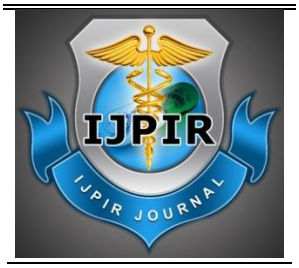
Research Article



ISSN Print 2231 – 3648
Online 2231 – 3656

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

STABILITY STUDIES OF EUDRAGIT LOADED BIOFLAVONOID NANOPARTICLES FOR ANTIDIABETIC AND ANTIOXIDANT ACTIVITY

S. Mohan[2], L. Nandhakumar*[1]

[1]Department of Pharmacy, Shri Jagadish Prasad Jhabharmal Tibrewala University, Churela, Jhujhunu-333001, Rajasthan, India.

[2]Department of Pharmacy, Shri Jagadish Prasad Jhabharmal Tibrewala University, Churela, Jhujhunu-333001, Rajasthan, India.

ABSTRACT

The primary objective of the present work was to evaluate the stability of the prepared Eudragit E 100 containing bioflavonoids Naringin and Hesperidin prepared by nanoprecipitation method. The nanoparticles were analyzed for the physical appearance, percentage drug content and percentage drug release. The prepared nanoparticles were subjected for long-term stability studies and accelerated stability study. The long-term stability studies were carried out for the batches FE1, FE2 and FE3 at $5^0C\pm3^0C$ and $30^0C\pm2^0C$, 65%±5% RH for initial, 1st, 3rd, 6th, 9th and 12th month. The accelerated stability study was conducted for the formulation FE1, FE2 and FE3 at $40^0C\pm2^0C$, 70%±5% RH for initial, 3rd and 6th month as mentioned in the ICH guidelines. The results depicts that the, prepared nanoparticles were stable at long term stability conditions as compared with the accelerated stability study

Keywords: Naringin, Eudragit polymer, Nanoprecipitation Method, Long Term Stability Studies, Accelerated stability study

INTRODUCTION

Diabetes mellitus arouse as a result of metabolic disarray consequently affects the carbohydrate, lipid and protein fate in the physiological system. It is also by and large responsible for the impaired insulin secretion and resistance to the pancreatic cell receptors. As result diabetes prone to cause various complications at cellular level encompass micro and macro vascular damages [1-5]. Basically diabetes is classified into following manner.

- Type-I : Insulin Dependent Diabetes Mellitus
- Type-II: Non-Insulin Dependent Diabetes Mellitus

In type 1 diabetes there is insufficient insulin production to suffice the need for glucose metabolism. Usually this type of diabetes starts at the age of twenties, the treatment protocol involves insulin supplement to have the control of blood sugar. On other hand type 2 diabetes is the most prevalent diabetes contributing 95% of the population. This type of diabetes is characterized by deprived generation of insulin or developing resistance to the insulin of pancreas. Usually this type of diabetes prevails at 40 years of age[6, 7].

Bioflavonoids having potential antidiabetic and antioxidant are the viable option for the management of diabetes and its complications. Very low aqueous solubility and bioavailability limits the use of such flavonoids in treating diabetes. To overcome the aforementioned

Author for Correspondence:
L. Nandhakumar
nandha_pharm@hotmail.com

problems, biodegradable polymers with solubility enhancing techniques considerably increase and improve the solubility and subsequent bioavailability[7-11]. One such technique is generation of nanoparticles by techniques like nanoprecipitation method. Such prepared nanoparticles have the option to get evaluated for its stability both in long term and accelerated conditions. A guideline from standard regulatory authorities like ICH (International Conference on Harmonization) provides a immense scope for the stability checking of the prepared nanoformulations[11-15].

MATERIALS AND METHODS

The Naringin and Hesperidin were purchased from Sigma Aldrich, Eudragit E 100 from Signet chemicals and all other chemicals used in the experiment of analytical grade.

Stability study of the prepared nanoformulation Naringin nanoparticles (Nr NP), Hesperidin nanoparticles (Hs NP) & Naringin-Hesperidin nanoparticles (Nr-Hs NP) were done as per ICH guidelines. The long term stability study was performed at $5^0C\pm3^0C$ and $30^0C\pm2^0C$, 65%±5% RH for initial, 1st, 3rd, 6th, 9th and 12th month. Accelerated stability study performed at $40^0C\pm2^0C$, 70%±5% RH for initial, 3rd and 6th month. The formulations are coded as FE1 Naringin nanoparticles (Nr NP), FE2 Hesperidin nanoparticles (Hs NP) & Naringin-Hesperidin nanoparticles (Nr-Hs NP)

The samples were stored at the ICH mentioned QA1(R) for 1 year and their drug content was determined for every 3 months. Likewise accelerated stability was carried out by storing the formulation for 6 months and observation at every 3 months.

In the long term stability study of 5ºC±3 ºC, 30 ºC±2 ºC, 65% ±5%RH there is a slight decrease in difference of the drug content was observed in the months of initial,3,6,9,12. But where as in the accelerated stability study of 40ºC±2 ºC, 70% ±5%RH there has been a significant decrease in the drug content was observed in initial,3 and 6 month. It may be due to the elevated temperature, because of drug degradation, the drug release decreased gradually. Hence in this study it has been observed that the formulated nanoparticles were quite stable in 5ºC±3 ºC and 30 ºC±2 ºC, 65% ±5%RH for a period of 12 months.

The formulated nanoparticles were subjected to both long term 5ºC±3 ºC, 30 ºC±2 ºC, 65% ±5%RH and accelerated stability study 40ºC±2 ºC, 70% ±5%RH as per ICH (International Conference on Harmonization) guidelines QA1(R). The results of drug release profile revealed that the prepared nanoparticles would be stable if stored at the temperature 5ºC±3 ºC and 30 ºC±2 ºC, 65% ±5%RH ideally. Hence the aforementioned two temperate conditions are ideal for the nanoparticles storage of FE1 (Naringin Nanoparticles), FE2 (Hesperidin Nanoparticles), FE3 (Naringin-hesperidin dual Nanoparticles).

RESULT AND DISCUSSION

Table 1. Stability study-cumulative release of FE1 (Naringin Nanoparticles)- Long term

Time (Min)	5ºC±3 ºC (Long term stability)					30 ºC±2 ºC, 65% ±5% RH(Long term stability)				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
0	0	0	0	0	0	0	0	0	0	0
40	39.83	39.61	39.52	39.36	39.00	39.83	39.41	39.00	38.65	38.30
80	65.27	65.01	64.91	64.72	64.56	65.27	64.82	64.46	64.14	63.78
120	75.17	75.00	74.82	74.61	74.45	75.17	74.92	74.65	74.27	74.00
160	83.03	82.81	82.64	82.43	82.25	83.03	82.88	82.47	82.11	81.96
200	89.00	88.82	88.59	88.35	88.17	89.00	88.62	88.29	88.01	87.75
240	94.97	94.72	94.56	94.27	94.13	94.97	94.66	94.23	93.95	93.60
280	98.53	98.38	98.21	98.01	97.96	98.53	98.15	97.85	97.44	97.09

Table 2. Stability study-cumulative release of FE1 (Naringin Nanoparticles)- Accelerated

Time (Min)	40ºC±2 ºC, 70% ±5% RH (Accelerated stability)		
	0	**3**	**6**
0	0	0	0
40	39.83	37.91	35.86
80	65.27	64.91	63.57
120	75.17	74.16	72.97
160	83.03	81.37	79.52
200	89.00	87.23	86.91
240	94.97	93.45	92.17
280	98.53	97.20	96.51

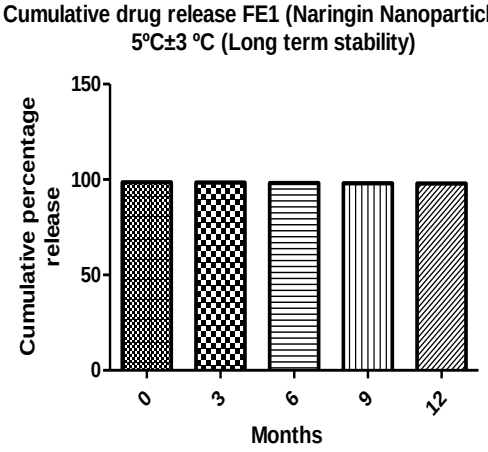


Figure 1. Release of Naringin NPs (FE1-long term)

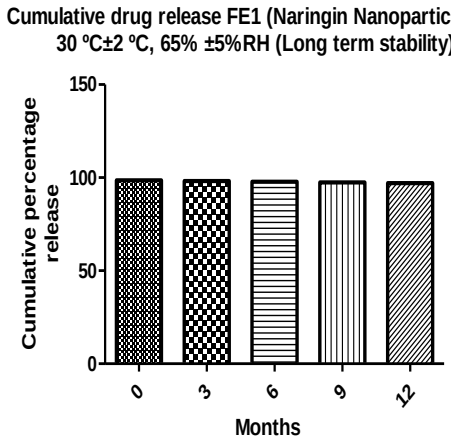


Figure 2. Release of Naringin NPs (FE1-long term)

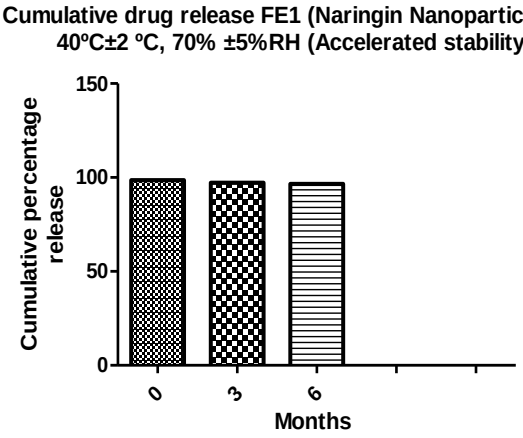


Figure 3. Release of Naringin NPs (FE1-Accelerated Stability)

Table 3. Stability study-cumulative release of FE2 (Hesperidin Nanoparticles)- Long term

Time (Min)	5ºC±3 ºC (Long term stability)					30 ºC±2 ºC, 65% ±5% RH (Long term stability)				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
0	0	0	0	0	0	0	0	0	0	0
40	33.67	33.42	33.25	33.10	32.97	33.67	33.23	32.93	32.57	32.30
80	52.23	52.02	51.90	51.80	51.67	52.23	51.92	51.49	51.20	50.95
120	62.23	62.03	61.85	61.63	61.43	62.23	61.92	61.56	61.10	60.82
160	71.03	70.83	70.58	70.42	70.21	71.03	70.82	70.41	70.06	69.78
200	80.37	80.21	79.87	79.75	79.56	80.37	80.00	79.62	79.26	78.93
240	86.87	86.62	86.39	86.18	86.01	86.87	86.53	86.09	85.72	85.33
280	94.90	94.68	94.52	94.32	94.26	94.90	94.51	94.10	93.75	93.47

Table 4. Stability study-cumulative release of FE2 (Hesperidin Nanoparticles)- Accelerated

Time (Min)	40ºC±2 ºC, 70% ±5% RH (Accelerated stability)		
	0	**3**	**6**
0	0	0	0
40	33.67	31.25	28.62
80	52.23	50.16	47.54
120	62.23	59.28	54.53
160	71.03	69.96	66.92
200	80.37	78.23	76.80
240	86.87	84.23	81.59
280	94.90	92.01	89.52

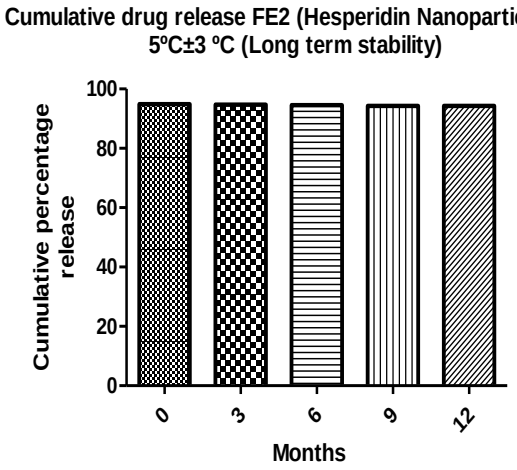


Figure 4. Release of Hesperidin NPs (FE2-long term)

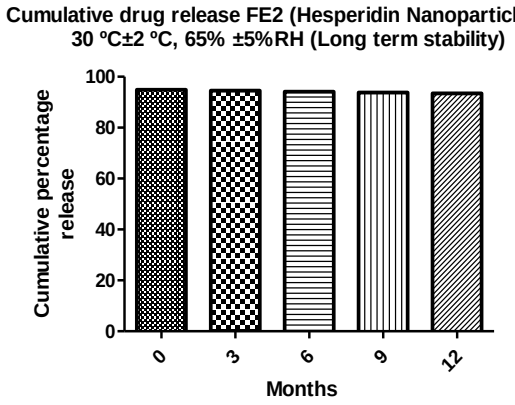


Figure 5. Release of Hesperidin NPs (FE2-long term)

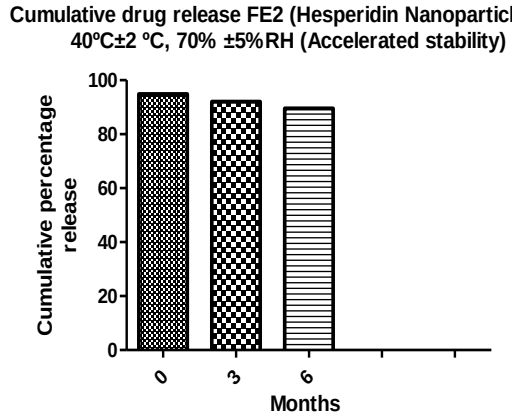


Figure 6. Release of Hesperidin NPs (FE2- Accelerated stability)

Table 5. Stability study-cumulative release of FE3 (Naringin Dual Nanoparticles)- Long term

Time (Min)	5ºC±3 ºC (Long term stability)					30 ºC±2 ºC, 65% ±5% RH(Long term stability)				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
0	0	0	0	0	0	0	0	0	0	0

40	37.03	36.82	36.59	36.38	36.19	37.03	36.70	36.27	35.95	35.56
80	60.00	59.82	59.60	59.38	59.25	60.00	59.62	59.18	58.75	58.42
120	70.63	70.40	70.17	70.01	69.83	70.63	70.19	69.82	69.39	68.98
160	79.97	79.73	79.56	79.34	79.08	79.97	79.52	79.10	78.70	78.26
200	88.10	87.92	87.70	87.56	87.34	88.10	87.76	87.34	86.97	86.65
240	92.93	92.70	92.48	92.19	91.99	92.93	92.57	92.05	91.73	91.47
280	94.57	94.32	94.15	93.97	93.75	94.57	94.19	93.76	93.35	93.02

Table 6. Stability study-cumulative release of FE3 (Naringin Nanoparticles)- Accelerated

Time (Min)	**40ºC±2 ºC, 70% ±5% RH (Accelerated stability)**		
	0	**3**	**6**
0	0	0	0
40	37.03	37.03	31.26
80	60.00	60.00	53.21
120	70.63	70.63	67.99
160	79.97	79.97	73.25
200	88.10	88.10	83.21
240	92.93	92.93	86.13
280	94.57	94.57	88.69

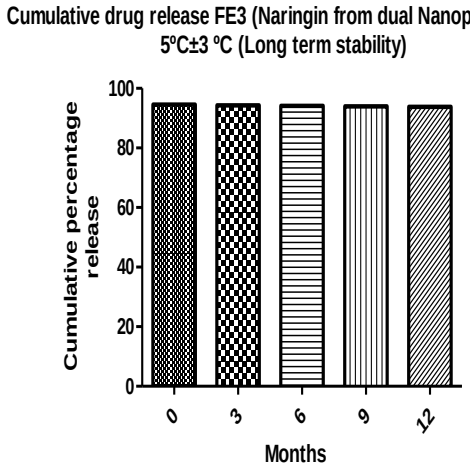


Figure 7. Release of Naringin Dual NPs (FE3-long term)

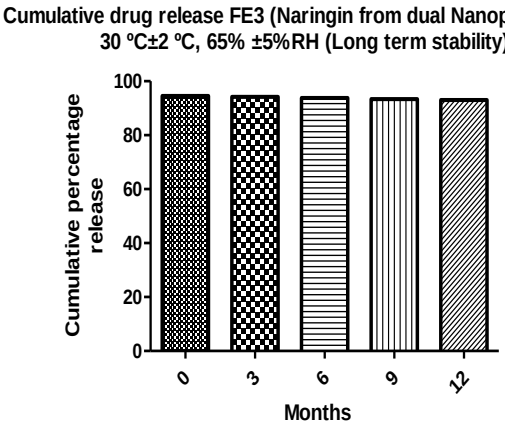


Figure 8. Release of Naringin Dual NPs (FE3-long term)

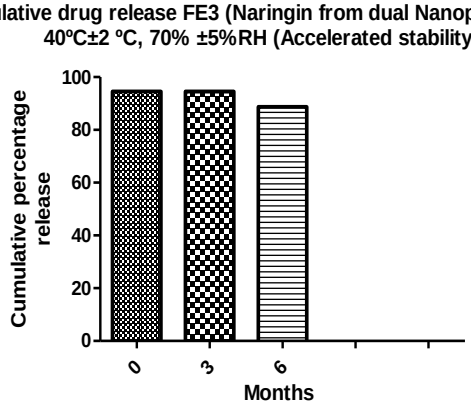


Figure 9. Release of Naringin Dual NPs (FE3- Accelerated stability)

Table 7. Stability study-cumulative release of FE3 (Hesperidin Dual Nanoparticles)- Long term

Time (Min)	5ºC±3 ºC (Long term stability)					30 ºC±2 ºC, 65% ±5% RH (Long term stability)				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
0	0	0	0	0	0	0	0	0	0	0
40	27.03	26.86	26.62	26.39	26.16	27.03	26.62	26.23	25.85	25.41
80	46.07	45.87	45.62	45.46	45.22	46.07	45.71	45.32	44.96	44.51
120	59.03	58.82	58.63	58.39	58.15	59.03	58.67	58.31	59.93	57.52
160	68.00	67.77	67.53	67.26	67.03	68.00	67.57	67.12	66.62	66.18
200	77.57	77.32	77.14	76.96	76.73	77.57	77.17	76.73	76.27	75.95
240	84.97	84.72	84.55	84.31	84.09	84.97	84.42	83.99	83.45	83.16
280	90.03	89.85	89.61	89.43	89.25	90.03	89.62	89.26	88.87	88.49

Table 8. Stability study-cumulative release of FE3 (Hesperidin Nanoparticles)- Accelerated

Time (Min)	40ºC±2 ºC, 70% ±5% RH (Accelerated stability)		
	0	**3**	**6**
0	0	0	0
40	27.03	25.26	23.63
80	46.07	43.01	41.08
120	59.03	57.26	55.56
160	68.00	66.26	64.87
200	77.57	75.13	72.26
240	84.97	82.13	80.19
280	90.03	88.53	85.62

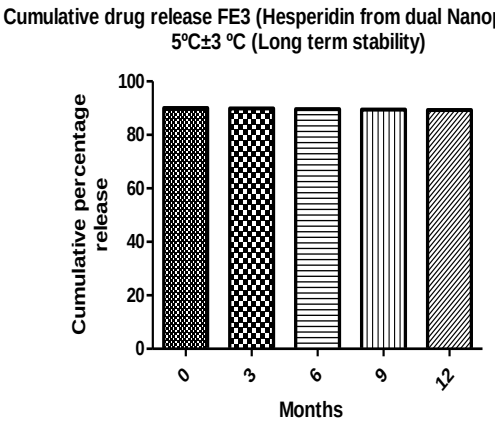


Figure 10. Release of Hesperidin Dual NPs (FE3-long term)

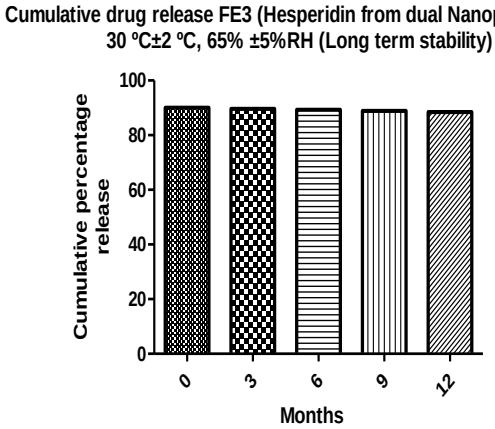


Figure 11. Release of Hesperidin Dual NPs (FE3-long term)

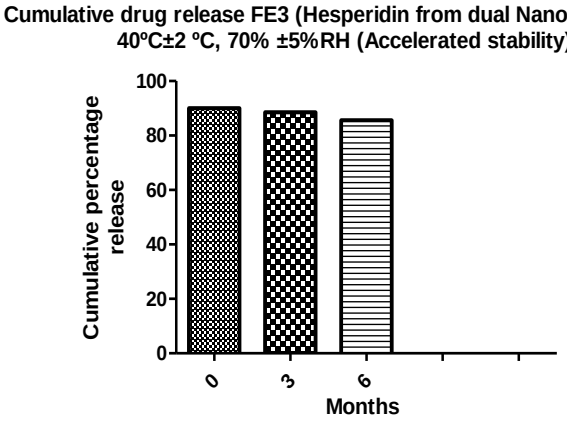


Figure 12. Release of Hesperidin Dual NPs (FE3-Accelerated stability)

The samples were stored at the ICH mentioned QA1(R) for 1 year and their drug content was determined for every 3 months. Likewise accelerated stability was carried out by storing the formulation for 6 months and observation at every 3 months.

In the long term stability study of 5ºC±3 ºC, 30 ºC±2 ºC, 65% ±5%RH there is a slight decrease in

difference of the drug content was observed in the months of initial,3,6,9,12. But where as in the accelerated stability study of 40ºC±2 ºC, 70% ±5%RH there has been a significant decrease in the drug content was observed in initial,3 and 6 month. It may be due to the elevated temperature, because of drug degradation, the drug content decreased gradually. Hence in this study it has been observed that the formulated nanoparticles were quite stable in 5ºC±3 ºC and 30 ºC±2 ºC, 65% ±5%RH for a period of 12 months.

The formulated nanoparticles were subjected to both long term 5ºC±3 ºC, 30 ºC±2 ºC, 65% ±5%RH and accelerated stability study 40ºC±2 ºC, 70% ±5%RH as per ICH (International Conference on Harmonization) guidelines QA1(R). The results from drug content revealed that the prepared nanoparticles would be stable if stored at the temperature 5ºC±3 ºC and 30 ºC±2 ºC, 65% ±5%RH ideally. Hence the aforementioned two temperate conditions are ideal for the nanoparticles storage of FE1 (Naringin Nanoparticles), FE2 (Hesperidin Nanoparticles), FE3 (Naringin-hesperidin dual Nanoparticles).

Table 9. Percentage Drug Content long term and accelerated stability

Code	5ºC±3 ºC (Long term stability)					30 ºC±2 ºC, 65% ±5% RH(Long term stability)					40ºC±2 ºC, 70% ±5% RH (Accelerated stability)		
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**	**0**	**3**	**6**
FE1	87.21	86.95	86.69	86.41	86.23	87.21	86.52	86.00	85.69	85.32	87.21	85.02	83.18
FE2	85.62	85.29	85.01	84.86	84.61	85.62	85.00	84.71	84.31	82.97	85.62	82.51	80.01
FE3 Nr	86.57	86.32	86.09	85.89	85.67	86.57	86.13	85.72	85.40	85.00	86.57	84.78	82.71
FE3 Hs	82.13	81.89	81.67	81.45	81.20	82.13	81.50	81.12	80.70	80.19	82.13	79.16	76.98

Nrn -→ Naringin ; Hsp -→ Hesperidin

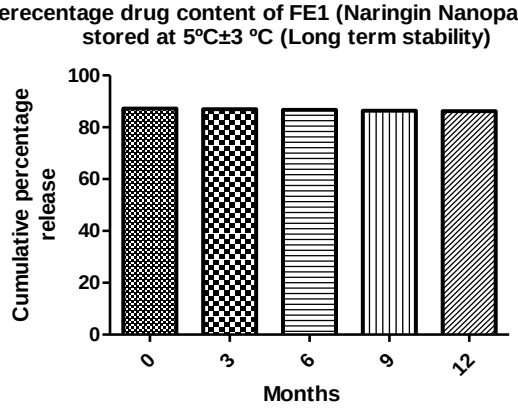


Figure 13. Percentage Drug Content FE1 Naringin (Long term)

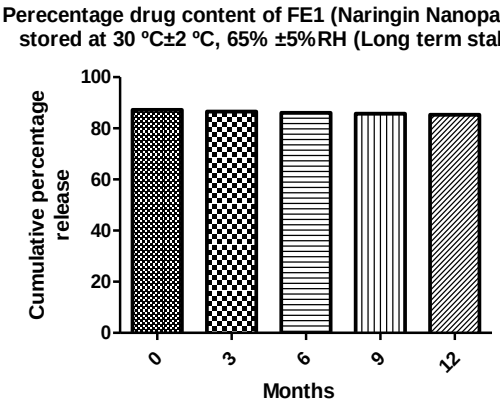


Figure 14. Percentage Drug Content FE1 Naringin (Long term)

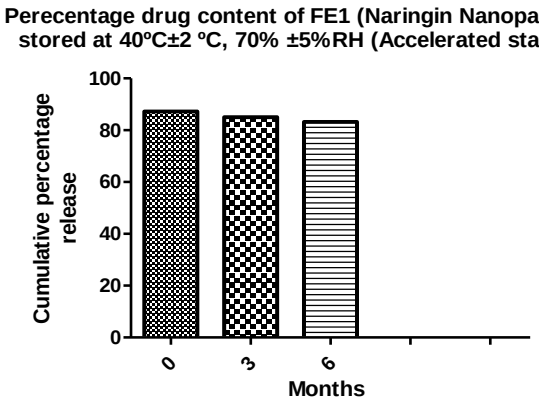


Figure 15. Percentage Drug Content FE1 Naringin (Accelerated)

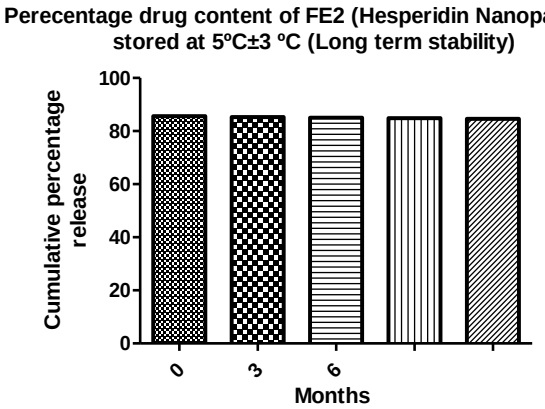


Figure 16. Percentage Drug Content FE2 Hesperidin (Long term)

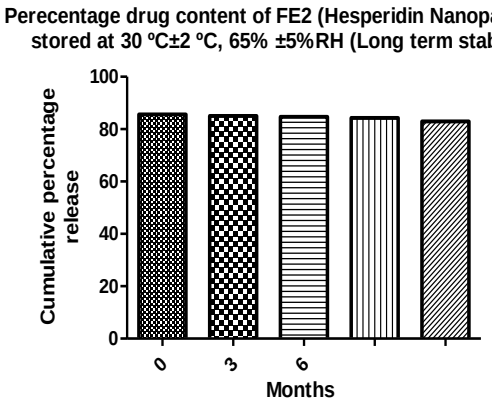


Figure 17. Percentage Drug Content FE2 Hesperidin (Long term)

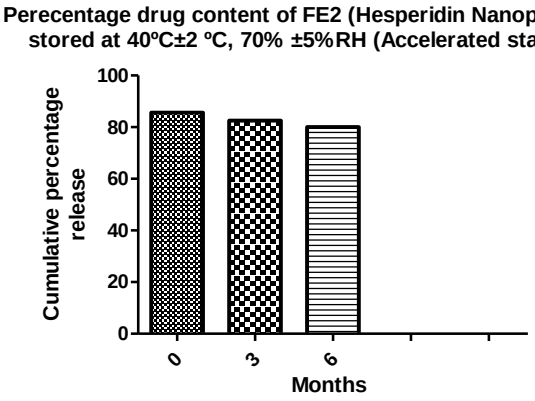


Figure 18. Percentage Drug Content FE2 Hesperidin (Accelerated)

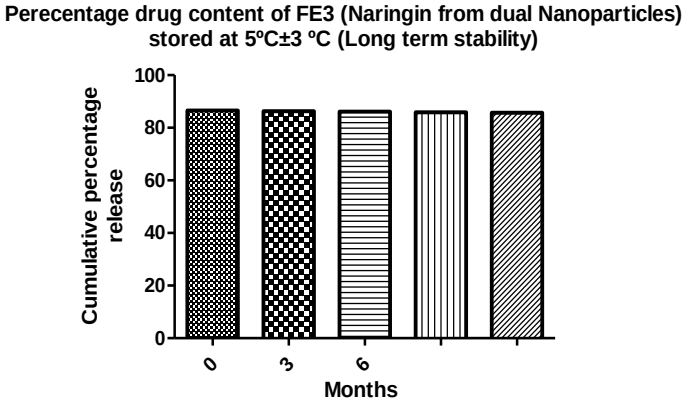


Figure 19. Percentage Drug Content FE3 Naringin (Long term)

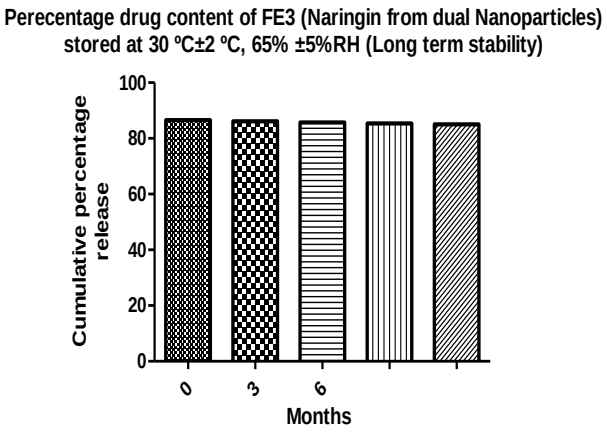


Figure 20. Percentage Drug Content FE3 Naringin (Long term)

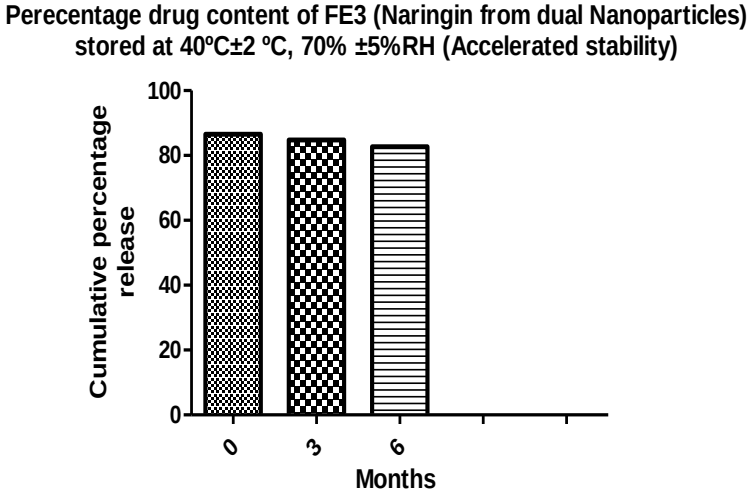


Figure 21. Percentage Drug Content FE3 Naringin (Accelerated)

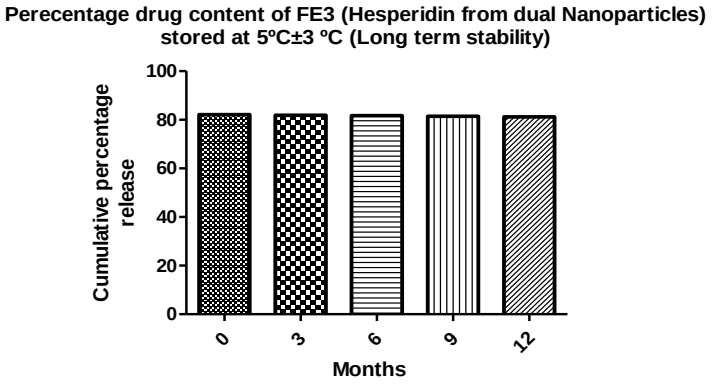


Figure 22. Percentage Drug Content FE3 Hesperidin (Long term)

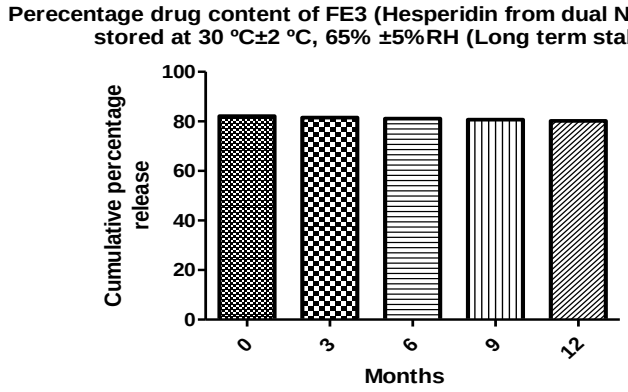


Figure 23. Percentage Drug Content FE3 Hesperidin (Long term)

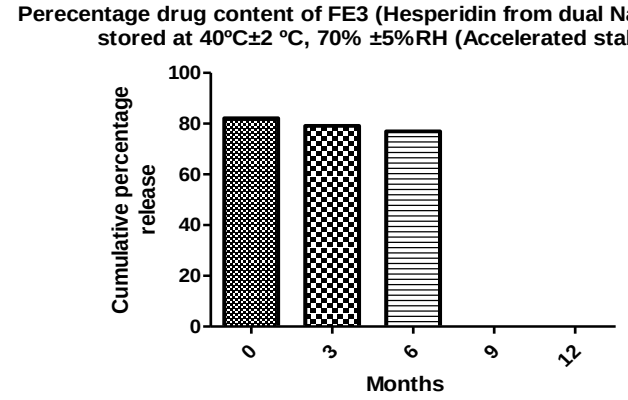


Figure 24. Percentage Drug Content FE3 Hesperidin (Accelerated)

CONCLUSION

In this study we conclude that the preparation of Eudragit nanoparticles containing bioflavonoids like Naringin and Hesperidin by Nanoprecipitation method has been found practical, conducive and feasible to produce by the proposed method. In the meantime, no significant physical changes of the samples were observed, which indicated the better stability of all the prepared nanoformulation. The stability study of the all the prepared nanoformulations FE1, FE2 and FE3 performed as per ICH guidelines, the results revealed that better long-term stability at $5^0C\pm3^0C$ and $30^0C\pm2^0C$, 65%±5% RH for initial, 1^{st}, 3^{rd}, 6^{th}, 9^{th} and 12^{th} month, as compared with the accelerated stability temperature stored at $40^0C\pm2^0C$, 70%±5% RH. Consequently it was found that, the long term conditions $5^0C\pm3^0C$ and $30^0C\pm2^0C$, 65%±5% RH are more stable temperature and relative humidity for the nanoformulations storage.

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