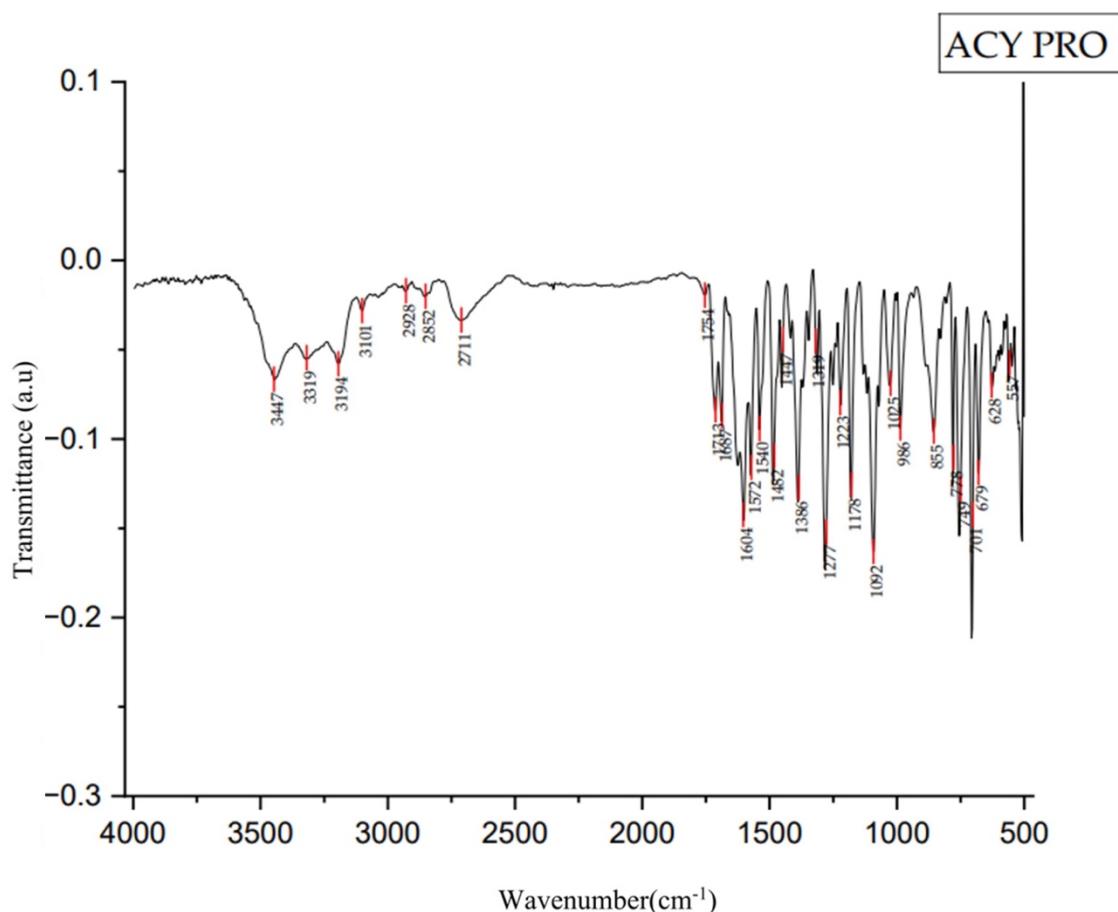
\*Corresponding author

Bharath Raja Guru

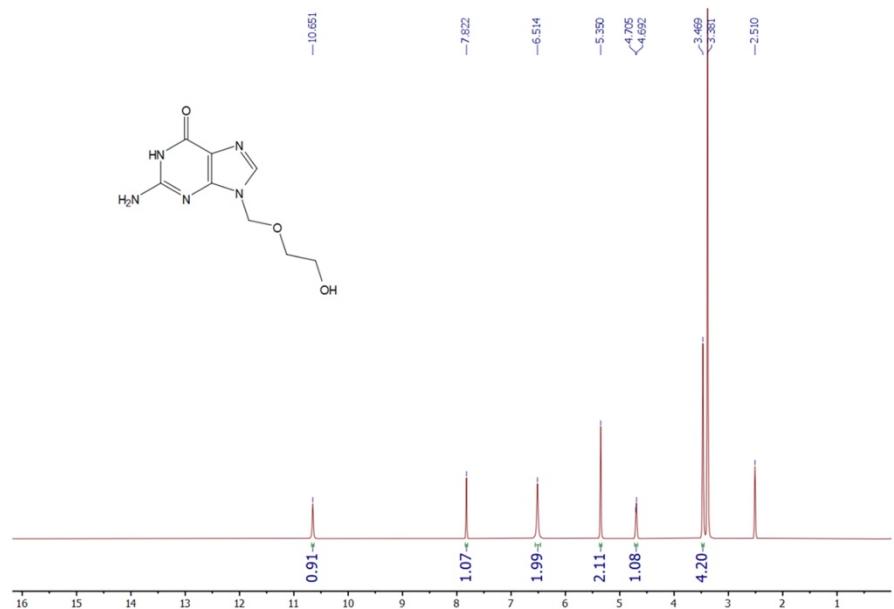
Bharath.guru@manipal.edu



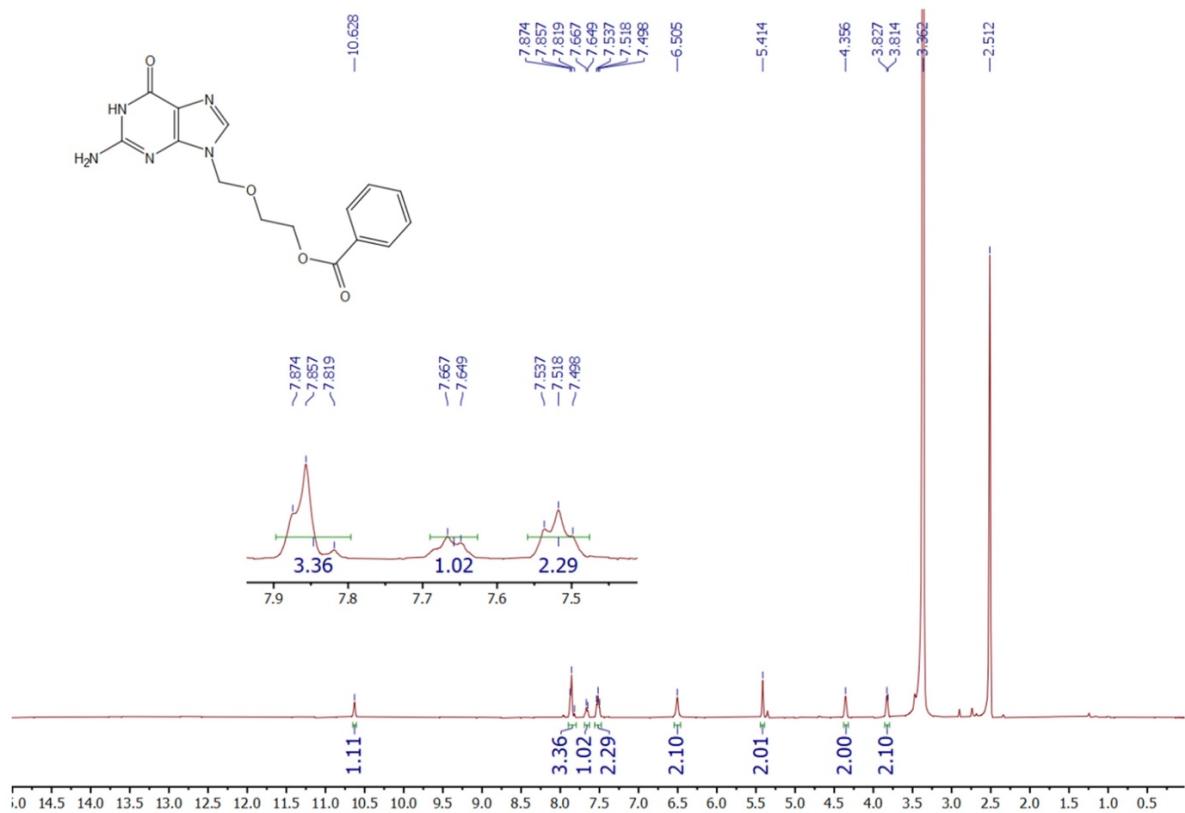
**Supplementary Fig. S1:** FTIR Spectra of ACY



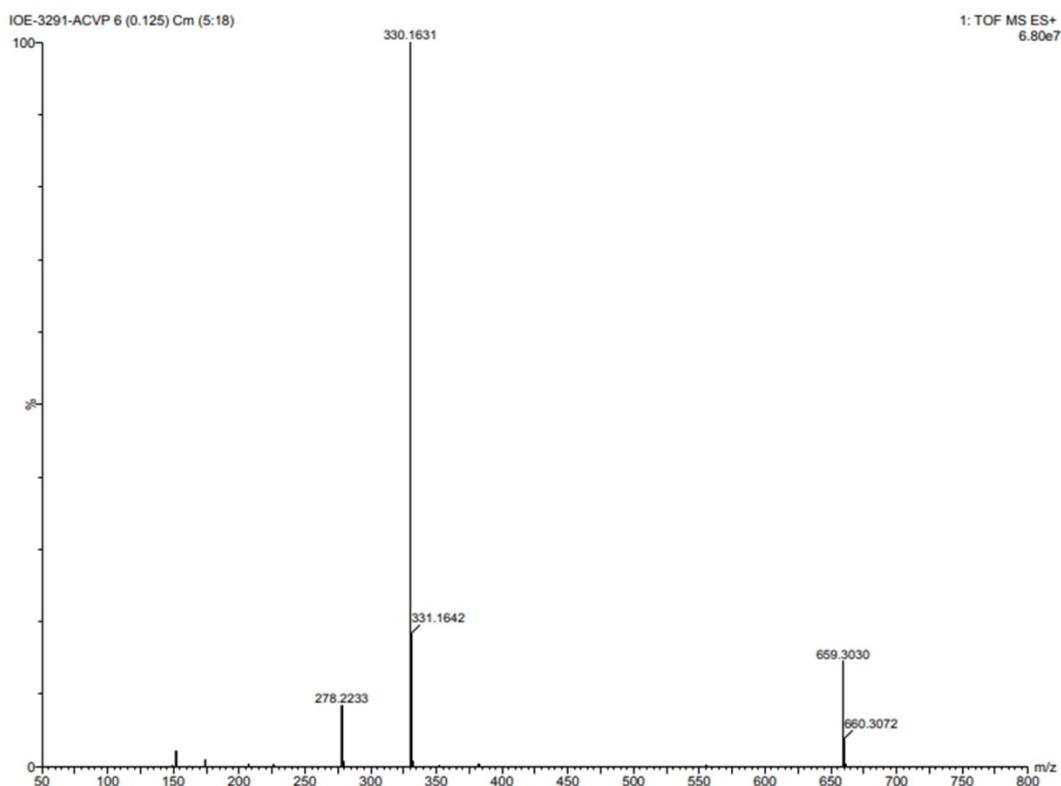
**Supplementary Fig. S2:** FTIR Spectra of ACPD



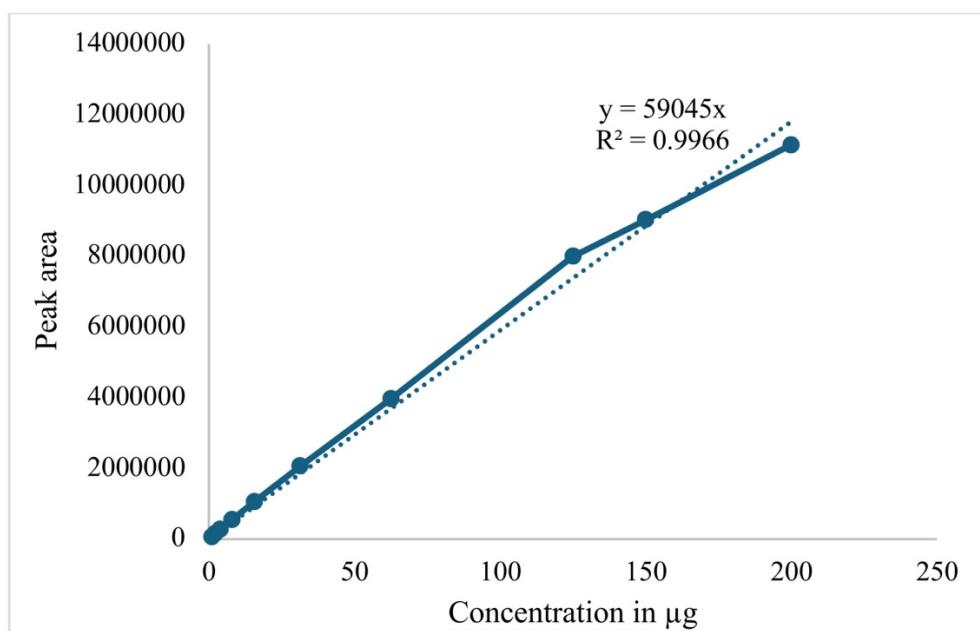
**Supplementary Fig. S3:** <sup>1</sup>H-NMR spectra of ACY



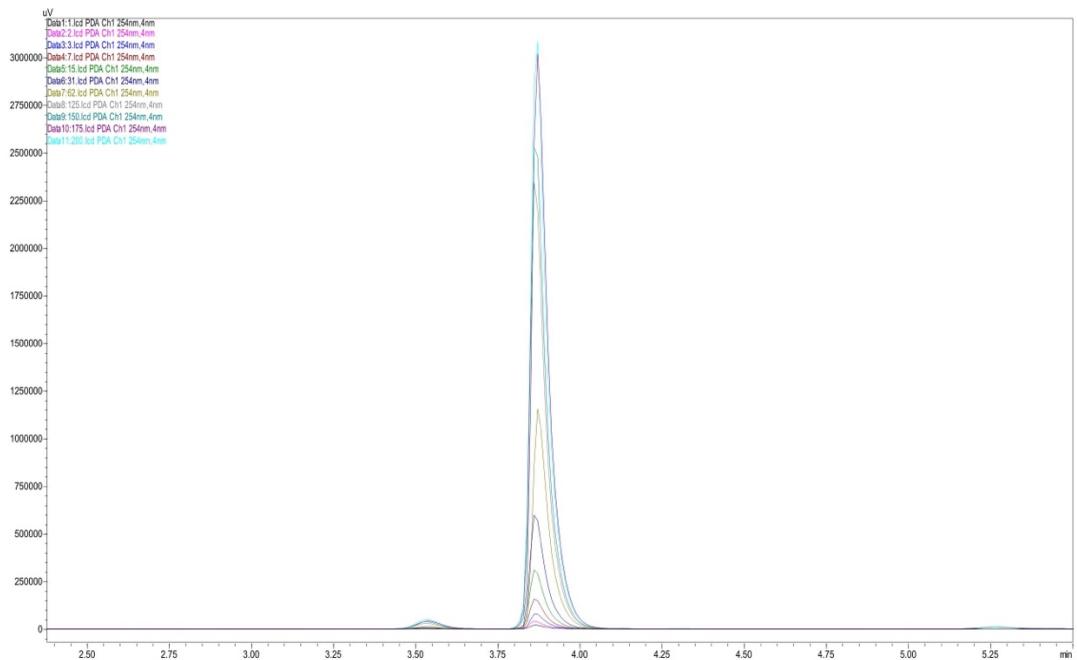
**Supplementary Fig. S4:** <sup>1</sup>H-NMR spectra of ACPD



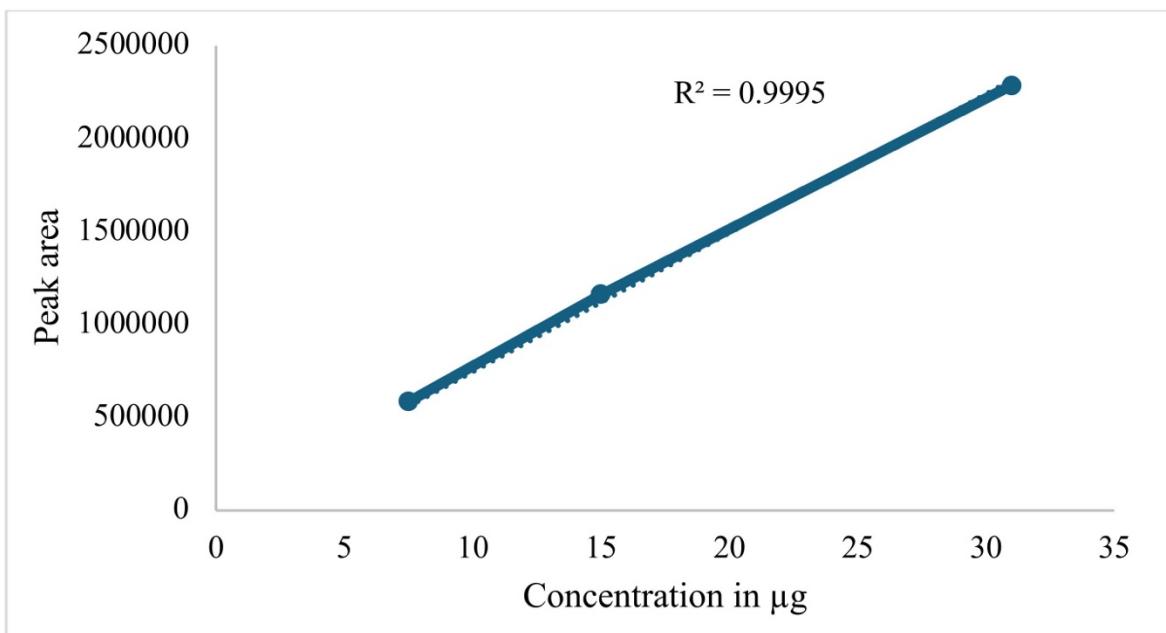
Supplementary Fig. S5: Mass spectra of ACPD



Supplementary Fig. S6: Calibration plot of ACY (0.976-200 $\mu\text{g}$ )



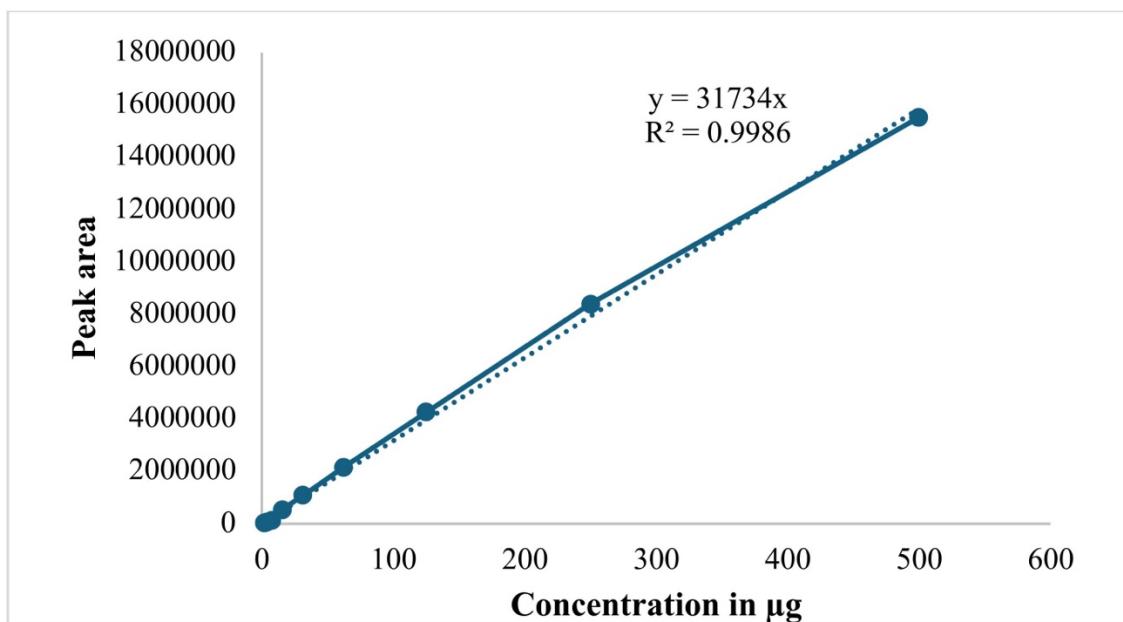
**Supplementary Fig. S7:** Graph of ACY (chromatogram stacked)

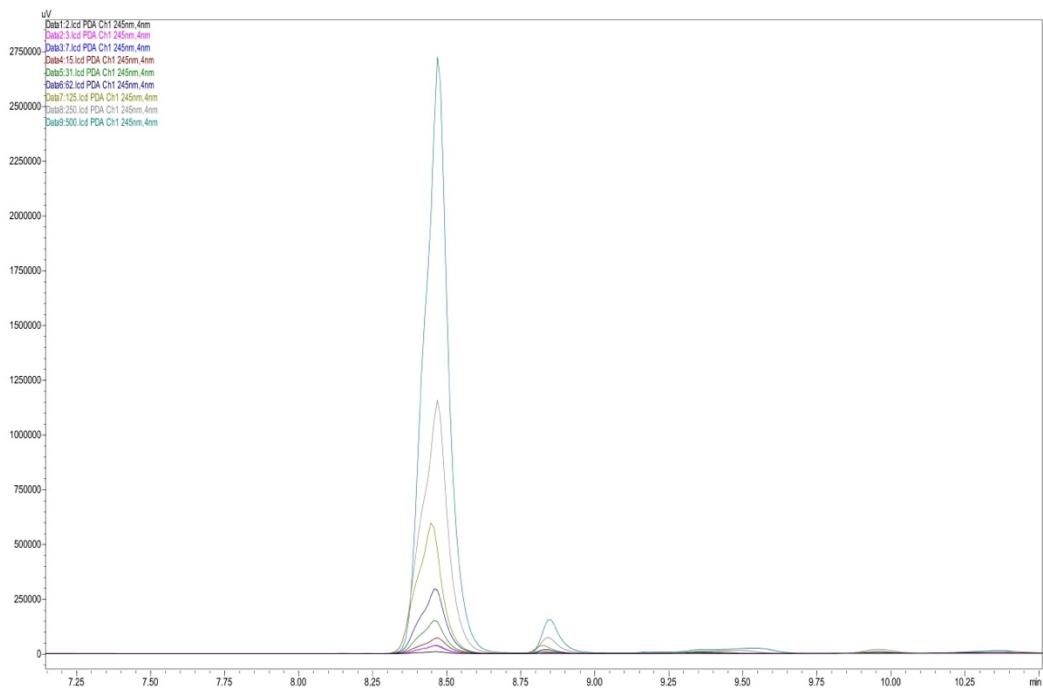


**Supplementary Fig. S8:** Precision plot Day 1

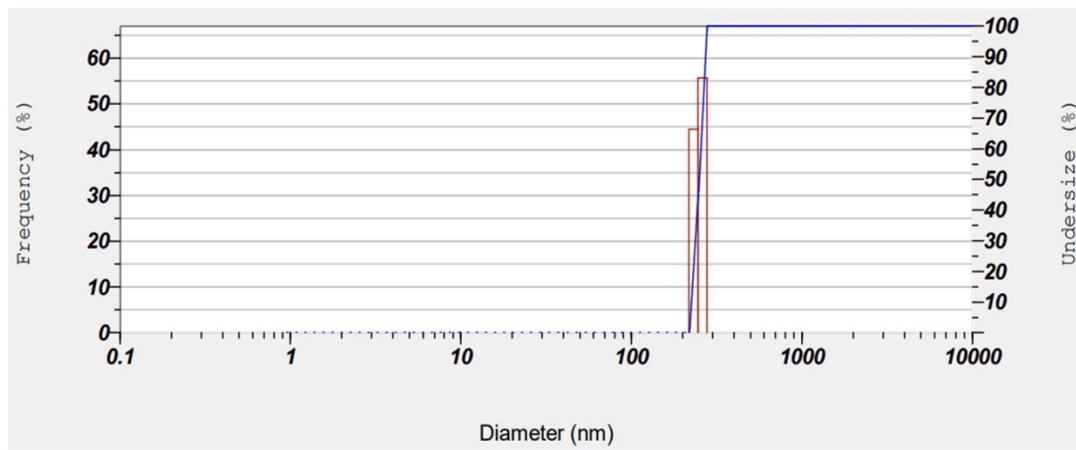
**Supplementary Table S1:** Precision Readings of 3 different concentrations Day 1

Analyte Level Concentration (ug/ml)	Trial 1	Mean	RSD	% RSD
7.81	585061	584366	1398.63	0.239
7.81	585281			
7.81	582756			
15.62	1163618	1163262	885.41	0.076
15.62	1162254			
15.62	1163914			
31.25	2287032	2287239	2039.91	0.089
31.25	2285311			
31.25	2289375			

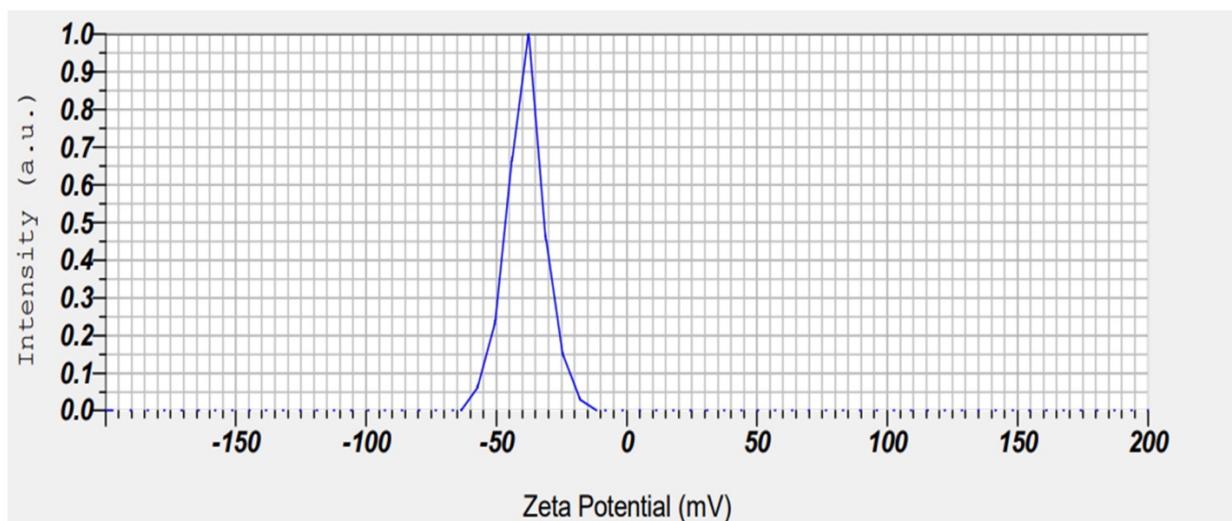
**Supplementary Fig. S9:** Calibration plot of ACPD (0.976-500 $\mu\text{g}$ )



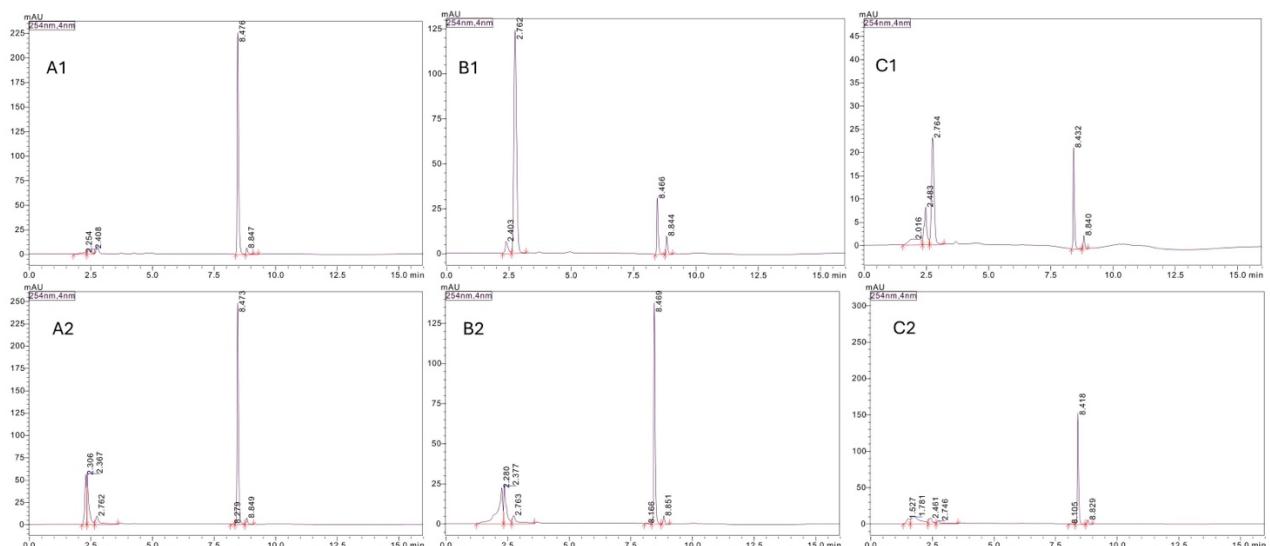
**Supplementary Fig. S10:** Stacked chromatogram of ACPD



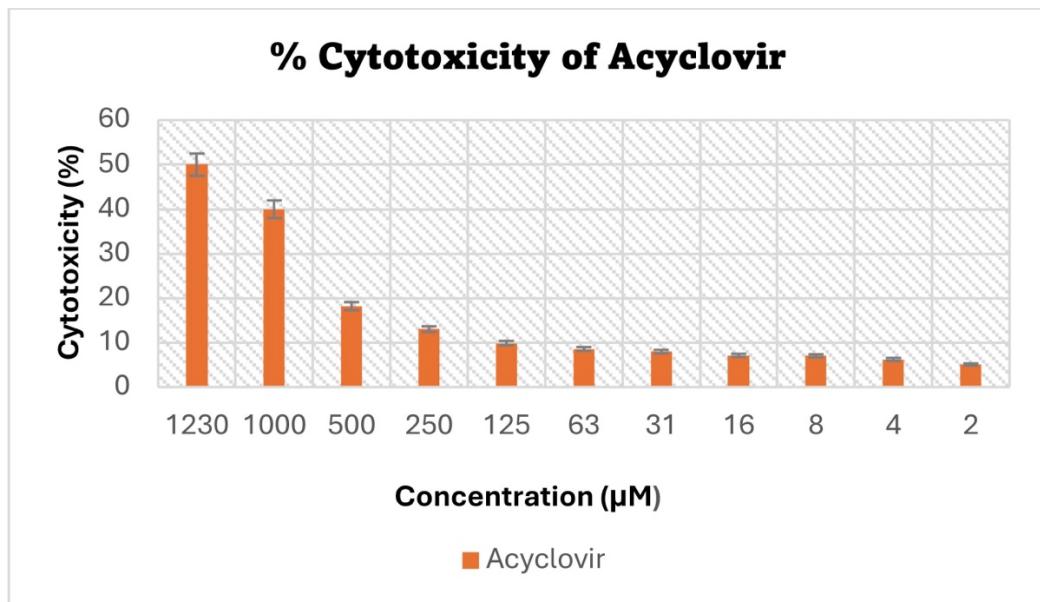
**Supplementary Fig. S11:** Particle size distribution graph of prepared plain PLGA nanoformulation



**Supplementary Fig. S12:** Zeta graph of prepared plain PLGA nanoformulation showing negative surface charge



**Supplementary Fig. S13:** RP-HPLC chromatograms show converted ACY (RT 2.7 min) from ACPD NPs in *in vitro* release studies: A1) 2 hours at pH 4.5, A2) 2 hours at pH 7.4, B1) Day 14 at pH 4.5, B2) Day 14 at pH 7.4, C1) Day 28 at pH 4.5, and C2) Day 28 at pH 7.4



**Supplementary Fig. S14:** Graph showing cytotoxicity of Acyclovir at different concentrations



**Supplementary Fig. S15:** Chromatogram showing breakdown of ACPD to ACY