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FORMULATION, DEVELOPMENT, OPTIMIZATION AND EVALUATION OF PARENTERAL PREPARATION FOR MIGRAINE TREATMENT

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Abstract

This study was aimed for the formulation development, optimization and evaluation of parenteral preparation for migraine treatment. A pre-formulation study for assay of model drug was conducted by characterization of active pharmaceutical ingredient done by IR showing numerous level of stretching between C-C, C-H, C=O, C-N and UV spectroscopy where calibration curve was prepared and R2 value was found 0.996, it shows the linearity between 10 µg/ml to 90 µg/ml. Forced degradation study was performed which concluded that model drug is susceptible for acidic, alkaline, oxidation and thermal. Drug excipient compatibility studies found that glycerin from spectrum and ethanol from Hayman is providing the satisfactory impurity profile while kept under stress condition. The tentative manufacturing process flow results reflected that there was significant increase in the Impurity C & D at 40/75%RH –1 month. Two batches were prepared by purging N_2 and CO_2 and it has been estimated that the Methane sulphonic acid (MSA) was consumed more in case of batch prepared under CO_2 purging. Hence N_2 as inert gas was recommended. Evaluation of the developed batch concluded that batch passes the osmolality test. However, the results of liquid particle count of developed batch also pass all the parameter of USP.

Keywords: Migraine, optimization, Methane sulphonic acid, degradation, Hayman.

Introduction

A complicated illness with genetic influences, migraine is typified by episodes of moderate-to-severe headache, usually unilateral, and is typically accompanied by nausea and enhanced light

and sound sensitivity. The Greek word "hemikrania," which was then translated into Latin as "hemigranea," is where the word migraine originates. One name that fits this description is "migraine" in French.(1). About 15% of people worldwide suffer from migraines, one of the most prevalent types of headaches that is regarded as a debilitating illness. (2.) As the most expensive neurological condition, migraine is a crippling condition that heavily affects sufferers both during the ictal and interictal phases (3). Based on the frequency of headaches and the presence or absence of an aura, migraines can be classified. Whether a patient has chronic migraine or episodic migraine depends on the number of headache days. 4.). Seventy-five percent of migraine cases are of the aura-free variety.(5). Antidepressants, anticonvulsants, antihypertensives, gepants, and calcitonin gene-related peptide (receptor) monoclonal antibodies (CGRP(r)mAbs) are among the drug types that are frequently used for migraine prevention (6).

According to epidemiological research, there was a 40.1% increase in the global incidence of migraine in 2019 to 87.6 million (95% UI: 76.6, 98.7), up from 1990. With 43.6% of all incidents worldwide, the countries with the highest number of incidences were Indonesia, China, India, and the United States of America. The incidence was higher in females than in males, with the 10–14 age group showing the highest incidence rate.(7)

The parenteral drugs (intravenous, intramuscular, and subcutaneous) available in the emergency department (ED) and in other clinical settings for the treatment of migraine offer several theoretical advantages over the (usually oral) formulations used by patients for self-treatment. Parenterally administered drugs may offer improved speed of onset of relief and, along with rectal formulations, can be used when severe nausea or vomiting preclude the use of oral medication. However, anecdotal research indicates that in emergency or urgent care settings, migraine is often treated insufficiently. A range of parenteral medications have undergone controlled testing to treat acute migraine headaches and their accompanying symptoms. Certain drugs, like NSAIDs and opiates, are commonly used to treat other illnesses, while others, like dihydroergotamine, are used almost exclusively or only to treat migraine. (8).

MATERIAL AND METHOD

Chemicals and drugs: All the chemicals which were used are of a ACS grade, excela R grade and procured from Sigma Chemical Co., USA, TEVA, Spectrum, Hayman limited, Spectrochem, Merck, Milli Q and Qualigens fine chemicals, Mumbai, India.

Pre-formulation study:

a) API characterization: Assay Detection of API was characterized by IR and UV spectroscopy using various ACS and excela R grade chemicals like, Milli Q water, p-dimethylaminobenzaldehyde, Ferric chloride, Sulfuric acid 98.08%, Tartaric acid at 583nm wavelength in 1cm cell.

Preparation of Diluent –10 g of tartaric acid was weighed and transfered to a bottle containing 1000 ml of water. Sonicate to dissolve completely at 20-25°C for about 5 minutes and mix well by shaking the bottle (9).

Preparation of Ferric chloride solution - 1 g of ferric chloride was weighed and transferred into a 20 ml volumetric flask. Add about 10ml of water and sonicate to dissolve at 20-25°C for about 5 minutes. Make up the volume upto the mark with water and mix well by shaking the flask in upside down and downside up movement for 4-5 times.

Preparation of Solution-A: 130ml of sulfuric acid was added and mixed in a 500 ml flask containing 70ml water. Cool the solution to room temperature. Weigh and transfer 250mg of p-dimethylaminobenzaldehyde to the above flask containing the solution. Sonicate to dissolve at 20-25°C for about 5 minutes. Add 0.4ml ferric chloride solution and mix well by shaking the flask in round movement of wrist for 4-5 times (10).

Preparation of blank solution: Transfer 5 ml of this Diluent to a 50 ml conical flask. Add 10 ml of solution-A. Shake well to mix by wrist movement for 4-5 times. Allow the solution to stand for 30 min and measure the absorbance.

Standard solution preparation-(50ppm): Weighed 50 mg of working standard and put in 50 ml volumetric flask. Added 30 ml diluent and sonicate to dissolve at 20-25°C for about 5 min. make up the volume with diluent and mix well by shaking the flask in upside down and downside up movement for 4-5 times. Dilute 5 ml of this solution to 100 ml with diluent and mix well by shaking the flask for 4-5 times. Transfer 5 ml of this standard solution to 50 ml conical

flask. Added 10 ml of solution A. shake well to mix by wrist movement for 4-5 times. Allow the solution to stand 30 min and measure the absorbance against blank.

Sample preparation (sample stock): Carefully mix the content of not less than five sample vials and dilute 5ml of this solution to a 100 ml with diluent and mix well by shaking the flask in upside down and downside up movement for 4-5 times. Transfer 5ml of this sample solution to a 50ml conical flask. Add 10ml of solution A. shake well to mix by wrist movement for 4-5 times. Allow the solution to stand for 30 min and measure the absorbance against the blank (11).

b) Forced degradation study of API

Forced degradation study was performed by providing stress thermal, acidic, alkali, oxidation condition and the nature of model drug was identified detected by HPLC.

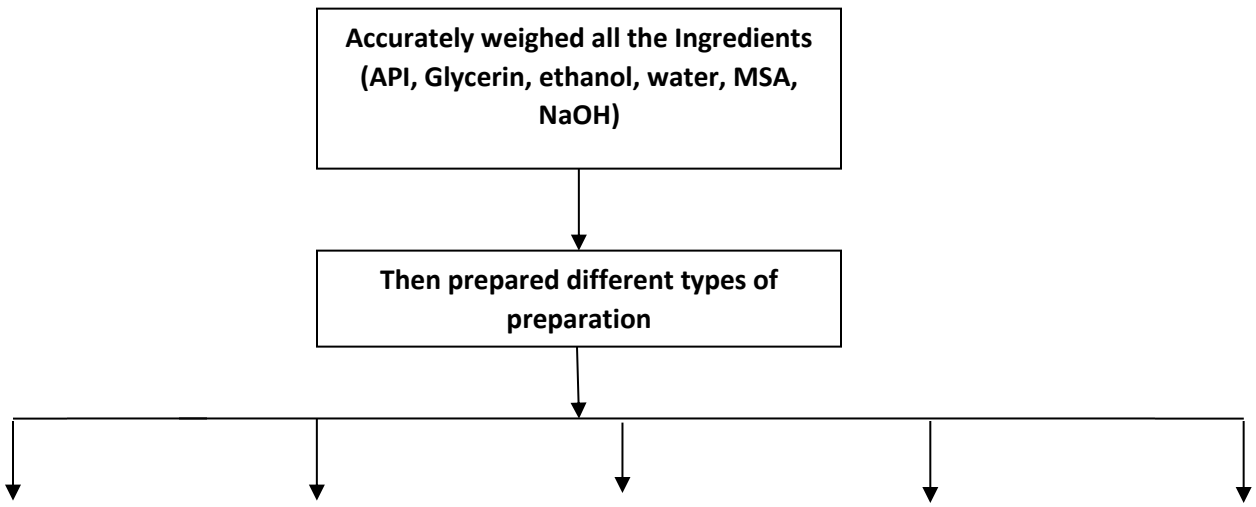
Mobile phase preparation: 700ml of water + 300 ml of Acetonitrile (ACN) + 5ml Triethylamine. Adjust the pH 2.00 with ortho-phosphoric acid.

Diluent mixture: 750ml of mobile phase + 150ml of propylene glycol.

Preparation of Standard solution: 100mg of API and dissolve in 100ml of diluent i.e. 1mg/ml. 1ml from the above prepared solution and diluent it up to 100ml i.e. 0.01mg/ml (10ppm).

c) Drug excipient compatibility study

Drug excipeint incompatibility study was performed using same grade of material from different vendor and finalized the best suited composition based on assessment of chemical attributes of product (12).



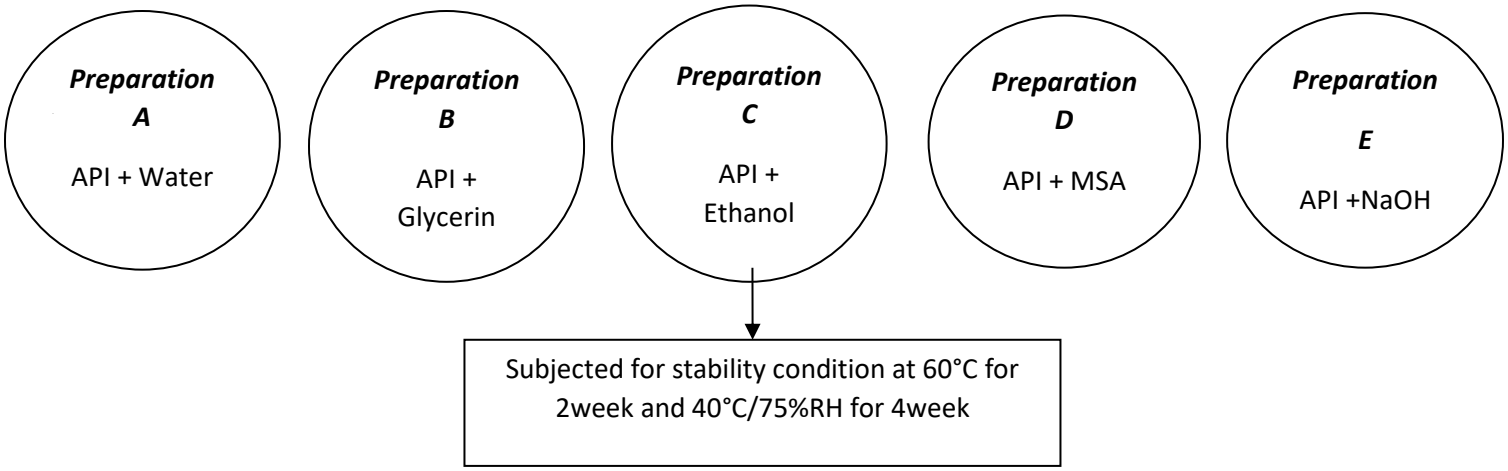


Figure 1: Procedure for Compatibility study

2) Formulation Development Trials

a) Feasibility study

i. **Order of addition optimization with process temperature condition:** Based on forced degradation study, it was found that model drug is susceptible for all the degradation (Acidic, alkaline, peroxide, photolytic and thermal). The order of addition was finalized by evaluating the impact on chemical attributes of drug product i.e. content of ethanol and related substance at initial and 2 week 60°C exposed.

3) Process optimization trials

- **Tentative manufacturing process flow:** Collected 80% water in schott bottle. And added weighed amount of API and Glycerin in the above collected water to be mixed properly. Added measured amount of ethanol in the above collected solution to make up the volume up to 100%. Filter and filled the bulk solution in the vials and submitted for stability at 40±2°C/75±5%RH and 25±2°C/60±5%RH.

i. **Effect of light during manufacturing:** Different trials were taken for evaluating the impact of light on drug product attributes. After preparation of bulk solution sample hold for 24 hr and 48 hr at ambient light and under sodium lamp to evaluate the impact on description, pH, assay and related substances of drug product.

ii. **Purging gas optimization trial:** Inert gas for purging, optimization was performed by preparation of bulk solution using carbon dioxide and nitrogen during manufacturing. Both

batches were subjected for stability. Selection of inert gas was performed on basis of volume of methane sulphonic acid consumed to attain the targeted pH of 3.6 during bulk solution manufacturing (13).

iii. **pH optimization trial:** pH optimization trial was executed with finalized order of addition. As the product pH range is 3.4 to 4.9. Batches were prepared at 3.4, 3.6, 4.0 and 4.5 and subjected for stress study for 2 week at 60°C and 40/75%RH for 1 month.

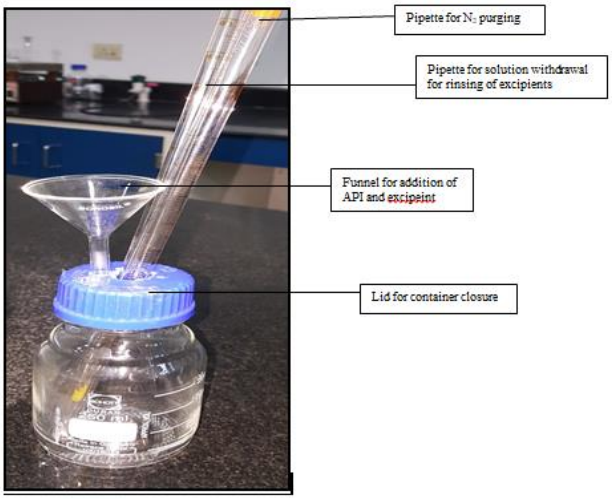


Figure 2: The final preparation of closed assembly for the manufacturing of product

iv. **Impact of manufacturing process temperature:** Manufacturing process temperature optimization was performed by preparation of bulk solution at room temperature (25°C) and 2-8°C and batches were subjected for stability study at accelerated condition.

v. **Impact of terminal sterilization or selection of sterilization process:** Terminal sterilization was done by both i.e. aseptic method (filtration) and moist heat method (autoclave at 121°C for 20 min). Batch was subjected for analysis at initial and 2 week 60°C exposed for description, pH, assay and related substance (14).

vi. **Filter flush study and primary packaging component finalization:** Filter flush study was performed to evaluate the suitable filter for drug product bulk solution manufacturing. -Batch was manufactured and filtered it by both PES and PVDF membrane (0.22µ). Collected all the samples and tested for analysis which includes Description, assay, pH, and related substance.

vii. **Hold time stability with process component:** Hold time study was performed with different process component which will come in contact with product solution throughout the

manufacturing process. Hold time study was performed with SS316L, glass, tubing (Silicone tubing and PTFE tubing).

viii. **Photostability study:** Photostability study was perform by following ICH Q1B guideline and product was exposed for sufficient time to get expose for recommended Photostability condition. As the marketed formulation was packed in amber color bottle. To conclude exactly the requirement for product, bulk solution were filled in transparent clear glass vial too for evaluating the impact of light on product. Plan of study are tabulated below (15).

ix. **Prototype development batch manufacturing:** Based on finalized order of addition, pH of final bulk, manufacturing process temperature, process component, other process related requirement, batch were prepared and subjected to stability study at accelerated (40±2°C\75±5%RH) and long term (25±2°C/60±5%RH) stability condition and liquid particle count at 1, 2, 3, 6 months. Batch was filled in amber color sulfur treated vial and non treated vial using both coated and uncoated stoppers.

x. **Additional stability study (Freeze thaw study):** Finalized batches after 3 month stability study data, additional stability data generation was performed which includes Freeze thaw, thermal cycling, short term temperature excursion study.

xi. Evaluation of developed batch

a) **Osmolality:** Freezing point depression Osmometer was used for measurement of osmolality by calibrating it with NaCl (0.9%) solution. Then takinh three reading, the value should be 290±2mosm. If meet the requirement then system is calibrated. Then take the product (1:10 diluted with WFI) batch of stability and find its osmolality.

b) Liquid Particle Count

- First calibrate the instrument with the WFI.
- If it will pass the calibration level then place the sample.
- Take 5 ml solution in the beaker and then calculate the liquid particle count (16).

RESULT

1) **Pre-formulation study:** Pre-formulation studies for assay of model drug was conducted by characterization of active pharmaceutical ingredient, Force degradation study of the drug, compatibility studies of the drug and excipient, compatibility studies of API with the other excipients and many others.

a) **API characterization:** characterization of active pharmaceutical ingredient (API) was performed through infrared spectroscopy by showing O-H, C-H, C=C, N-H, C-O, C=O

stretching at different peaks and UV spectrophotometer by interpreting calibration curve of Model drug in water at 583 nm wavelength having Correlation coefficient, r^2 = 0.996 as shown in figure__ below.

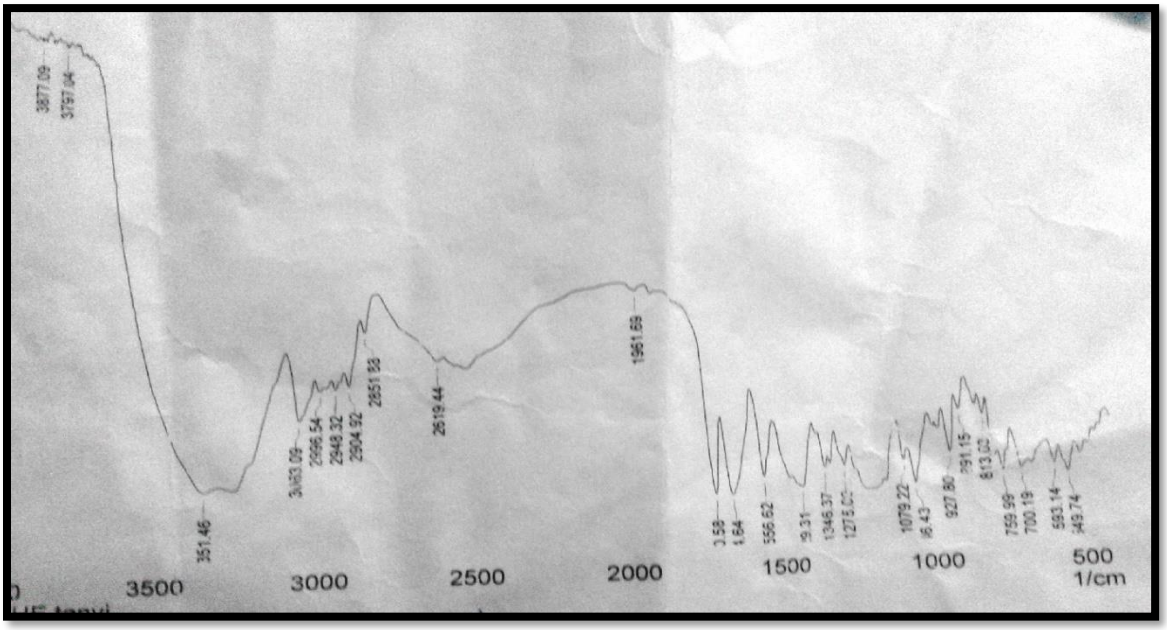


Figure 3: I.R of API

Table 1: Interpretation of I.R data

Peaks	**Peaks**
549.74	1556.62
593.14	1664.64
700.19	1720.58
759.99	1961.69
813.03	2619.44
891.5	2851.88
927.8	2904.92
1046.43	2948.32
1079.22	2996.54
1275	3063.09
1346.37	3351.46
1429.31	3797.04
3877.09	

1.	O-H	stretch.	3797.04 cm-1
2.	C-H	Bending	2851.88 cm-1
3.	C=C	stretch	1556.62 cm-1
4.	N-H	stretch	3351.46 cm-1
5.	C-O	stretch	1046.43 cm-1
6.	C=O	stretch	1664.64 cm-1

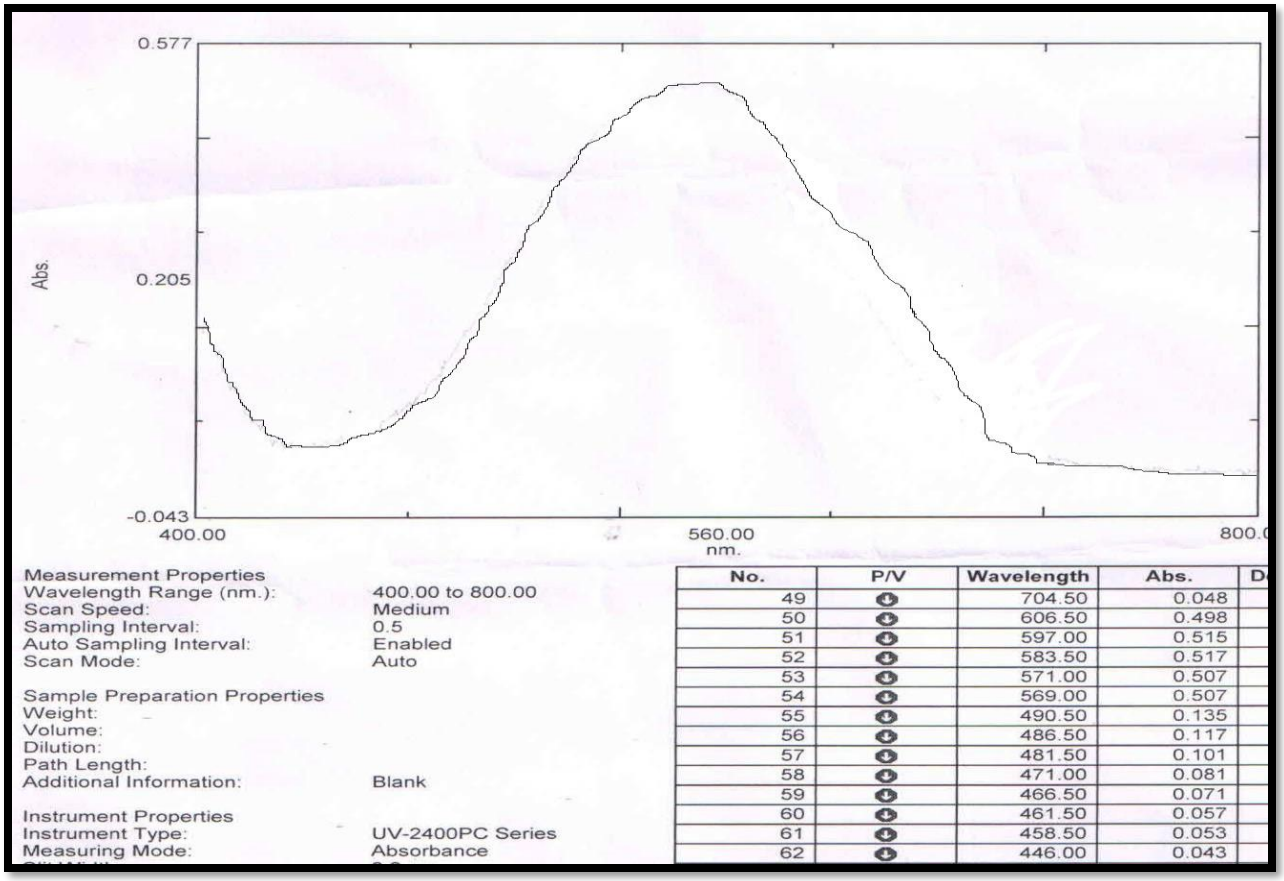


Figure 4: Preparation of calibration curve of Model drug in water by UV spectrophotometer

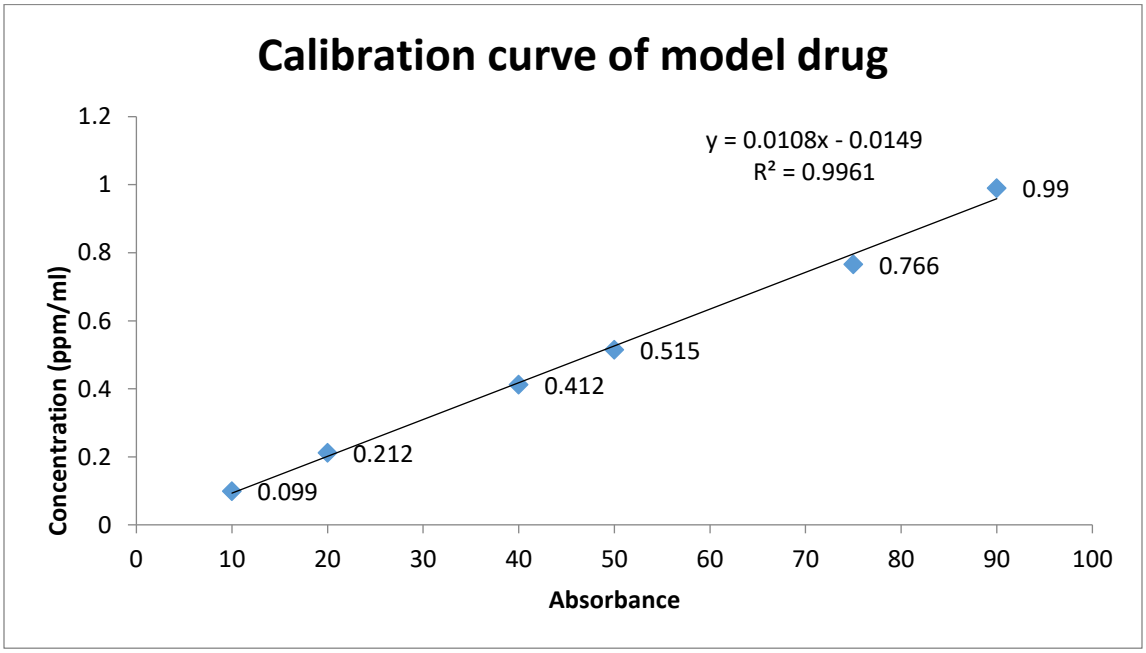


Figure 5: Calibration curve of model drug in water

Table 2: Assay and Impurities (RS) of API [ref. COA of API]

Parameters	Observed Value (%)	Range (%)
Assay	100%	97.0 to 103%
RS		
Impurity A	0.05%	NMT 0.10%
Impurity B	0.19%	NMT 0.30%
Impurity C	0.15%	NMT 0.50%
Any unspecified impurity	0.05%	NMT 0.10%
Total impurity	0.44%	NMT 1%

b) Force degradation of drug study

Table 3: Force degradation data of model drug

Model drug									
		Known Impurities					**Unknown Impurities**		
Mode of degradation	**Condition**	**Compound 1 RRT 1.0**	**A RRT (0.83)**	**B RRT (0.95)**	**C RRT (0.97)**	**D RRT (1.07)**	**Max. Unk-1**	**Max. Unk-2**	**Max. Unk-3**
Control	-	99.14	0.13	0.15	0.23	ND	0.13 (RRT-0.47)	0.05 (RRT- 0.53)	0.04 (RRT- 0.29)
Thermal degradation	70°C /120 min	98.87	0.57	0.14	0.24	ND	0.10 (RRT- 0.53)	0.06 (RRT- 0.46)	0.05 (RRT- 0.79)
Acid degradation 5 N HCl	1 ml / 70°C / 120 min	37.17	40.15	0.47	0.26	21.69	0.26 (0.29)	ND	ND
Alkali degradation 5 N NaOH	1 ml / 70°C / 120 min	14.14	ND	ND	ND	ND	85.71 (RRT-0.6)	0.15 (RRT-1.09)	ND
Peroxide degradation 30 % w/v H2O2	1 m l/ 70°C / 120 min	73.7	11.26	ND	ND	ND	4.54 (RRT-0.36)	3.41 (RRT-1.10)	3.11 (RRT-0.63)
Acid degradation 1 N HCl	1 ml / 70°C / **180 min**	41.24	51.51	0.19	0.08	6.41	0.33 (RRT-0.41)	0.12 (RRT-0.76)	0.13 (RRT-1.27)
Alkali degradation 1 N NaOH	1 m l/ 70°C / **180 min**	14.11	0.1	ND	ND	ND	34.17 (RRT-0.30)	49.40 (RRT-0.36)	2.08 (RRT-0.26)
Peroxide degradation 30 % w/v H2O2	0.5 ml / 70°C / **180 min**	82.42	6.58	0.14	0.18	ND	0.81 (RRT-0.36)	0.49 (RRT-0.41)	4.87 (RRT-1.04)

ND-Not Detected

c) Drug excipient compatibility

Table 4: The result of best grade of material for our formulation

Condition for stability (40±2°C/75±5%RH / 4 weeks)

Excipient name	Finar								Spectrum							
	Known (NMT 1%)						Unknown (NMT 0.5%)	Total (%)	Known (NMT 1%)					Unknown (NMT 0.5%)		Total (%)
	A	B	C	D	E	UN 1	UN 2	2.95	A	B	C	D	E	UN 1	UN 2	*1.19*
Glycerin	0.23	0.12	0.65	0.75	0.45	0.45	0.30		*0.03*	*0.15*	*0.34*	*0.09*	*0.34*	*0.04*	*0.20*	
	Merck								Hayman							
Ethanol	0.54	0.24	0.08	0.45	0.76	0.07	0.34	2.48	*0.20*	*0.10*	*0.39*	*0.06*	*0.33*	*0.07*	*0.38*	*1.53*

Table 5: The compatibility of API with the excipients

Preparations	Conditions	
	60°C / 2 weeks	40°C/75%RH /4 weeks

	Known (NMT 1%)					**Unknown (NMT 0.5%)**		**Total (%)**	**Known (NMT 1%)**					**Unknown (NMT 0.5%)**		**Total (%)**
	A	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**		**A**	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**	
Preparation A (API+water)	0.53	0.12	0.05	0.30	0.05	0.15	0.01	1.21	0.03	0.15	0.44	0.09	0.30	0.04	0.20	1.25
Preparation B (API+glycerin)	0.54	0.14	0.18	0.15	0.66	0.07	0.24	1.98	0.20	0.19	0.39	0.16	0.33	0.07	0.38	1.72
Preparation C (API+ethanol)	0.28	0.32	0.29	0.34	0.19	0.41	0.03	1.86	0.28	0.40	0.36	0.41	0.17	0.26	0.05	1.93
Preparation D (API+MSA)	0.20	0.06	0.78	0.20	0.49	0.39	0.29	2.41	0.75	0.54	0.39	0.08	0.65	0.37	0.62	3.40
Preparation E (API+NaOH)	0.19	0.24	0.39	0.33	0.23	0.29	0.19	1.86	0.01	0.09	0.05	0.29	0.29	0.02	0.32	1.07

2) Formulation Development Trials

a) Feasibility study

i) Order of addition optimization with process temperature condition

Table 6: Results for different order of addition of excipients at various conditions

Conditions		**At 25°C**									**At 2-8°C**								
No. of Trials	**Study Condition**	**Ethanol content (NLT 90%)**	**Total impurity (NMT 8%w/w)**								**Ethanol content (NLT 90%)**	**Total impurity (NMT 8%w/w)**							
			Known (NMT 1%)					**Unknown (NMT 0.5%)**		**Total (%)**		**Known (NMT 1%)**					**Unknown (NMT 0.5%)**		**Total (%)**
			A	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**			**A**	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**	
T1	Initial	87.96	0.37	0.65	0.34	0.33	0.08	0.08	0.05	1.90	85.78	0.65	0.34	0.35	0.05	0.70	0.04	0.30	2.43
	60°C 2 weeks	85.78	0.53	0.12	2.05	0.30	0.05	0.15	0.01	3.21	85.00	0.24	0.43	0.22	4.23	0.11	0.65	0.25	6.13
T2	Initial	90.76	0.40	0.09	0.45	0.38	0.22	0.47	0.60	2.61	97.03	0.10	0.14	0.53	0.14	0.12	0.04	0.15	1.22
	60°C 2 weeks	89.65	0.17	0.05	0.54	1.29	0.49	0.09	0.38	3.01	85.09	0.30	0.55	1.00	0.27	0.33	0.18	0.15	2.78
T3	Initial	92.00	0.13	0.09	1.30	0.06	0.55	0.34	0.87	3.34	88.5	0.35	0.87	0.22	0.23	0.22	0.10	0.11	2.10
	60°C 2 weeks	84.36	0.63	0.29	0.20	0.17	0.40	1.98	0.98	4.65	70.20	0.43	0.33	0.65	0.65	0.22	0.32	0.32	2.92
T4	Initial	88.45	0.54	0.56	0.38	0.16	0.45	0.30	0.34	2.73	97.60	0.10	0.34	0.95	0.34	0.07	0.23	0.17	2.20
	60°C 2 weeks	79.56	0.26	0.38	0.40	0.66	0.10	1.78	0.08	3.66	90.43	0.89	0.43	0.66	0.45	0.11	1.07	0.54	4.15

T5	Initial	90.56	0.37	0.22	0.08	0.59	0.30	0.34	1.00	2.90	89.98	0.54	0.07	0.32	0.10	0.55	0.35	0.54	2.47
	60°C 2 weeks	90.17	0.38	0.10	0.83	0.09	0.11	0.35	0.44	2.30	88.09	0.87	0.34	0.87	0.22	0.32	0.32	0.34	3.26
T6	Initial	95.78	0.45	0.09	0.19	0.20	0.11	0.02	0.32	1.38	***97.56***	0.26	0.09	0.47	0.20	0.09	0.41	0.01	1.53
	60°C 2 weeks	95.00	0.56	0.07	0.39	0.14	0.87	0.30	0.05	2.38	***95.34***	0.29	0.14	0.11	0.34	0.56	0.15	0.22	1.81
T7	Initial	98.45	0.95	0.18	0.43	0.55	0.01	0.54	0.10	2.76	97.45	0.20	0.07	0.92	0.55	0.65	0.64	0.43	3.46
	60°C 2 weeks	93.56	1.98	0.11	0.54	ND	0.64	0.54	0.29	4.1	93.90	0.30	0.45	0.66	0.44	0.25	0.19	2.10	4.39
T8	Initial	90.78	0.55	0.75	0.55	0.33	0.44	0.30	0.23	3.15	92.76	0.33	ND	0.66	0.20	0.07	0.34	0.66	2.26
	60°C 2 weeks	85.67	0.59	0.54	0.43	0.64	0.43	0.64	0.06	3.33	89.00	1.89	0.50	0.65	0.49	0.54	0.07	0.53	4.67

ND- Not Detected

2) Process optimization trials

i. Tentative manufacturing process flow

Table 7: Result of tentative manufacturing process flow

Study Condition	Study parameters									
	Assay (%)	pH	Total impurity (NMT 8%w/w)							Total (%)
			Known (NMT 1%)					Unknown (NMT 0.5%)		
			A	B	C	D	E	UN 1	UN 2	
Initial	91.05	4.03	0.23	0.12	6.65	0.75	0.45	0.45	0.30	8.95
25±2°C/60±5%RH	87.76	4.53	0.56	1.98	0.73	*7.33*	0.67	0.04	0.93	12.24
40±2°C/75±5%RH	93.52	4.60	0.54	0.24	0.08	0.45	0.76	4.07	0.34	6.48

ii. Effect of light during manufacturing

Table 8: The effect of light on model drug

Trials	Study Condition	Study parameters										
		Description	Assay (%)	pH	Total impurity (NMT 8%w/w)							
					Known (NMT 1%)					Unknown (NMT 0.5%)		Total (%)
					A	B	C	D	E	UN 1	UN 2	
Batch A	**Initial**	Clear colorless,	91.35	3.84	0.23	0.12	6.65	0.75	0.45	0.45	ND*	8.65

(Ambient light)	**24 hrs**	free from any visible particulate matter	89.76	3.98	1.56	1.98	0.73	4.33	0.67	0.04	0.93	10.24
	48 hrs		89.62	4.54	0.54	0.24	0.08	7.35	0.76	1.07	0.34	10.38
Batch B	**Initial**		***97.84***	***3.62***	***0.06***	***0.67***	***0.54***	***0.25***	***0.27***	***0.29***	***0.08***	***2.16***
(sodium vapour lamp)	**24 hrs**		***97.00***	***3.68***	***0.83***	***0.34***	***0.33***	***0.76***	***0.45***	***0.06***	***0.23***	***3.00***
	48 hrs		***97.65***	***3.68***	***0.40***	***0.54***	***0.11***	***0.70***	***0.06***	***0.04***	***0.50***	***2.35***

ND*- Not Detected

iii. Purging gas optimization trial

Table 9: The difference in the quantity used for MSA in batch manufacturing

No. of Batches	**Amount of MSA to reach pH 3.6 (ml)**
Batch A Purged by CO_2	1.2
Batch B Purged by N_2	0.6

iv. pH optimization trial

Table 10: Result of Batches at different pH

Study condition	**Study Period**	**pH**	**Assay (%)**	**Total impurity (NMT 8%)**		
				Known (NMT 1%)	**Unknown (NMT 0.5%)**	**Total**

				A	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**	**(%)**
Trial 1(at pH 3.4)											
Initial	Initial	3.41	98.18	0.34	0.65	0.06	ND	0.24	0.27	0.27	1.82
40±2°C/75±5%RH	1M	3.53	97.43	0.64	0.64	0.04	0.10	0.75	0.07	0.33	2.57
60°	4 week	3.70	97.40	0.20	0.36	0.07	0.33	0.80	0.39	0.45	2.60
Trial 2 (at pH 3.6)											
Initial	Initial	***3.60***	***98.00***	***0.30***	***0.45***	***0.07***	***0.03***	***0.74***	***0.05***	***0.36***	***2.00***
40±2°C/75±5%RH	1M	***3.83***	***97.99***	***0.48***	***0.43***	***0.30***	***0.20***	***0.20***	***0.20***	***0.20***	***2.01***
60°	4 week	***3.94***	***97.99***	***0.28***	***0.44***	***0.19***	***0.18***	***0.64***	***0.10***	***0.18***	***2.01***
Trial 3 (at pH 4.0)											
Initial	Initial	4.08	98.00	0.74	ND	0.05	0.06	0.54	0.54	0.07	2.00
40±2°C/75±5%RH	1M	4.30	96.34	0.49	0.07	0.07	0.08	0.40	0.54	2.01	3.66
60°	4 week	4.48	94.61	0.74	0.29	0.96	1.08	0.33	1.89	0.10	5.39
Trial 4 (at pH 4.5)											
Initial	Initial	4.51	94.33	1.09	0.27	0.19	0.11	3.10	0.90	0.01	5.67
40±2°C/75±5%RH	1M	4.90	95.97	0.77	0.82	0.64	0.07	0.63	0.37	0.73	4.03
60°	4 week	4.91	88.08	0.19	0.54	0.47	ND	9.64	0.54	0.54	11.92

v. Impact of manufacturing process temperature

Table 11: Temperature affect the ethanol content, Assay of the product batch

Manufacturing condition	Storage Condition	Study parameters									
		Ethanol (90-110%)	Assay (%)	Total impurity (NMT 8%w/w)							
				Known (NMT 1%)					Unknown (NMT 0.5%)		Total (%)
				A	B	C	D	E	UN 1	UN 2	
Room temp (20-25°C)	**Initial**	94.56	97.95	0.23	0.12	0.65	0.05	0.45	0.45	0.10	2.05
	40±2°C/75±5%RH 1 month	84.03	96.06	0.56	1.08	0.03	1.30	0.67	0.04	0.53	3.94
	60°C /2 weeks	90.34	94.62	0.54	0.24	0.08	3.35	0.76	0.07	0.34	5.38
At 2-8°C	**Initial**	***98.78***	97.74	0.06	0.67	0.54	0.35	0.27	0.29	0.08	2.26
	40±2°C/75±5%RH 1 month	***97.67***	97.60	0.43	0.14	0.33	0.76	0.45	0.06	0.23	2.40
	60°C /2 weeks	***97.67***	97.45	0.40	0.54	0.61	0.80	0.06	0.04	0.10	2.55

vi. Impact of terminal sterilization or selection of sterilization process

Table 12: Result of sterilization process that affects the product quality

Sterilization type	Assay (%)	Ethanol content	Total impurity (NMT 8%w/w)		
			Known (NMT 1%)	Unknown (NMT 0.5%)	Total

		(90-110%)	A	B	C	D	E	UN 1	UN 2	(%)
Aseptic sterilization (filtration)	***98.46***	***95.76***	**0.01**	**0.19**	**0.49**	**0.10**	**0.43**	**0.05**	**0.27**	1.54
Terminal sterilization	87.25	94.65	0.23	0.12	3.65	0.75	7.45	0.45	0.10	12.75

vii. Filter flush study and primary packaging component finalization

Table 13: Result of Filter flush study and primary packaging component

Study condition	Ethanol (90-110%)	Assay (%)
0.22µ hydrophilic PES Membrane Filter		
Unfiltered bulk	***97.5***	100.00
Flush 1	***96.3***	97.67
Flush 2	***96.0***	97.65
Flush 3	***96.1***	96.65
Flush 4	***96.0***	96.57
0. 22µ hydrophilic PVDF Membrane Filter		
Flush 1	93.9	99.98
Flush 2	93.7	98.00
Flush 3	87.7	97.20
Flush 4	80.6	95.74

viii. Hold time stability with process component

Table 14: Effect of material that comes in direct contact of the product during manufacturing.

Study condition		pH	Assay	Ethanol content (90-110%)	Total impurity (NMT 8%w/w)							
					Known (NMT 1%)					Unknown (NMT 0.5%)		Total (%)
					A	B	C	D	E	UN 1	UN 2	
SS316L	**Initial**	3.64	100.95	96.46	0.23	0.11	0.65	0.75	0.45	0.45	0.10	2.74
	After 24 hrs	3.65	100.89	95.20	0.54	0.05	0.75	0.37	0.44	0.28	0.27	2.65
	After 48 hrs	3.65	100.89	95.54	0.56	0.54	0.73	1.33	0.67	0.04	0.53	4.40
Glass	**Initial**	3.63	100.98	97.95	0.54	0.09	0.08	0.35	0.76	0.07	0.34	2.23
	After 24 hrs	3.66	100.76	96.46	0.32	0.54	0.43	0.54	0.04	0.20	0.25	2.32
	After 48 hrs	3.65	100.90	95.00	0.56	0.32	0.54	ND	0.27	0.29	0.08	2.06

ND- Not Detected

Table 15: Compatibility results for the selection of tube for the product filling.

St.udy condition	pH	Total impurities (NMT 8%)								Assay (%)	Ethanol content (90-110%)
		Known (NMT 1%)					Unknown (NMT 0.5%)		Total (%)		
		A	B	C	D	E	UN 1	UN 2			

Dynamic tubing compatibility study (silicon)											
Initial	3.67	0.43	0.39	0.64	0.29	0.54	0.04	0.10	2.43	97.57	96.87
First pass	3.00	0.64	0.39	0.54	0.54	0.20	0.22	0.20	2.73	97.27	96.09
Second pass	3.78	0.06	0.49	0.39	0.30	0.20	3.00	0.50	4.94	95.06	9487
Third pass	4.01	0.54	0.06	0.05	ND	2.40	0.30	0.38	3.73	96.27	96.00
Static tubing compatibility study (silicon)											
24 Hrs	4.08	0.57	0.29	0.64	0.29	0.54	0.26	0.20	2.79	97.21	95.98
48 Hrs	4.11	0.36	0.36	0.04	0.74	0.28	0.64	0.54	2.96	97.04	94.00
Dynamic tubing compatibility study (PTFE)											
Initial	3.67	0.29	0.30	0.15	0.20	0.33	0.04	0.28	1.59	98.41	96.86
First pass	4.00	0.20	0.45	0.20	0.44	0.54	0.14	0.24	2.21	97.79	96.80
Second pass	3.98	0.49	0.72	0.29	0.37	ND	0.23	0.29	1.90	98.10	96.80
Third pass	4.01	0.20	0.47	0.56	0.74	0.64	0.64	0.04	3.29	96.71	96.67
Static tubing compatibility study (PTFE)											
24 Hrs	4.08	0.57	0.29	0.64	0.29	0.54	0.26	0.20	2.79	97.21	96.98
48 Hrs	4.09	0.36	0.36	0.04	0.74	0.28	0.64	0.54	2.96	97.04	96.00

ix. Photostability study

Table 16: Results for the Photostability study

Sample exposure procedure	Parameters evaluated										
	Description	pH	Assay (%)	Total impurity (NMT 8%)							
				Known (NMT 1%)					Unknown (NMT 0.5%)		Total (%)
				A	B	C	D	E	UN 1	UN 2	
Control(Vial covered with foil)	colorless solution free from any visible particulate matter	8.65	82.5	2.85	0.53	7.73	3.00	1.67	1.09	0.63	17.5
Directly exposed in transparent vial without label	slight pale yellow color observed	6.56	90.12	0.83	0.35	0.27	0.43	5.04	0.98	1.98	9.88
Directly exposed in transparent vial with label	slight pale yellow color observed	3.76	89.98	0.35	0.46	0.43	0.63	2.87	0.29	0.28	5.31
Exposed in primary packaging in amber color vials without label.	colorless solution free from any visible particulate matter	3.69	100.84	0.52	0.23	0.73	0.07	0.66	0.35	0.27	2.83
Exposed in primary packaging in amber color vials with label.	colorless solution free from any visible particulate matter	3.60	100.56	0.52	0.63	0.52	0.26	0.67	0.34	0.06	3.00
Exposed in secondary packaging material in amber color vials with label and carton.	colorless solution free from any visible particulate matter	3.78	97.65	0.24	0.05	0.52	0.36	0.63	0.35	0.20	2.35

Exposed in secondary packaging material in transparent vials with label and carton.	colorless solution free from any visible particulate matter	3.68	98.34	0.05	0.28	0.97	0.28	0.65	0.34	0.06	2.63

x. Prototype development batch for stability study

Table 17: Stability parameters result after finalization of the manufacturing procedure.

Stability conditions		**Study parameters**										
		pH	**Assay**	**Ethanol content (90-110%)**	**Total impurity (NMT 8%w/w)**							
					Known (NMT 1%)					**Unknown (NMT 0.5%)**		**Total (%)**
					A	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**	
Initial		3.65	98.51	97.51	0.43	0.23	0.11	0.06	0.42	0.19	0.05	1.49
Accelerated 40±2°C\75±5 %RH	1 month	3.76	97.10	95.98	0.11	0.65	0.75	0.45	0.45	0.10	0.39	2.90
	2 month	3.76	97.75	93.98	0.05	0.75	0.37	0.44	0.08	0.27	0.29	2.25
	3 month	3.73	96.58	96.99	0.54	0.73	1.33	0.67	0.04	0.03	0.08	3.42
	6 month	4.04	98.45	90.38	0.09	0.08	0.35	0.36	0.07	0.34	0.26	1.55
Long term	1 month	3.65	97.58	95.98	0.54	0.43	0.54	0.04	0.20	0.25	0.42	2.42

25±2°C/60±5 %RH	2 month	3.75	97.97	93.98	0.32	0.54	0.25	0.27	0.29	0.08	0.28	2.03
	3 month	3.75	98.39	96.43	0.28	0.27	ND	0.37	0.22	0.19	0.28	1.61
	6 month	4.03	97.47	95.88	0.05	0.45	0.73	0.54	0.03	0.32	0.41	2.53

xi. **Additional stability study (Freeze thaw study)**

Table 18: Results of freeze thaw study

No. of cycles	**Temperature(°C) and time period (2 days at each station)**	**Study parameters**											
		pH	**Assay (%)**	**Ethanol (%)**	**Total impurity (NMT 8%)**							
					Known (NMT 1%)					**Unknown (NMT 0.5%)**		**Total (%)**
					A	**B**	**C**	**D**	**E**	**UN 1**	**UN 2**	
Cycle 1	-20 25	3.76	97.83	96.87	0.45	0.18	0.45	0.05	0.66	0.05	0.33	2.17
Cycle 2	-20 25	3.69	98.26	90.05	0.06	0.48	0.29	0.29	0.53	0.06	0.03	1.74
Cycle 3	-20 25	3.75	97.60	96.08	0.37	0.45	0.35	0.40	0.43	0.16	0.24	2.40

Table 19: Results of thermal cycling study

No. of cycles	Temperature(°C) and time period (-20° 2 days followed by 40°)	Study parameters										
		pH	Assay (%)	Ethanol (%)	Total impurity (NMT 8%)							
					Known (NMT 1%)					Unknown (NMT 0.5%)		
					A	B	C	D	E	UN 1	UN 2	Total
Cycle 1	-20	3.76	98.36	96.50	0.35	0.18	0.45	0.05	0.16	0.05	0.13	1.37
	40											
Cycle 2	-20	3.69	98.74	95.78	0.06	0.40	0.29	0.29	0.03	0.16	0.03	1.26
	40											
Cycle 3	-20	3.75	97.60	95.08	0.37	0.45	0.35	0.40	0.43	0.16	0.24	2.40
	40											

Table 20: Result of Short term temperature excursion study.

No. of cycles	Temperature(°C) and time period (2 days at each station)	Study Parameters										
		pH	Assay (%)	Ethanol (%)	Total Impurity (NMT 8%)							
					Known (NMT 1%)					Unknown (NMT0.5%)		
					A	B	C	D	E	UN 1	UN 2	Total%

Cycle 1	-20	3.70	97.84	96.87	0.04	0.46	0.34	0.54	0.63	0.11	0.04	2.16
	60	4.29	97.34	94.00	0.43	0.56	0.73	0.25	0.25	0.33	0.36	2.66

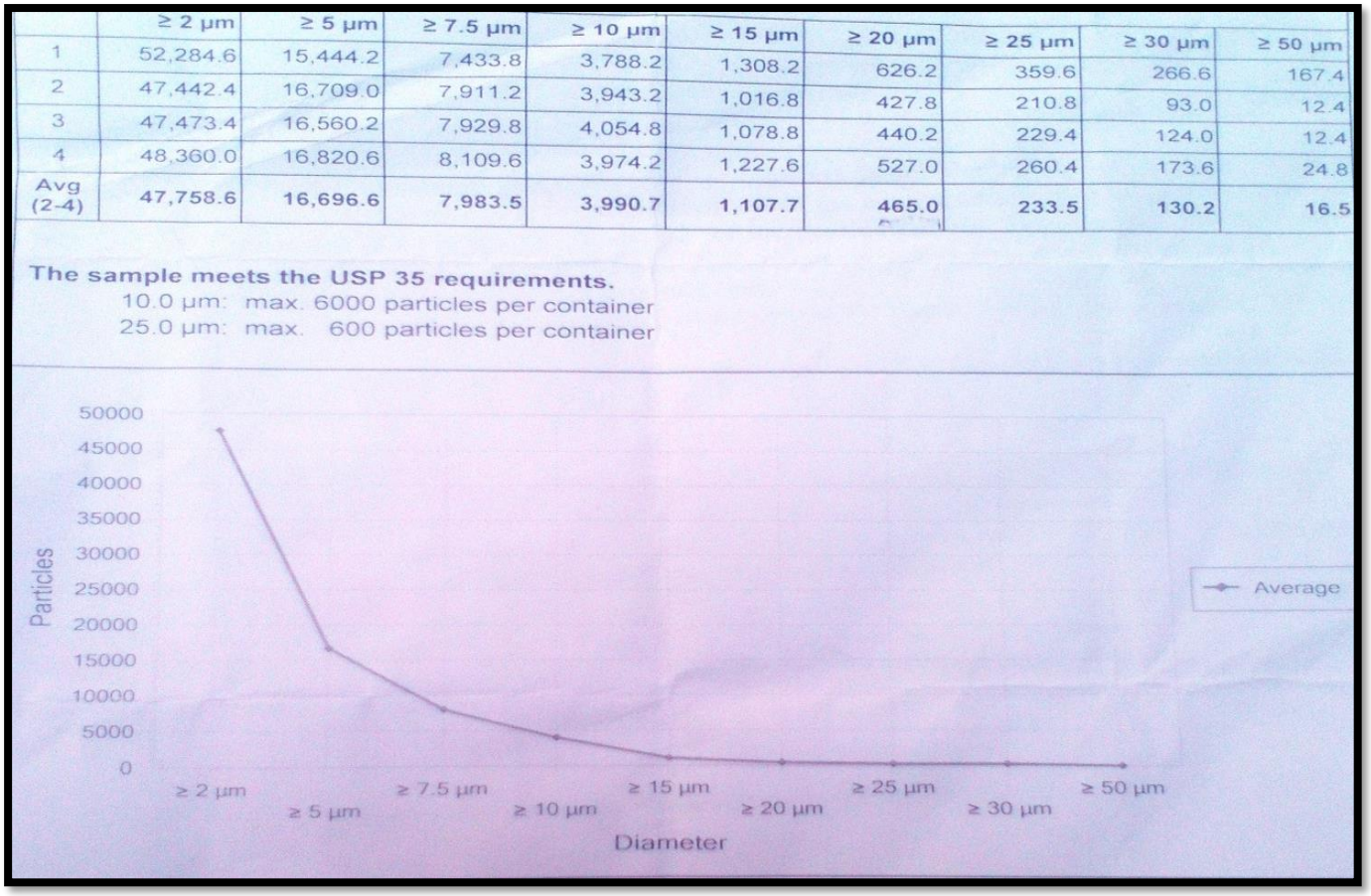
xii. Evaluation of developed batch

a) Osmolality

Table 21: Result of osmolality of developed batch

Sample	**Condition**	**Osmolality (milliosmoles)**
Sample 1	40˚C/75%RH,Initial	273mOsm
Sample 2	40˚C/75%RH,1M	NR
Sample 3	40˚C/75%RH,2M	NR
Sample 4	40˚C/75%RH,3M	278mOsm

b) Liquid particle count



	≥ 2 µm	≥ 5 µm	≥ 7.5 µm	≥ 10 µm	≥ 15 µm	≥ 20 µm	≥ 25 µm	≥ 30 µm	≥ 50 µm
1	52,284.6	15,444.2	7,433.8	3,788.2	1,308.2	626.2	359.6	266.6	167.4
2	47,442.4	16,709.0	7,911.2	3,943.2	1,016.8	427.8	210.8	93.0	12.4
3	47,473.4	16,560.2	7,929.8	4,054.8	1,078.8	440.2	229.4	124.0	12.4
4	48,360.0	16,820.6	8,109.6	3,974.2	1,227.6	527.0	260.4	173.6	24.8
Avg (2-4)	47,758.6	16,696.6	7,983.5	3,990.7	1,107.7	465.0	233.5	130.2	16.5

Figure 6: The results of liquid particle count of developed batch

Discussion and conclusion

Assay Detection of API was characterized by IR and UV spectroscopy showing numerous level of stretching between C-C, C-H, C=O, C-N and a calibration curve of the model drug in water at 583 nm wavelength by UV using various ACS and excela R grade chemicals. Calibration curve

was prepared and R2 value was found 0.996, it shows the linearity between 10 µg/ml to 90 µg/ml. As the tabulated data indicates in Table 3, it can be concluded that model drug is susceptible for acidic, alkaline, oxidation and thermal. While designing the manufacturing process, optimum parameters to be optimized for stable formulation. Based on drug excipient compatibility study as shown in table 4, it was found that glycerin from spectrum and ethanol from Hayman is providing the satisfactory impurity profile while kept under stress condition. Hence same shall be further undertaken for development activity. Above tabulated data in Table 5 results showed that selected excipient was found compatible under stress study and same shall be further taken for process development and process optimization.

Based on results of order of addition optimization with process temperature condition as indicated in table 6, it can be concluded that T6 trial is providing satisfactory results for ethanol content; however there was no significant difference in the impurity profile of rest of the trials. Hence process adopted in T6 trial shall be undertaken for process optimization study. However, the tentative manufacturing process flow results reflected in table 7 that there was significant increase in the Impurity C & D at 40/75%RH –1 month. It may be happened because of no inert gas was used during manufacturing. Effect of light during manufacturing was evaluated based on comparison of batch executed under sodium lamp and ambient light as shown in table 8. Assay of batch that was prepared in the presence of sodium vapour lamp is found on higher side in comparison to batch prepared in ambient light along with pH deflection from the initial value is also seen in ambient light. Hence sodium lamp during manufacturing is recommendable, as impurity was found on the lower side.

Batches A and B were prepared by purging N_2 and CO_2 and it has been estimated that the Methane sulphonic acid (MSA) was consumed more in case of batch prepared under CO_2 purging as shown in table 9. Based on forced degradation, it was clear that model drug is susceptible to acidic condition. At molecular level degradation may occur more in case batch prepared under CO_2. Hence N_2 as inert gas was recommended for batch manufacturing. It has been observed that while manufacturing of these batches that there is slight decrease in pH when CO_2 was purged in the batch solution from its initial stage, and slight increase in pH while N_2 was purged. Table 10 shows that different pH of Trials show different trend of impurity, pH, Assay. The best result that we get is from Trial 2 i.e. at pH 3.6. So pH of 3.6 is recommendable for final product to maintain the product attributes. The impact of manufacturing process temperature on product batch concluded that there is no wide difference in the assay between these two batches but there is huge difference seen in the ethanol content as depicted in table 11. As ethanol tend to evaporate at room temperature condition which leads to reduction in the ethanol content in finished product. Hence 2-8°C temperature is recommended for

manufacturing. Nevertheless, good result of ethanol content has been seen in the case of filter the solution by PES filter membrane as compared to PVDF. It could be due adsorption of ethanol in PES. Although there is no variation seen in the assay content, so PES filter membrane is recommended for aseptic filtration of test product.

Based on results of selection of sterilization process it can be concluded that impurity C and E was significantly increases at initial in comparison to batch prepared by aseptic filtration as discussed in table 12. Moreover assay was also found on the lower side in comparison to aseptic filtration. Hence aseptic filtration will be the method of choice.

Based on results of hold time stability with process component it can be concluded that product was found compatible with both glass and SS316L up to 48 hr for chemical attributes of drug product as shown in table 14.

Compatibility results for the selection of tube for the product filling (table 15) concluded that adsorption of ethanol was found more in silicone tubing in comparison to PTFE tubing however the related substance also well within the specification while comparing the data of PTFE v/s silicone. Hence PTFE tubing shall be used for finished product manufacturing. Based on tabulated results for the photostability study as shown in table16, it was found that product showed a significant change in the assay and related substance in transparent vial in comparison to product stored in amber color vial. Hence amber color vial shall be used for conducting stability study. Prototype batch prepared after finalization of formulation composition and process parameters charged in stability at long term and accelerated condition showed that the entire chemical attributes complying with the targeted specification (table 17). Hence the composition, process parameter and primary packaging material used in above said batch can be recommendable for attaining the reproducibility in batches manufacturing and quality attributes throughout the shelf life.

However, additional stability study (Freeze thaw study) concluded from the results that product is stable in freeze thaw condition because there is no variation seen in the chemical attributes of product after exposure as indicated in table 18. Thermal cycling study result in table 19 showed that product is stable in thermal cycling condition because there is no variation seen in the chemical attributes of product after exposure. It was concluded from the results of Short term temperature excursion study (table 20) that product is stable in short term temperature excursion because there is no variation seen in the chemical attributes of product after exposure.

Evaluation of the developed batch concluded from the result that batch passes the osmolality test (table 21). However, the results of liquid particle count of developed batch also pass all the parameter of USP (figure 6).

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Conflict of Interest

The authors declare no conflicts of interest.

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