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Formulation and evaluation of oral suspension of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran using suspending agent

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Abstract:

The anti-cancer properties of 2-Butyl-3-(3, 5-Diiodo-4-Hydroxybenzoyl) Benzofuran have been determined in earlier *in vitro & insilico* studies. In the present investigation, an attempt has been made for formulation and evaluation of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran suspension by adding acacia powder in different ratio in all five formulations. The five suspensions (F1 to F5) of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran were prepared by using different ratio of acacia powder (1,2,3,4 and 5% w/v). These formulations were evaluated for sedimentation volume, pH measurement, viscosity measurement, particle size, and drug release at various time intervals for 2 months. The 2- Butyl-3- (3, 5-diiodo-4-hydroxybenzoyl) benzofuran was found to be compatible with different excipients. The results of the study indicated on increasing the concentration of acacia powder in suspensions improved the physical stability. Formulation F3 and F5 shows satisfactorily physical stability while F5 containing 5.0% w/v concentration of acacia showed better physical stability, and was found to be optimum concentration. Among the formulated suspensions F5 showed better in vitro drug release profile as well as better physical stability compared to other formulated suspensions.

Keywords: 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran, suspension, excipients, viscosity, anti-cancer, *insilico, invitro.*

Introduction:

A pharmaceutical suspension, similar to other disperse systems, is inherently unstable from a thermodynamic perspective. Therefore, it is essential to incorporate a stabilizer or suspending agent into the formulation. This component serves to decrease the rate of settling and enables convenient redispersion of any particles that may have settled. The settled particulates are both prevented from settling further through protective colloidal action and by increasing the viscosity of the liquid in which they are suspended [1],[2].

In our previous investigations, we identified a compound called 2-Butyl-3-(3,5- diiodo-4-hydroxybenzoyl)benzofuran as a ligand that demonstrated strong anticancer activity against HT-29 (Human Colon Cancer) and A549 (Human Lung Cancer) cells. This molecule adheres to Lipinski's fifth rule. It has a molecular weight of 546 and a partition coefficient (log P) of 5.49, indicating its solubility in octanol and water. It has one hydrogen bond donor and three hydrogen bond acceptors. DataWarrior v04.04.04 software predicts that this substance does not pose significant risks of toxicity, such as mutagenicity, tumorogenicity, gonadal toxicity, and irritant effects.

According to the insilico method, 2-Butyl-3-(3,5-diiodo-4-hydroxybenzoyl)benzofuran has a greater tendency to attach to tubulin protein compared to EGFR, ER, and anaplastic lymphoma kinase. During the in vitro MTT assay, the compound demonstrated a greater cytotoxic impact on A549, MCF-7, HT-29, and DU145 cancer cells, with IC50 values of 10.66, 6.05, 5.17, and 7.02 µg/mL, respectively. This effect was more pronounced compared to the standard doxorubicin, which had IC50 values of 14.54, 5.96, 2.4, and 6.3 µg/mL for the same cell lines. This compound exhibited substantial cytotoxicity against A549, MCF-7, HT-29, and DU145 cancer cells, resulting in a decrease in cell viability that was dependent on the concentration of the compound. This substance exhibited substantial dose-dependent anti-proliferative properties in the growth of cancer cells. At the

IC50 concentration of 5.17 µg/mL, 80% of HT-29 cells died during the early stage of the cell cycle, and 50% of cells were arrested in the G0/G1 phase. This compound induces programmed cell death by regulating the ratio of Bax to Bcl-2 and enhancing the activation ofcaspase-3. Enzymes such as DNase become active following caspase-3 activation, resulting in the fragmentation of DNA and the initiation of apoptosis. The level of DNA fragmentation exhibited a 6.5-fold increase compared to cells that were not treated [3].

One significant obstacle in the development of oral suspensions is their inherent thermodynamic instability. Consequently, it becomes imperative to incorporate a stabilizer or suspending agent in the formulation to decrease the rate of settling and facilitate effortless redispersion. The settled particulate matter is both protected by colloidal action and its consistency in the suspending medium is increased [4]. Suspending agents can consist of inorganic substances, synthetic compounds, or polysaccharides. 2-Butyl-3- (3, 5-diiodo-4-hydroxybenzoyl) benzofuran was selected for this study due to its characteristics as a practically insoluble drug in wateer, necessitating the use of a suspending agent in order to formulate it as a liquid dosage form. This study examines the impact of 2-Butyl-3- (3, 5- diiodo-4- hydroxybenzoyl) benzofuran suspension on its physical stability, including formulation and evaluation.

Materials and methods:

Materials: 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran was procured from Avra chemicals Pvt. Limited, Hyderabad, India. The Polyvinyl alcohol (PVA), Methylparaben, Hydroxypropyl methylcellulose (HPMC) and Acacia were procured from UV scientifics Pvt Ltd.

Preparation of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran suspensions:

Compound acacia powder 1g, HPMC 1.0g, PVA 0.5g and 100mg of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran were triturated together in mortar pestle to formsmooth paste. Methyl parabean (1g) was added gradually with constant stirring and then mixed with 1% tween 80 solution. This was followed by addition of quantity sufficient peppermint oil and purified water. The mixture was transferred into a 100 ml amber bottle, made-up volume with distilled water and then shaken vigorously for 2 min (thus making0.5% w/v of the gum in the preparation). The procedure was repeated using 1.0, 2.0, 3.0, and 2.5% w/v of compound acacia powder [5,6,7]. All formulation are shown in table 1.

Table no:1 Formulations of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran oral suspension

S.no	Ingredient	F1	F2	F3	F4	F5
1	**Drug**	100mg	100mg	100mg	100mg	100mg
2	**Tween 80**	1gm	1gm	1gm	1gm	1gm
3	**Methyl paraben**	1gm	1gm	1gm	1gm	1gm
4	**PVA**	1gm	1gm	1gm	1gm	1gm
5	**Acacia**	1gm	2gm	3gm	4gm	5gm
6	**HPMC**	1.5gm	2gm	2.5gm	3gm	3.5gm
7	**Purified water**	q.s	q.s	q.s	q.s	q.s

Evaluation of suspension

Sedimentation volume: Sedimentation volume (F) is a ratio of the final volume of sediment (Vu) to the original volume of sediment (Vo) before settling. 50ml of each suspension were transferred to 50 ml measuring cylinders and the volume of sediment formed was noted at every 24 hr for 7 days. The sedimentation volume F (%), was calculated using the formula:

$F = 100\ Vu/\ Vo$

Viscosity measurement: The viscosity of the samples was determined at 25ºC using the Brookfield Synchro- lectic viscometer, model LVF at 30 revolution/min.

Particle size measurement: The particle size of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran particles in the prepared suspensions was measured by optical microscopy using a trinocular microscope at 100x (10×10) magnification. The size of 100 particles were measured and the average particle size of was determined.

Drug release: The release studies were carried out at 37± 0.5º C by using a beaker method rotating cellophane membrane apparatus. A 1000 ml volume of the 0.1N HCL of the release media. The cellophane membrane containing 5.00 ml of solution or a suspension of the suspension salt was placed inside the vessel at time zero. Release of the drug salt from the cellophane membrane into the aqueous sink condition studied from the following type of test preparation; (i) a solution of the salt, (ii) salt suspensions, (iii) suspensions formed in situ in cellophane membrane cell. Salt solutions were obtained from dissolving in 0.1 N HCL. After

15 min taking 5 ml solution was withdrawn and maintained sink condition. Samples were withdrawn after time interval 30, 45, 60, 75, 90, 105, 120 and 135 min. Maintaining sink condition, the taking solutions were further diluted with 0.1 N HCL and absorbance measured in double beam UV-spectrophotometer[8,9,10,11,12,13]

Results:

Evaluation of suspension Sedimentation volume: The sedimentation volume of F1 to F5 was found to be 99.01%, 96.86%, 97.50%, 96.50% and 97% respectively at the end of 24 hours (Table 2). After one week suspension F1 found in a clumpy mass which was not pourable from the container, while F2 resulted clumpy mass after one month. After long time it was found that, in F3 to F5 the sedimentation volume gradually decreases from 97.50% to 52%, 96.50% to 84% and 97% to 85% respectively. The formulation F3 to F5 revealed good flow from container. This indicates that on increasing the concentration of suspending agentit increases the stability of suspensions.

Table 2: Values of sedimentation volume (%) suspension using different concentration of suspending agent.

Formulation	24hr	1 week	2 week	1-month	2-month
F1	99.01	CM	CM	CM	CM
F2	96.86	84.45	75	CM	CM
F3	97.50	59	54	53	52
F4	96.50	84	85	83.50	84
F5	97	86	85.5	85.5	85.5

Viscosity measurement: The formulation F1 has clumpy mass so that it fails to measure the viscosity, while F2 shows decrease in viscosity during 24 hrs to 2 weeks but it fail to measure the viscosity of suspension after one month. When the concentration of suspending agent increased from F3 to F5, a slight increase in viscosity was found (Table 3). When solution kept for long time the viscosity of F3, F4 and F5 has decreased from 44, 50, and 54 to 41, 44 and 53 respectively. The change in viscosity in case of F5 was less indicating that F5 is relatively a stable formulation.

Table 3: Viscosity values of different formulations for 2 month period

Formulation	24hr	1 week	2 week	1 month	2 month

F1	**38**	**CM**	**CM**	**CM**	**CM**
F2	**40.50**	**39.50**	**40**	**CM**	**CM**
F3	**44**	**43.45**	**42**	**43.50**	**41**
F4	**50**	**49**	**48**	**45**	**44**
F5	**54**	**53.50**	**52**	**51**	**53**

Particle size analysis: Highly viscous mass particle size analysis was not possible in case of F1. In the case of F2 the particle size slightly change over a period of two week, but after one month due to formation of clumpy mass particle size measurement was not possible. In formulation F3, F4 and F5 there was slightly decrease in the particle size (Table 4).

Table 4: Particle size determination values of different formulations for 2 month period

Formulation	24hr	1 week	2 week	1 month	2 month
F1	**49**	**CM**	**CM**	**CM**	**CM**
F2	**46**	**39.50**	**38**	**CM**	**CM**
F3	**44**	**43.45**	**42**	**41**	**41**
F4	**39**	**37**	**36**	**35**	**33**
F5	**39**	**37.50**	**36**	**35.50**	**35**

PH: The pH value of F1 was found to be 7.12, when it kept for long time formulation exhibited in a clumpy mass so that determination of pH was not possible. The other formulations F2 to F5 showed a more or less constant pH value. This indicates that there is nochemical change when kept for long time except F2 (Table 5).

Formulation	24hr	1 week	2 week	1 month	2 month
F1	**7.0**	**CM**	**CM**	**CM**	**CM**
F2	**6.5**	**6.16**	**6.25**	**CM**	**CM**
F3	**6.27**	**6.80**	**6.47**	**6.97**	**6.5**
F4	**6.60**	**6.50**	**6.25**	**6.12**	**6.40**
F5	**6.50**	**6.40**	**6.37**	**6.60**	**6.50**

Drug release: All the formulations showed acceptable properties as shown in table 6. The result of the drug release study indicating that F1 and F2 released 81 and 84 at the end of 135 min, respectively. Formulation F3, F4 and F5 released 85, 85.50 and 87 at the end of 135 min (Table 6). The results indicated that F5 release maximum drug in media.

Table 6: Drug release of 2-Butyl-3- (3, 5-diiodo-4- hydroxybenzoyl) benzofuran suspension in 0.1N HCl

Time withdrawn (mins)	F1	F2	F3	F4	F5
30	4	4.57	6	6.8	7
45	11	12	12.5	13	14
60	18	18.90	21	22	22.50
75	25	26	26.50	28	28.50
90	35	35.50	37	36	38
105	49	51	52.50	53	54
120	60	62	63	61	64
135	81	84	85	85.80	87

Discussion:

The suspension F1 to F5 was prepared by adding different concentration such as 1. 0, 2.0, 3.0, 4.0 and 5.0% w/v of compound acacia powder. These formulations were evaluated for various quality parameters to determine their stability such as sedimentation volume, viscosity, particle size, pH and drug release for 2 months' time in regular intervals. The data obtained from the determination of sedimentation rates revealed that the formulations prepared with higher concentration of acacia possessed higher sedimentation volume when compared with other formulations. Among the five formulations of suspension, the F5 showed nearby constant sedimentation volume after two week, this indicates more stable suspensions. When the concentration of suspending agent increases in suspensions a slight increase in viscosity was found. When kept the suspension for long time, the change in viscosity in case of F5 was less indicating that F5 is relatively a stable formulation. With

increasing the concentration of suspending agent, the particle size of the suspension was found to be decreased except F5. The pH values of all the formulations were compiled as per I.P. requirements. Suspensions formulation (F5) employing 5.0% concentration acacia gave higher drug release rate among all the formulations. Hence 5.0% concentration of acacia was found to be optimum concentration.

Conclusion:

The results of the study indicated on increasing the concentration of acacia powder in suspensions improved the physical stability. Among the formulated suspensions F5 showed better in vitro drug release profile as well as better physical stability compared to other formulated suspensions.

Conflict of interest; NO

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