Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Co-delivery of hesperetin enhanced bicalutamide induced apoptosis by exploiting mitochondrial membrane potential via polymeric nanoparticles in PC-3 cell line

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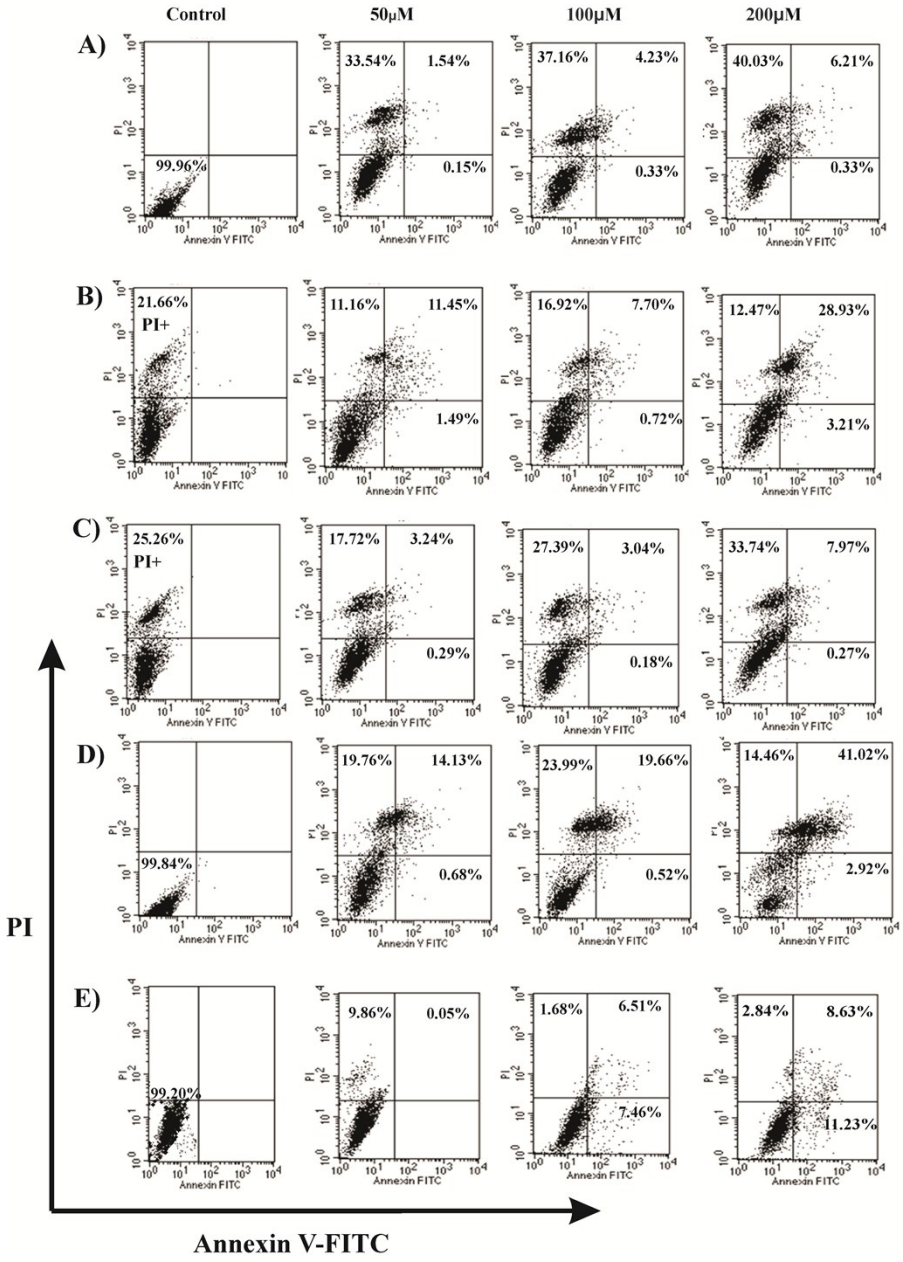


Figure S1. Dot plot representing levels of apoptosis after treatment with A) BCT, B) HSP, C) BCT plus HSP, D) PCL-BCT-HSP-CS NPs, and E) Blank NPs when treated with 50μM, 100μM and 200μM. Cell apoptosis analysis was performed after dual staining with PI (vertical axis) and annexin V-FITC (horizontal axis).

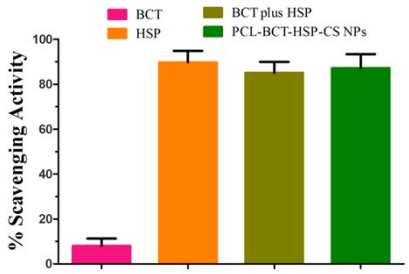


Figure S2. % Scavenging Activity of BCT, HSP, BCT plus HSP (1:1) and PCL-BCT-HSP-CS NPs. Data represented as Mean ± SD (n=3).

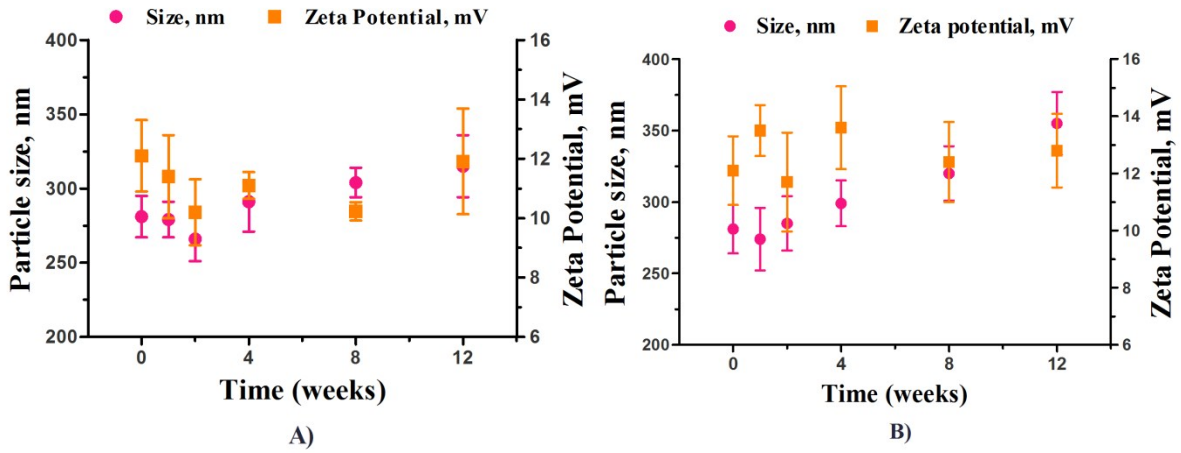
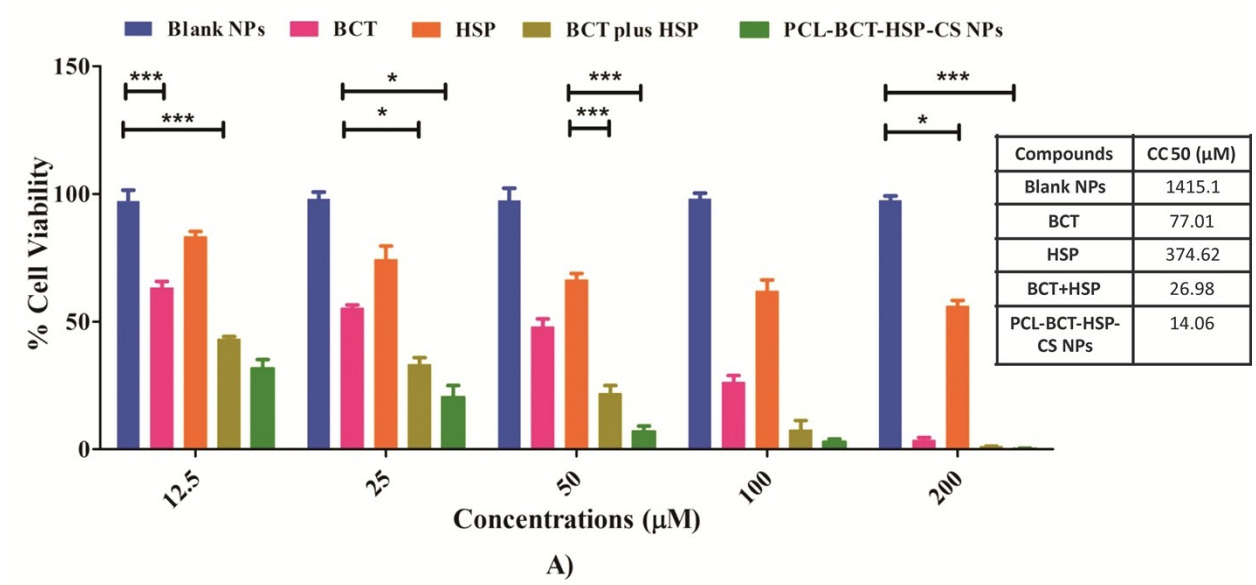


Figure S3. Stability Study of PCL-BCT-HSP-CS NPs at A) 4 ^{o}C, and B) 25 ^{o}C for 3 months. Data represented as Mean ± SD (n=3).



Compounds	CC50 (µM)
Blank NPs	1415.1
BCT	77.01
HSP	374.62
BCT+HSP	26.98
PCL-BCT-HSP-CS NPs	14.06

Figure S4. Representative percent cell viability of PC-3 cells after incubation for 48hr with BCT, HSP, BCT plus HSP, PCL-BCT-HSP-CS NPs, and Blank NPs when treated with 50µM, 100µM and 200µM. Inset is the CC_{50} value of the respective treatment.

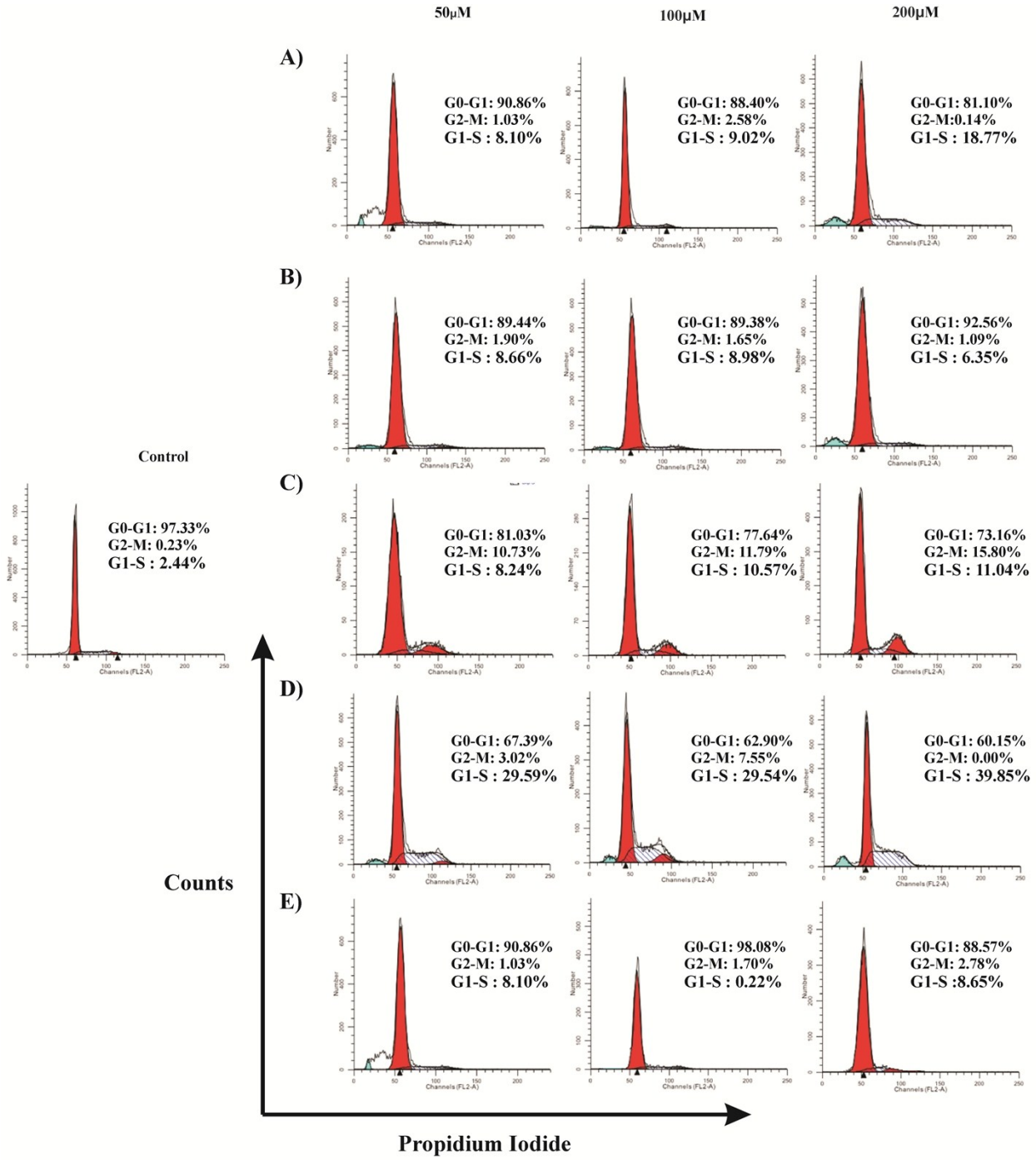


Figure S5. Representative cell cycle distribution in PC-3 cells after incubation with A) BCT, B) HSP, C) BCT plus HSP, D) PCL-BCT-HSP-CS NPs, and E) Blank NPs when treated with 50µM, 100µM and 200µM. Analysis was performed using flow cytometry and each figure represents the population present in G0-G1, G1-S and G2-M phase.

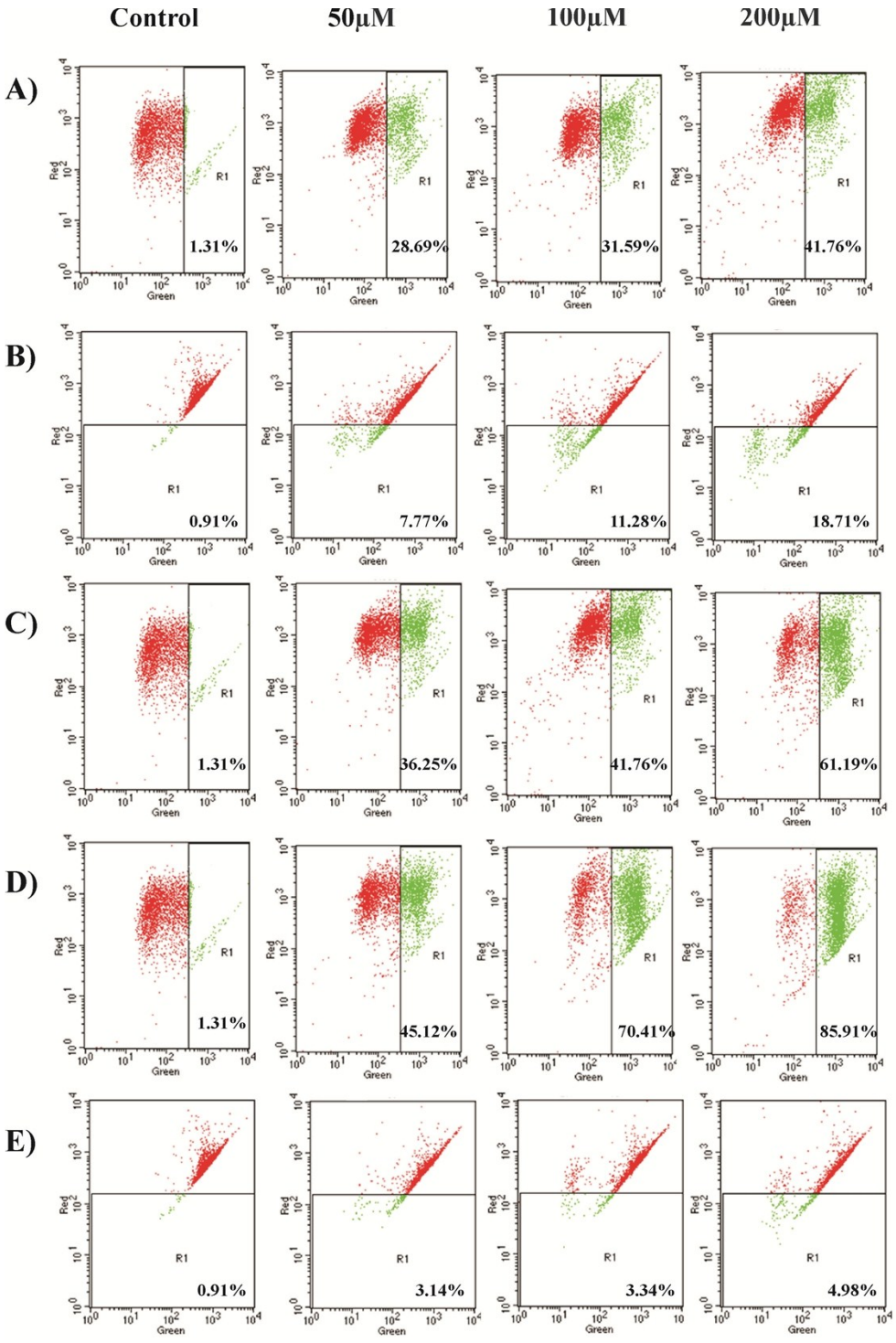


Figure S6. Representative dot plot of disruption of mitochondrial potential generated after incubation with A) BCT, B) HSP, C) BCT plus HSP, D) PCL-BCT-HSP-CS NPs, and E) Blank NPs when treated with 50µM, 100µM and 200µM. The red color in quadrant of the dot plot symbolizes the cells with polarized mitochondria whereas green color in quadrant showed percent cell population with depolarized mitochondria.

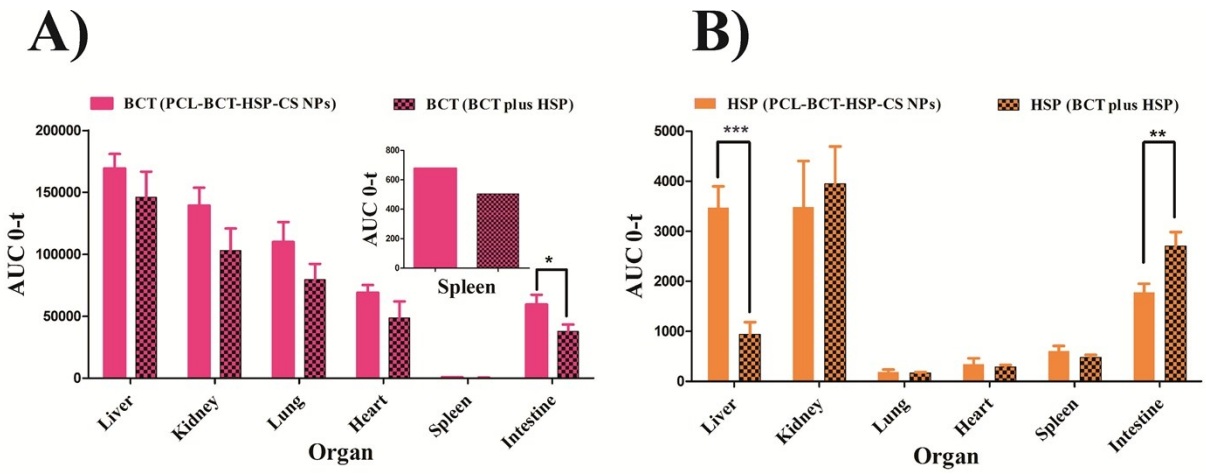


Figure S7. Representative AUC $_{0\text{-}t}$ of; **(A)** BCT and inset is the enlarged portion $AUC_{0\text{-}t}$ of Spleen, and **(B)** HSP from various organs from PCL-BCT-HSP-CS NPs at 20mg/kg in SD male rats compared with equivalent dose of BCT plus HSP aqueous suspension after oral administration.. Data represented as Mean ± SEM (n=3).

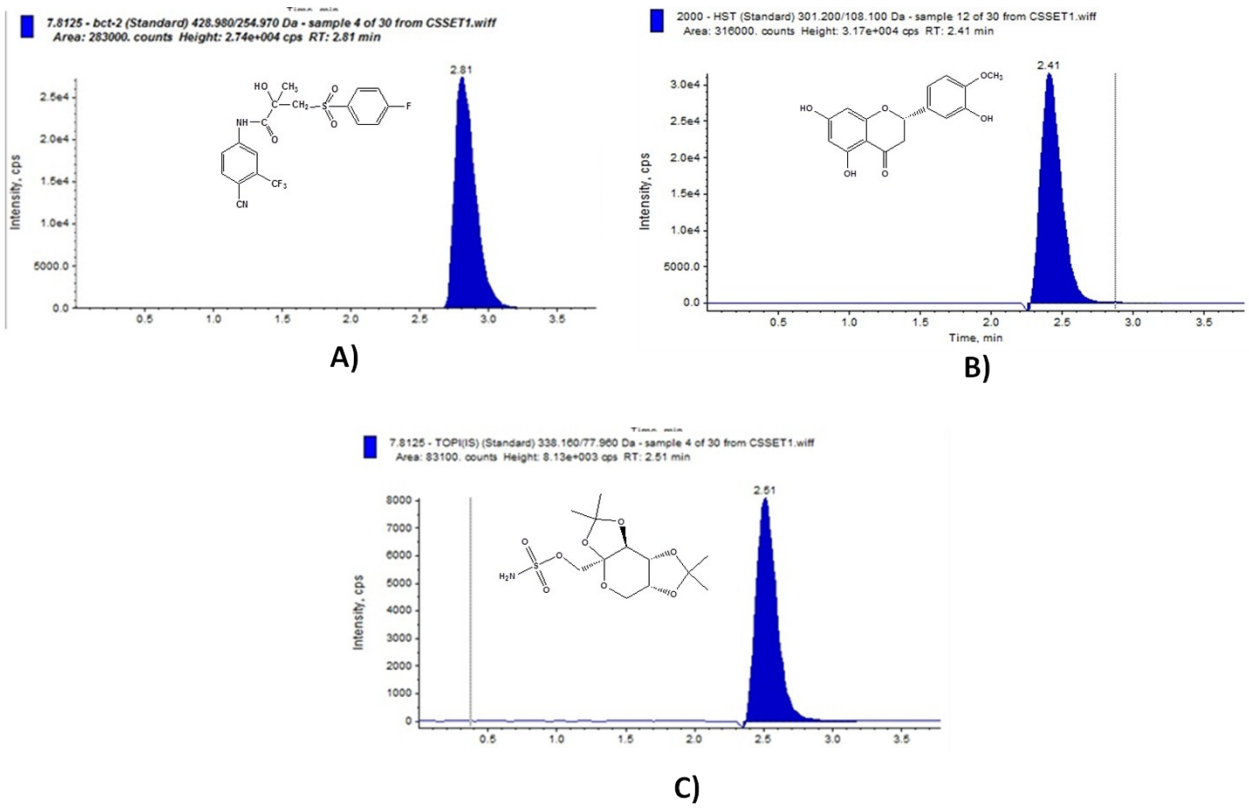


Figure S8. MRM Chromatograms of; A) Bicalutamide, B) Hesperetin, C) Topiramate.

Table SI. Fitting of drug release data to various models to predict the mechanism of drug release from PCL-BCT-HSP-CS NPs.

PCL-BCT-HSP-CS	Zero order		First Order		Higuchi		Korsmeyer-Peppas		
NPs	**R^2**	**k_0**	**R^2**	**k_1**	**R^2**	**k_2**	**R^2**	**K_3**	**n**
BCT	0.9728	0.889	0.9787	0.013	0.9870	6.389	0.9872	6.083	0.513
HSP	0.9654	1.363	0.9975	0.027	0.9931	9.680	0.9982	6.371	0.612