Formulation and stability evaluation of immediate release antioxidant tablet

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Abstract:Oxidative stress plays an important part in the development of human diseases. Pharmaceutical strategies are required to be work out in order to fight against such oxidative damages. Designing of new formulations that can protect human beings from the undesirable effects, consequence of oxidative stress, the crucial cellular and molecular processes, along with recurring oxidative damage and diseases is to be expedited. The main objective of present work was to design a rapidly releasing synthetic antioxidant tablet dosage form comprising of vitamin A, vitamin C, vitamin E and zinc in combination with lecithin (a phospho-lipid) that can fulfill human health and nutritional requirement and to perform stability studies. Beside active ingredients, the excipients used in present formulation were; Avicel pH 102, starch pregelatinized, silicon dioxide colloidal and polyethylene glycol 8000 milled magnesium stearate, acid stearic fine powder and aq.opa dry coating material. The immediate release formulation of antioxidant was prepared by wet granulation method. Three different trials were developed. Vitamin C was selected as tracer for detection and evaluation of tablet dosage form. When the resulting formulation was evaluated by USP 24 / NF 19, 2000 guidelines and later by stability studies, it was found that their quality can be maintained over a storage period of 24 months.

Keywords: Antioxidants, immediate release tablets, lecithin,vitamin A,vitamin C, vitamin E, wet granulation, zinc.

INTRODUCTION

The immediate release formulation of antioxidant tablets gaining popularity and acceptance as a drug delivery system, mainly due to easy to administer and it leads to better patient compliance (Lester and Enrique, 1997).Antioxidants reduce the chances of gradual deterioration in the structure of human body parts with a consequent loss of ability of parts to function in a normal and healthy way. These antioxidants also defend body cells from the oxidative DNA destruction. Examples of such deteriorating pathophysiological conditions include atherosclerosis, cardiovascular, cancer diseases, etc. The antioxidant acts as substance that can greatly reduce the adverse effects of reactive molecules found in human body. These antioxidants protect cells from oxidative damage by eliminating free radicals (Pryor, 1976).

Vitamin E acts as an antioxidant by breaking chains of oxidative reaction chains and can decrease the spread of free radical chain reactions (McCay *et al.*, 1980). Other studies have been performed to observe effect of soy lecithin and it was concluded that level of cholesterol and triglycerides is decreased by soy lecithin in a remarkable manner, together with increase in level of High Density Lipoprotein in blood (Iwata *et al*., 1993; Jimenez *et al*., 1990).

Treatment with vitamin C, or with Vitamin C and Vitamin A significantly lowered the urea and creatinine levels, improvement of antioxidant enzyme activities, and prevention of renal tissue damage in endotoxemic rats (Kanter *et al.*, 2005). Antioxidants are effective in preventing ovaries from oxidative damage and thus eliminating the possibilities of ovarian cancer (Tung *et al*., 2005).

Antioxidants are mainly available in the market as capsules. However, capsules are generally more costly, their filling is relatively slower and it may cause gastric irritation (DeVilliers, 2005). Therefore present work was designed with the following objectives (i) to formulate an immediate release antioxidant tablet comprising of vitamin A, vitamin C, vitamin E and Zinc in combination with lecithin (a phospho-lipid) (ii) to study the effect of formulation factors on the stability of vitamin C as a tracer and index vitamin for evaluation of tablet dosage form and (iii) to perform stability studies of formulated antioxidant tablets.

MATERIALS AND METHODS

Materials

The active ingredients purchased were; vitamin A acetate powder and vitamin E, dl alpha tocopherylacetate (BASF, Germany), ascorbic acid (Alland, China), zinc sulphate (Dr. Paul Lohmann, GMBH, Germany) and lecithin (Shanghai Youngsun foods Co., Ltd., China).

The excipients purchased included; Avicel pH 102 (F.

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M.C.USA), starch pregelatinized (Cerestar, Germany), silicon dioxide colloidal and polyethylene glycol 8000 milled (Degussa, Germany and Clariant, Germany), alcohol isopropyl anhydrous (Leechang, Taiwan), magnesium stearate (Dr. Paul Lohmann, GMBH, Germany), acid stearic fine powder (Merck, Germany) and aq. opa dry coating material (Colorcon, UK).

Methods

Tablet formulation

Antioxidant tablets were manufactured by wet granulation method (table 1).

Table 1: Composition of antioxidant tablet manufacture by wet granulation method

S. No	Material name	Quantity per tablet	Quantity per 5000 tablets
1.	Vitamin A Acetate Powder (25% Excess)	1.9 mg	9.5 g
2.	Ascorbic Acid (10% Excess)	550.0 mg	2.75 kg
3.	Vitamin E ,dl Alpha Tocopheryl Acetate (05% Excess)	63.0 mg	315.0 g
4.	Lecithin Powder	100.0 mg	500.0 g
5.	Zinc Sulphate (0% Excess)	10.0 mg	50.0 g
6.	Polyvinyl Pyrolidone (PVP)	28.4 mg	142.0 g
7.	Cellulose Microcrystalline Avicel PH 102	124.0 mg	620.0 g
8.	Starch Pregelatinized	50.0 mg	250.0 g
9.	Silicon Dioxide Colloidal	6.5 mg	32.5 g
10.	Polyethylene Glycol 8000 Milled	6.9 mg	34.5 g
11.	Alcohol Isopropyl, Anhydrous	150.0 ml	750.0 ml
12.	Magnesium Stearate Powder	2.5 mg	12.5 g
13.	Acid Stearic Powder	30.0 mg	150.0 g
	Coating:		
14.	Aqueous, Dry Powder Coating Solution	1.03 gm	5.15 kg
14.1	Dry powder Pink	0.23 gm	1.15 kg
14.2	Water Purified (Deionized)	0.8 ml	4.0 L
Total Tablet Weight		973.2 mg	4.866 kg

At first step of manufacturing Vitamin A, C, E, lecithin, zinc, starch pregelatinized, microcrystalline cellulose, polyethylene glycol 8000, colloidal silicon dioxide were passed through 30 mesh screen. Dry mixing of sieved materials was done in a Multi function mixer THP-4 (STC- China) for five minutes. Polyvinyl pyrolidone (PVP) was dissolved in isopropyl alcohol (IPA) and the resulting solution was use as granulating solution. The PVP-IPA solution was then added slowly to dry mix powders in same mixer and mixed for five minutes. Wet mass was then spread in trays and dried in open air at room temperature for one hour. Moisture content was checked by using moisture analyzer (MOC-120H, Shimadzu, Japan).

Dried granules were then passed through 10 mesh screen. Blending of screened dried granules was done in a blender (THP-4, STC, China), after adding magnesium stearate and stearic acid (previously passed through 30 mesh screen).

Evaluation of flow properties of granules

Evaluation of flow properties of blended granules were done by determining the angle of repose, bulk density, tapped density, Hausner's ratio and compressibility index (Carr's index) as per USP 24/ NF 19, 2000 requirements.

Compression of granules

Compression of blended granules were done by using single punch tablet press (THP-4, STC, China) to produce oblong shaped tablets weighing 973.2 mg each with a thickness of 5.53mm - 5.82mm.About 5000 tablets were prepared for each batch.

Physical evaluation of tablets

Compressed tablets were evaluated according to USP24/NF 19, 2000 guidelines. Tablet weight variation was checked by weighing balance (BX-300, Shimadzu, Japan). Tablet hardness and thickness were checked by using tablet hardness tester (PTB-311E, Germany) and vernier caliper (CD-6, CSX, Mitutoyo, Japan) respectively. Friability was assessed by using friability tester (PTS-10E, Germany).

Disintegration test of tablets

Disintegration of compressed tablets was performed by using disintegration apparatus (PTZ-S, Germany) according to USP (USP 24/NF 19, 2000) in distilled water at 37ºC± 2ºC.

Coating of compressed tablets

Core tablets were coated by using aqueous coating solution (table 1) and spray coating technique in a multifunction experimental machine coating pan (THP-4, STC- China). Coating solution was applied till the tablets attained a weight of about 1002 mg. (limit= 992-1012mg).

Chemical evaluation of coated tablets

Dissolution test: Dissolution test was performed as per USP24/NF19, 2000 by using Erweka USP Dissolution apparatus II fitted with a peddle. Dissolution requirements as stated for index vitamin and index mineral for Vitamin C are:

Dissolution medium:	0.1 N hydrochloric acid
Volume:	900 ml
Speed:	75 rpm

Initial testing/assay

Assay of vitamin A, vitamin C, vitamin E, zinc and lecithin was performed for each batch individually as standard test methods according to USP24/ NF19, 2000.

Packaging of antioxidant tablets

Once the initial evaluation results of coated tablets were found satisfactory, the final product was packed in clear, transparent foil, grammage: 310 g/m^2-361 g/m^2 and plain, cold forming aluminum foil, grammage: 225g/m^2-275g/m^2 for stability studies.

Stability testing

After getting satisfactory initial testing results, the tablets in final packing were kept on stability studies for a period of 3, 6, 9, 12, 18 and 24 months under conditions of tropical (ICH Zones 3 and 4: 30°C ±2°C /65% ±5% RH) and at 40°C ±2°C /75% ±5% RH.

Tablets under test were stored in thermostatically controlled stability chamber maintained at 30°C±2°C/ 65% RH ±5% RH. Physical and chemical testing/assays were performed after 3 months, 6 months, 9 months, 12 months, 18 months and 24 months.

RESULTS

The various formulations of antioxidant tablets are prepared by wet granulation methods and results are given below:

Pre-formulation studies

Pre-formulation studies of granules like flow properties including angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio were studied and found satisfactory (table 2).

Evaluation of tablets

Uniformity of weight

Weight variation among three batches was found within the range of 978.8 mg - 979.3 mg (average wt. of each batch). All formulation batches were in compliance within USP 24 weight variation limits (table 3).

For batch No. 1and 2 all tablets were within the range of ± 3S.D, where as in batch No.3, 18 tablets out of 20 tablets were within the range of ± 3S.D and all readings of three batches were found within ±1% from target weight of tablet (i.e. +982.93 mg and -963.46mg). There was no chipping/capping observed during compression of tablets.

Tablet thickness

Thickness of all three batches shows that all tablets were found within the range of ±3S.D(range:0.08-0.09)(table 3).

Tablet hardness

Hardness was found in the range of 5.51-5.65 with standard deviation 0.11-0.19 within the range ±3 (table 3).

Tablet friability

The friability of tablets was checked for all of three batches and was found to be less than 1% (table 3).

Disintegration test

Results of disintegration test of compressed tablets met the USP official requirements in water and were within the range of 15.6-17.3 minutes and appears to be satisfactory.

Coated tablets were also studied for their disintegration and were found to be within the range of 26.3 minutes - 27.3 minutes in simulated gastric fluid (table 3).

Dissolution test

Dissolution test was performed as per USP 24 /NF 19, 2000 (Dissolution of nutritional supplements) on coated tablets. Results met the requirements of USP tolerance limit, i.e. NLT 75% of the assayed content of the index vitamin (Vitamin C) or the index element from the units tested is dissolved in one hour as presented in table 3.

***Ref. USP 24 <2040>, Dissolution of nutritional Supplements*:** All nutritional supplements belonging to USP Class II to VI, prepared as tablet or capsules, are subject to the dissolution test and criteria described for index vitamins and index minerals as mentioned below:

USP CLASS	Combination of Vitamins or Minerals present	Dissolution Requirement
I	Oil-Soluble vitamins	Not applicable
II	Water -Soluble vitamins	One index vitamin; Folic Acid (if present)
III	Water -Soluble vitamins with Minerals	One index vitamin and one index elemental; Folic Acid (if present)
IV	Oil and Water - Soluble vitamins	One index water soluble vitamin; Folic Acid (if present)
V	Oil and Water - Soluble vitamins with Minerals	One index water soluble vitamin and one index elemental; Folic Acid (if present)
VI	Minerals	One index element

Table 2: Evaluation of flow properties of blended granules

Powder Blend	Angle of repose Mean ± SD	Bulk Density Mean ± SD	Tapped Density Mean ± SD	Carr's Index Mean ± SD	Hausner's Ratio Mean ± SD
	(degrees)	g/ml	g/ml	(%)	Ratio
Batch 1	35.30±0.04	0.73±0.01	0.95±0.01	22.35±0.01	1.29±0.02
Batch 2	34.99±0.11	0.74±0.01	0.95±0.01	22.48±0.02	1.29±0.02
Batch 3	34.72±0.03	0.74±0.01	0.94±0.01	21.29±0.02	1.27±0.03
Flowability	Good	Good	Good	Passable	Passable

All results were average of three observations

Table 3: Pharmaceutical evaluation of trial batches

Batch No.	Weight (mg) (Core Tab) Mean ± SD	Thickness (mm) (Core Tab) Mean ± SD	Hardness (kp) Mean ± SD	Volume of Tablet mm^3 Mean ± SD	Friability (%) Mean ± SD	Disintegration Time (Core Tab) (min) Mean ± SD	Disintegration Time (Coated Tab) (min) Mean ± SD	Dissolution Assayed content of Vit.C dissolved in one hour (% Release) Mean ± SD
1	979.3 ±1.14	5.68 ± 0.08	5.54 ± 0.19	267.6±1.18	0.028±0.15	16.6±1.36	27.3±1.87	80.34±1.31
2	978.9 ±0.92	5.67 ± 0.09	5.51 ± 0.14	266.5±1.09	0.027±0.12	17.3±1.35	26.3±1.88	79.25±1.50
3	978.8 ±1.01	5.65 ± 0.08	5.65 ± 0.11	267.2±0.95	0.027±0.13	15.6±1.33	26.3±1.90	81.15±1.88

Table 4: Percent drug content (assay) of antioxidant tablets

Active Ingredients	Specification (USP-24, NF-19 2000)	Batch 1 Percent drug content (mg/tab)	Batch 2 Percent drug content (mg/tab)	Batch 3 Percent drug content (mg/tab)
Vitamin A	1.5 mg/5000 I.U.	102.63% / (1.95)	102.105% / (1.94)	103.16% / (1.96)
Vitamin C	500 mg	99.8% /(548.9)	100.35% / (551.92)	100.57% / (553.14)
Vitamin E	60.0 mg/30 I.U.	104.25% (65.68)	104.2% (65.64)	103.7% (65.33)
Lecithin	100 mg	98.566% (98.56)	98.531 % (98.53)	98.515% (98.51)
Zinc	10 mg	99.89% (9.98)	100.03% (10.0)	100.04% (10.0)

Table 5: Stability of Antioxidant tablets –maximum release /assay results

Months	Physical Appearance	Vitamin C Labelled amount mg /tablet (% Potency)	Vitamin A Labelled amount mg /tablet (% Potency)	Vitamin E Labelled amount mg /tablet (% Potency)	Zinc Labelled amount mg /tablet (% Potency)	Lecithin Labelled amount mg /tablet (% Potency)	Disintegr-ation time (minutes)	Dissolution Index Vit. C (SinglePoint)
0	Complies	553.14mg (100.57%)	1.96mg (103.16%)	65.33mg (103.70%)	10.0mg (100.03%)	98.566mg (98.566%)	25	80.24%
3	Complies	550.0mg (100.0%)	1.93mg (101.58%)	64.14mg (101.81%)	9.968mg (99.685%)	98.371mg (98.371%)	25	79.26%
6	Complies	545.0mg (99.09%)	1.91mg (100.53%)	63.56mg (100.89%)	9.948mg 999.48%)	98.36mg (98.36%)	26	80.88%,
9	Complies	543.57mg (98.83%)	1.89 mg (99.47%)	62.82mg (99.71%)	9.928mg (99.285%)	98.258mg (98.258%)	25	84.11%,
12	Complies	536.69mg (97.58%)	1.87 mg (98.42%)	61.85mg (98.175%)	9.91mg (99.10%)	98.217mg (98.217%)	25	82.49%,
18	Complies	532.73mg (96.86%)	1.85 mg (97.37%)	61.13mg (97.03%)	9.884mg (98.84%)	98.205mg (98.205%)	23	79.26%
24	Complies	530.75mg (96.50%)	1.82 mg (95.79%)	60.95mg (96.75%)	9.858mg (98.58%)	98.122mg (98.122%)	21	80.88%

(Condition: 30°C ± 2°C / 65% ± 5% RH)

Table 6: Stability of Antioxidant tablets- Assay results

Months	Physical Appearance	Vitamin C Labelled amount (% Potency)	Vitamin A Labelled amount (% Potency)	Vitamin E labelled amount (% Potency)	Zinc Labelled amount (% Potency)	Lecithin Labelled amount (% Potency)
6	Complies	95.4%	99.7%	98.6%	98.2%	94.5%

(Condition: 40°C ± 2°C / 75% ± 5% RH)

Table 7: Percent Degradation of vitamin A, C, E, zinc and lecithin

Months	Vit.A (% Degradation)	Vit.C (% Degradation)	Vit.E (% Degradation)	Zinc (% Degradation)	Lecithin (% Degradation)
3	0.010	0.100	0.397	0.115	0.065
6	0.008	0.237	0.295	0.0917	0.034
9	0.008	0.163	0.279	0.083	0.034
12	0.007	0.220	0.290	0.077	0.029
18	0.006	0.168	0.233	0.066	0.020
24	0.005	0.147	0.182	0.061	0.0184

(Condition: 30°C ± 2°C /65% + 5% RH)

Assay of tablets

Assay results of Vitamin A, E and chemical test results of vitamin C, zinc and lecithin were found as per acceptance requirement of USP24 / NF19, 2000 (table 4).

Statistical analysis of vitamin A, C, E, zinc and lecithin was performed by One-way ANOVA at statistical level of significance equal to 0.05 or 5%. The test of significance gives a p-value lower than the significance level, the null hypothesis is rejected at that level. These results are found to be significant ($p<0.05$).

Stability Studies

The% degradation of vitamin A, C, E, lecithin & zinc follow zero order reaction and it is calculated by using following zero order equation;

$$K = \frac{C_o - C}{t}$$

$$t = \frac{C_o - C}{k}$$

$$C_o - C = kt$$

Where:

k = Rate constant
t = Time in weeks/months/years
C = Concentration in % time t
C_o = Concentration in % at time 0

Evaluation of stability data

Evaluation of the data obtained from 24 months stability studies was performed and each attribute was evaluated in sequence as mentioned in the ICH guideline "Q1A(R) stability testing of new drug substances and products". (European Medicines Agencies, 2003 CPMP/ICH/2736 /99).

The purpose of stability studies is to provide the facts about the quality of active ingredients of formulation varies with time under the influence of a variety of environmental factors. After comparison of stability data of three batches the proposed shelf life of product is 24 months, as % degradation is found to be NLT 90% of initial assay/test results of all active ingredients (tables 5-7). Half Life ($t_{1/2}$) was also calculated by using formula, $t_{½} = 0.693/k$ (figs. 1-2).

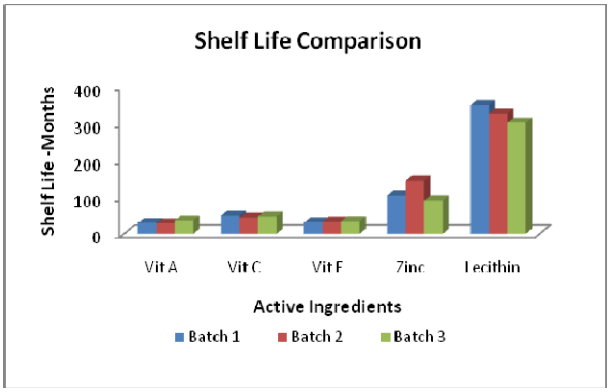


Fig. 1: Shelf-life comparison of active ingredients among three batches

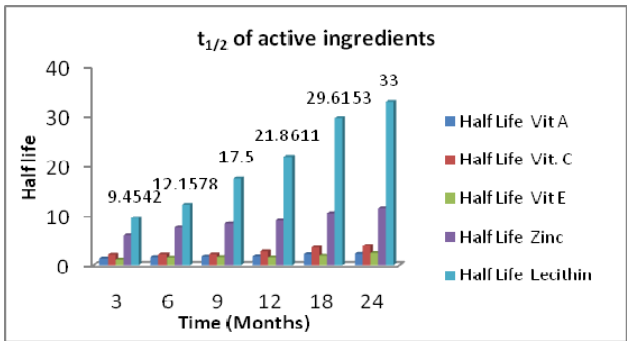


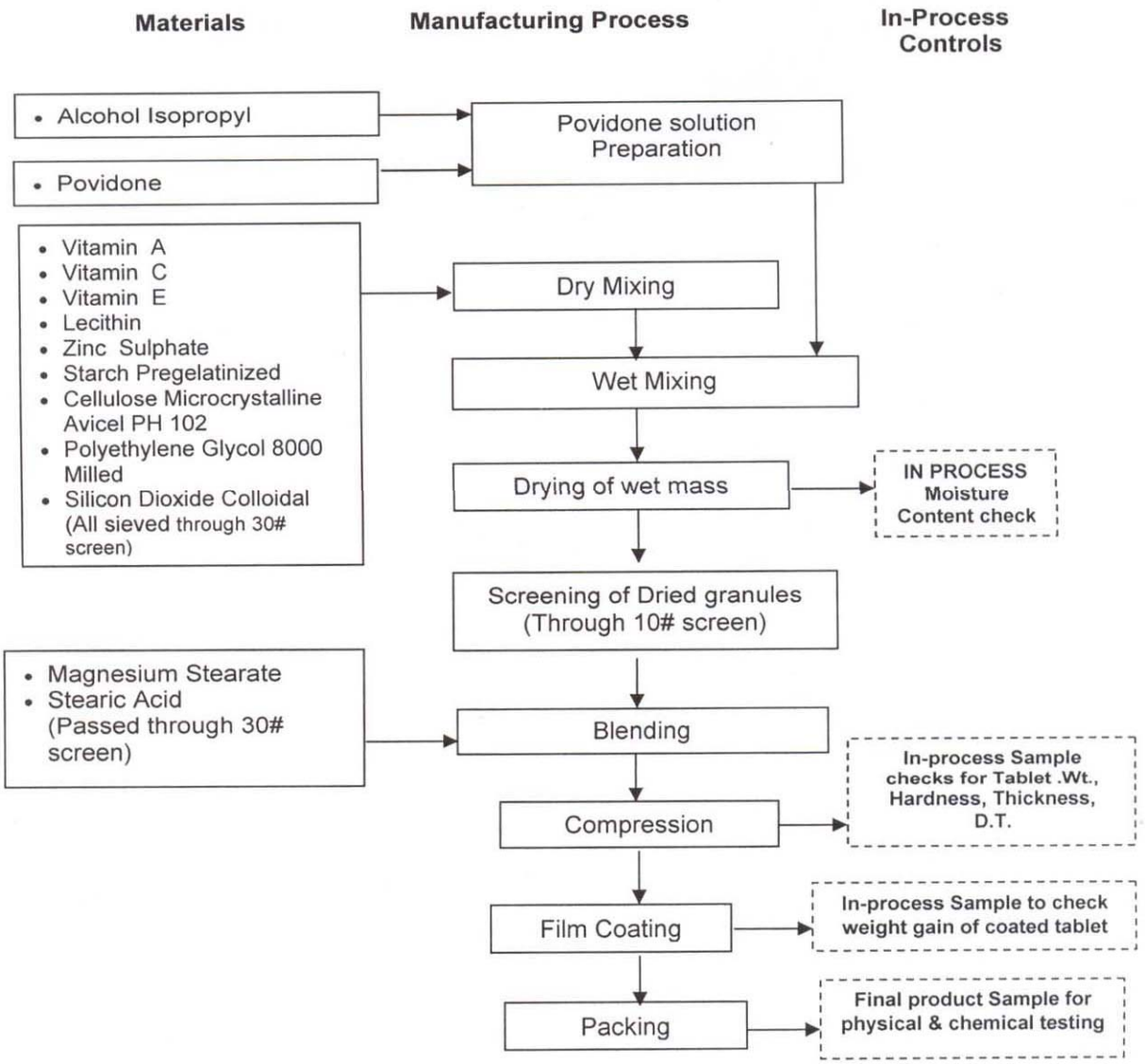
Fig.2: Half-life ($t_{1/2}$) of active ingredients

DISCUSSION

Antioxidant tablets are important for health benefits, considering many diseases as a dietary supplements in the case of every day free radical damage protection and prevention (Milosevic *et al*, 2011). In the present study, the immediate release antioxidant tablet with vitamin A, vitamin C, vitamin E, lecithin and zinc in one formulation was successfully manufactured by employing wet granulation method. This method of granulation has been employed earlier in designing rapidly releasing dosage forms (Jampani *et al*., 2012). The powder blend showed good flow properties as evaluated by various tests (table 2). Results of all physical parameters were found satisfactory during evaluation of compressed and coated tablets. Disintegration time is very important for immediate release tablets as it assists swallowing and increasing drug absorption (Gowtham *et al*., 2011). Disintegration time of (compressed and coated) tablets was found within the USP specification range (table 3). The percent cumulative as ascorbic acid was found to be within the range i.e., NLT 75% in 60 minutes (table 3).

For stability studies antioxidant tablets were packed in PVC/PVDC al. foil and kept for tropical conditions (ICH Zones 3 and 4) at 30°C±2°C/65%±5% RH and at 40°C±2°C/75% ±5% RH,conducted as per I.C.H. Guidelines QIA (R2). Final product assay results were evaluated for 24 months stability period. Percent degradation of Vitamin A, C, E, lecithin & zinc was

Flow Diagram of Manufacturing of Antioxidant Tablet



determined as zero order reaction and calculated at each time interval completion during 24 months. Results confirmed that percent degradation of individual active ingredients is meeting acceptance limit of NLT 90% availability of each active ingredient vs. initial amount at 0 month time period (tables 5 and 6). However, it has been reported that the vitamin C presented stability for 48 hours at 25°C, with or without photo protection in parenteral nutrition for neonatal use (Daniela *et al*., 2011). Another study revealed that the percent loss of vitamin C in sustained release antioxidant tablet coated with ethyl cellulose at pH 1.2 during 8 hours attained 0.68% at ambient temperature and 2.50% at 37°C (Cantoni *et al*, 2010).

Research work on statistical evaluation of pharmaceutical stability data have been performed earlier to achieve improvement in the current statistical model of the stability study and for the sake of the reliable shelf-life estimation (Komka and Kemeny, 2003). Statistical evaluation of stability data was done to propose shelf life. According to ICH, the shelf life of any formulation estimated as the period time at which concentration of the components are not reduced more than 10% (I.C.H. QIA R2). For this purpose calculation of X-axis at point of intersect of lower 95% confidence line with 90% drug activity, t_{90}, of each active ingredient was done. On the basis of data available, the proposed shelf life is at least 24 months, with no intermediate significant change during stability period (figs. 1 and 2). Analysis of stability data generated was performed by using computer software program, Sigma plot (Systat Software Inc. exact graphs and data analysis) for each active ingredient. After verification of physical and chemical parameters, it is concluded that this manufacturing process is reliable and reproducible. All the parameters were found to be unchanged significantly over a long-term study period of 24 months.

CONCLUSION

Antioxidant tablet comprising of Vitamin A, C, E and zinc in combination with lecithin were satisfactorily manufactured by using wet granulation method. Results of stability testing of all three batches were satisfactory, showing no significant changes in physical characteristics, disintegration, dissolution and content assays of coated tablets. The shelf life was found to be 24 months Therefore, these finding suggest that the quality of the formulated product can be maintained over a storage period of at least 24 months.

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