CHAPTER 4

DEVELOPMENT OF READY TO INFUSE DOSAGE FORM

"Learn everything that is good from others, but bring it in, and in your own way absorb it; do not become other."

- *Swami Vivekananda*

Design and development of RTI injection for oxytocin, vasopressin and angiotensin

4.1 Introduction

Large number of molecules (peptide and other sensitive chemical moieties) are unstable in aqueous and diluted form [1,2]. Thus, these molecules are available in lyophilized and concentrated injection form respectively. Oxytocin, vasopressin and angiotensin II are available in the market as concentrated injection dosage form. But they need to be diluted in suitable diluents such as dextrose or sodium chloride or dextrose in sodium chloride injection or lactate ringer's solution or other compatible diluents prior to administration. The dilution of injection involves aseptic preparation procedures, which incorporate additional step in delivery of medicine to patient [3]. This is very critical stage when drug is delivered in emergency situation. In addition, physicochemical stability of drug in diluted formulation is limited up to 24 h at room temperature. The aim of ready to infuse dosage form development is to provide ready dosage form to patient without any intermediate steps prior to administration. The Ready to infuse dosage form for all three selected peptides was developed as per FDA recommended strength and characterized for all Critical Quality Attributes (CQAs). The developed formulations were also subjected to stability study as per ICH Guidelines.

4.2 Materials and Instruments

The details of materials which were used in the current research work are portrayed in Table 4.1. The details of instruments used are displayed in Table 4.2.

Table 4.1: Raw materials, chemicals and reagents employed in the current research work

Items	Source
0.2 µ PVDF Capsule filter	Pall, USA
2-Hydroxypropyl-β-cyclodextrin	Roquette, France
100 mL sterile PHC bag	Hosokawa, Japan
Acetonitrile for HPLC	Spectrochem Pvt. Ltd., Mumbai, India
Advantaflex tube	Advantapure, USA
Aluminum overwrap pouch	Hosokawa, Japan
Angiotensin-II	Sun Pharmaceutical Industries Ltd, Ahmednagar, India
Dextrose anhydrous	J T Baker, USA
Disodium hydrogen phosphate	Merck, USA

Items	Source
Dextrose	J T Baker, USA
Hydrochloric acid	J T Baker, USA
Mannitol	Roquette, France
Methanol	Merck India Ltd., Mumbai, India
Methanol for HPLC	Spectrochem Pvt. Ltd., Mumbai, India
Minitulip (Stopper)	Sippex, France
Needles (Hypodermic-BD)	Beckton Dickinson and Co., USA
Nylon filters	Advanced Microdevices Pvt. Ltd., Ambala, India
Octane	S.D. Fine-Chem Ltd., Mumbai, India
Orthophosphoric acid	Fisher Scientific, Mumbai, India
Oxygen Scavenger (Ageless ZPT-200MBC)	Mitsubishi, Japan
Oxytocin	Sun Pharmaceutical Industries Ltd, Ahmednagar, India
Potassium Bromide	SRL Pvt. Ltd., Mumbai, India
Potassium Dihydrogen Phosphate	Fuligins Fine Chemicals Ltd., Mumbai, India
Propidium iodide (PI) staining kit	eBioscience, USA
Sodium Chloride	Merck, USA
Sodium Citrate	Merck, USA
Sodium Hydroxide	Merck, USA
Sucrose	Merck, USA
Teflon coated rubber stopper	West, USA
Vasopressin USP	Polypeptide laboratories Pvt. Ltd., India
Water for Injection	Mili-Q
Water, for HPLC	Merck Specialties Pvt. Ltd., Mumbai, India

Table 4.2: Details of various instruments employed during current research work

Instruments	Make
pH meter	Mettler Toledo, USA
Analytical balance	Mettler Analytical Balances, Switzerland
HPLC	Shimadzu, Japan
Osmometer	Advance instrument 3250, Belgium, Netherlands
PMT analyser (Liquid particulate counter)	HIAC 9730+, Beckman Coulter, USA
Mechanical Stirrer	RQ 122, Remi Motors, Mumbai, India

4.3 Formulation Development

4.3.1 Development and Optimization of Ready to Infuse formulation of Oxytocin

4.3.1.1 Manufacturing procedure

I. Preparation of Oxytocin Stock

10 mg oxytocin was dissolved in WFI adjusted to pH 4.0 with Glacial acetic acid.

II. Preparation of bulk solution and filling:

I). An appropriate volume of water for injection (WFI), comprising 60-80% of the total batch size, was carefully collected in a sterile container, and nitrogen gas was purged into the container to effectively lower the dissolved oxygen (DO) level to below 1 ppm.

II). A precisely measured quantity of glacial acetic acid was introduced into the solution and thoroughly dissolved, followed by a meticulous assessment of the pH to ensure it met specified criteria.

III). An accurately weighed portion of Osmogen was then added to the solution, which was gently stirred to ensure complete dissolution while continuously monitoring the pH level.

IV). Following this, a carefully weighed amount of additional excipient(s) was added into the mixture, ensuring it dissolved completely under stirring condition.

V). The pH of the resulting solution was methodically adjusted to fall within the range of 3.0 to 5.0 through the gradual addition of glacial acetic acid (10% v/v) or sodium hydroxide (10% w/v), with constant pH monitoring.

VI). The calculated volume of oxytocin stock was then added to the solution under continuous nitrogen purging to ensure effective mixing and minimize oxygen exposure.

VII). Subsequently, the volume of the solution was adjusted to the final batch size by adding additional water for injection, followed by thorough mixing under stirring to achieve uniformity.

VIII). The prepared solution was then aseptically filtered through a 0.2 µm filter to eliminate any particulates and was subsequently filled into sterilized infusion bags.

IX). Throughout the entire process, nitrogen gas was continuously purged to keep the DO level consistently below 1 ppm, and the bags were promptly stoppered to maintain sterility.

Overwrap: Finally, the infusion bags were meticulously overwrapped with a nitrogen blanket and an oxygen scavenger to ensure their integrity and prolong the shelf life of product.

4.3.1.2 Preliminary Feasibility and Developmental trials

Various formulation variables for development of RTI formulation for Oxytocin were evaluated to acquire a final formulation with assay ranging 90-110% and osmolarity range of 250-350 mOsmol/Kg. Therefore, different buffering agents (sodium acetate, tartaric acid, citric acid), amino acids (Glycine, L-methionine, L-arginine), metal ions ($MgCl_2$), osmogens (mannitol, sucrose, Trehalose, Lactose, dextrose, sodium chloride), stabilizer (HPβCD) and antioxidants (SMBS) were evaluated. The different compositions in various packaging materials (PM) are described in below tables along with their assay and osmolarity value. The details of PM are tabulated in Table 4.3 and data obtained is tabulated in Table 4.4.

Table 4.3: Details of Packaging materials used in RTI formulation

Brand Name	MOC	Product Contact Layer	Thickness of Film (micron)
Polyelite PHC	Polypropylene (Outer layer)- Linear Low-Density Polyethylene- Cycloolefin polymer (Inner layer)	COP	200
Polyelite AOB	Vacuum Metalized PET (outer layer)-adhesive Linear Low-Density Polyethylene -Tie -Active barrier-Tie- Linear Low-Density Polyethylene -Adhesive High Density Polyethylene - Linear Low Density Polyethylene - Cycloolefin polymer Linear Low Density Polyethylene- High Density Polyethylene (Inner layer)	HDPE	245
TH82	High Density Polyethylene (outer layer) - Linear Low-Density Polyethylene- High Density Polyethylene (Inner layer)	HDPE	200

Table 4.4 : Initial data of various preliminary developmental trials

Formulation Code	Compositions	Packaging Description	Batch No.	Assay (%)	Osmolarity (mOsmol/Kg)
FA-1	Each mL contains: Oxytocin 0.02 IU, Citric acid 2 mg, $MgCl_2$ 2.033 mg, NaOH q.s. to adjust pH 4	100 mL PHC	FP015_A-4	88.13±1.06	46±1.26
		100 mL AOB	FP015_A-5	89.47±1.03	46±1.11
		100 mL TH82	FP015_A-6	83.12±1.12	46±1.93

Formulation Code	Compositions	Packaging Description	Batch No.	Assay (%)	Osmolarity (mOsmol/Kg)
FA-2	Each mL contains: Oxytocin 0.02 IU, Citric acid 2 mg, $MgCl_2$ 2.033 mg, L-methionine 3 mg, L-arginine 1.5 mg, SMBS 3.2 mcg	100 mL PHC	FP015_B-4	69.44±1.89	63±1.34
		100 mL AOB	FP015_B-5	68.63±1.12	63±1.15
		100 mL TH82	FP015_B-6	60.77±1.37	63±1.13
FA-3	Each mL contains: Oxytocin 0.02 IU, Citric acid 2 mg, $MgCl_2$ 2.033 mg, Mannitol 50 mg, NaOH q.s. to adjust pH 4	100 mL PHC	FP015_C-4	83.56±1.46	318±1.96
		100 mL AOB	FP015_C-5	82.07±1.18	318±1.17
		100 mL TH82	FP015_C-6	72.42±1.46	318±1.96
FA-4	Each mL contains: Oxytocin 0.02 IU, Citric acid 2 mg, $MgCl_2$ 2.033 mg, Dextrose 50 mg, NaOH q.s. to adjust pH 4	100 mL PHC	FP015_D-4	68.14±1.15	327±1.76
		100 mL AOB	FP015_D-5	65.34±1.36	327±1.16
		100 mL TH82	FP015_D-6	60.27±1.76	327±1.63

$MgCl_2$: Magnesium chloride; NaOH: Sodium Hydroxide; q.s.: quantity sufficient (n=3).

In chapter 2, literature on the topic of oxidation in protein formulations was explored. It is often possible to regulate oxidation by carefully managing the redox potential of formulation. This can be achieved through the inclusion of specific amino acids, which help to stabilize proteins. Furthermore, the use of antioxidants and metal chelating agents is recommended to effectively inhibit oxidation in these formulations.

To investigate this further, a series of experimental trials were done. The details of which are summarized in Table 4.4. The formulation process adhered to specific methodology outlined in section 4.3.1.3 of RTI formulation guidelines. In total, we crafted four distinct formulations labelled FA-1 through FA-4 by experimenting with different combinations of ingredients. Each of these formulations was packaged into three types of materials, as detailed in Table 4.4, to assess their effectiveness and stability.

The assay was selected as main CQA in preliminary developmental trials. The assay was found to be below 90% in all trials taken in three different packaging materials, although, formulation FA-1 was found to be better compared to other formulations. Hence from above results, we

concluded that PHC and AOB bag are better than TH82 bag due to lower assay values of formulations stored in TH82 compared to both bags. Hence, we selected PHC and AOB bag for further study for sequential screening of osmogens, buffers and stabilizers.

4.3.1.3 Selection of Osmogens

In order to select the buffering agent and osmogen, different formulation trials were conducted with different osmogens and buffers in their different concentration.

Table 4.5: Initial data of various preliminary development trials for selection of Osmogens for Oxytocin

Formulation Code	Compositions (Each mL contains)	Packaging Description	Batch No	Assay (%)	pH	Osmolarity
FB-1	Oxytocin 0.02 IU, Sod Acetate 1 mg, Mannitol 50 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP064-A2	88.27±1.13	3.96±0.06	391±1.12
		AOB	FP064-A3	84.26±1.41	3.96±0.07	388±1.12
FB-2	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Mannitol 50 mg, Glycine 1 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP064-B2	87.32±1.88	3.99±0.08	398±1.65
		AOB	FP064-B3	86.94±1.16	4.0±0.09	398±1.93
FB-3	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Dextrose 50 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP065-A2	80.07±1.12	3.96±0.05	364±1.71
		AOB	FP065-A3	82.74±1.34	3.97±0.06	363±1.51
FB-4	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Dextrose 50 mg, Glycine 1 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP065-B2	80.89±1.16	3.95±0.08	383±1.83
		AOB	FP065-B3	81.70±1.93	3.96±0.06	385±1.56
FB-5	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Sucrose 50 mg GAA q.s. to pH 4, WFI q.s.	PHC	FP063-A2	100.21±1.11	3.97±0.09	280±1.12
		AOB	FP063-A3	100.50±1.03	3.97±0.05	279±1.56
FB-6	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Sucrose 50 mg, Glycine 1 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP063-B2	97.98 ±1.13	3.98±0.09	280±1.08
		AOB	FP063-B3	99.67±1.45	3.97±0.08	282±1.16

Formulation Code	Compositions (Each mL contains)	Packaging Description	Batch No	Assay (%)	pH	Osmolarity
FB-7	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Trehalose 50 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP032D	83.84±1.09	3.99±0.06	218±1.87
		AOB	FP032E	75.10±1.09	4.0±0.05	218±1.91
FB-8	Oxytocin 0.02 IU, Sod. Citrate 4.9 mg Lactose 50 mg Citric acid 6.4 mg GAA q.s. to pH 4 WFI q.s.	PHC	FP030D	96.94±1.23	4.02±0.09	246±1.12
		AOB	FP030E	96.65±2.65	4.03±0.08	247±1.36
FB-9	Oxytocin 0.02 IU, Sod. Acetate 1 mg Sodium Chloride 4.50 mg GAA q.s. to pH 4 WFI q.s.	PHC	FP067-A2	80.85±2.16	3.96±0.06	227±1.19
		AOB	FP067-A3	66.27±1.13	3.95±0.03	226±1.67
FB-10	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Sodium Chloride 4.50 mg, HPβCD 1 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP063-E2	98.08±2.07	3.98±0.05	223±1.98
		AOB	FP063-E3	98.79±1.98	3.97±0.02	222±1.22
FB-11	Oxytocin 0.02 IU, Sod. Acetate 1 mg, Sodium Chloride 9.0 mg, HPβCD 1 mg, GAA q.s. to pH 4, WFI q.s.	PHC	FP069-A	100.76±1.32	3.95±0.04	293±2.41
		AOB	FP069-B	101.01±1.72	3.89±0.03	291±1.94

Sod. Acetate: Sodium acetate; GAA: Glacial acetic acid; WFI: Water for Injection; HPβCD: Hydroxy propyl beta cyclodextrin (n=3)

All formulations were prepared with sodium acetate and citrate buffers with various stabilizers and filled in two different packaging materials (table 4.5). The assay in FB-5, FB-6, FB-8 & FB-10 was found to be above 90% and within proposed specification (90-110%). % of assay in FB-5, FB-6 & FB-10 was found to be 98-100% in both bags (PHC & AOB), while 96 % assay was found in FB-8 in both filled bags. Formulation FB-5 and FB-6 contains sucrose as osmogen while FB-10 contains sodium chloride. The formulations which were formulated with lactose, sucrose and sodium chloride (with HPβCD) have shown assay value within the limits. However, in 4.50 mg/mL concentration of sodium chloride, the obtained value of osmolarity

was slightly low. But FB-11 found osmolality with in desired value. Based on the results portrayed in Table 4.5, sodium acetate as buffer, sodium Chloride as osmogen, while HPβCD as stabilizer were found to be prominent compared to others.

Additionally, on the basis of reported literature, sodium acetate and acetic acid were found to improve stability of peptides [4], but after experimental outcome with these buffers in oxytocin formulations, sodium acetate buffer was found to be prominent compared to others. Therefore, sodium acetate was selected as a buffering agent to stabilize the formulation. Henceforth, we decided to select Sodium chloride as osmogen, sodium acetate as buffer and HPβCD as stabilizer for further optimization studies.

4.3.1.4 Optimization of selected excipients

The optimization of selected excipients was done in the current study. Sodium chloride was fixed at 0.9 mg/mL as optimum osmogen, while sodium acetate and HPβCD were optimized from 0.01 and 0.5 mg/mL and 0.1 mg to 0.5 mg/mL respectively. Osmolality of solution for infusion should be isotonic and should be in range from 250 to 350mOsmo/kg, as per the literature and regulatory recommendation, while the pH of solution should be from 3 to 5. The pH study has been described separately in section 4.3.1.5.

Table 4.6: Development trials for selection of excipients for Oxytocin RTI

Compositions (Each mL contains)	Bag description	Form Code	Stability Station	Assay (%)	pH	Osmolarity
Oxytocin 0.02 IU, Sod acetate 0.5 mg, Sodium chloride 9 mg, HPβCD 0.5 mg Sodium Hydroxide q.s. to pH 4, WFI q.s.	PHC	F1	Initial	101.24±1.02	3.98±0.09	307±1.58
			25°C/40%RH-3M	97.76±1.01	3.97±0.08	306±1.93
	AOB		Initial	100.81±1.06	3.98±0.09	308±1.69
			25°C/40%RH-3M	97.58±1.07	4.0±0.06	305±1.13
Oxytocin 0.02 IU, Sod acetate 0.5 mg, Sodium	PHC	F2	Initial	100.41±1.12	3.97±0.07	306±1.56
			25°C/40%RH-3M	96.57±1.31	3.98±0.08	307±1.61

Compositions (Each mL contains)	Bag description	Form Code	Stability Station	Assay (%)	pH	Osmolarity
chloride 9 mg, HPβCD 0.1 mg Sodium Hydroxide q.s. to pH 4, WFI q.s	AOB		Initial	100.45±1.21	4.06±0.09	308±1.46
			25°C/40%RH-3M	97.23±1.14	4.10±0.08	311±1.63
Oxytocin 0.02 IU, Sod acetate 0.01 mg, Sodium chloride 9 mg, HPβCD 0.5 mg Sodium Hydroxide q.s. to pH 4, WFI q.s	PHC	F3	Initial	100.48±1.41	4.06±0.06	298±1.51
			25°C/40%RH-3M	97.66±1.19	4.10±0.06	299.±1.65
	AOB		Initial	99.02±1.16	4.06±0.09	296±1.87
			25°C/40%RH-3M	97.55±1.61	4.10±0.08	296±1.56
Oxytocin 0.02 IU, Sod acetate 0.01 mg, Sodium chloride 9 mg, HPβCD 0.1 mg Sodium Hydroxide q.s. to pH 4, WFI q.s	PHC	F4	Initial	100.06±1.09	3.99±0.05	294±1.34
			25°C/40%RH-3M	98.26±1.08	4.02±0.06	291±1.21
	AOB		Initial	99.48±1.27	4.02±0.06	294±1.67
			25°C/40%RH-3M	97.88±1.34	3.94±0.08	293±1.91

In previous section, the concentration of both sodium acetate and HPβCD was 1 mg/mL (FB-10). To optimize their concentration, we took trials for both in combination of 0.01 and 0.5 mg/mL and 0.1 mg and 0.5 mg /mL respectively. On the basis of results depicted in Table 4.6, all the formulations prepared with different concentration of HPβCD and Sod acetate combination have shown assay values within the limits in both bags at initial stage.

HPβCD was also evaluated to find its role in the stability of oxytocin. The effect can be clearly seen in the formulation with (F1-4) and without (F5, as mentioned in Table 4.5) HPβCD, where it increases the stability of oxytocin after 3M at 25°C/40%RH. The assay of formulation remained same when concentration of HPβCD increased from 0.1 to 0.5 mg/mL. Therefore, selection of lower concentration i.e., 0.1 mg/mL for further study was done.

The pH and osmolality of formulations prepared by various excipients remained unchanged and within the specifications. The pH of all formulations was found to be from 3.95 to 4.1,

while osmolality was from 287 to 308 mOsmol/Kg. As the assay, pH, and osmolality of formulations filled in PHC and AOB remained same, it was decided to select PHC bag due to lower cost compared to AOB bag. Despite the intense competition between the plastic infusion bottle and the infusion bag, it's impossible to resist the trend of flexible plastic injection liquid packaging. Infusion bags are safer, healthier, more affordable, and more environmentally friendly than standard glass packaging [5]. In addition to above, plastic infusion packages and containers have a significant market share in the United States and European Union. The following tests are required for infusion bag: barrier property testing, sealing performance testing, mechanical performance testing (heats seal strength testing, suspension force testing, pull open force testing, piercing force testing). On the basis of results obtained in above studies (Table 4.5 and Table 4.6), PHC bag was selected for further product related studies and stability studies.

4.3.1.5 pH Stability Study

Stability of any aqueous formulation depends on its pH. For a stable product, the pH of formulation should be within limit during stability studies [6]. Before initiation of long-term stability of optimized formulation and prototype finalization, a pH stability study was conducted to optimize the suitable pH of formulation. With the optimized composition, five formulations of different pH (5.0, 4.5, 4.0, 3.5 and 3.0) were prepared by method described in section 4.3.1.1. Formulations were tested at initial stage and for 3M at 25°C/40%RH. After 3M, results were compared with their respective initial results (Table 4.7; Figure 4.1 and 4.2). pH variation of formulation over the period may change the physical and chemical properties of oxytocin RTI formulation. So, as per Pharmacopeial recommendation, in addition to assay, pH and osmolality, PMT, absorbance and transmittance as CQAs in all pH stability studies were considered. Initially, there were no remarkable difference in assay values of all formulations, formulated at different pH. However, over the period of 3M at 25°C/40%RH, the assay value was decresed remarkably at pH 3.0 and 5.0. The formultion prepared at pH 4.0 had shown better stability after 3M among all tested formulations.

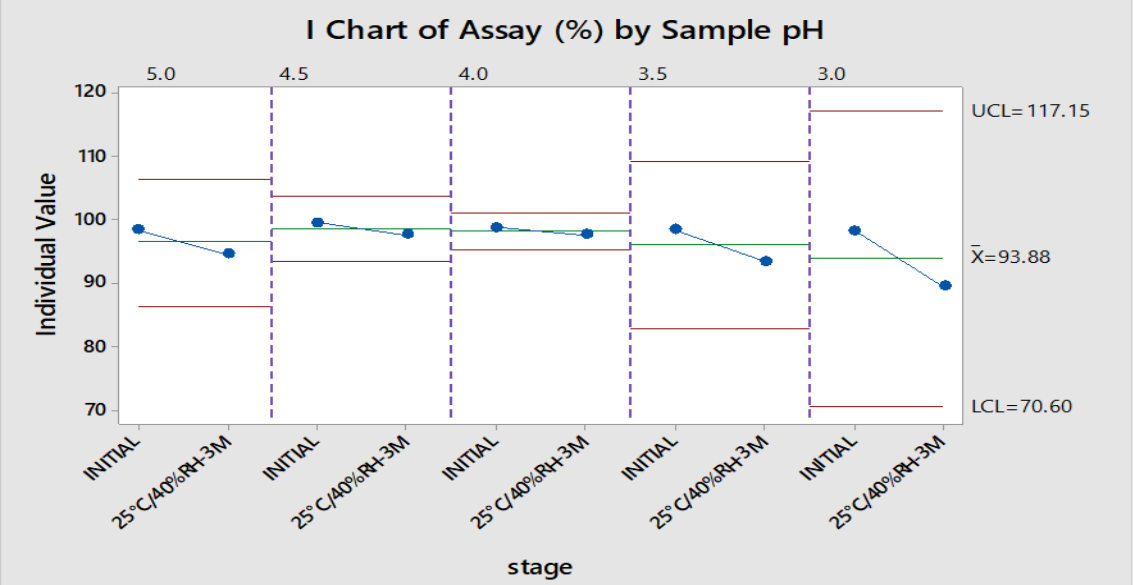

Figure 4.1: Assay of Oxytocin in RTI formulation at different pH at Initial time point and after 3M at 25 °C/40% RH. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

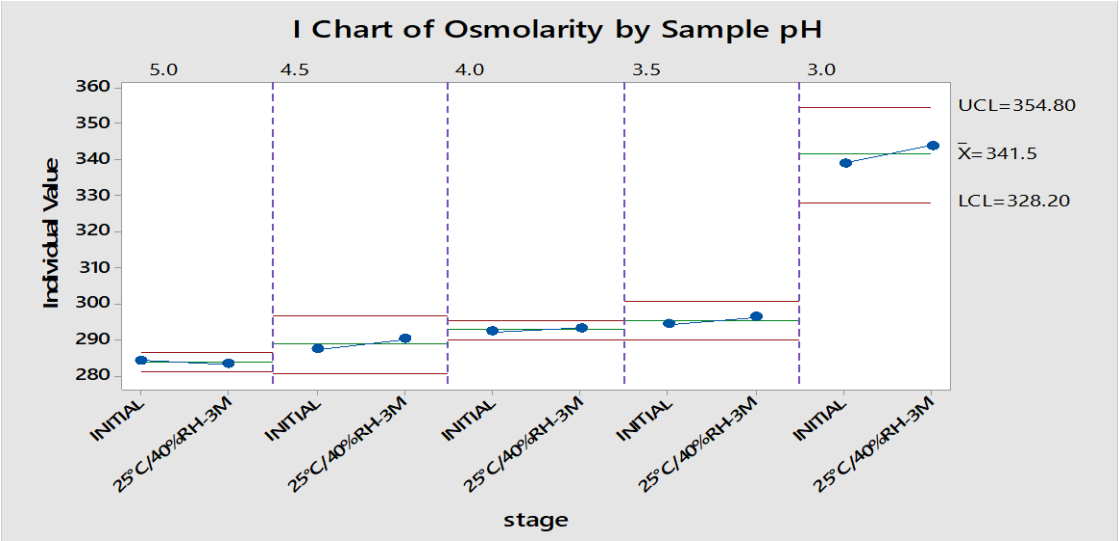

Figure 4.2: Osmolarity of Oxytocin RTI formulation at different pH at Initial time point and after 3M at 25 °C/40% RH. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

Table 4.7: pH study data of experimental trials

Formulation Code	Sample pH (Target)	Batch No	Stage	Assay (%)	pH (Observed)	Osmolarity (mOsm/kg)	PMT		% Transmittance at 650 nm	% Absorbance at 420 nm
							≥10µm	≥25µm		
Specification	3-5			90-110		250-350	6000	600	>95	<1
FD-1	5.0	FP094B	Initial	98.37±1.08	4.88±0.06	284±1.34	87.33±1.56	13.33±1.09	99.656±0.09	0.01±0.003
	5.0	FP094B	25°C/40%RH-3M	94.58±0.82	4.93±0.09	283±1.21	66.5±2.34	15.6±0.61	99.656±0.06	0.012±0.001
FD-2	4.5	FP094C	Initial	99.52±1.03	4.44±0.11	287±1.82	37.33±2.15	3.66±1.34	99.446±0.05	0.04±0.002
	4.5	FP094C	25°C/40%RH-3M	97.58±1.02	4.48±0.09	290±1.73	11.6±0.94	6.87±2.05	99.656±0.04	0.011±0.002
FD-3	4.0	FP094D	Initial	99.78±1.01	3.91±0.06	292±1.89	56.67±2.31	6.67±2.14	99.408±0.08	0.01±0.003
	4.0	FP094D	25°C/40%RH-3M	98.66±1.06	3.97±0.09	293±1.09	10.98±2.76	5.76±1.76	99.656±0.07	0.02±0.001
FD-4	3.5	FP094E	Initial	98.42±1.24	3.41±0.05	294±2.14	58.76±1.81	6.67±2.87	99.370±0.12	0.013±0.004
	3.5	FP094E	25°C/40%RH-3M	93.43±1.32	3.46±0.04	296±.2.36	12.76±2.01	11.31±2.07	98.67±0.15	0.03±0.003
FD-5	3.0	FP094F	Initial	98.25±1.16	2.92±0.02	339±2.94	30.54±1.52	14.76±3.54	99.415±0.19	0.014±0.001
	3.0	FP094F	25°C/40%RH-3M	89.5±1.11	2.94±0.03	344±2.87	56.87±2.02	6.4±2.86	99.670±0.23	0.016±0.002

Although, decreasing assay for all pH formulations was observed after 3M, other CQAs i.e., pH, osmolality, PMT, % of transmittance & absorbance was within specifications. The trend of assay and osmolality can be seen in Figure 4.1 & 4.2. Regarding control of method capability, upper control limit (UCL) and lower control limit (LCL) value can be easily found in the figure 4.1 and 4.2. If, LCL and UCL values are close to target value, then method is considered as more capable due to less variability. Here, target value of assay is 100% and it can be seen that UCL and LCL is close to 100 %. So, we confirmed that our method is capable and controlled for formulation with pH 4.0, while not in case of formulations with other values. Therefore, it was decided to keep pH 4.0 in our formulation for further studies [7].

4.3.1.6 Sterilization method selection for Oxytocin RTI

The selection of an appropriate sterilization method or aseptic processing technique must be thoroughly justified to ensure the safety and efficacy of pharmaceutical products. All sterilization processes should adhere strictly to the guidelines established by the European Pharmacopoeia (Ph. Eur.), unless there are compelling reasons to deviate from these protocols. This documentation must encompass details about the sterilization procedures employed for various components, including the finished product, active substances, excipient(s), and the containers used for these materials. Additionally, the name and specific location of the sterilization facility must be provided to maintain transparency and traceability.

A comprehensive description of the chosen sterilization method or aseptic processing approach is essential, including detailed information about in-process controls and validation data that ensures the method's reliability. Furthermore, bioburden control criteria should be clearly defined prior to commencing any sterilization processes. It is critical to note that high acceptance criteria for bioburden should never be justified solely on the capabilities of the sterilization process or any measures taken to reduce bioburden prior to sterilization.

To assist manufacturers in selecting the most effective sterilization method, the decision trees presented in Figures 4.3 serve as a valuable resource. These trees are designed to guide users through the evaluation process by considering various key factors. As one navigates through the decision trees, it becomes evident that the sterilization methods typically offer a progressively lower assurance of sterility. Consequently, it is advisable

to select the first viable option available, as this often represents the best choice for ensuring product safety. Although these decision trees primarily cater to finished products containing chemical active substances, they are also relevant for other types of products, including active substances and excipients, thereby broadening their applicability in the field of pharmaceutical manufacturing.

There are two methods for sterilising injectable drugs: either by terminal sterilization or via an end-to-end aseptic production process. A steam autoclave is frequently used in terminal sterilization to expose a medication to heat and destroy any microorganisms [8]. However, in many of cases where the formulation is heat sensitive and sterilization at standard conditions is not possible, there is a recommendation of European Medical Agency [9] to evaluate the drug product according to below mentioned sterilization decision tree. As per regulatory recommendation, autoclave cycles need to run and based on results outcome, we need to take decision accordingly [10]. In order to evaluate a suitable sterilization method, Oxytocin RTI formulation was sterilized at different recommended sterilization conditions and analysed thereafter (Table 4.8).

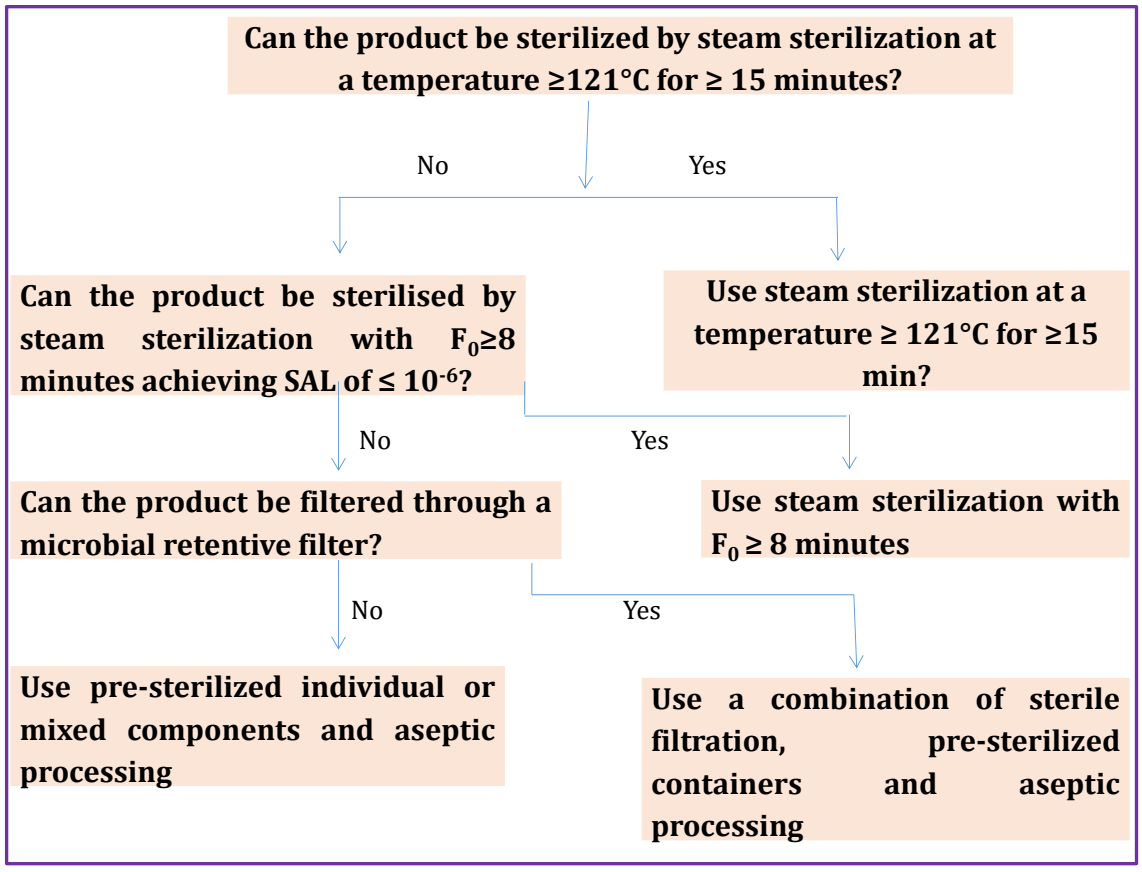

Figure 4.3: Decision tree for sterilization choices for aqueous based products

Table 4.8: Effect of sterilization process on the Oxytocin RTI formulation by autoclaving

Sterilization parameter	Assay (%)	pH
Initial (Un-autoclaved)	100.19±1.76	4.05±0.06
15 Min_121°C	64.12±1.65	4.04±0.08
F0 12 Min_121°C	82.79±1.21	4.03±0.07
F0 08 Min_121°C	85.56±1.06	4.06±0.05
F0 08 Min_116°C	73.81±1.18	4.05±0.08
F0 08 Min_111°C	30.21±1.93	4.05±0.09

In all recommended sterilization conditions mentioned above, assay of Oxytocin in RTI formulation was found to be reduced. There was no effect on pH and osmolarity of formulation. Therefore, the aseptic process comprising sterile filtration using pre-sterilized container and aseptic processing was chosen for sterile formulation development.

4.3.1.7 Evaluation of optimized formulation prepared by aseptic filtration method

The developed formulation was tested for critical quality attributes (Assay, pH, osmolality, PMT, % transmittance & absorbance) till 6 months at 25 °C/40%RH and 5 ± 3 °C. Formulation was found stable after 6M at both storage conditions. The stability study's results are compiled in Table 4.9 and in Figure 4.4 to 4.6.

From the assay values, the formulation stability at tested storage conditions was found in following order 2-8°C> 25°C> 30°C. The control of assay value at 2-8°C was found with narrow window of upper and lower control value (red line) in figure 4.4, while wide window was observed for 30°C. From the figure 4.4, the variation of all storage conditions can be clearly predicted and we find that product is more stable at 2-8°C and least at 30°C, while intermediate at 25°C. For pH and osmolality, formulation showed no change at all storage conditions during 6M stability, and USL and LSL showed very close window in figure 4.5 & 4.6. In conclusion, no impact of storage condition on pH and osmolality, was found during stability, while decrease in assay was observed.

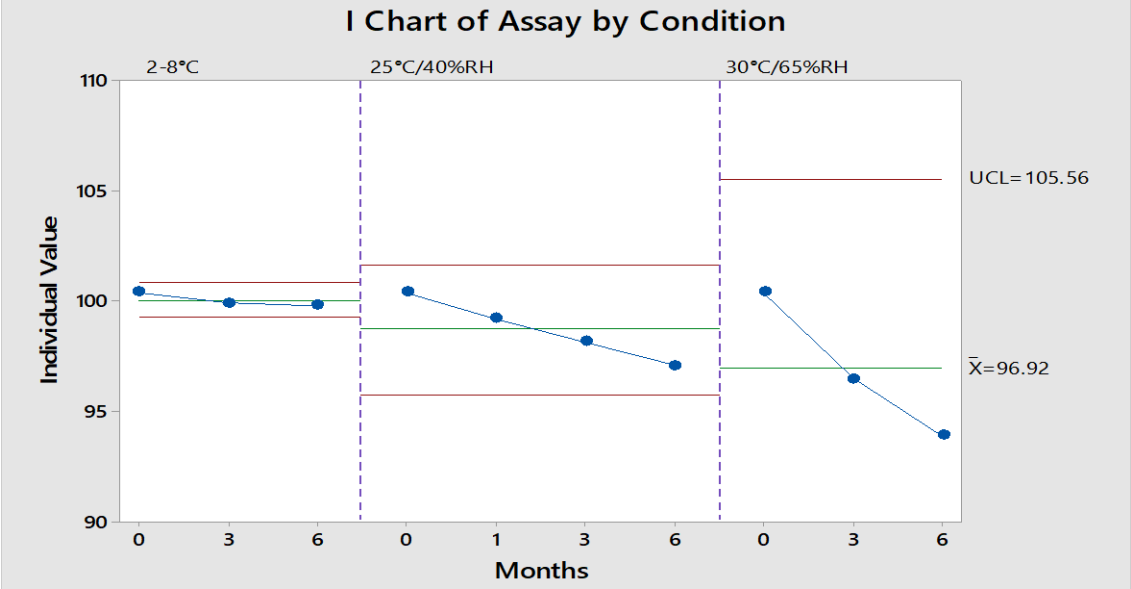

Figure 4.4: Short term Stability Plot of Assay (mean) at different conditions and at different time point for optimized Oxytocin formulation (Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

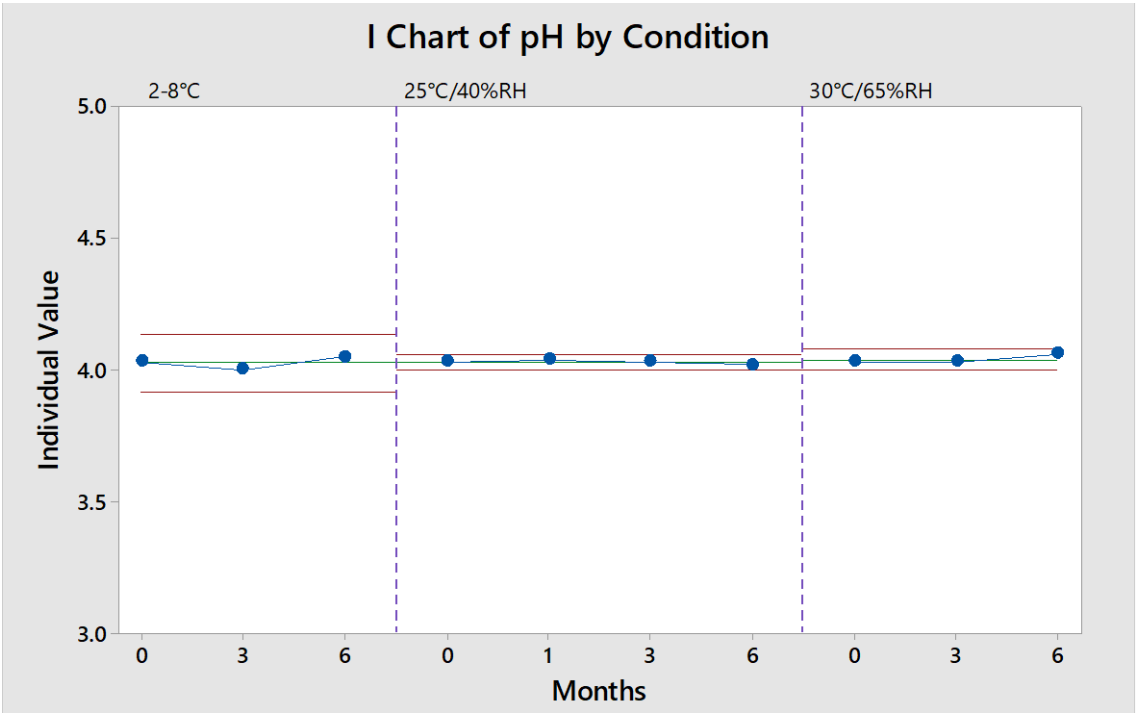

Figure 4.5: Short term stability plot of pH at different conditions and at different time point. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

Table 4.9: Short term stability data of optimized Oxytocin RTI formulation

Condition	Assay	pH	Osmolality (mOsm/kg)	Particulate matter (PMT)		% Transmittance at 650 nm	Absorbance at 420 nm
				≥10µm	≥25µm		
Specification	**90-110**	**3-5**	**250-350**	**6000**	**600**	**>95**	**<1**
Initial	100.39±1.06	4.03	295±1.32	23.33±1.03	40.00±1.13	99.814±1.06	0.010±0.03
5 ± 3 °C_3M	99.90±1.07	4.00	290±1.41	22.66±2.81	13.00±0.41	99.937±1.07	0.011±0.004
5 ± 3 °C_6M	99.80±1.11	4.05	292±1.35	40.00±1.56	20.0±0.23	99.377±1.67	0.01±0.003
25 °C / 40%RH_1M	99.20±1.23	4.04	291±161	73.00±4.05	7.00±0.03	99.944±1.45	0.01±0.002
25 °C / 40%RH_3M	98.13±1.46	4.03	296±1.23	20.67±3.41	66.67±0.12	98.844±1.89	0.01±0.001
25 °C / 40%RH_6M	97.05±1.18	4.02	291±1.56	22.00±3.67	3.20±0.03	99.073±1.13	0.012±0.003
30 °C / 65%RH_3M	96.47±1.61	4.03	292±1.07	180.00±2.87	7.00±0.03	100.210±1.21	0.01±0.001
30 °C / 65%RH_6M	93.89±1.34	4.06	293±1.98	80.00±2.76	2.00±0.08	99.570±1.56	0.0±0.00

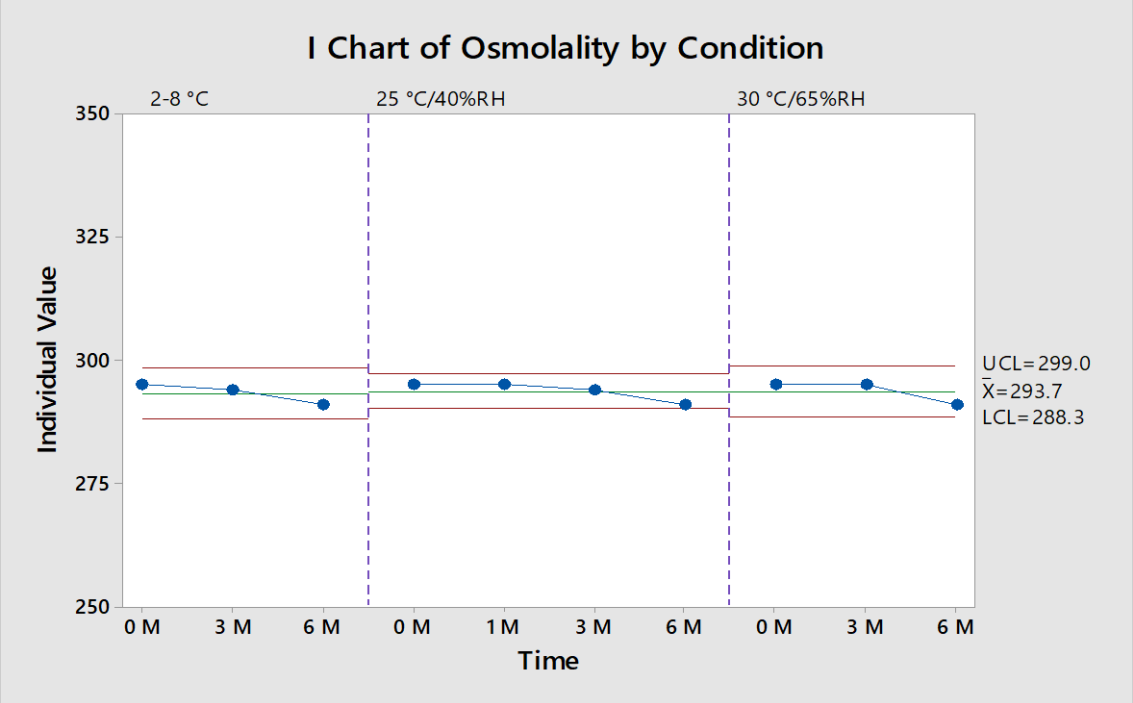

Figure 4.6: Short term stability plot of osmolarity at different conditions and at different time point. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

4.3.1.8 Temperature cycling study

Freeze thaw study is important to find out the excursion storage condition during transport. Moreover, sometimes, products may come in contact in higher and lower temperature during storage and transport. Therefore, we need to evaluate developed products to ensure the drug product quality, efficacy, and safety are not compromised when materials are subjected to short term temperature excursions from intended storage that may occur during e.g., shipping, transport, or patient use [11]. RTI formulations of Oxytocin were placed on stability storage at -20°C for 24 h. Samples were then transferred to 40°C / 75%RH for 24 h. Procedure was repeated for a total of 14 cycles. Samples were analysed for Critical quality attributes (CQAs), assay, pH and osmolality after 7 and 14 cycles respectively. The outcomes after 7 and 14 cycles were compared with initial results (Table 4.10 and Figure 4.7).

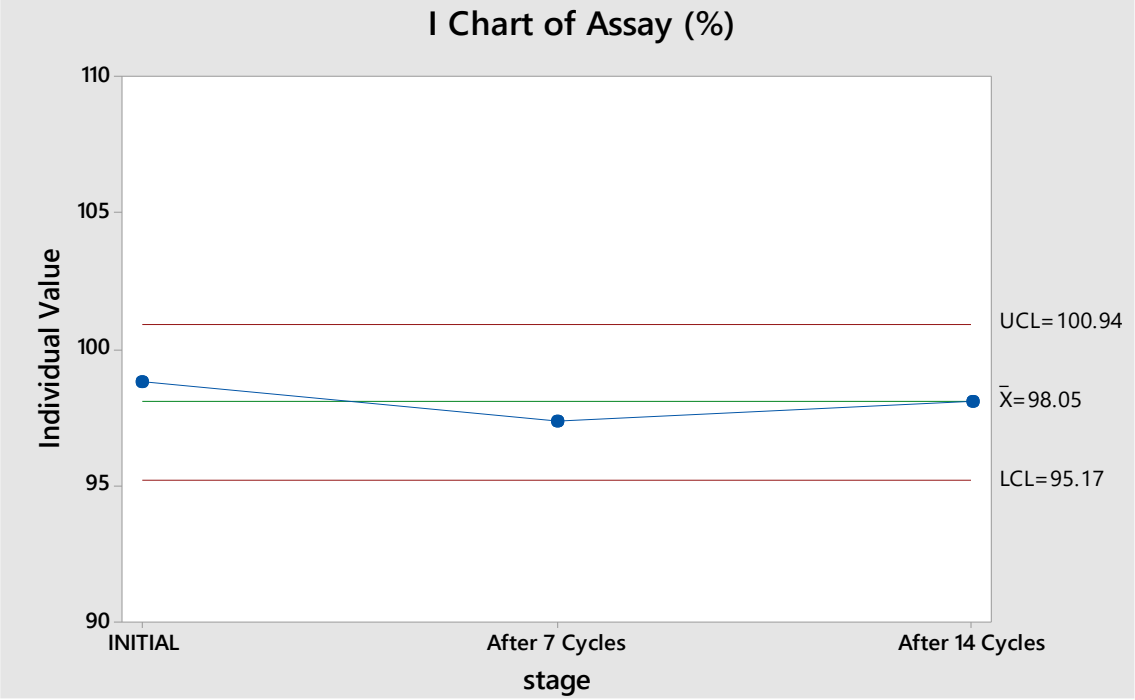

Figure 4.7: Assay of Oxytocin in RTI formulation after different freeze (-20 °C) thaw (+40 °C) cycles. Graph presents mean of individual value. Red line in graph presents upper control limit (UCL) and lower control limit (LCL)

There was no remarkable difference in the assay, pH value, and osmolality of the formulation after 7 and 14 days of study. This signifies the robustness and stability of developed formulation.

Table 4.10: Experimental data of freeze thaw cycle study

Formulation Code	Stage	Assay (%)	pH	Osmolality
FD-3	Initial	98.78±1.12	3.90±0.02	292±1.92
	After 7 Cycles	97.33±1.56	3.87±0.06	296±1.82
	After 14 Cycles	98.05±1.09	3.85±0.05	295±1.65

4.3.1.9 Oxygen Sensitivity Study

Much consideration was taken in selecting the packaging, given the fact that the main resource used to protect medications from the harmful effects of oxygen is the packing material. In addition, there are steps that must be taken in the development and manufacturing phases to ensure that excess oxygen isn't simply bottled or packaged with the medication [12]. Formulations must be evaluated and characterized for long-term

stability when exposed to oxygen throughout research and development before being included in the Critical Quality Attributes (CQAs) for the medicinal product. Therefore, effect of nitrogen, oxygen and air was evaluated on stability of developed RTI formulation of Oxytocin. Three formulations were prepared using aforementioned procedure and in presence of different gases viz air, nitrogen and oxygen. The formulations were filled and packed in respective environment.

Table 4.11: Effect of different gases on Oxytocin assay

Formulation Code	Sample description	Batch No	stage	Assay (%)	pH	Osmolarity
FD-6	Nitrogen	FP097A	Initial	100.39±1.21	4.03±0.09	295±1.16
	Nitrogen	FP097A	25°C/40%RH-3M	98.13±1.811	4.13±0.07	292±1.87
FD-7	Air	FP097B	Initial	101.49±1.03	4.03±0.08	290±1.23
	Air	FP097B	25°C/40%RH-3M	85.82±1.21	4.03±0.09	296±1.81
FD-8	Oxygen	FP097C	Initial	100.74±1.28	4.03±0.06	288±1.78
	Oxygen	FP097C	25°C/40%RH-3M	46.06±1.34	4.03±0.09	293±1.59

All the formulations were tested at initial stage and were kept on stability at 25°C/40%RH for 3M. After 3M, samples were withdrawn and analysed. Results of the study are presented in Table 4.11 and Figure 4.8.

The developed formulation was found to be stable in presence of nitrogen. However, formulations, prepared in presence of air and oxygen had shown prominent degradation with most significant degradation observed in formulation that was prepared in presence of oxygen. The outcome the current study will help to control process during formulation by continuous N_2 purging and secondary packaging in which oxygen scavenger can be used to reduce O_2 present between primary packaging and secondary packaging material.

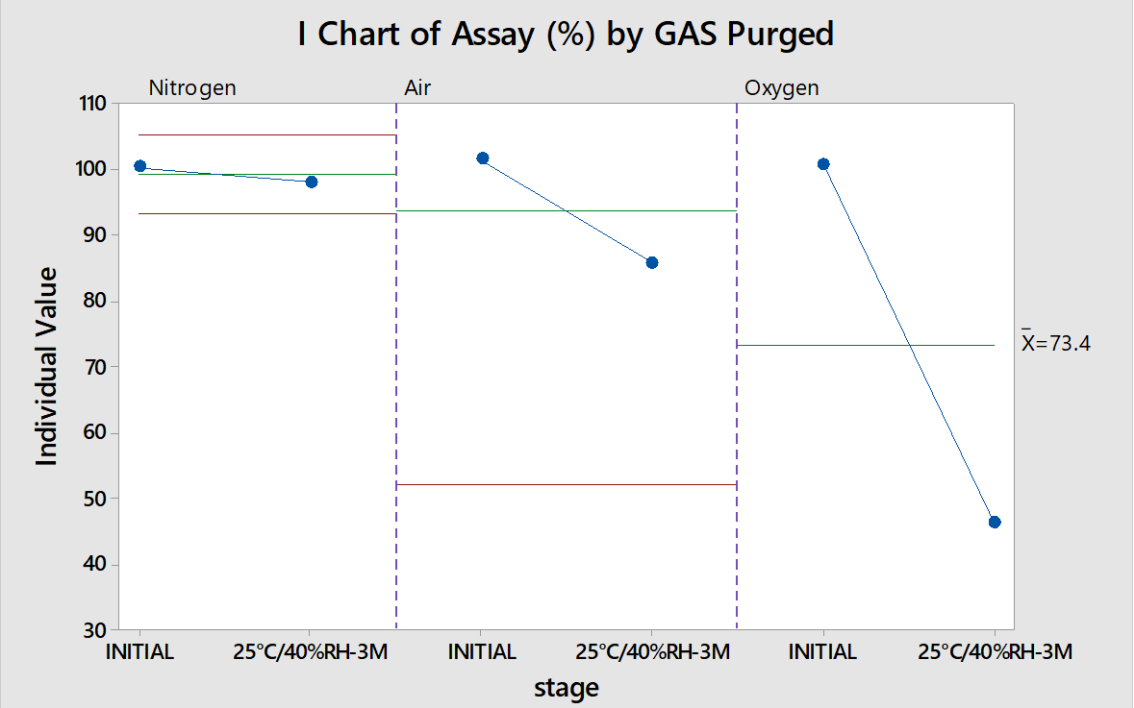

Figure 4.8: Sensitivity to gas: Assay of Oxytocin in RTI formulation after purging with different gases. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

4.3.1.10 Photostability study for Oxytocin RTI

Photostability testing was undertaken to provide information necessary for manufacturing, handling, packaging, and labelling [13]. US-FDA in 1996 issued ICH guidance Q1B for industry and stated that "the intrinsic photostability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change" [14]. Considering this fact, photostability testing was recommended to be carried out as a tool to evaluate the suitability of packaging materials in providing protection to the drug formulation. In this regard, a photostability study was conducted to evaluate photosensitivity of oxytocin RTI formulation. Oxytocin RTI samples (in infusion bag, in final pack and a control sample) were placed in photostability chamber and exposed to light of 20 million lux. After exposure, the samples were withdrawn and analysed. The results of photostability study are tabulated in Table 4.12.

Table 4.12: Effect of light on assay of Oxytocin

Sample description	Assay (%)	pH	Osmolarity (mOsmol/Kg)
Controlled (Infusion bag wrapped in aluminium sheet)	100.19±1.16	4.05±0.09	295±2.81
Market pack (Infusion bag placed inside the overwrap pouch)	100.13±1.87	4.04±0.11	295±2.13
Direct exposed (infusion bag only)	94.49±1.25	4.03±0.15	294±2.35

The assay of Oxytocin in RTI formulation was found reduced when it was exposed to light. There was no effect on pH and osmolarity of formulation. Therefore, utmost care should be taken during manufacturing, handling and packaging of Oxytocin RTI formulation. The outcome the current study will be help to control process during formulation by protecting the formulation by direct exposure to light and selection of secondary packaging which will protect the product from direct exposure to light during storage.

4.3.1.11 Hold time/Manufacturing vessel compatibility

This study was conducted to evaluate the suitability of material of construction (MOC) of manufacturing vessel (SS316L) for bulk solution manufacturing. The study is usually executed by preparing a bulk solution and subsequently stored in manufacturing vessels at room temperature (~20-25°C) for predefined period of time [15]. Samples are withdrawn intermittently and submitted for evaluation of CQAs of drug product. Hold time recommendation for time of Bulk solution (Filtered & Unfiltered) will be used during commercial batches.

To evaluate the RTI formulations, a bulk solution of drug product was prepared by using previously described manufacturing process. The bulk was distributed into four equal parts into four separate SS-manufacturing tanks as per the following details.

A) Non filtered bulk, without nitrogen purging
B) Non-filtered bulk with nitrogen purging and solution remained in nitrogen blanket all through the study period
C) Bulk was filtered through 0.2-micron filter without nitrogen purging
D) Bulk was filtered through 0.2-micron filter with nitrogen purging and solution remained in nitrogen blanket all through the study period

At predetermined time intervals i.e., Initial (0 h), 8 h, 24 h, 48 h, 72 h, 96 h, the samples were withdrawn and analysed for. Description, pH, Assay, osmolarity and particulate matters. The results of SS-vessel compatibility study are tabulated in Table 4.13.

Table 4.13: Stainless steel (SS316L) compatibility data at room temperature (~20-25°C)

Sample withdrawal time (h)	Treatment	Assay	pH	Osmolarity (mOsmol/Kg)	Particulate matters	
					≥10µm	≥25µm
0	Non-filtered without Nitrogen	99.13±1.87	3.95±0.11	296±1.65	1.87±0.05	0.40±0.05
8		98.78±1.06	3.92±0.08	295±1.76	0.067±0.08	0.0±0.05
24		96.95±1.05	3.96±0.12	297±1.94	0.067±0.04	0.0±0.05
48		95.85±1.04	3.96±0.08	295±1.67	0.20±0.09	0.067±0.05
72		92.98±1.07	3.93±0.09	291±1.71	1.11±0.03	0.0±0.05
96		79.32±1.07	3.95±0.10	295±1.83	3.66±0.15	0.0±0.05
0	Non-filtered with Nitrogen environment	99.41±1.87	3.96±0.09	296±1.82	4.40±0.28	0.0±0.05
8		98.98±1.87	3.94±0.10	297±1.62	2.80±0.06	0.0±0.05
24		97.49±1.87	3.97±0.07	294±1.81	0.267±0.09	0.0±0.05
48		96.95±1.87	3.95±0.07	291±1.96	0.467±0.04	0.0±0.05
72		93.76±1.87	3.94±0.06	299±1.56	1.06±0.03	0.13±0.05
96		80.53±1.87	3.96±0.08	294±2.05	0.53±0.02	0.0±0.05
0	Filtered without Nitrogen	99.53±1.87	3.96±0.07	292±2.12	0.267±0.06	0.0±0.05
8		99.11±1.87	3.93±0.09	297±2.56	0.26±0.08	0.067±0.05
24		97.91±1.87	3.95±0.08	295±1.59	0.66±0.02	0.0±0.05
48		96.35±1.87	3.95±0.09	294±2.01	0.73±0.01	0.20±0.05
72		92.76±1.87	3.96±0.11	295±2.15	0.20±0.04	0.0±0.05
96		79.32±1.87	3.97±0.08	294±2.08	0.80±0.07	0.0±0.05
0	Filtered with Nitrogen environment	99.63±1.87	3.96±0.07	297±2.08	21.27±0.05	6.67±0.09
8		99.41±1.87	3.96±0.08	296±2.07	21.13±0.06	0.80±0.08
24		98.94±1.87	3.95±0.10	294±1.49	0.68±0.07	8.67±0.05

Sample withdrawal time (h)	Treatment	Assay	pH	Osmolarity (mOsmol/Kg)	Particulate matters	
					≥10µm	≥25µm
48		96.85±1.87	3.98±0.09	295±1.93	0.87±0.08	3.66±0.65
72		94.58±1.87	3.96±0.08	293±2.85	0.067±0.01	7.76±0.15
96		85.32±1.87	3.97±0.07	295±1.74	2.40±0.03	9.73±1.65

The outcome of vessel compatibility study suggested that in all four conditions, the product was compatible with manufacturing vessel up to 24 h. After 24 h and till end of study, the assay of product was found in decreasing trend. The rate of degradation of oxytocin was higher in case of nonfiltered and without nitrogen environment. However, filtered solution which was kept in nitrogen environment has shown lesser rate of degradation. Moreover, there was not any remarkable changes in the pH, osmolarity and particulate matters in all four conditions. The outcome the current study will help to control process during formulation of product in plant where stainless-steel container/vessel is used to formulate large quantity from 500L to 2000L. Due to large quantity formulation at commercial stage, it will take around 24 hr to complete the activity from blending to filling and packaging. From above study, we can conclude that formulation can be held for 24 hr in SS container without loss of potency of oxytocin/API.

4.3.1.12 Filter membrane compatibility study

The purpose of filter membrane compatibility study was to evaluate different available filter membranes for their physical and chemical compatibility with product [16]. A study was conducted to evaluate the compatibility and adsorption of oxytocin by filter membrane used and selection. A bulk solution was prepared and passed through filter membranes (Nylon, PES, PVDF) separately and kept at room temperature (~20-25°C). Samples were withdrawn at Initial (0 h), 24 h, 48 h, and evaluated for drug product. Description, pH, Assay, particulate matters. The results of filter compatibility study are tabulated in Table 4.14.

Table 4.14: Filter membrane compatibility data at room temperature (~20-25°C)

Sample withdrawal time (h)	Treatment	Assay	pH	Osmolarity	Particulate matters	
					≥10µm	≥25µm
0	PES membrane	100.62±1.12	3.97±0.08	291±2.35	3.87±0.05	0.67±0.01
24		99.99±1.05	3.97±0.07	289±2.41	0.267±0.05	0.067±0.09

Sample withdrawal time (h)	Treatment	Assay	pH	Osmolarity	Particulate matters	
					≥10µm	≥25µm
48		99.89±1.13	3.97±0.09	290±2.34	1.73±0.05	0.033±0.08
0	PVDF membrane	100.41±1.25	3.97±0.06	291±2.185	1.40±0.05	0.067±0.07
24		99.49±1.61	3.96±0.04	290±1.00	1.267±0.05	0.033±0.04
48		98.05±1.07	3.97±0.08	291±1.14	0.367±0.05	0.20±0.05
0	Nylon membrane	100.11±1.08	3.96±0.03	289±1.13	0.467±0.05	0.33±0.00
24		98.71±1.34	3.97±0.07	291±2.00	0.67±0.05	0.67±0.00
48		96.64±1.05	3.95±0.08	290±2.5	1.67±0.05	0.33±0.05

The outcome of filter compatibility study suggested that the product is most compatible with PES membrane up to 48 h. After 24 h and till end of study, assay of the product was found in decreasing trend with Nylon and PVDF filter. The assay of oxytocin was decreased most, when drug product was stored after filtration through nylon membrane. Moreover, there was not any remarkable changes in pH, osmolarity and particulate matters when solution was stored with all three filters.

4.3.1.13 Stability study of optimized RTI formulation of Oxytocin

The stability of the API in drug formulations is a significant concern, as it is a key requirement in the formulation development process. Therefore, a thorough stability testing plan was devised, where the manufactured RTI formulations were evaluated for storage stability at 5°C ±3°C and at 25°C/40% relative humidity, in accordance with ICH guidelines.

RTI formulations were filled in PHC bag (non-PVC infusion) and subsequently stoppered. Bags were then overwrapped in aluminium pouches followed by labelling. The packed formulations were subjected to storage stability in 5°C ±3°C and 25°C/40% RH stability chamber. At selected intervals, the samples were analysed for %drug assay, pH, osmolarity, particulate matters, %transmittance and absorbance at 420 nm. The analytical test results for RTI formulation are illustrated in Table 4.15, and Figure 4.9 to 4.11.

The stability evaluation of the oxytocin RTI formulation, set at a concentration of 0.02 IU/mL, was meticulously conducted under specific environmental conditions: 5 ± 3 °C, 25°C with 40% relative humidity, and 30°C with 65% relative humidity, all over a period

of six months. The stability data gathered was subsequently subjected to comprehensive analysis using Minitab, allowing for an in-depth assessment of the formulation's integrity over time. The RTI formulations were systematically stored at the designated temperatures and humidity levels for the stability studies, during which they were rigorously tested for several critical parameters, including assay potency, pH level, osmolality, PMT, percentage of absorbance, percentage of transmittance, and sterility. The evaluation of the formulation's storage conditions involved analysing the percentage assay at specified time intervals, revealing important insights into its stability.

Remarkably, the percentage of assay was found to decline from an initial value of 102.2±1.76 to 95.73±0.95 after 24 months at the controlled temperature of 5±3 °C. In contrast, the assay value showed a decrease to 93.13±1.01 after 12 months in the 25°C/40%RH environment, and further declined to 91.09±1.06 after just six months at the 30°C/65%RH condition. Notably, the oxytocin formulation displayed no significant changes when stored at 5 ± 3 °C, whereas the assay values at 25°C/40%RH and 30°C/65%RH indicated measurable changes over six months. Throughout the stability period, the effects of varying environmental conditions on the assay stability were closely monitored, revealing that the percentage assay consistently decreased under the higher temperature and humidity conditions of 25°C/40%RH and 30°C/65%RH. Conversely, the formulation stored at 5 ± 3 °C exhibited minimal degradation, suggesting that maintaining this temperature is effective in preserving the integrity of the formulation.

Additionally, there were no noteworthy differences in the initial baseline measurements of osmolality, PMT, percentage absorbance, percentage transmittance, and sterility when compared to the values recorded throughout the various stability conditions. Thus, the data gathered from the stability testing strongly indicated that the RTI formulations of oxytocin stored at the cooler temperature of 5±3 °C exhibited a significantly higher degree of stability over time.

Table 4.15: Stability data of Oxytocin injection RTI 0.02 IU/mL

Stage	Assay (%)	pH	Osmolality (mOsm/kg)	PMT		% Transmittance at 650 nm	Absorbance at 420 nm	Sterility
				≥10µm	≥25µm			
Specification	90-110	3-5	250-350	6000	600	>95	<1%	No evidence of microbial growth should be found
Initial	102.2±1.76	4.01±0.03	290±2.67	33.00±0.03	13.00±0.03	99.467±1.10	0.015±0.01	Complies
5 ± 3°C-3M	98.33±1.03	4.12±0.04	288±1.13	53.33±0.03	0.00±0.03	99.23±0.03	0.03±0.01	-
5 ± 3°C-6M	97.99±1.09	4.06±0.08	285±1.18	33.00±0.03	7.00±0.03	99.467±0.04	0.012±0.01	Complies
5 ± 3°C-9M	97.52±1.15	4.12±0.06	287±1.23	49.33±0.03	3.70±0.03	99.835±0.05	0.0±0.00	Complies
5 ± 3°C-12M	97.27±1.10	4.00±0.09	291±1.23	30.70±0.03	2.00±0.03	99.944±0.06	0.0±0.00	Complies
5 ± 3°C-24M	95.73±0.95	4.10±0.07	295±2.15	23.33±0.03	3.33±0.03	99.853±0.07	0.0±0.00	Complies
25°C/40%RH-1M	100.63±0.91	4.03±0.08	284±2.00	48.7±0.03	00±0.03	99.700±0.08	0.01±0.00	Complies
25°C/40%RH-3M	98.22±1.05	4.05±0.06	288±2.15	53.33±0.03	6.67±0.03	99.965±0.07	0.0±0.00	Complies
25°C/40%RH-6M	95.53±1.01	4.12±0.09	285±2.13	72.3±0.03	00±0.03	99.772±0.04	0.015±0.00	Complies
25°C/40%RH-9M	94.11±1.03	4.18±0.05	287±2.13	67.77±0.03	6.7±0.03	99.921±0.07	0.0±0.00	Complies
25°C/40%RH-12M	93.13±1.01	4.03±0.05	293±1.95	33±0.03	0.0±0.03	99.512±0.05	0.012±0.01	Complies
30°C/65%RH-3M	95.33±1.06	4.07±0.07	287±1.65	47.7±0.03	7±0.03	99.667±0.06	0.011±0.01	Complies
30°C/65%RH-6M	91.09±1.06	4.05±0.08	286±1.76	43.33±0.03	0.67±0.03	99.872±0.05	0.010±0.01	Complies
Note: Extrapolation and stability study report generated for 25°C/40%RH Container content @ initial, 3M, 6M & 12 M & 12M done and found NLT 100mL								

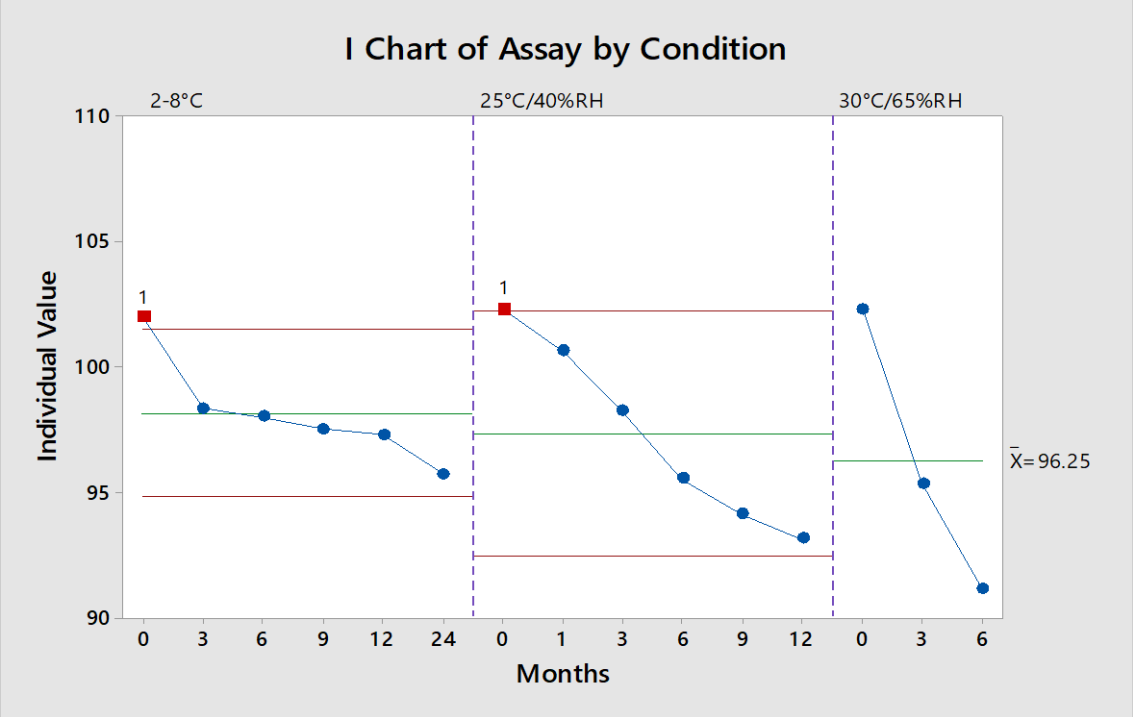

Figure 4.9: Assay of Oxytocin at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control**.**

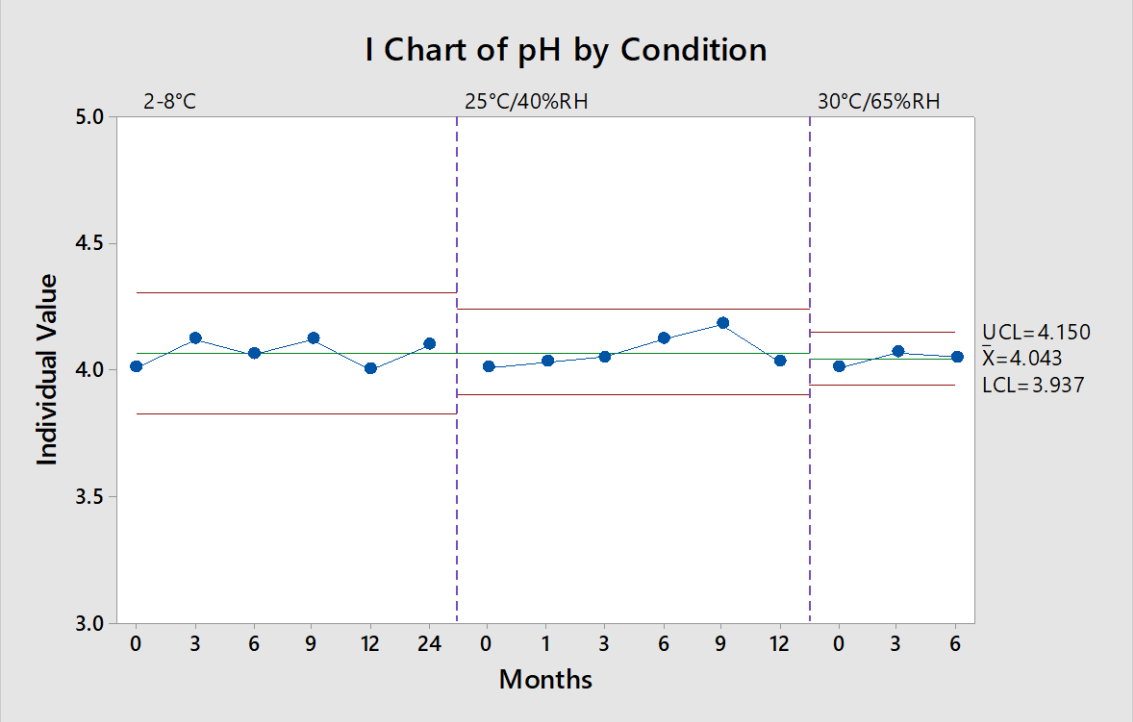

Figure 4.10: pH of Oxytocin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

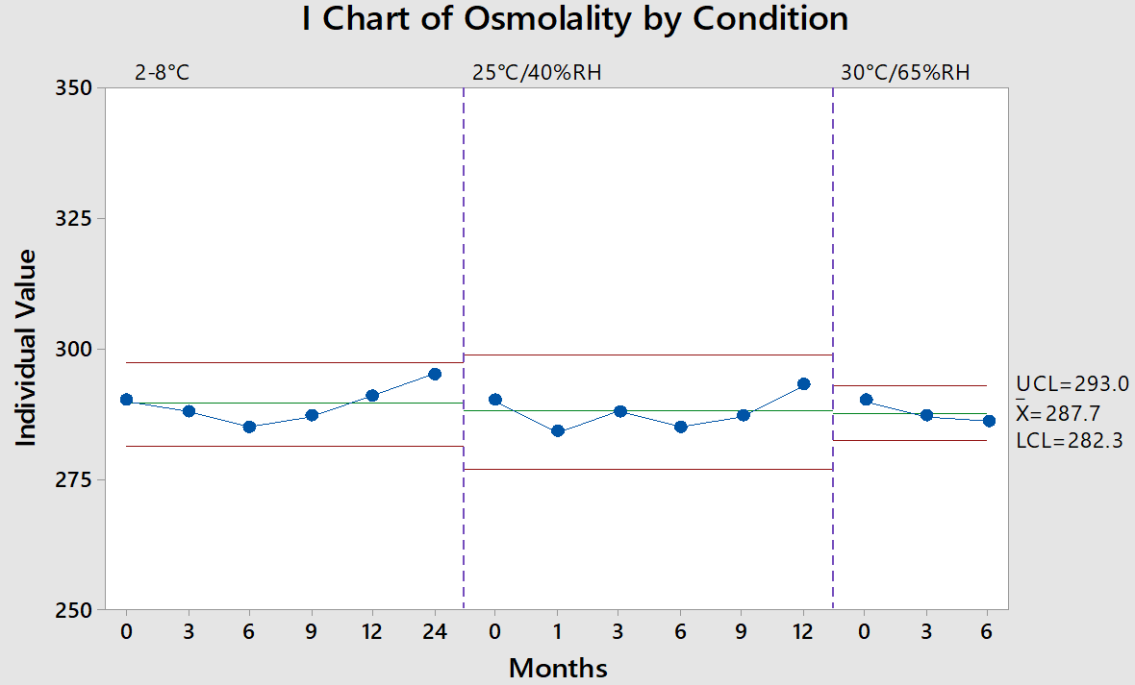

Figure 4.11: Osmolarity of Oxytocin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

4.3.1.14 Stability study with higher strength

In the same line, stability study of Oxytocin injection of higher strength was also carried out. The analytical outcomes of 0.08 IU/mL RTI formulation of oxytocin are presented below (Table 4.16 and Figure 4.12 to 4.14).

The stability evaluation of the oxytocin RTI formulation (0.08 IU/mL) was conducted at 5 ± 3 °C, 25°C/40%RH, and 30°C/65%RH over a period of 6 months. The stability data obtained was analysed using Minitab to assess its relation to time. RTI formulations were maintained at 5 ± 3 °C, 25°C/40%RH, and 30°C/65%RH for stability analysis, and were examined for assay, pH, osmolality, PMT, % absorbance, % transmittance, and sterility. The formulation's storage conditions were evaluated by analysing the percentage of assay after designated time intervals. The percentage of assay changed from 100.68±1.06 to 98.66±1.67 after 24 months at 5±3 °C, while it was 96.39±1.41 after 12 months at 25°C/40%RH and 93.78±1.14 after 6 months at 30°C/65%RH. Oxytocin exhibited no significant changes at 5 ± 3 °C, whereas changes in assay were noted at 25°C/40%RH and 30°C/65%RH after 6 months.

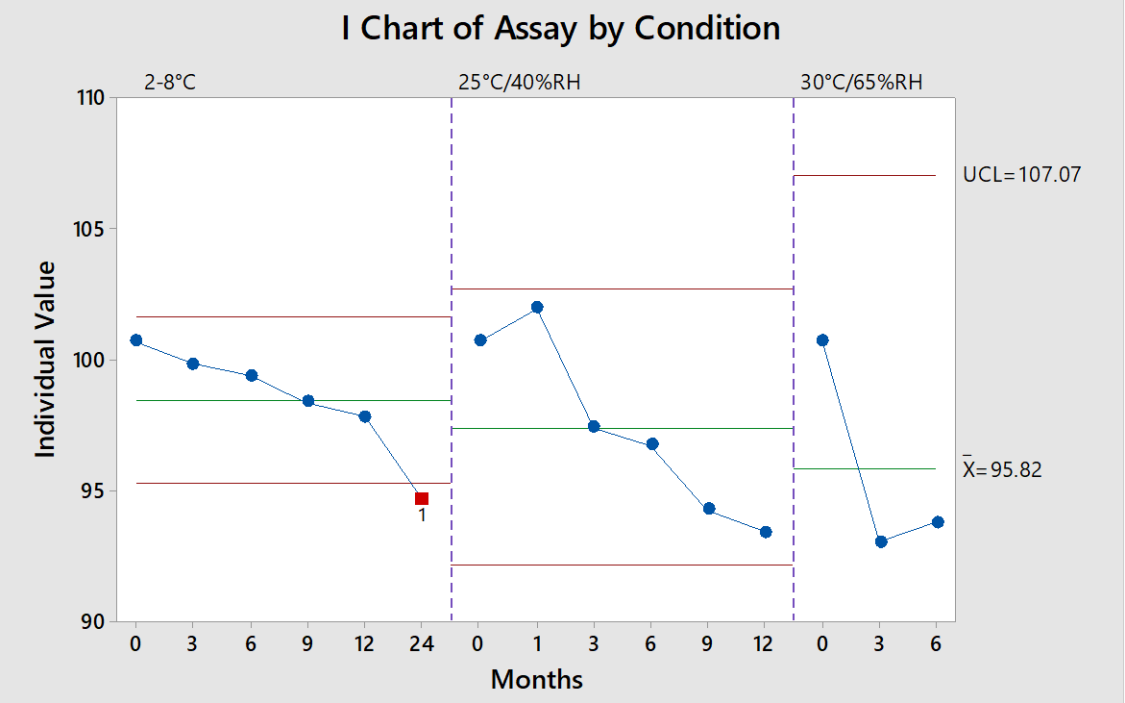

Figure 4.12: Assay of Oxytocin in final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

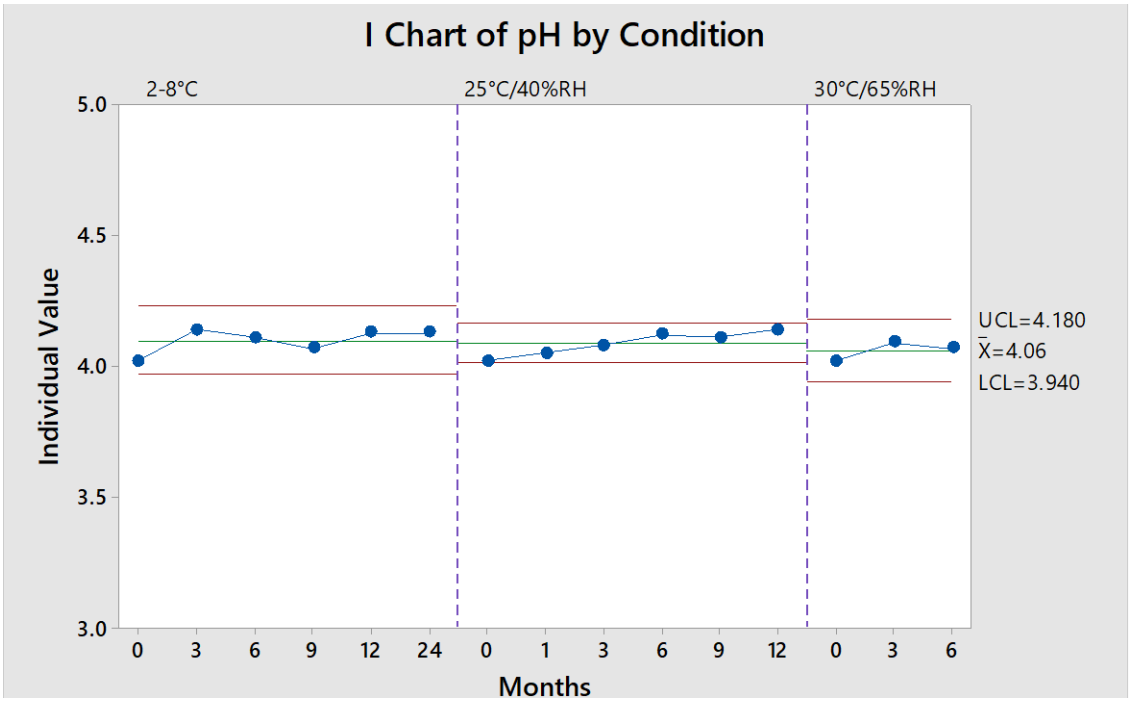

Figure 4.13: pH of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

Table 4.16: Stability data of Oxytocin injection 0.08 IU/mL

Stage	Assay (%)	pH	Osmolality (mOsm/kg)	PMT (Per container)		% Transmittance	Absorbance ≥10µm	Sterility
				≥10µm	≥25µm			
Specification	90-110	3-5	250-350	6000	600	>95	<1%	No evidence of microbial growth should be found
Initial	100.68±1.06	4.02±0.04	290±1.06	53.3±0.08	0.0±0.00	99.76±0.03	0.011±0.001	Complies
5 ± 3°C-3M	98.83±1.13	4.14±0.08	291±1.06	33.3±0.13	0.33±0.01	99.65±0.03	0.012±0.001	Complies
5 ± 3°C-6M	100.38±1.51	4.11±0.06	289±1.13	66.7±1.21	6.7±0.04	100.0±0.03	0.0±0.001	Complies
5 ± 3°C-9M	97.37±1.31	4.07±0.07	287±1.91	29.3±1.34	3.67±0.02	99.63±0.03	0.015±0.001	Complies
5 ± 3°C-12M	101.80±1.21	4.13±0.08	286±1.14	70.0±1.22	10±0.01	99.44±0.03	0.016±0.001	Complies
5 ± 3°C-24M	98.66±1.67	4.13±0.09	297±1.17	23.2±1.21	7.0±0.06	99.36±0.03	0.023±0.00	Complies
25°C/40%RH-1M	101.98±1.01	4.05±0.05	289±1.18	20.0±1.81	0.67±0.03	99.69±0.01	0.010±0.00	Complies
25°C/40%RH-3M	97.39±1.09	4.08±0.07	288±2.01	33.1±1.72	0.0±0.07	99.99±0.01	0.01±0.00	Complies
25°C/40%RH-6M	97.72±1.06	4.12±0.08	286±1.12	40.0±1.62	0.33±0.00	99.27±0.01	0.019±0.00	Complies
25°C/40%RH-9M	94.26±1.32	4.11±0.06	289±1.16	47.7±1.61	0.67±0.01	99.38±0.01	0.01±0.0	Complies
25°C/40%RH-12M	96.39±1.41	4.14±0.07	290±1.15	47.7±1.82	7.0±0.02	99.24±0.02	0.01±0.00	Complies
30°C/65%RH-3M	93.00±1.21	4.09±0.05	288±1.17	33.0±1.13	0.0±0.00	99.76±0.01	0.01±0.00	Complies
30°C/65%RH-6M	93.78±1.14	4.07±0.05	286±1.18	6.77±1.13	0.33±0.01	99.98±0.03	0.01±0.00	Complies

Note: Extrapolation and stability study report generated for 5 ± 3°C & 25°C/40%RH.

- Container content @ initial, 3M, 6M & 12 M & 12M done and found NLT 100mL

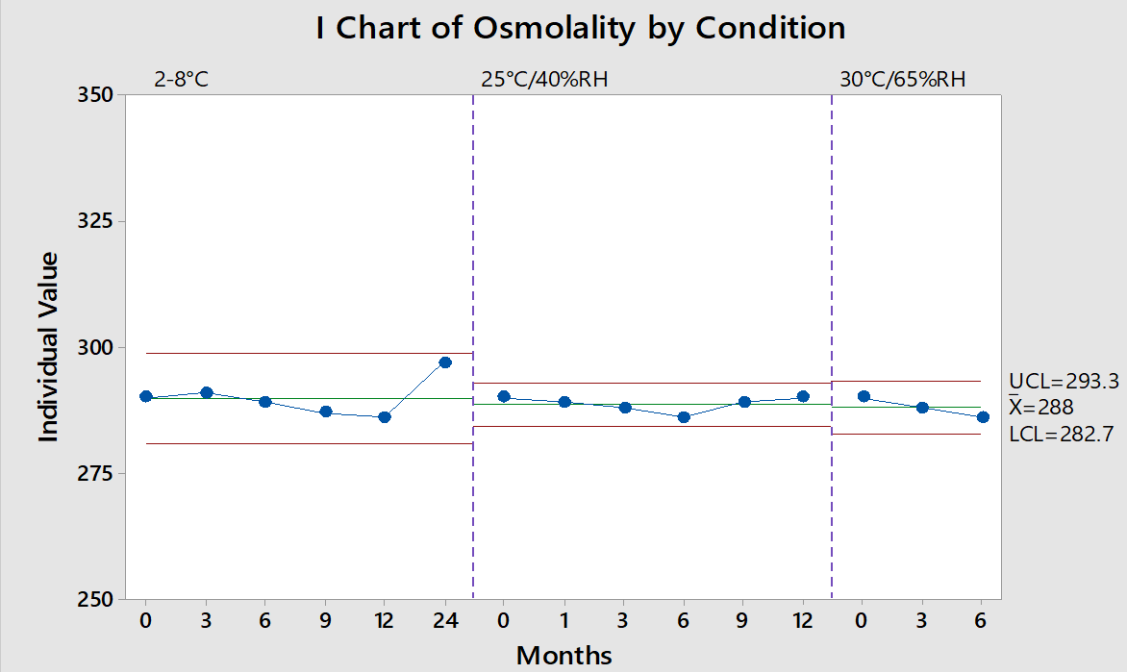

Figure 4.14: Osmolarity of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

The effects of different conditions on the assay were noted throughout the stability period, while others remained unaffected. The percentage assay was observed to decline when stored at 25°C/40%RH and 30°C/65%RH. This degradation effect was minimal for the formulation kept at 5±3 °C, suggesting that maintaining the formulation at this temperature can help control degradation. There were no significant differences in the initial values of osmolality, PMT, % absorbance, % transmittance, and sterility when compared to the values noted throughout the stability testing under all storage conditions. Therefore, the stability testing results indicated that the RTI formulations of oxytocin stored at 5±3 °C exhibited greater stability.

4.3.1.15 Stability Study Report at 5 ± 3 °C & 25°C/40%RH for final formulation as per ICH Q1E

The stability study of oxytocin RTI formulation for two strength (0.02 IU/mL & 0.08 IU/mL) was done at 5±3 °C, 25°C/40%RH & 30°C/65%RH-6M. However, as per regulatory recommendation, the stability extrapolation of products is required to find the best real time storage (long term storage) condition. Since, the stability of products was found promising at 5±3 °C and 25°C/40%RH, while at 30°C/65%RH-6M was least

amongst all tested storage conditions. Henceforth, we decided to generate stability data report of both strengths for 5±3 °C and 25°C/40%RH storage condition. To generate stability report, identification of CQAs and risk assessment was needed. In the same context, as per ICH Q1E, a risk assessment was performed in order to identify CQAs (Table 4.17) of drug product. Then after, stability report was generated employing Minitab, Version 21 software. Drug product quality attributes were assessed for likely impact on product safety and efficacy. Variation in drug product quality attributes like Assay of oxytocin, pH and Osmolality can impact product safety and efficacy and hence, were categorized as "CQAs" and were monitored in development batches. The oxytocin shows non-significant change at 5±3°C, while at 25°C/40%RH, it shows changes in assay. The summary of shelf life is summarised in Table 4.18 & 4.19 (Figure 4.15 to figure 4.21), and the calculations are represented in Annexure 1. Obtained stability data was also processed with Minitab in order to evaluate statistically and finding out the suitable best fit model. The statistical outcomes of data are also presented in subsequent sections.

4.3.1.16 Stability Study: Assay vs Months

- **Stability Study: pH versus Months, Strength**

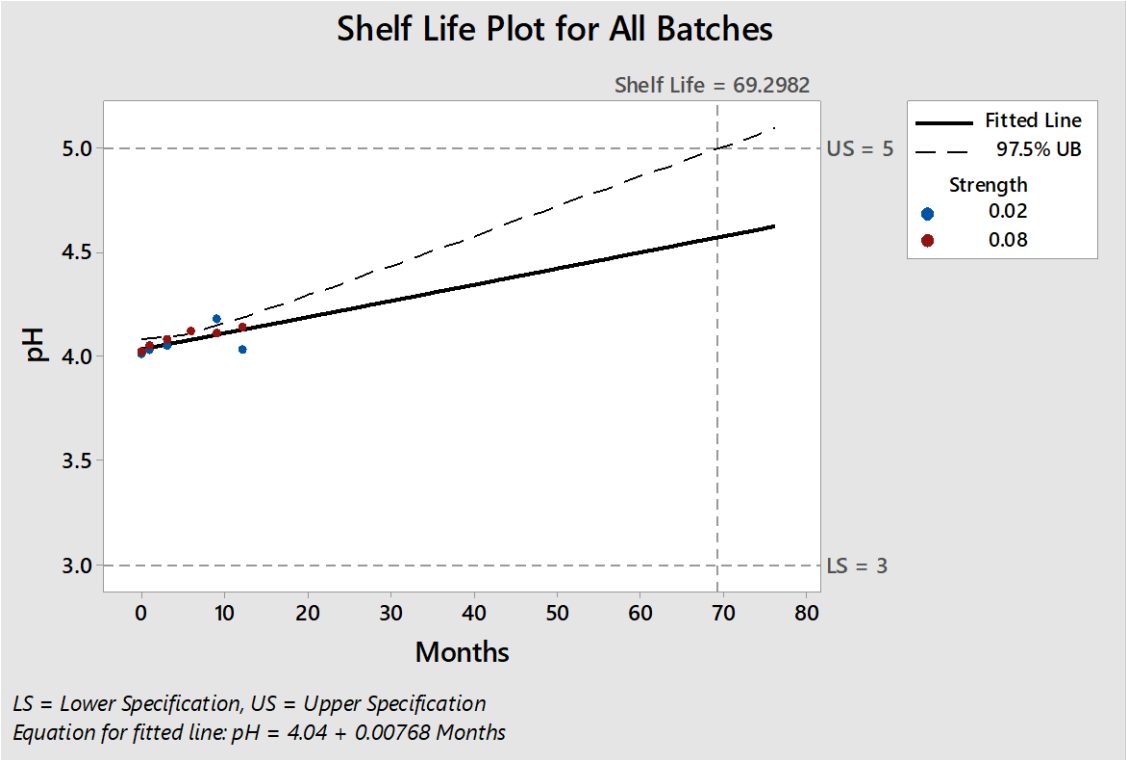

Figure 4.15: Shelf-Life Plot for All Batches

- **Stability Study: Osmolality versus Months, Strength**

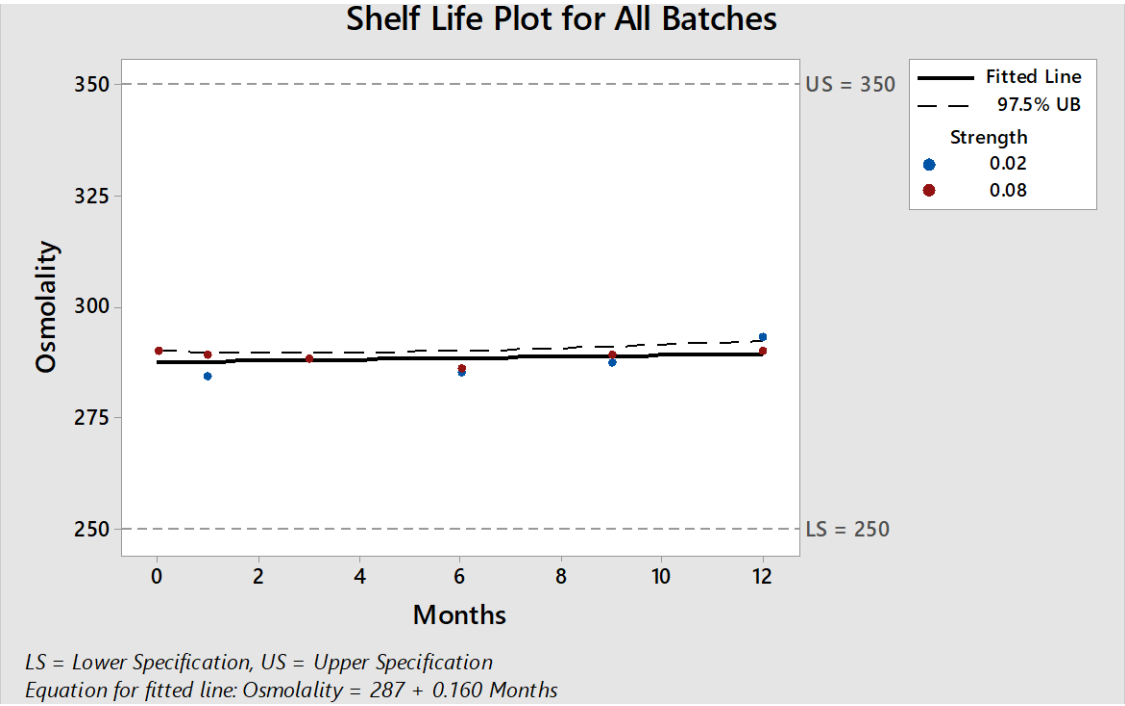

Figure 4.16: Shelf Life Plot for All Batches

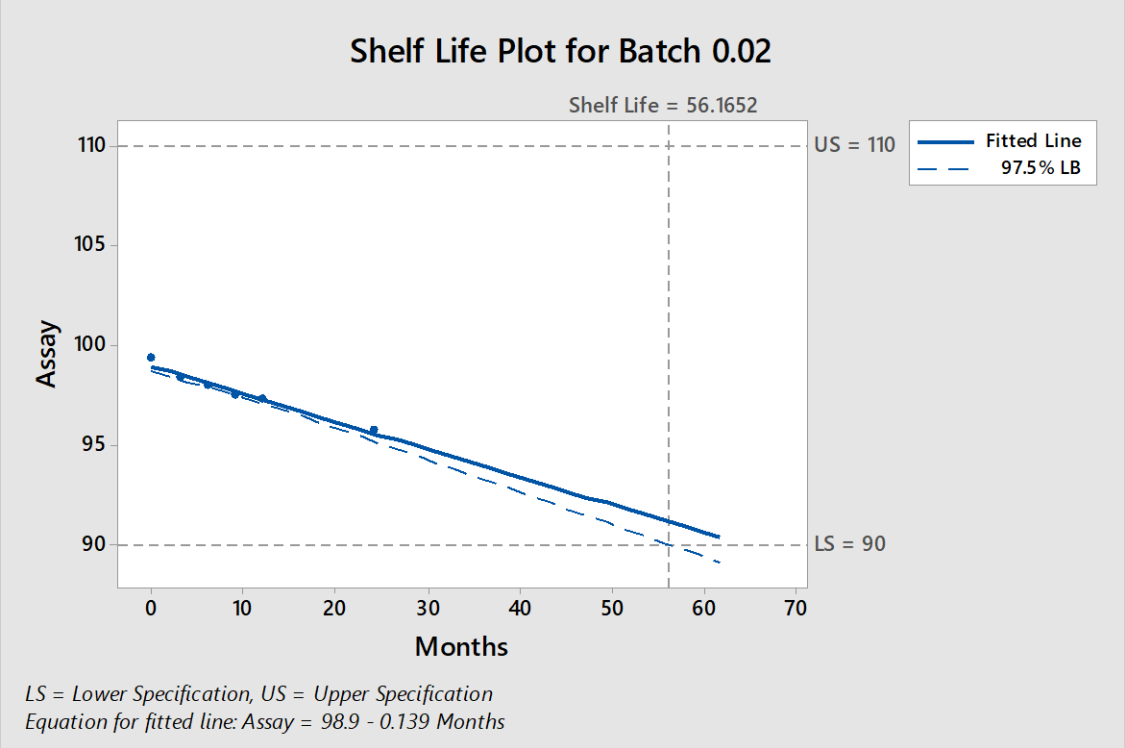

Figure 4.17: Shelf Life Plot for Batch 0.02

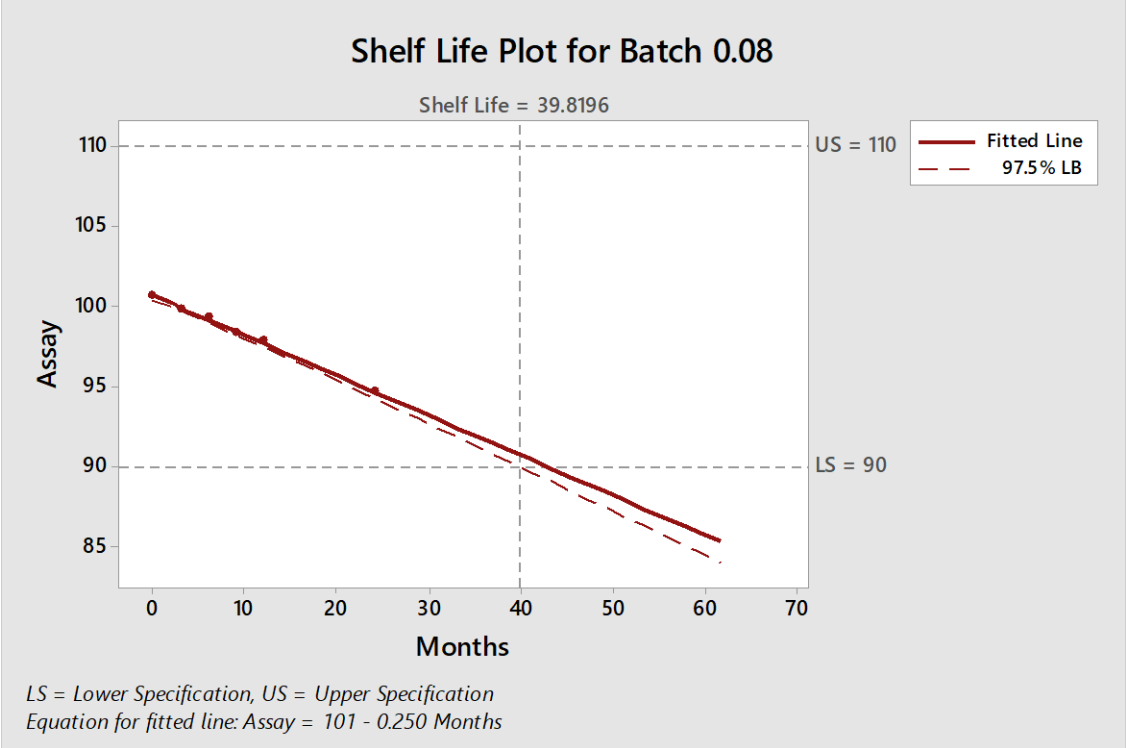

Figure 4.18: Shelf Life Plot for Batch 0.08

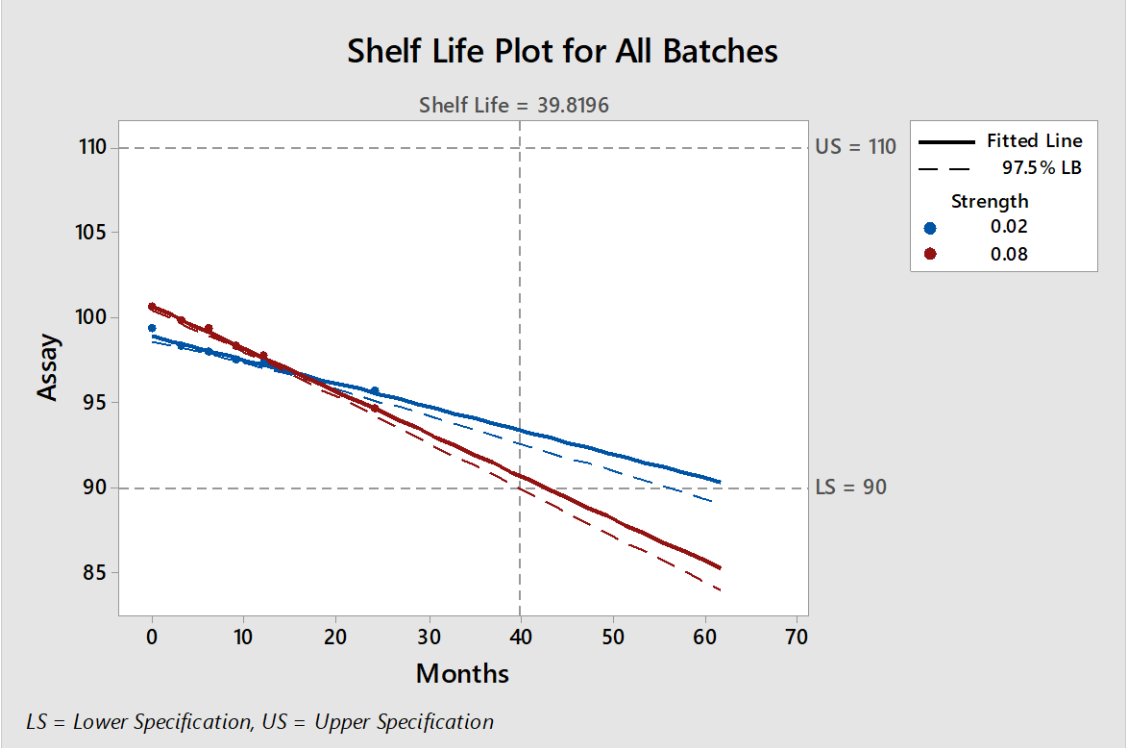

Figure 4.19: Shelf Life Plot for All Batches

- **Stability Study: pH versus Months, Strength**

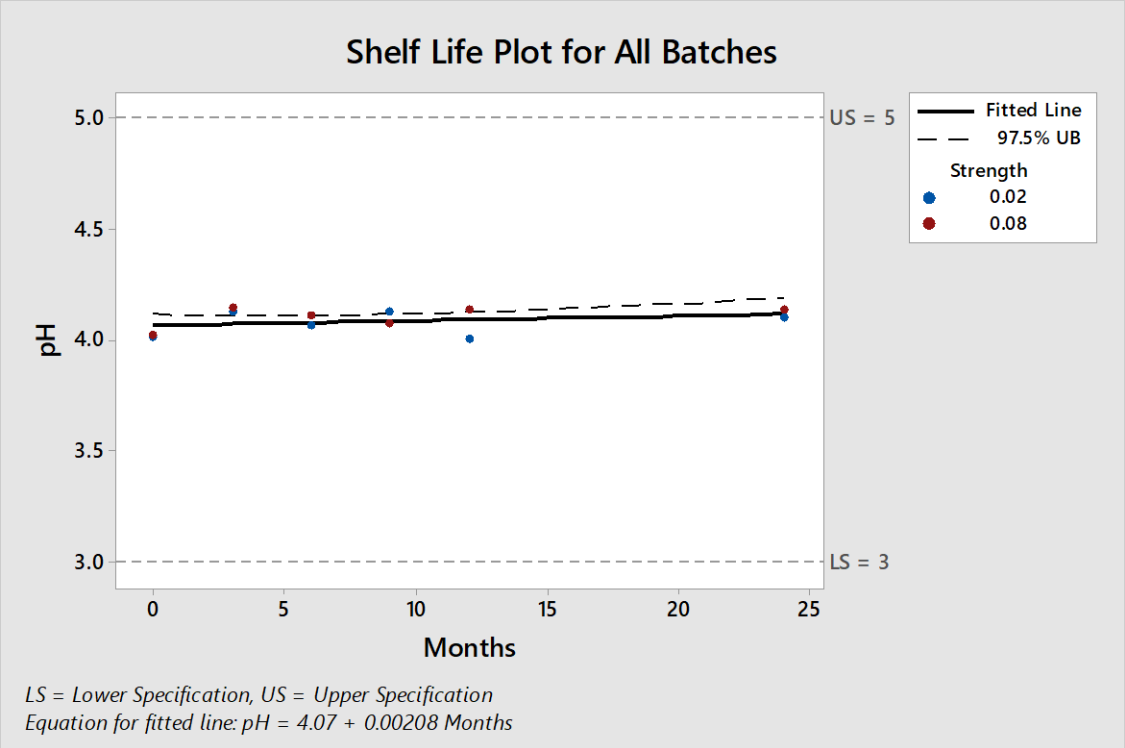

Figure 4.20: Shelf Life Plot for All Batches

- **Stability Study: Osmolality versus Months, Strength**

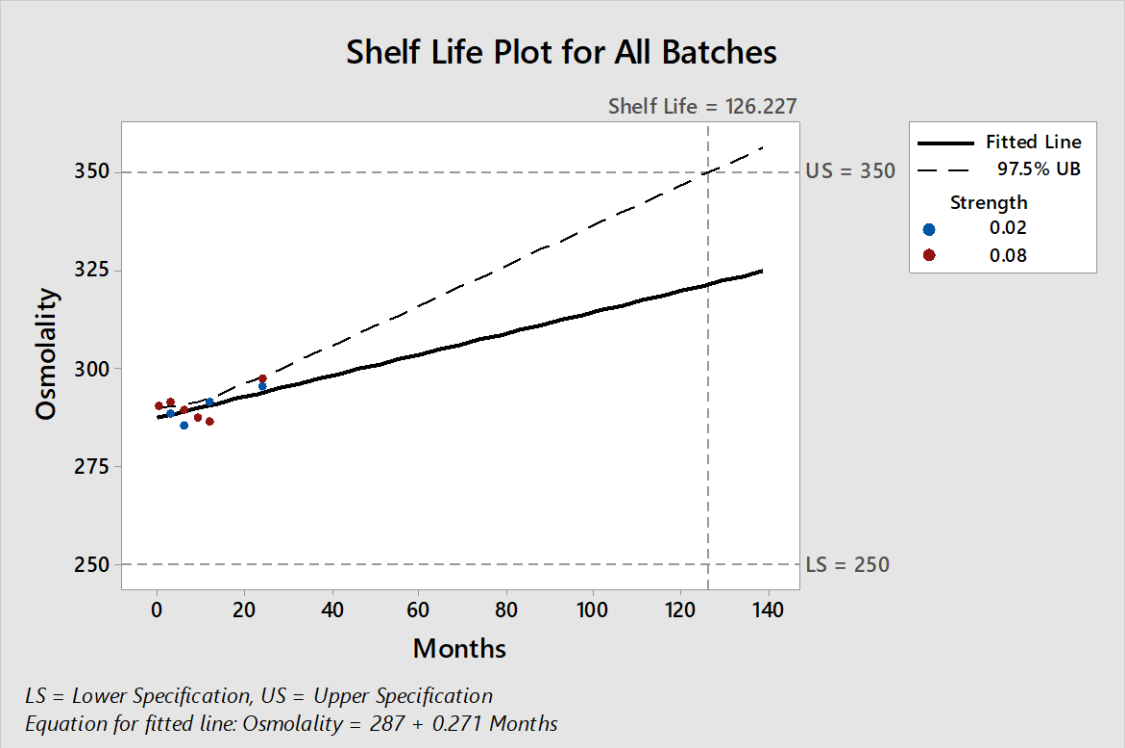

Figure 4.21: Shelf Life Plot for All Batches

4.3.1.16.1 Stability Report at 5 ± 3°C & 25°C/40%RH

RTI formulations were kept at temperatures of 5±3°C, 25°C/40%RH, and 30°C/65%RH for stability evaluation and were tested for various parameters, including assay, pH, osmolality, PMT, % absorbance, % transmittance, and sterility. The influence of different storage conditions on the assay was monitored throughout the stability assessment, while some factors showed no impact. The storage conditions of the formulations were evaluated by analysing the percentage of assay after specified time intervals. It was observed that the percentage of assay decreased when stored at 25°C/40%RH and 30°C/65%RH, likely due to the instability of peptide molecules at elevated temperatures. The formulation stored at 5±3°C exhibited the least degradation, suggesting that maintaining this temperature can help control the degradation of the formulation. No significant differences were identified in the initial pH and osmolarity values when compared to those noted during the stability testing at all storage conditions. Therefore, the stability testing results demonstrated that RTI formulations of oxytocin kept at 5 ± 3°C displayed greater stability. However, the formulations could also be stored at temperatures not exceeding 25°C/40%RH for up to 12 months without significant drug loss. The strength of the RTI formulation was found to have no notable effect on the product's shelf life, as seen in Tables 4.19 and 4.20.

The extrapolation and stability report were generated as per ICH Q1E and assay was found shelf life determining or indicating as there was statistically significant change happened during stability in both storage conditions, which is critical CQA to determine over all shelf life. Shelf life of another CQAs like Osmolality (mOsm/kg) and pH were found to be more compared to assay in both storage conditions. But, based on the stability data prediction as per ICH Q1E, overall shelf life was found to be 13.49 months and 39 months for 25°C/40%RH and 5±3°C respectively. The more shelf life of oxytocin RTI is at 5±3°C. Thus, it can be concluded that even in the worst-case scenario the stability of oxytocin RTI formulation in sodium chloride injection is expected to be more than 12M at 25°C/40%RH and 36M at 5±3°C for proposed specification.

Table 4.17: Drug Product CQAs

Drug product quality attributes		Target	Is it critical?	Justification of critically
Description		**For 0.02 IU/mL (2 IU/100 ml):** A clear, colourless solution, free from visible particulate matter, in Infusion bag Pre-printed with strength 0.02 IU/mL (2 IU/100 ml): **For 0.08 IU/mL (2 IU/100 ml):** A clear, colourless solution, free from visible particulate matter, in Infusion bag Pre-printed with strength 0.8 IU/mL (8 IU/100 ml)	Yes	Critical for safety & efficacy, but formulation & process variables are unlikely to impact on it because raw materials are soluble in water and effectively controlled by the quality management system. It will be monitored at release & on stability.
Identification	By HPLC	The retention time of the Oxytocin peak in the chromatogram of the test preparation corresponds to that of the chromatogram of the standard preparation, as obtained in the test for assay Oxytocin	Yes	Critical for safety & efficacy, but formulation & process variables are unlikely to impact on it because addition of API is ensured by the quality management system. It will be monitored at release & on stability.
	By PDA spectra (HPLC)	The PDA spectrum, in the range of 200 -400 nm of Oxytocin peak in the test preparation corresponds to that of Oxytocin peak in standard preparation, as obtained in the test for assay of Oxytocin		Critical for safety & efficacy, but formulation & process variables are unlikely to impact on it because addition of API is ensured by the quality management system. It will be monitored at release.
Assay of Oxytocin		Between 90.0 % and 110.0 % of Label claim	Yes	Critical; Variability in assay will affect safety & efficacy. Both formulation and process parameters may impact assay. It will be monitored at release and on stability.
pH		Between 3 and 5	Yes	Critical for patient safety and efficacy. Formulation and process variables may impact pH. It will be monitored at release and on stability.
Osmolality		Between 250 and 350 mOsm/kg	Yes	Critical to patient safety. Formulation variables may impact the Osmolality. It will be monitored at release and on stability

Drug product quality attributes	Target	Is it critical?	Justification of critically
Container content	**For 100 ml fill volume (100 mg/100 ml):** NLT 100 ml	Yes	Critical as low volume units may affect efficacy. Filling process may impact Volume in Container but will be adequately controlled by quality management system at intended scale. It will be monitored at release.
Particulate Matter	**For 100 ml fill volume (100 mg/100 ml):** Particles greater than or equal to 10 µm – NMT 6000/container Particles greater than or equal to 25 µm – NMT 600/container	Yes	Critical to patient safety. Formulation and process parameters may impact Particulate Matter. It will be monitored at release and on stability at reduced time points.
Sterility	No evidence of microbial growth should be found	Yes	Sterility is a microbial attribute and critical to patient safety. As the product is filtered, filled into bag, and terminally sterilized, the risk is low. It will be monitored at release and reduced time points on stability.

Table 4.18: Shelf-life estimation of developmental batches of Oxytocin RTI @ 25°C/40%RH

<table>
<tr><th rowspan="2">CQAs</th><th rowspan="2">Proposed shelf-life specification</th><th colspan="3">Result</th><th colspan="3">Inference</th><th rowspan="2">Selected model</th><th rowspan="2">Shelf life indicating?</th></tr>
<tr><th>Time, p-value</th><th>Strength & Time interaction, p value</th><th>Intercept, p value</th><th>Effect of Time</th><th>Slope (Batch & Time interaction) Poolability</th><th>Intercept Poolability</th></tr>
<tr><td>Assay</td><td>90-110</td><td>0.000</td><td>0.647</td><td>0.901</td><td><0.05; Significant</td><td>>0.25 Poolable</td><td>>0.25 Poolable</td><td><table><tr><th>Strength</th><th>Intercept</th><th>Slope</th><th>Shelf life</th></tr><tr><td>0.02 IU/mL</td><td>101.093</td><td>- 0.7219</td><td>13.39</td></tr><tr><td>0.08 IU/mL</td><td>101.093</td><td></td><td>13.39</td></tr><tr><td colspan="3">Over all</td><td>13.39</td></tr></table></td><td>Yes</td></tr>
<tr><td>Osmolality (mOsm/kg)</td><td>250-350</td><td>0.392</td><td>0.472</td><td>0.591</td><td>>0.05; Not Significant</td><td>>0.25 - Poolable</td><td>>0.25 Poolable</td><td><table><tr><th>Strength</th><th>Intercept</th><th>Slope</th><th>Shelf life</th></tr><tr><td>0.02 IU/mL</td><td>287.42</td><td>0.16</td><td rowspan="3">*The mean response slope is not significantly larger than zero</td></tr><tr><td>0.08 IU/mL</td><td>287.42</td><td>0.16</td></tr><tr><td>Over all</td><td></td><td></td></tr></table></td><td>NO</td></tr>
<tr><td>pH</td><td>3.0-5.0</td><td>0.045</td><td>0.704</td><td>0.546</td><td>>0.05; non-Significant</td><td>>0.25 Poolable</td><td>>0.25 Poolable</td><td><table><tr><th>Batch No</th><th>Intercept</th><th>Slope</th><th>Shelf life</th></tr><tr><td>26201276FP033B</td><td>4.038</td><td>0.0078</td><td rowspan="2">69.29 M</td></tr><tr><td>26201276FP040B</td><td>4.038</td><td>0.0078</td></tr><tr><td colspan="3">Over all</td><td>69.29 M</td></tr></table></td><td>Yes</td></tr>
<tr><td colspan="10">Over all stability is 13.49M: Data of particulate matter were observed well within the specification limit for all the time points hence not considered for extrapolation</td></tr>
</table>

Table 4.19: Shelf-life estimation of developmental batches of Oxytocin RTI @ 5 ± 3°C

CQAs	Proposed shelf-life specification	Result			Inference			Selected model	Shelf life indicating?
		Time, p-value	Strength & Time interaction, p value	Intercept, p value	Effect of Time	Slope (Batch & Time interaction) Poolability	Intercept Poolability		
Assay	90-110	0.000	0.000	0.000	<0.05; significant	<0.25 Not Poolable	<0.25 Not Poolable	**Strength** / **Intercept** / **Slope** / **Shelf life** 0.02 IU/mL / 98.945 / - 0.1391 / 56.165 0.08 IU/mL / 100.702 / -0.2498 / 39.82 **Over all** / 39.82	Yes
Osmolality (mOsm/kg)	250-350	0.055	0.867	0.715	>0.05; Not Significant	>0.25 Poolable	>0.25 Poolable	**Strength** / **Intercept** / **Slope** / **Shelf life** 0.02 IU/mL / 287.23 / 0.271 / 126M 0.08 IU/mL Over all	Yes
pH	3.0-5.0	0.316	0.741	0.298	>0.05; non-Significant	>0.25 Poolable	>0.25 Poolable	**Strength** / **Intercept** / **Slope** / **Shelf life** 0.02 IU/mL / 4.0654 / 0.00208 / *The mean response slope is not significantly larger than zero 0.08 IU/mL / 4.038 Over all	No
Over all stability is 39.82M: Data of particulate matter were observed well within the specification limit for all the time points hence not considered for extrapolation									

4.3.2 Development and Optimization of Ready to Infuse formulation of Vasopressin

4.3.2.1. Formulation development for Selection of Osmogens

In view of knowledge gathered during formulation development of Oxytocin RTI and considering effect of concentration of different osmogens on osmolarity of formulation, various developmental trials have been taken in order to stabilize the Vasopressin in large volume aqueous based formulations. For this purpose, osmogens such as Sodium chloride, Mannitol, Lactose, Sucrose, Trehalose and Dextrose were evaluated [18]. Sodium acetate buffer with 0.01 mg/mL was selected due to prominent result in previous part (Oxytocin). Oxytocin and vasopressin are nonapeptides and similar in structure [17]. Therefore, sodium acetate buffer in same concentration was selected in vasopressin RTI development. In initial screening, pH was kept 3.7 as per concentrate vasopressin data. The details of different developmental trials are presented below (Table 4.20).

Table 4.20: Development trials with different osmogens for Vasopressin RTI formulation

Formulation Code	Compositions (Each mL Contains)	Sample description	Stage	Assay (%)	pH	Osmolarity (mOsmol/Kg)
FB-1	Vasopressin USP 1.887 µg, Sod Acetate 0.01 mg, Mannitol 50 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	PHC	Initial	96.29±1.31	3.69±0.07	298±1.31
		AOB	Initial	94.14±1.25	3.70±0.05	298±1.87
FB-2	Vasopressin USP 1.887 µg, Sod Acetate 0.01 mg, Dextrose 50 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	PHC	Initial	90.71±1.42	3.66±0.06	293±1.65
		AOB	Initial	92.42±1.65	3.70±0.09	290±1.74
FB-3	Vasopressin USP 1.887 µg, Sod Acetate 0.01 mg, Sucrose 50 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	PHC	Initial	91.01±1.16	3.71±0.08	295±1.45
		AOB	Initial	90.5±1.06	3.67±0.06	299±1.05
FB-4	Vasopressin USP 1.887 µg,	PHC	Initial	97.98±1.07	3.74±0.07	280±1.21

Formulation Code	Compositions (Each mL Contains)	Sample description	Stage	Assay (%)	pH	Osmolarity (mOsmol/Kg)
	Sod Acetate 0.01 mg, Lactose 50 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	AOB	Initial	95.67±1.24	3.65±0.08	282±1.31
FB-5	Vasopressin USP 1.887 µg, Sod Acetate 0.01 mg, Trehalose 50 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	PHC	Initial	93.84±1.54	3.68±0.09	288±1.87
		AOB	Initial	96.2±1.65	3.65±0.05	288±1.34
FB-6	Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	PHC	Initial	100.85±1.29	3.66±0.09	297±1.81
		AOB	Initial	101.07±1.24	3.71±0.07	298±1.07
Note: All batches Drug Product containers passed the leak test						

All formulations were prepared with sodium acetate buffer (0.01mg/mL) with different osmogens and filled in two different packaging materials (PHC & AOB). The assay of formulations prepared with mannitol, dextrose sucrose, lactose, trehalose, and sodium chloride (FB-1 to FB-6) was found to be above 90% and within proposed specification (90-110%). But, % of assay in FB-6 containing sodium chloride as osmogen was found to be 100% in both bags (PHC & AOB), while others prepared by lactose, sucrose, mannitol, trehalose, dextrose were found to be below 98%. Further, to check the effect of HPβCD as stabilizer, same formulation (FB6) with HPβCD was formulated (FB-6) in both bags and kept for 3M at 25°/40%RH. However, the effect of HPβCD was not significant compared to without HPβCD (FB-7) after 3M at 25°/40%RH (Table 4.21). Therefore, we decided that FB-6 is optimum formulation for further study. Based on the results portrayed in Table 4.21, sodium chloride was selected as osmogens for further development.

Table 4.21: Trials with HPβCD for Vasopressin RTI formulation

Formulation Code	Compositions (Each mL Contains)	Sample description	Stage	Assay (%)	pH	Osmolarity (mOsmol/Kg)
FB-6	Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 3.7, WFI q.s.	PHC	Initial	100.85±1.29	3.66±0.09	297±1.81
			25°/40%RH-3M	96.25±1.13	3.69±0.02	298±1.01
		AOB	Initial	101.07±1.24	3.71±0.07	298±1.07
			25°/40%RH-3M	96.87±1.14	3.73±0.04	299±1.13
FB-6A	Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HPβCD 0.1 mg/mL, HCl/NaOH q.s. to pH 3.7, WFI q.	PHC	Initial	98.29±1.05	3.69±0.05	302±1.04
			25°/40%RH-3M	95.32±2.15	3.72±0.045	303±2.13
		AOB	Initial	99.14±1.03	3.70±0.06	303±1.02
			25°/40%RH-3M	96.89±1.31	3.73±0.04	302±2.02
Note: All batches Drug Product containers passed the leak test						

4.3.2.2. pH Stability Study

With the optimized composition, five formulations of different pH (5.0, 4.5, 4.0, 3.5 and 3.0) were prepared. All the formulations were kept for 3M at 25°C/40%RH. After 3M, the formulations were analysed for CQAs and results were compared with their respective initial results (Table 4.22).

Table 4.22: pH study data of experimental trials (Vasopressin)

Formulation Code	Sample pH (Target)	Batch No	Stage	Assay (%)	pH (Observed)	Osmolarity (mOsm/kg)	PMT		% Transmittance at 650 nm	% Absorbance at 420 nm
							≥10µm	≥25µm		
Specification	3-5			90-110		250-350	6000	600	>95	<1
FD-1	3.0	FP064A	Initial	99.31±1.71	3.01±0.08	283±2.12	12.76±1.71	6.7±1.11	99.712±0.07	0.012±0.004
	3.0	FP064A	25°C/40%RH-3M	92.18±0.96	3.06±0.07	284±2.02	56.87±2.04	17.8±0.91	99.681±0.08	0.011±0.004
FD-2	3.3	FP064B	Initial	99.17±1.48	3.31±0.09	285±1.98	10.98±2.09	13.66±0.34	99.571±0.06	0.03±0.003

Formulation Code	Sample pH (Target)	Batch No	Stage	Assay (%)	pH (Observed)	Osmolarity (mOsm/kg)	PMT		% Transmittance at 650 nm	% Absorbance at 420 nm
							≥10µm	≥25µm		
Specification	3-5			90-110		250-350	6000	600	>95	<1
	3.3	FP064B	25°C/40%RH-3M	93.60 ±1.14	3.35± 0.08	286± 1.93	67.66 ±1.04	3.33± 1.48	99.758 ±0.05	0.014 ±0.003
FD-3	3.8	FP064C	Initial	100.51±1.32	3.82± 0.07	285± 2.19	20.33 ±1.89	12.76 ±1.14	99.708 ±0.07	0.02± 0.005
	3.8	FP064C	25°C/40%RH-3M	98.38 ±1.36	3.87± 0.06	286± 2.09	30.57 ±1.98	6.67± 1.07	99.856 ±0.09	0.019 ±0.002
FD-4	4.3	FP064D	Initial	99.25 ±1.19	4.30± 0.04	282± 2.19	67.66 ±1.48	13.89 ±1.05	99.468 ±0.11	0.016 ±0.003
	4.3	FP064D	25°C/40%RH-3M	96.12 ±1.41	4.34± 0.05	289± 2.41	20.38 ±2.24	14.71 ±1.19	98.712 ±0.14	0.029 ±0.004
FD-5	4.6	FP064E	Initial	98.98 ±1.11	4.62± 0.03	284± 2.82	47.92 ±1.87	15.20 ±0.64	99.598 ±0.18	0.019 ±0.002
	4.6	FP064E	25°C/40%RH-3M	93.65 ±1.32	4.69± 0.04	287± 2.78	38.48 ±2.14	19.68 ±0.81	99.771 ±0.22	0.017 ±0.003
FD-6	5.0	FP064E	Initial	98.80 ±1.28	5.02± 0.05	285± 2.38	20.67 ±2.12	12.48 ±1.01	99.448 ±0.21	0.021 ±0.004
	5.0	FP064E	25°C/40%RH-3M	91.19 ±1.21	5.06± 0.04	288± 2.51	48.54 ±2.54	6.77± 1.05	99.603 ±0.24	0.022 ±0.004

A decrease in assay for all pH formulations was observed after 3M, while other CQAs i.e., pH, osmolality, PMT, % transmittance & absorbance were well in control. The assay difference after 3M for formulation with pH 3.8 was found least compared to others. Therefore, we decided to keep pH 3.8 in our formulation for further studies.

4.3.2.3. Manufacturing Process for RTI formulation of Vasopressin

i. 80% batch size of WFI was collected in SS vessel

ii. Temperature 5 ± 3°C was achieved and maintained throughout process. N_2 was purged to achieve DO <1ppm.

iii. pH was adjusted to 3.8 with 0.1% v/v HCl and/or 0.1% w/v NaOH

iv. Vasopressin was added and dissolved with continuous stirring

v. Solution was purged to achieve DO< 1ppm

vi. Solution was diluted with 0.9% NaCl and volume was made up to 100% with WFI and pH was adjusted to 3.8 with 0.1% v/v HCl and/or 0.1% w/v NaOH, if needed.

Overwrap: Infusion bags were overwrapped with nitrogen blanketing and an oxygen scavenger

4.3.2.4. Sterilization method selection for Vasopressin RTI

In order to evaluate a suitable sterilization method, Vasopressin RTI formulation was sterilized at different recommended sterilization conditions (Table 4.23) and analysed thereafter.

Table 4.23: Effect of sterilization process on the Vasopressin RTI formulation

Compositions (Each mL Contains)	Sterilization parameter	Assay (%)	pH	Osmolarity (mOsmol/Kg)
Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 3.7), WFI q.s.	Without autoclave	100.32±0.95	3.69± 0.05	294±4.52
	15 Min_121°C	74.21 ± 2.14	3.70±0.08	296±4.39
	F0 12 Min_121°C	87.12 ± 1.95	3.68±0.06	295±3.89
	F0 08 Min_121°C	88.86 ± 2.13	3.71±0.05	291±5.36
	F0 08 Min_116°C	79.13 ± 1.34	3.67±0.04	292±4.12
	F0 08 Min_111°C	51.21± 3.56	3.69±0.06	296±3.28

In all sterilization conditions mentioned above, assay of Vasopressin in RTI formulation was found drastically reduced. However, no effect on pH and osmolarity of formulation was observed. Therefore, the process comprising sterile filtration, pre-sterilized container and aseptic processing was chosen for formulation.

4.3.2.5. Photostability study for Vasopressin RTI

To evaluate the photosensitivity of Vasopressin RTI, the samples (in infusion bag, in final pack and a control sample) were placed in the photostability chamber and exposed to light of 20 million lux. After exposure, the samples were withdrawn and analysed. The results of photostability are tabulated in Table 4.24.

Table 4.24: Effect of light on Vasopressin RTI

Compositions (Each mL Contains)	Sample description	Assay (%)	pH	Osmolarity
Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 3.7), WFI q.s.	Controlled (Infusion bag wrapped in aluminium sheet)	100.32±0.81	3.69±0.04	294±4.50
	Market pack (Infusion bag placed inside the overwrap pouch)	100.24±0.93	3.67±0.06	296±3.22
	Direct exposed (infusion bag only)	96.91±1.13	4.03±0.02	295±4.12

The assay of Vasopressin in RTI formulation was found slightly reduced when it was exposed to light. There was no effect on pH and osmolarity of formulation. Therefore, utmost care should be taken while manufacturing, handling and packaging of Vasopressin formulation. The outcome the current study will be help to control process during formulation by protecting the formulation by direct exposure of light and selection of secondary packaging which will protect the product direct exposure of light during storage.

4.3.2.6. Stability study of Vasopressin RTI formulation

The stability of the API in drug formulations is a significant concern, as it is a key requirement in the formulation development process. Therefore, a thorough stability testing plan was devised, where the manufactured RTI formulations were evaluated for storage stability at 5°C ±3°C and at 25°C/40% relative humidity, in accordance with ICH guidelines.

RTI formulations were filled in PHC bag (non-PVC infusion) and subsequently stoppered. Bags were then overwrapped in aluminium pouches followed by labelling. The packed formulations were subjected to storage stability in 5°C ±3°C and 25°C/40% RH stability chamber. At selected intervals, the samples were analysed for %drug assay, pH, osmolarity, particulate matters, %transmittance and absorbance at 420 nm. The stability data of final RTI formulation of vasopressin is tabulated in Table 4.25 and depicted in figure 4.22 – 4.27. The stability data of first and second batch of Vasopressin RTI formulation is tabulated in Table 4.25 & Table 4.26, and depicted in Figures 4.23 to 4.28.

The stability evaluation of the vasopressin RTI formulation was conducted at 5 ± 3 °C and 25°C/40%RH over a period of 12 months. The stability data obtained was analysed

using Minitab to assess its relation to time. RTI formulations were maintained at 5 ± 3 °C and 25°C/40%RH for stability analysis, and were examined for assay, pH, osmolality, PMT, % absorbance, % transmittance, and sterility. The formulation's storage conditions were evaluated by analyzing the percentage of assay after designated time intervals. The percentage of assay of batch 1 changed from 99.54±0.69 to 97.406±1.02 after 12 months at 5 ± 3 °C, while it was 93.64±1.04 after 12 months at 25°C/40%RH. Vasopressin exhibited no significant changes at 5 ± 3 °C, whereas changes in assay were noted at 25°C/40%RH. Other tested parameters of RTI formulation remained nearly same from their initial values.

Further, the % assay of batch 2 changed from 99.91±0.86 to 96.17±1.05 after 12 months at 5 ± 3 °C, while it was 94.50±1.25 after 12 months at 25°C/40%RH. Vasopressin exhibited no significant changes at 5 ± 3 °C, whereas changes in assay were noted at 25°C/40%RH. Other tested parameters of RTI formulation remained nearly same from their initial values.

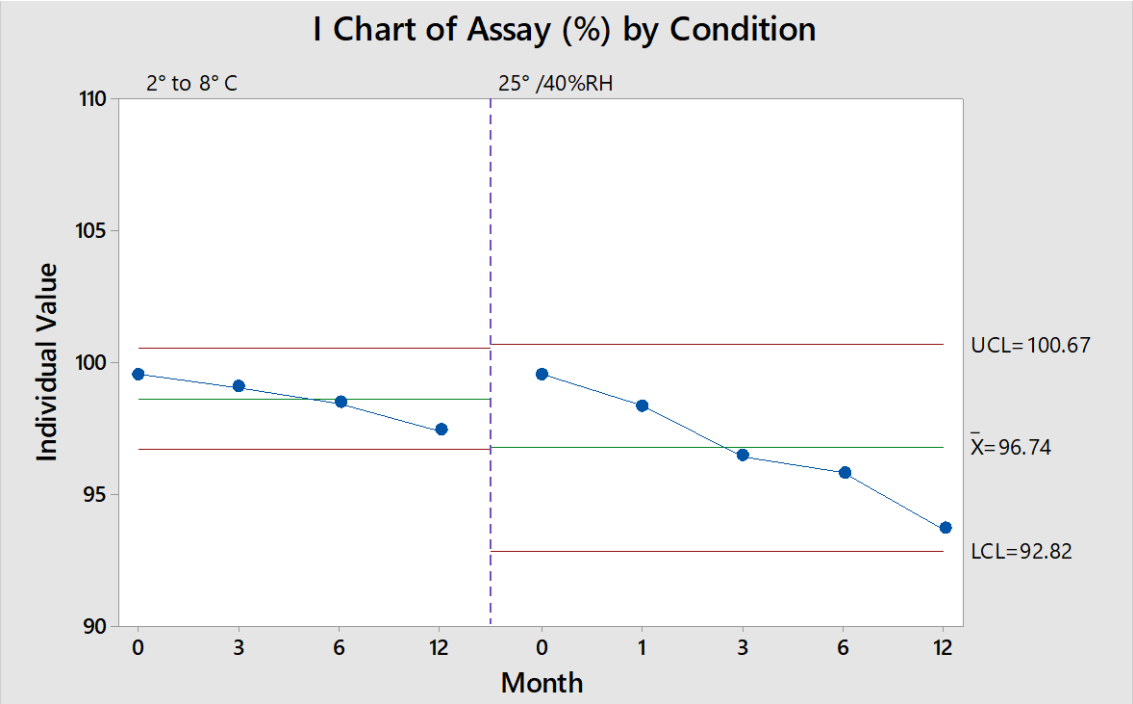

Figure 4.22: Assay of Vasopressin at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

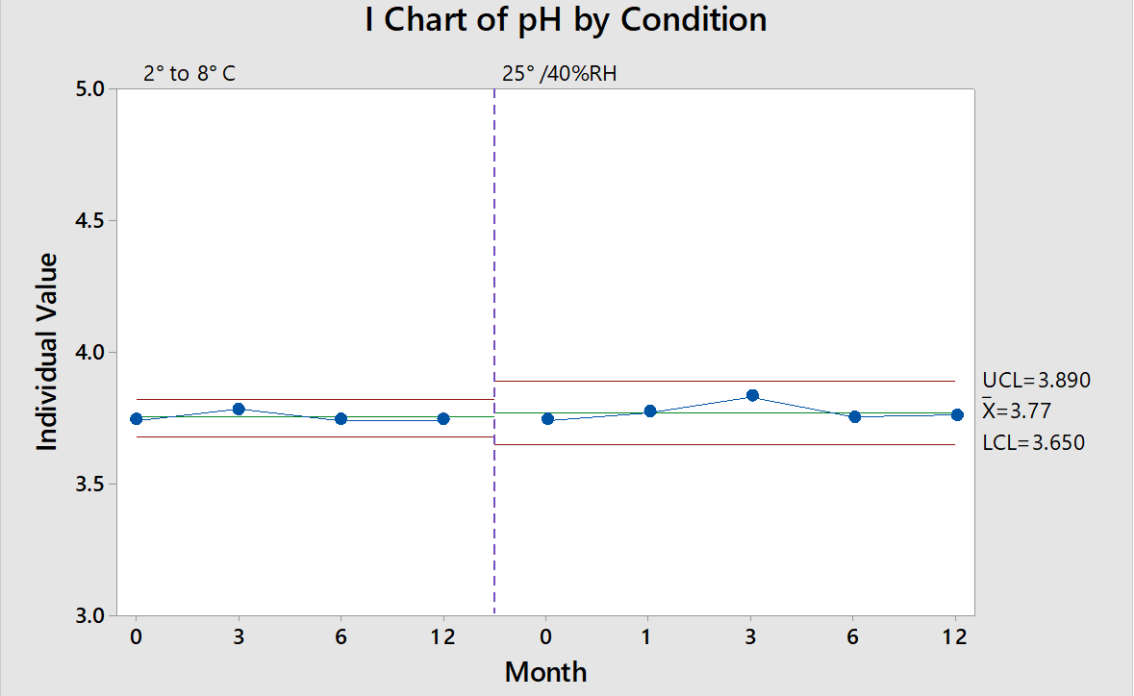

Figure 4.23: pH of vasopressin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

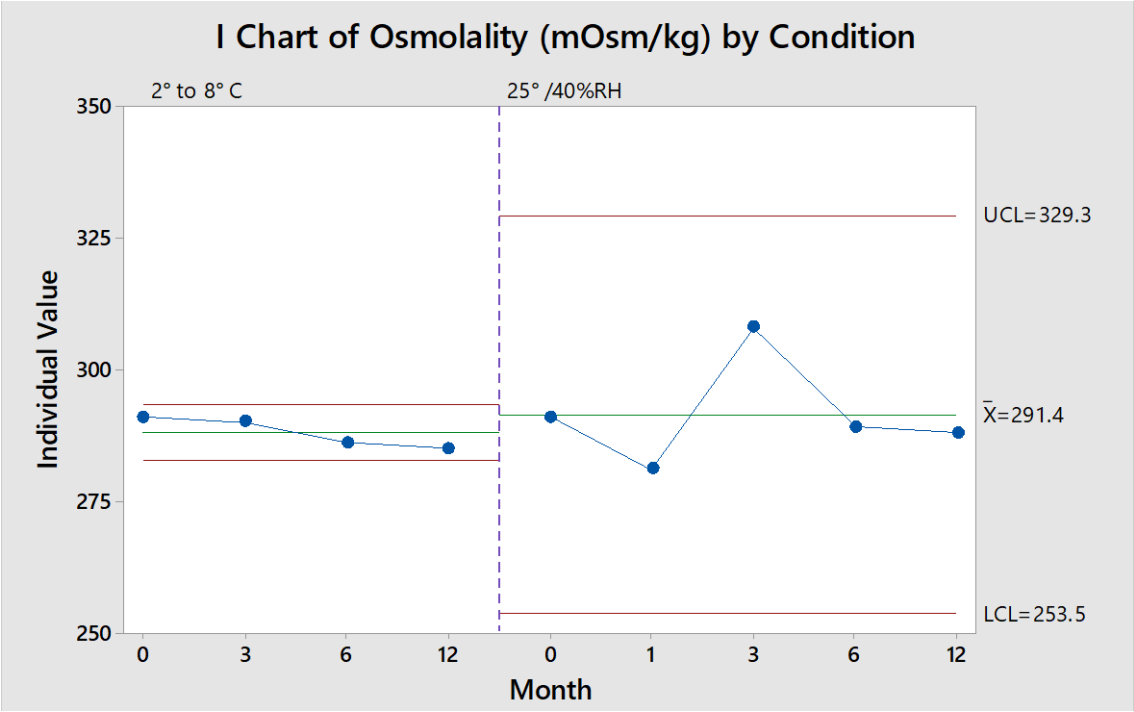

Figure 4.24: Osmolarity of vasopressin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

Table 4.25: Stability of formulations in infusion bags at initial stage and after 12M at different temperature conditions (Batch 1)

Condition	Time	Assay (%)	pH	Osmolality (mOsm/kg)	PMT		% Transmittance at 650 nm	Absorbance at 420 nm	Sterility
					≥10µm	≥25µm			
Specification		90-110	3-5	250-350	6000	600	>95	<1%	No evidence of microbial growth should be found
Initial	0	99.54±0.69	3.74±0.06	291±3.12	93.33±8.53	4.00±0.5	99.889±0.56	0.01±0.005	Complies
2° to 8° C	3M	99.03±0.45	3.78±0.03	290±5.00	23.3±6.48	13±0.76	99.912±0.23	0.0	Complies
2° to 8° C	6M	98.44±0.89	3.71±0.04	286±5.32	12.00±3.95	20.00±0.56	99.768±0.45	0.012±0.008	Complies
2° to 8° C	12M	97.40±0.1.02	3.76±0.05	285±4.16	67.73±7.66	07±0.33	99.961±0.87	0.0	Complies
25° /40%RH	1M	98.32±0.76	3.77±0.02	281±6.23	20..67±4.96	6.67±0.25	98.889±0.49	0.0	Complies
25° /40%RH	3M	96.43±0.59	3.77±0.06	308±2.11	22.0±8.46	0.0	99.673±0.29	0.023±0.004	Complies
25° /40%RH	6M	95.79±1.11	3.83±0.05	289±3.45	33.33±7.23	0.0	99.961±0.46	0.01±0.002	Complies
25° /40%RH	12M	93.64±1.04	3.75±0.04	288±3.89	67..67±4.56	0.67±0.05	98.889±0.78	0.0	Complies

Note:

- Each mL contains: Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 3.7), WFI q.s.
- Extrapolation and stability study report generated for 25°C/40%RH
- Container content @ initial, 3M, 6M & 12 M & 12M done and found NLT 100 mL

Table 4.26: Stability of formulations in infusion bags at initial stage and after 12M at different temperature conditions (Batch 2)

Condition	Time	Assay (%)	pH	Osmolality (mOsm/kg)	PMT		% Transmittance at 650 nm	Absorbance at 420 nm	Sterility
					≥10µm	≥25µm			
Specification		90-110	3-5	250-350	6000	600	>95	<1%	No evidence of microbial growth should be found
Initial	0	99.91±0.86	3.73±0.05	291±4.89	93.33±8.56	4.00±0.13	100±0.56	0.01±0.004	Complies
2° to 8° C	3M	98.25±0.56	3.71±0.04	290±4.63	23.3±4.99	13±1.24	99.9±0.78	0.0	Complies
2° to 8° C	6M	97.52±1.02	3.75±0.04	286±5.00	12.00±2.5	20.00±1.55	100±0.45	0.012±0.003	Complies
2° to 8° C	12M	96.17±1.05	3.76±0.05	285±3.89	67.73±4.66	07±0.89	99.9±0.44	0.0	Complies
25° /40%RH	1M	99.20±0.86	3.79±0.06	281±7.56	20.67±3.12	6.67±0.53	100±0.56	0.0	Complies
25° /40%RH	3M	97.67±0.88	3.78±0.02	308±2.69	22.0±3.45	0.0	99.9±0.33	0.023±0.005	Complies
25° /40%RH	6M	96.52±1.13	3.71±0.03	289±3.55	33.33±3.89	0.0	100±0.52	0.01±0.001	Complies
25° /40%RH	12M	94.50±1.25	3.72±0.07	288±4.13	67..67±4.26	0.67±0.08	99.9±0.89	0.0±0.001	Complies

Note:

- Each mL contains: Vasopressin USP 1.887 µg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 3.7), WFI q.s.
- Extrapolation and stability study report generated for 25°C/40%RH
- Container content @ initial, 3M, 6M & 12 M & 12M done and found NLT 100 mL

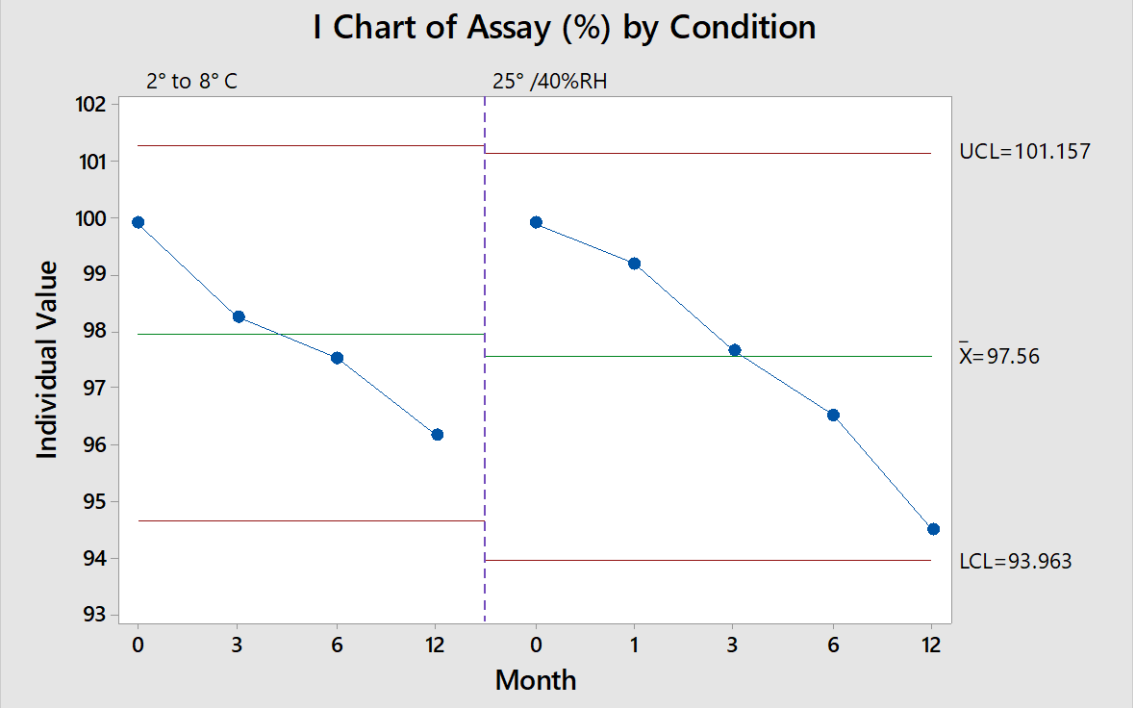

Figure 4.25: Assay of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit

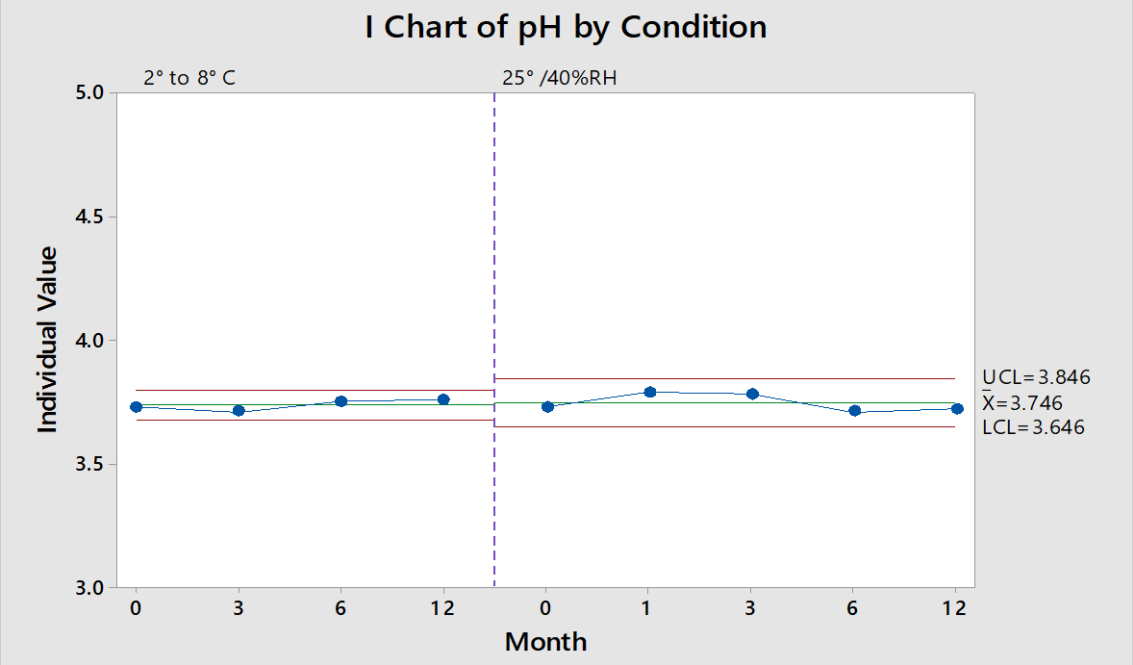

Figure 4.26: pH of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit

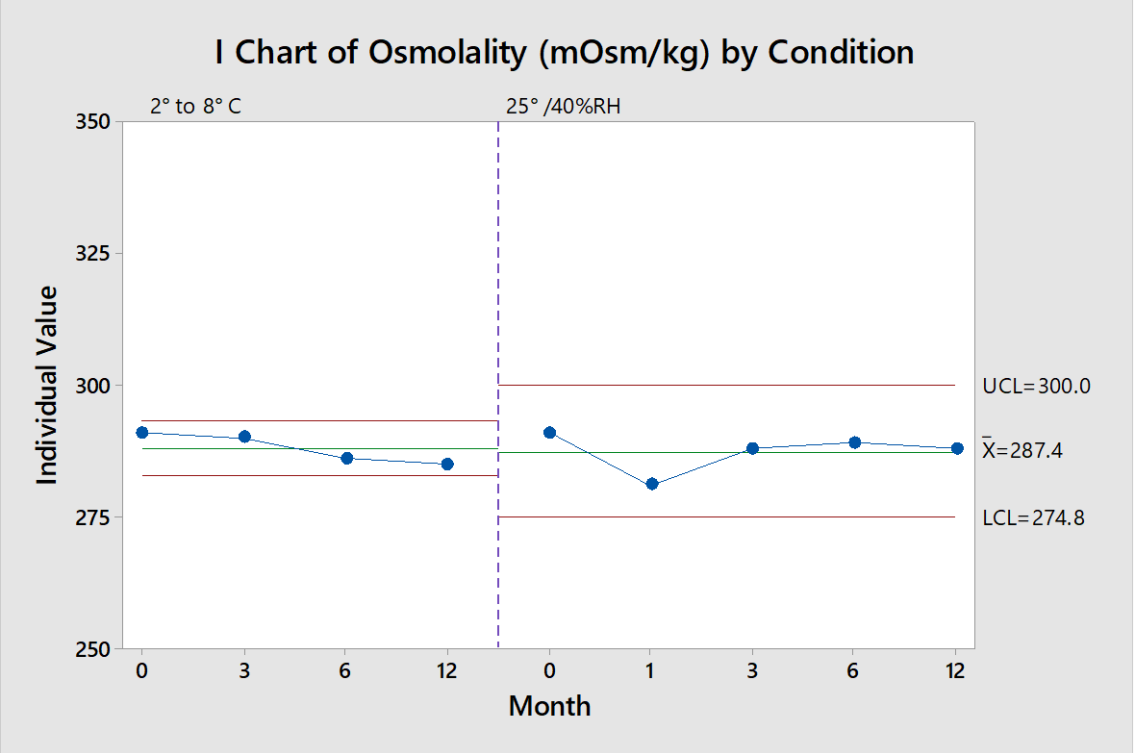

Figure 4.27: Osmolality of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

4.3.2.7. Stability Study Report at 5±3°C & 25°C/40%RH for final formulation as per ICH Q1E

The stability study of vasopressin RTI formulation 1.887 µg/mL (1 IU/mL) was done at 5±3 °C and 25°C/40%RH. A risk assessment was performed in order to identify the CQAs of drug product. Drug product quality attributes were assessed for likely impact on product safety and efficacy. Variation in drug product quality attributes like Assay of vasopressin, pH and Osmolality can impact product safety and efficacy and hence, were categorized as "CQAs" and were monitored in development batches. The stability data was processed with statistical tool (Minitab, version 21) to find out appropriate statistical model and to extrapolate shelf-life of drug product while considering all major CQAs of formulation. The vasopressin showed non-significant change at 5±3°C, while it significantly decreased at 25°C/40%RH. The summary of shelf life is tabulated in Table 4.27 and 4.28. The graphs are portrayed in Figure 4.28 to 4.41 and calculations are represented in Annexure 1. CQAs of formulations are same as discussed in case of Oxytocin.

Stability Study: Assay vs Months at 25 °C and 40 % RH

- **Stability Study: Assay (%) versus Month, Batch**

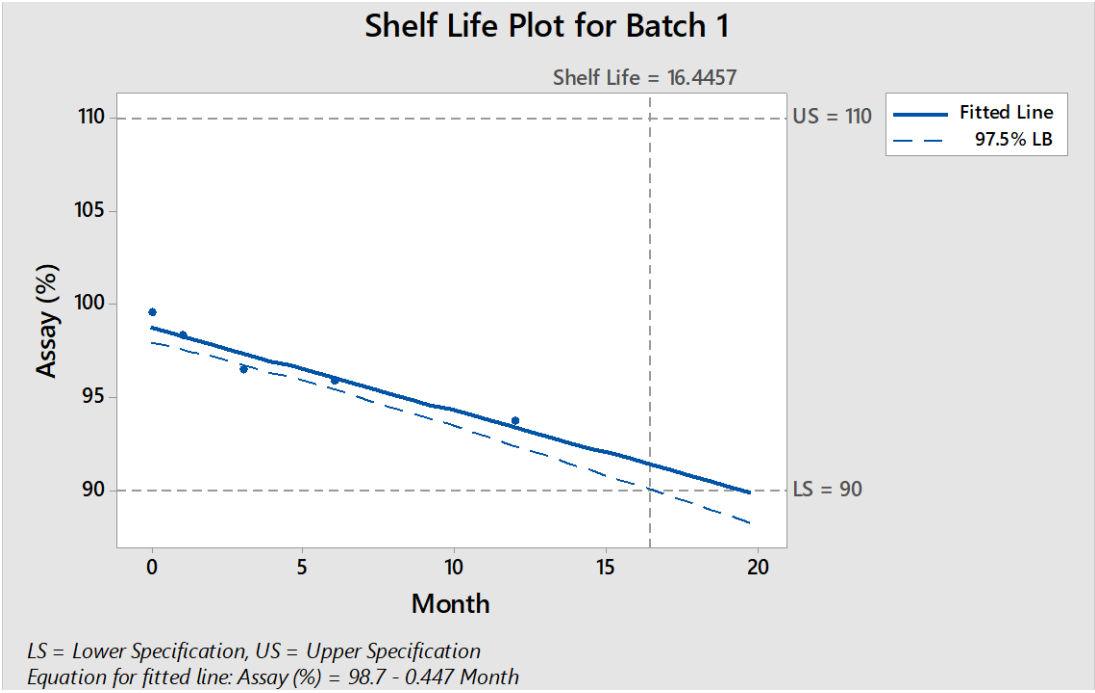

Figure 4.28: Shelf-Life Plot for Batch 1

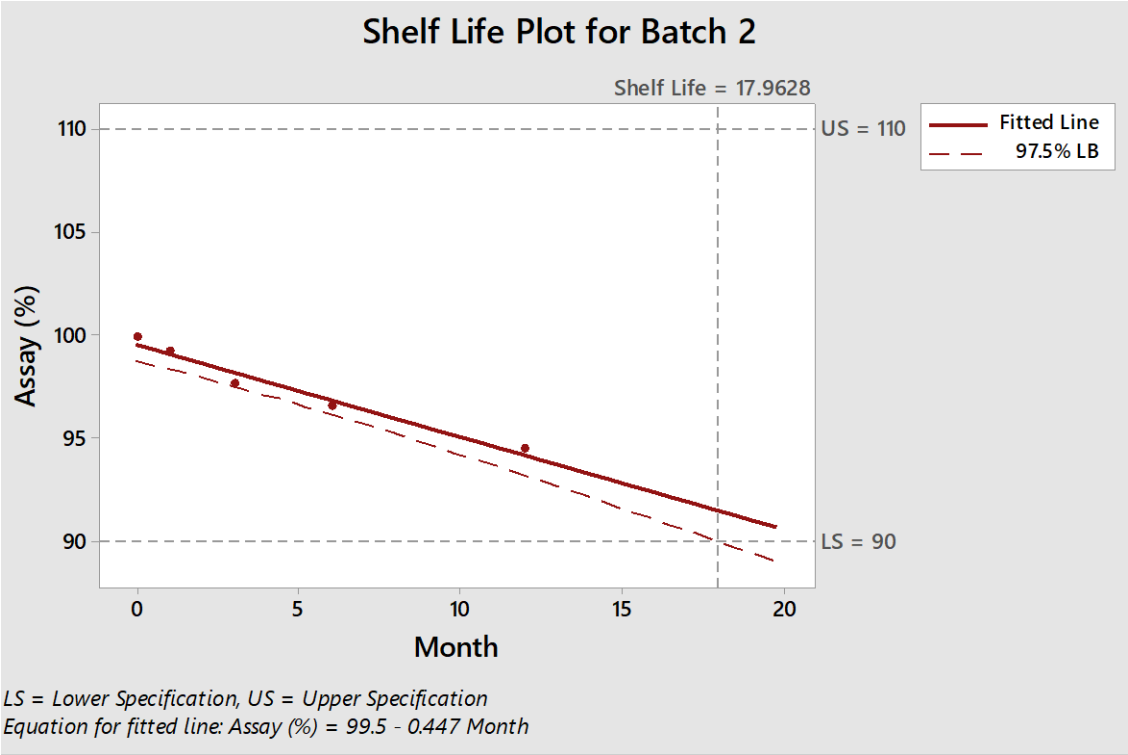

Figure 4.29: Shelf-Life Plot for Batch 2

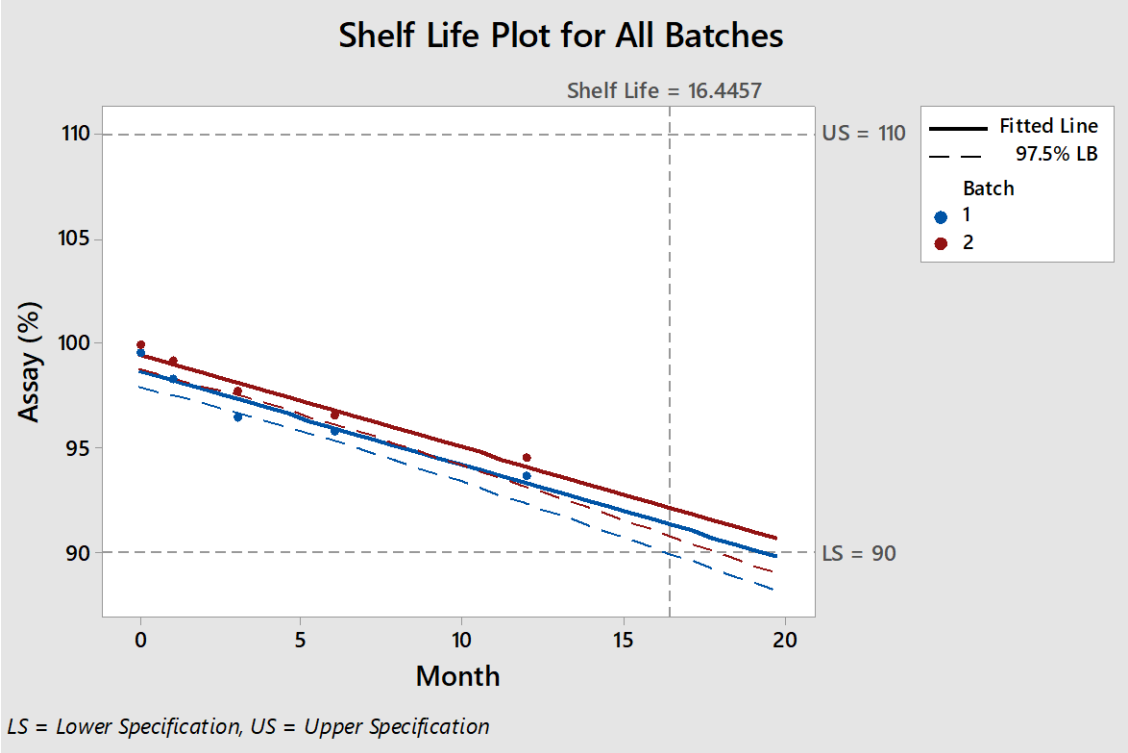

Figure 4.30: Shelf-Life Plot for Batch 1 & 2

- **Stability Study: pH versus Month, Batch**

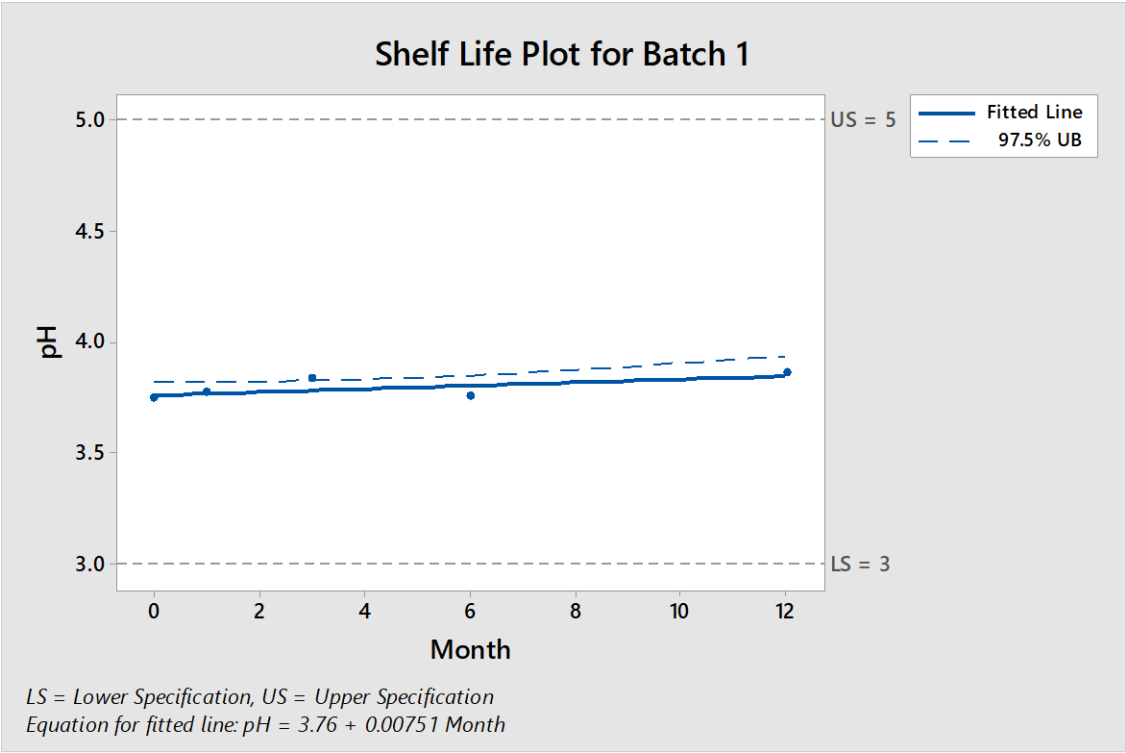

Figure 4.31: Shelf-Life Plot for Batch 1

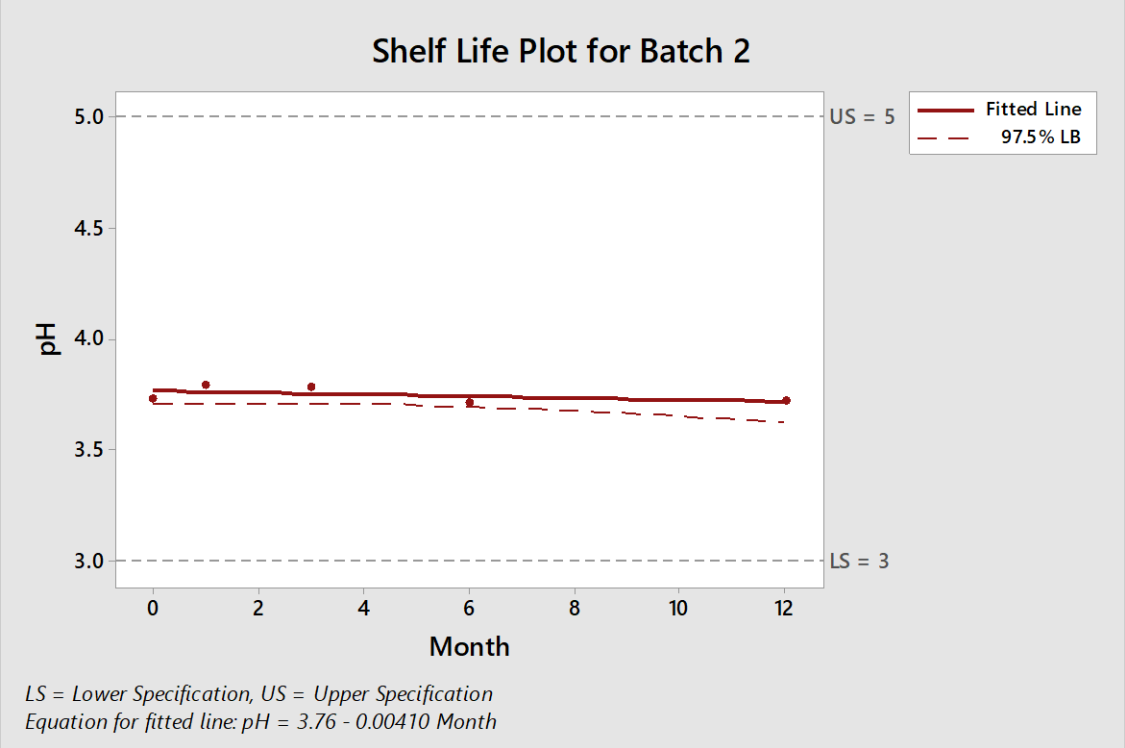

Figure 4.32: Shelf-Life Plot for Batch 2

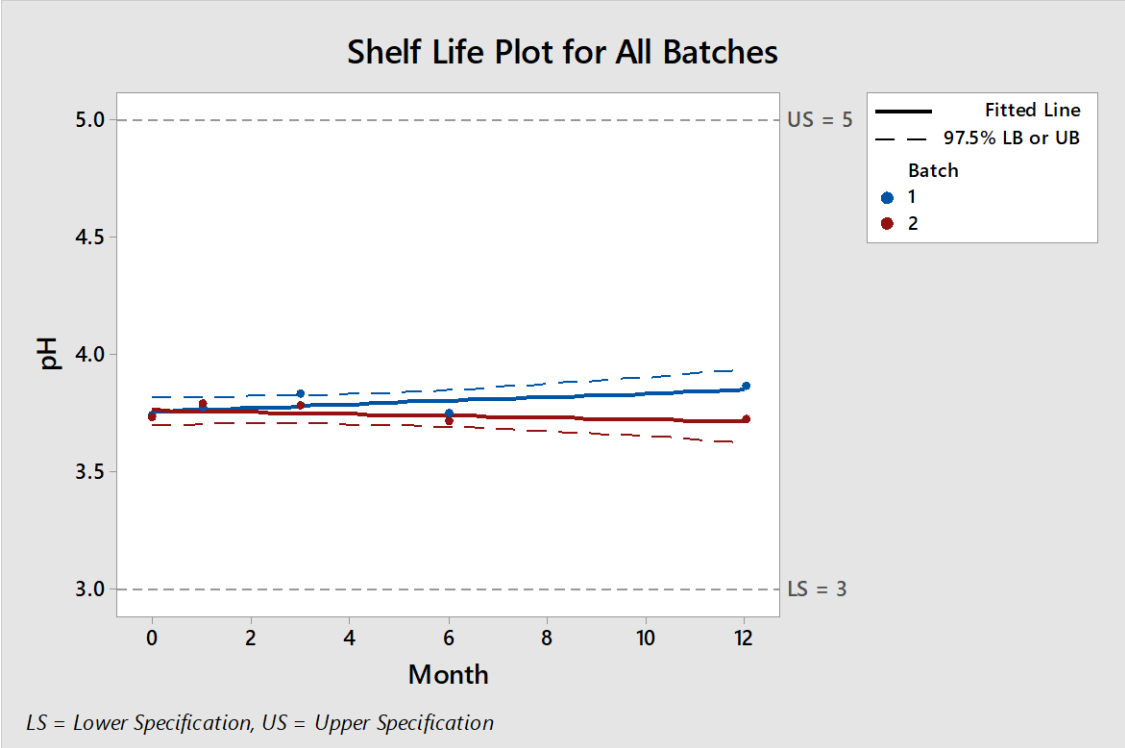

Figure 4.33: Shelf-Life Plot for Batch 1 & 2

- **Stability Study: Osmolality (mOsm/kg) versus Month, Batch**

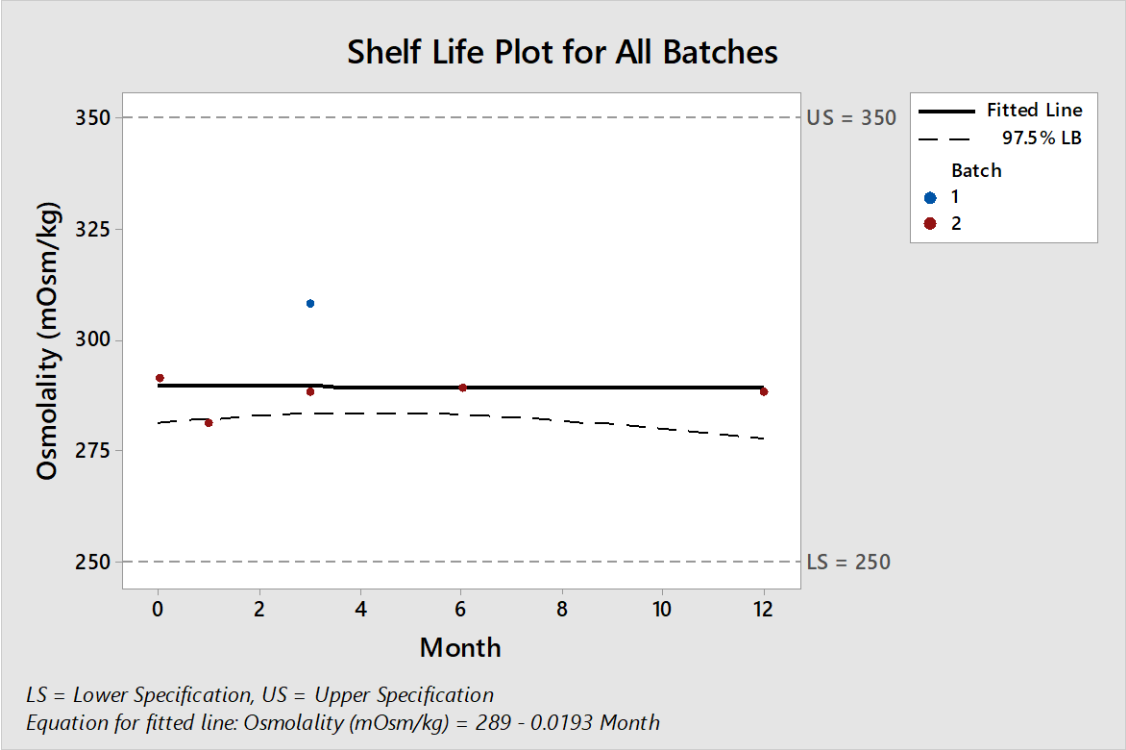

Figure 4.34: Shelf-Life Plot for All Batches

Stability Study Report of Vasopressin RTI at 5 ± 3 °C

- **Stability Study: Assay (%) versus Month, Batch**

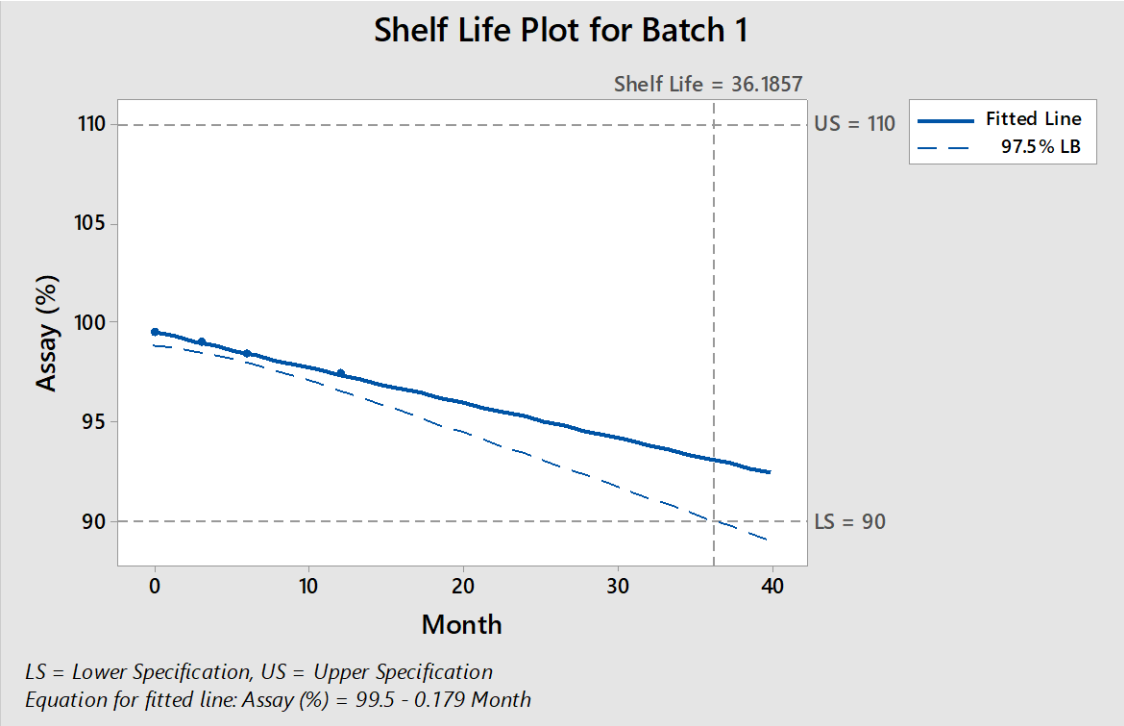

Figure 4.35: Shelf-Life Plot for Batch 1

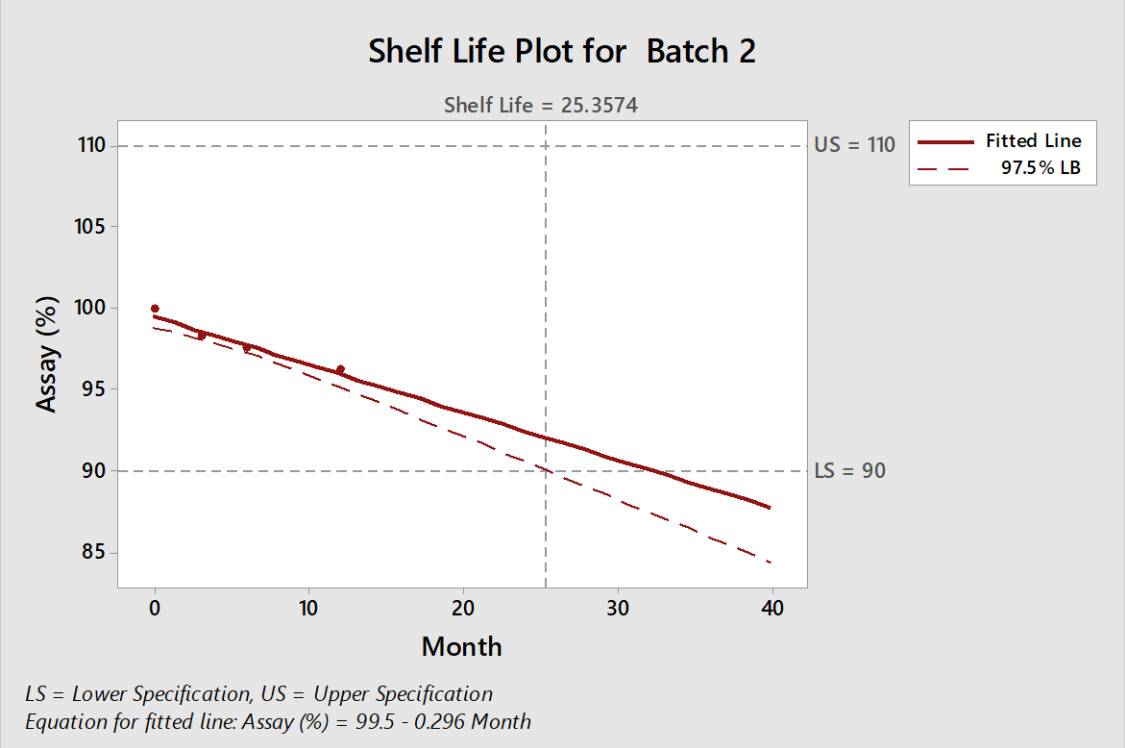

Figure 4.36: Shelf-Life Plot Batch 2

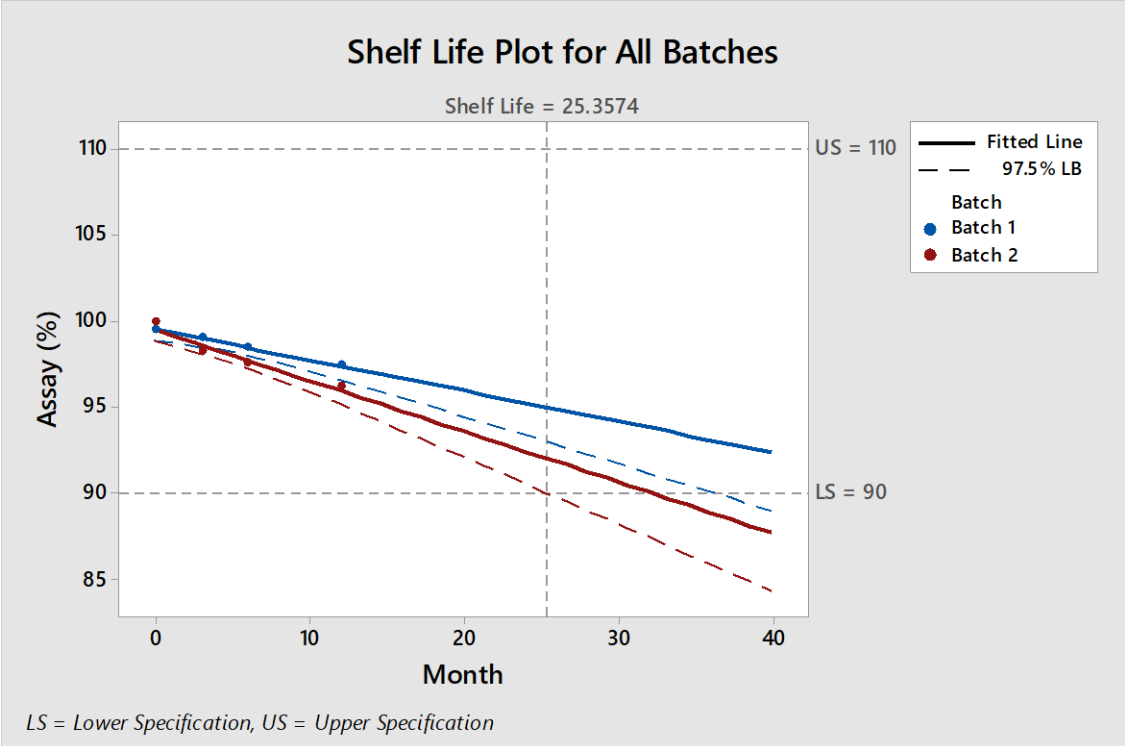

Figure 4.37: Shelf-Life Plot for All Batches

- **Stability Study: pH versus Month, Batch.**

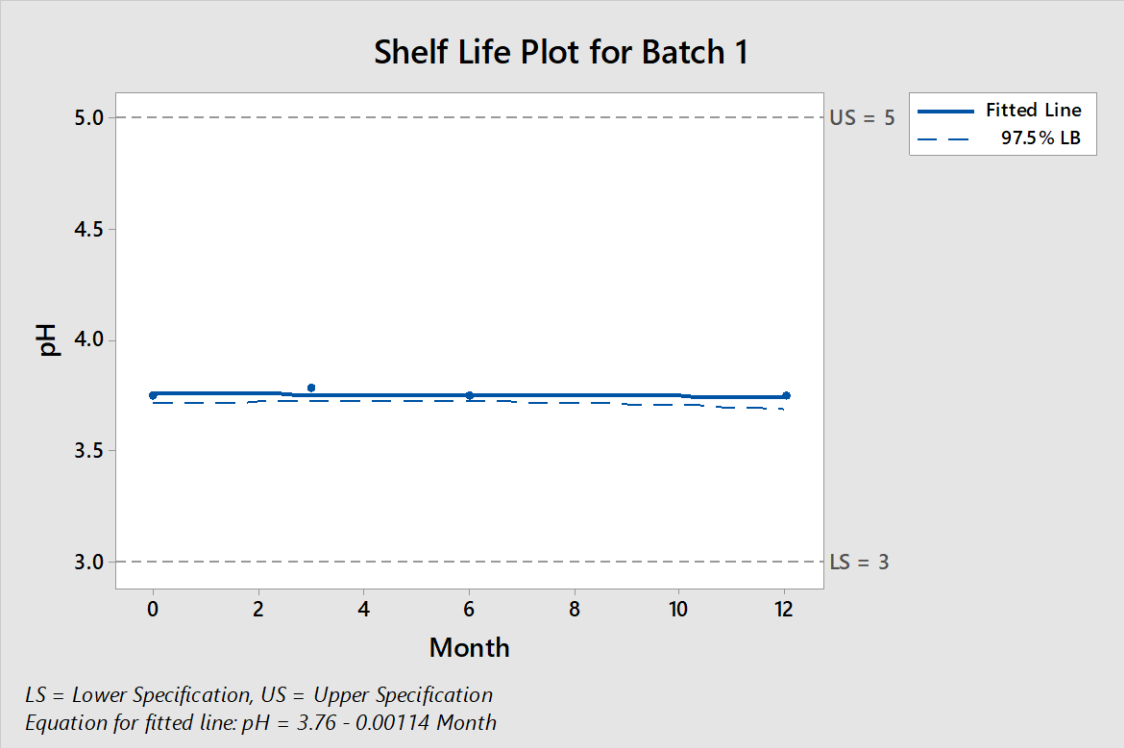

Figure 4.38: Shelf-Life Plot for Batch 1

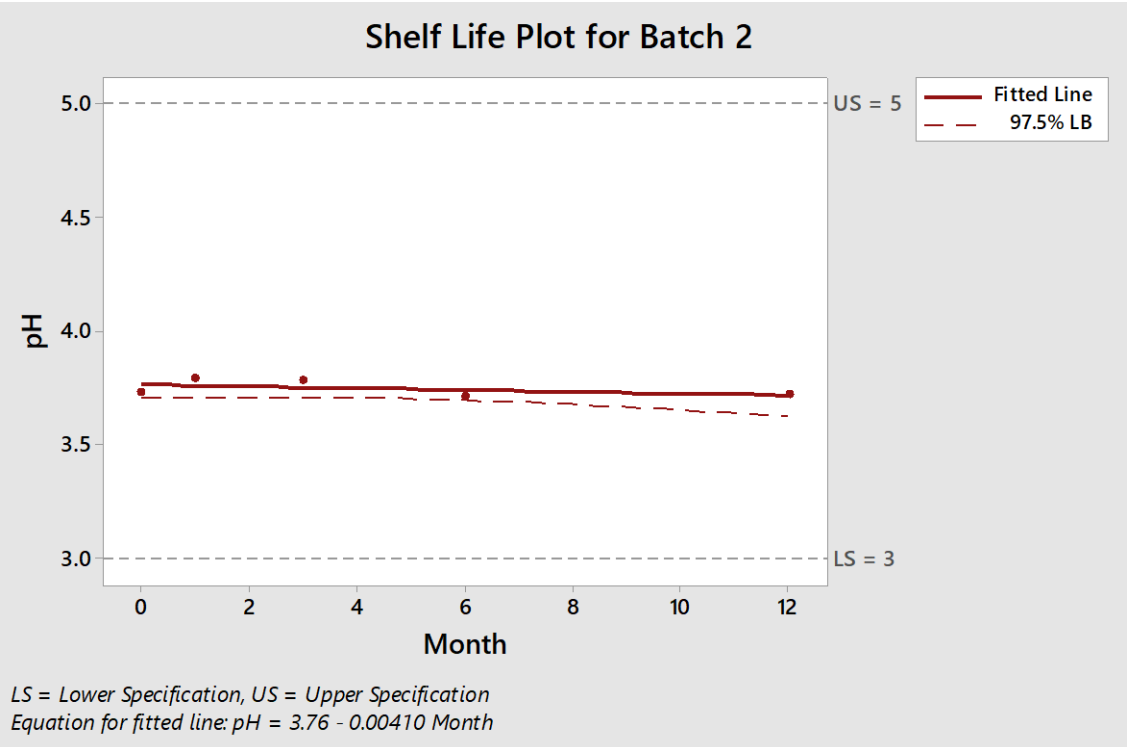

Figure 4.39: Shelf-Life Plot for Batch 2

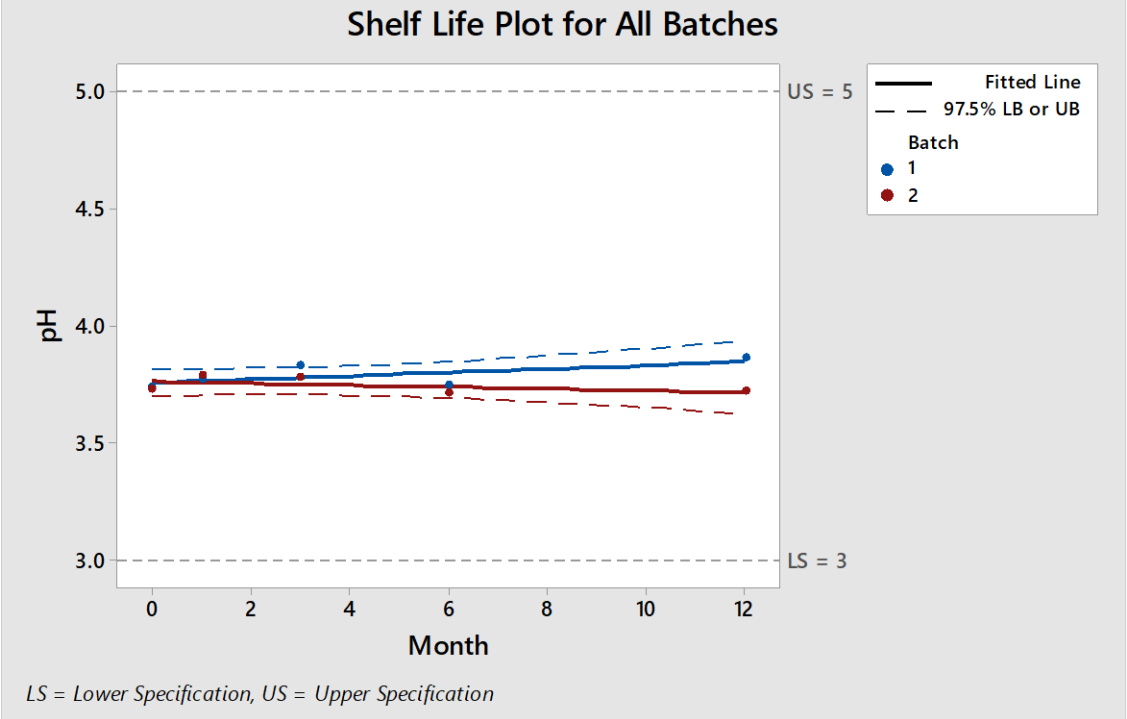

Figure 4.40: Shelf-Life Plot for All Batches

- **Stability Study: Osmolality (mOsm/kg) versus Month, Batch**

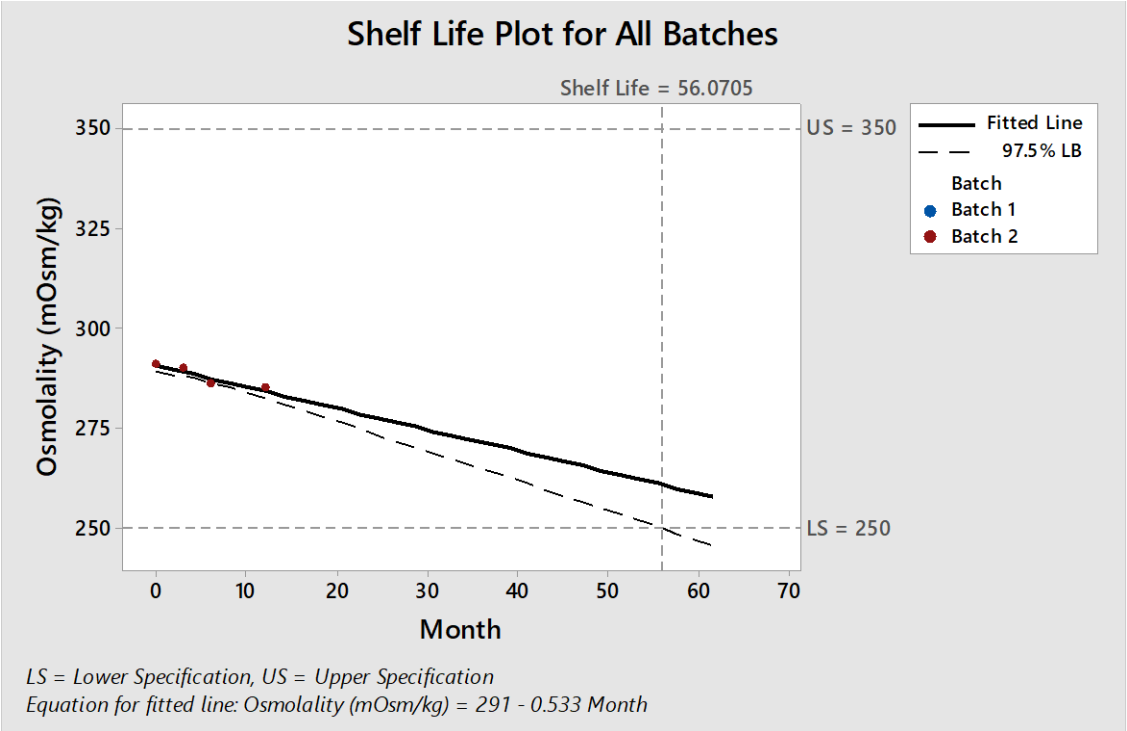

Figure 4.41: Shelf-Life Plot for All Batch

Table 4.27: Shelf-life estimation of developmental batches of Vasopressin RTI @ 25°C/40%RH

CQAs	Proposed shelf-life specification	Result			Inference			Selected model	Shelf life indicating?
		Time, p-value	Strength & Time interaction, p value	Intercept, p value	Effect of Time	Slope (Batch & Time interaction) Poolability	Intercept Poolability		
Assay	90-110	0.000	0.880	0.062	<0.05; Significant	>0.25 Poolable	>0.25 Not Poolable	**Batch** / **Intercept** / **Slope** / **Shelf life** 1 / 98.711 / - 0.447 / 16.446 2 / 99.527 / - 0.447 / 17.96 **Over all** / 16.446	Yes
Osmolality (mOsm/kg)	250-350	0.977	0.821	0.46	>0.05; Not Significant	>0.25 Poolable	>0.25 Poolable	**Batch** / **Intercept** / **Slope** / **Shelf life** 1 / 289.48 / -0.01 / *The mean response slope is not significantly larger than zero 2 / 289.48 / -0.01 Over all	NO
pH	3.0-5.0	0.580	0.093	0.131	>0.05; non-Significant	>0.25 Poolable	<0.25 Not Poolable	**Batch** / **Intercept** / **Slope** / **Shelf life** 1 / 3.757 / 0.00751 / * The mean response slope is not significantly larger than zero 2 / 3.76 / -0.00410 Over all	No

Table 4.28: Shelf-life estimation of developmental batches of Vasopressin RTI @5 ± 3°C

CQAs	Proposed shelf-life specification	Result			Inference			Selected model	Shelf life indicating?
		Time, p-value	Strength & Time	Intercept, p value	Effect of Time	Slope (Batch & Time interaction) Poolability	Intercept Poolability		
Assay	90-110	0.001	0.079	0.040	<0.05; Significant	<0.25 Not-Poolable	<0.25 Not Poolable	**Strength** / **Intercept** / **Slope** / **Shelf life** 1 / 99.544 / -0.1793 / 36.186 2 / 99.516 / - 0.2959 / 25.357 **Over all** / 25.357	Yes
Osmolality (mOsm/kg)	250-350	0.008	1.0	1.0	<0.05; Significant	>0.25 Poolable	>0.25 Poolable	**Strength** / **Intercept** / **Slope** / **Shelf life** 1 / 290.8 / -0.533 / 56 2 / 290.8 / -0.533 Over all	Yes
pH	3.0-5.0	0.541	0.244	0.439	>0.05 Non-Significant	<0.25 Not Poolable	>0.25 Poolable	**Strength** / **Intercept** / **Slope** / **Shelf life** 1 / 3.75 / -0.00114 / * The mean response slope is not significantly larger than zero 2 / 3.72 / 0.00333 Over all	No

4.3.2.8. Stability Report at 5 ± 3°C & 25°C/40%RH

The extrapolation and stability report were generated as per ICH Q1E, and assay was found shelf life determining or indicating as there was statistically significant change happened during stability in both storage conditions, which is critical CQA to determine over all shelf life. Shelf life of other CQAs like Osmolality (mOsm/kg) and pH were found to be more compared to assay in both storage condition. But, based on the stability data prediction as per ICH Q1E, overall shelf life was found to be 16.5 months and 25.357 months for 25°C/40%RH and 5±3°C respectively. The more shelf life of Vasopressin RTI is at 5±3°C. Thus, it can be concluded that even in the worst-case scenario the stability of vasopressin RTI formulation in sodium chloride injection is expected to be more than 12M at 25°C/40%RH and 36M at 5±3°C for proposed specification. Peptides are available in small volume and stored at 5 ± 3°C, but our developed formulation is large volume, so we recommend 25°C/40%RH storage condition for better feasibility and easily transportation.

2.3.3 Development and Optimization of Ready to Infuse formulation of Angiotensin-II

4.3.3.1 Formulation development

In view of knowledge gathered during formulation development of Oxytocin RTI, and Vasopressin RTI and considering effect of concentration of different osmogens on osmolarity of formulation, various developmental trials were taken in order to stabilize Angiotensin-II in large volume aqueous formulations. For this purpose, osmogens such as Sodium chloride, mannitol and Dextrose were evaluated. The details of different developmental trials are presented in Table 4.29.

Table 4.29: Development trials with different osmogens for angiotensin RTI formulation

Formulation Code	Compositions (Each mL contains)	Packaging Description	Stage	Assay (%)	pH	Osmolarity
FB-1	Angiotensin-II USP 0.01 mg, Citric acid 0.1 mg, Sodium Citrate Dihydrate 0.242 mg, Sodium Chloride 9 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	PHC	Initial	91.39±1.08	5.45±0.14	298±2.65
		AOB	Initial	90.13±1.98	5.46±0.11	297±2.53

Formulation Code	Compositions (Each mL contains)	Packaging Description	Stage	Assay (%)	pH	Osmolarity
FB-2	Angiotensin-II USP 0.01 mg, Citric acid 0.1 mg, Sodium Citrate Dihydrate 0.242 mg, Mannitol 50 mg, HCl/NaOH q.s. to 5.5, WFI q.s.	PHC	Initial	81.98±1.85	5.51±0.12	295±2.18
		AOB	Initial	83.47±1.75	5.50±0.11	294±2.38
FB-3	Angiotensin-II USP 0.01 mg, Sod Acetate 1 mg, Dextrose 50 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	PHC	Initial	88.74±1.86	5.56±0.13	291±2.83
		AOB	Initial	89.74±1.71	5.62±0.12	288±2.15
FB-4	Angiotensin-II USP 0.01 mg, Sod Acetate 1 mg, Sucrose 50 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	PHC	Initial	90.41±1.52	5.49±0.07	298±2.74
		AOB	Initial	89.15±1.84	5.47±0.09	297±2.72
FB-5	Angiotensin-II USP 0.01 mg, Sod Acetate 1 mg, Sucrose 50 mg, Glycine 1 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	PHC	Initial	87.09±1.86	5.54±0.13	289±2.54
		AOB	Initial	89.32±1.15	5.55±0.12	292±1.73
FB-6	Angiotensin-II USP 0.01 mg, Sod Acetate 1 mg, Trehalose 50 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	PHC	Initial	78.54±1.92	5.49±0.11	293±2.13
		AOB	Initial	77.92±1.71	5.52±0.07	294±2.98
FB-7	Angiotensin-II USP 0.01 mg, Sodium Chloride 9 mg, Mannitol 1 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	PHC	Initial	100.12±1.08	5.44±0.09	309±2.76
		AOB	Initial	100.67±1.05	5.45±0.08	310±2.18
Note: All batches Drug Product containers passed the leak test						

All formulations were prepared with various osmogens and buffers, and filled in two different packaging materials (PHC & AOB). The assay of formulation prepared by mannitol in combination of sodium chloride in FB-7 was found to be 100% and within proposed specification (90-110%). While, in formulations prepared with other excipients, assay was not found to satisfactory. Therefore, we decided FB-7 is optimum formulation for further study.

4.3.3.3 pH Stability Study

For pH stability study, batches were formulated at pH 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5, and kept for 3M at 25°C/40%RH. The obtained results are tabulated in Table 4.30. The decreasing assay for all pH formulations was observed after 3M, while other CQAs i.e., pH, osmolality, PMT, % of transmittance & absorbance was well in controlled. The assay difference after 3M for formulation with pH 5.5 was least compared to others. Therefore, we decided to keep pH 5.5 in our formulation for further studies.

4.3.3.4 Sterilization method selection for Angiotensin-II RTI

In order to evaluate a suitable sterilization method, Angiotensin-II RTI formulation was sterilized at different recommended sterilization conditions and analysed thereafter (Table 4.31). The details are as mentioned in section 4.3.1.6.

In all sterilization conditions mentioned above (See section 4.3.1.6), the assay of Angiotensin-II in RTI formulation was found reduced. There was no effect on pH and osmolarity of formulation. Therefore, the process comprising sterile filtration, pre-sterilized container and aseptic processing was found suitable for further use.

4.3.3.5 Photostability study for Angiotensin-II RTI

To evaluate the photosensitivity of Angiotensin-II RTI, the samples (in infusion bag, in final pack and a control sample) were placed in the photostability chamber and exposed to light of 20 million lux. After exposure, the samples were withdrawn and analysed. The outcomes of photostability study are presented in Table 4.32. The assay of Angiotensin-II in RTI formulation was found slightly reduced when it was exposed to light. There was no effect on pH and osmolarity of formulation. Therefore, utmost care should be taken to manufacturing, handling and packaging of formulation of Angiotensin-II.

Table 4.30: pH study data of experimental trials (Angiotensin II)

Formulation Code	Target pH	Stage	Assay (%)	pH (Observed)	Osmolarity (mOsm/kg)	PMT		% Transmittance at 650 nm	% Absorbance at 420 nm
						≥10µm	≥25µm		
Specification			90-110		250-350	6000	600	>95	<1
FD-1	4.0	Initial	99.52±1.52	4.02±0.07	285±2.36	1.3.79±1.12	13.66±1.02	99.365±0.09	0.038±0.005
	4.0	25°C/40%RH-3M	87.02±0.78	4.07±0.09	286±2.06	87.12±2.19	6.77±0.61	99.448±0.12	0.036±0.006
FD-2	4.5	Initial	99.75±1.32	4.52±0.07	287±2.38	20.33±2.36	11.65±0.56	99.532±0.19	0.073±0.004
	4.5	25°C/40%RH-3M	92.45±1.28	4.56±0.09	284±2.36	10.13±1.82	13.24±1.65	99.325±0.25	0.064±0.005
FD-3	5.0	Initial	100.12±1.64	5.02±0.06	283±2.43	66.67±1.65	19.78±1.42	99.124±0.24	0.061±0.007
	5.0	25°C/40%RH-3M	94.18±1.72	5.07±0.05	282±2.27	60.48±2.32	18.37±1.28	99.605±0.31	0.076±0.003
FD-4	5.5	Initial	99.68±1.38	5.51±0.05	287±2.57	40.33±2.20	24.35±1.36	99.608±0.41	0.066±0.004
	5.5	25°C/40%RH-3M	96.52±1.62	5.55±0.04	281±2.28	30.38±2.64	34.52±1.32	98.652±0.48	0.049±0.008
FD-5	6.0	Initial	98.51±1.33	6.03±0.08	289±2.53	57.85±2.71	42.12±1.11	99.702±0.29	0.039±0.005
	6.0	25°C/40%RH-3M	92.89±1.48	6.08±0.07	281±2.34	65.23±1.89	38.68±0.98	99.571±0.28	0.023±0.006
FD-6	6.5	Initial	97.65±1.46	6.53±0.03	289±2.19	40.67±1.98	36.23±1.02	99.357±0.37	0.039±0.007
	6.5	25°C/40%RH-3M	89.77±1.43	6.58±0.08	281±2.69	57.89±2.12	24.77±1.01	99.319±0.44	0.035±0.006

Over the period of 3M at 25°C/40%RH, the assay value was decresed remarkably at all pH excep the formulation prepared at pH 5.5

Table 4.31: Effect of sterilization process on the Angiotensin-II RTI formulation

Compositions (Each mL Contains)	Sterilization parameter	Assay (%)	pH	Osmolarity
Angiotensin-II USP 0.01 mg, Sodium Chloride 9 mg, Mannitol 1 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	Initial (Un-autoclaved)	99.97±1.01	5.55±0.12	309±2.16
	15 Min_121°C	81.98±1.87	5.53±0.07	310±2.87
	F0 12 Min_121°C	89.56±1.67	5.54±0.08	309±2.95
	F0 08 Min_121°C	90.29±1.87	5.57±0.13	309±2.87
	F0 08 Min_116°C	82.97±1.43	5.56±0.12	310±2.76
	F0 08 Min_111°C	66.91±1.31	5.54±0.09	311±2.43

Table 4.32: Effect of light on Angiotensin-II RTI

Compositions	Sample description	Assay (%)	pH	Osmolarity
Angiotensin-II USP 0.01 mg/mL, Sodium Chloride 9 mg, Mannitol 1 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.	Controlled (Infusion bag wrapped in aluminium sheet)	99.97±1.01	5.55±0.09	309±2.21
	Market pack (Infusion bag placed inside the overwrap pouch)	100.04±1.12	5.57±0.08	310±2.03
	Direct exposed (infusion bag only)	97.12±1.43	5.53±0.04	308±2.97

4.3.3.6 Manufacturing process and Stability study for RTI formulation of Angiotensin-II

Manufacturing process followed for drug product manufacturing is given below:

1. WFI (Approx. 80% of batch size) was collected in SS vessel.
2. Temperature 5 ± 3ºC was achieved and maintained throughout process.
3. N_2 was purged to achieve DO <1ppm.
4. Mannitol was added and dissolved with continuous stirring and purging.
5. pH was adjusted to 5.5 using 0.1%w/v HCl and/or 0.1%w/v NaOH.
6. Purging was stopped. Angiotensin-II acetate was added and dissolved with continuous stirring.

7. Sodium chloride and mannitol was added and dissolved with continuous stirring and purging.
8. pH was again adjusted to 5.5 with 0.1% HCl and/or 0.1% NaOH, if needed.
9. Volume was made up to 100% with WFI.
10. Solution was purged to achieve DO< 1ppm.
11. Filtration was performed using 0.2 µ PES capsule filter and non-siliconized tubing.
12. Solution was filled in container and suitably stoppered.
13. Containers were then overwrapped in aluminium pouch with Nitrogen blanketing and oxygen scavenger.

Prepared optimized RTI formulation was tested initially and at stability time points. The analytical test results for RTI formulation are illustrated below (Table 4.33, Figure 4.42 to 4.44)

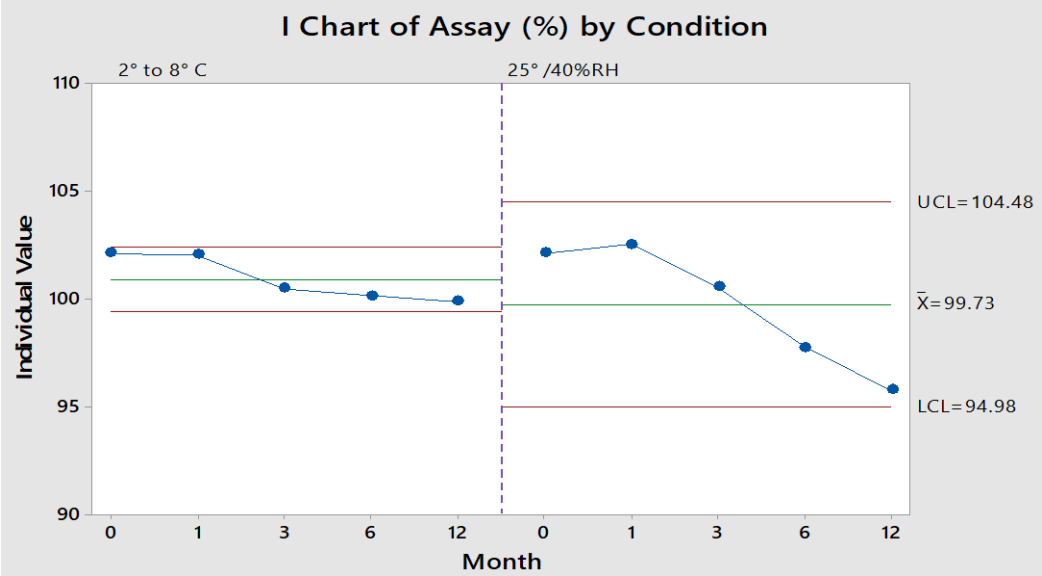

Figure 4.42: Assay of Angiotensin-II at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

The stability of the API in drug formulations is a significant concern, as it is a key requirement in the formulation development process. Therefore, a thorough stability testing plan was devised, where the manufactured RTI formulations were evaluated for storage stability at 5°C ±3°C and at 25°C/40% relative humidity, in accordance with ICH

guidelines. RTI formulations were filled in PHC bag (non-PVC infusion) and subsequently stoppered. Bags were then overwrapped in aluminium pouches followed by labelling. The packed formulations were subjected to storage stability in 5°C ±3°C and 25°C/40% RH stability chamber. At selected intervals, the samples were analysed for %drug assay, pH, osmolarity, particulate matters, %transmittance and absorbance at 420 nm. The analytical test results for RTI formulation are illustrated in Table 4.15, and Figure 4.42 to 4.46.

The percentage of assay changed from 102.12±1.867 to 99.66±1.06 after 12 months at 5±3 °C, while it was 95.76±1.04 after 12 months at 25°C/40%RH. Angiotensin exhibited no significant changes at 5±3 °C, whereas changes in assay were noted at 25°C/40%RH after 12 months.

Additionally, there were no noteworthy differences in the initial baseline measurements of osmolality, PMT, pH, percentage absorbance, percentage transmittance, and sterility when compared to the values recorded throughout the various stability conditions. Thus, the data gathered from the stability testing strongly indicated that the RTI formulations of angiotensin stored at the cooler temperature of 5±3 °C exhibited a significantly higher degree of stability over time.

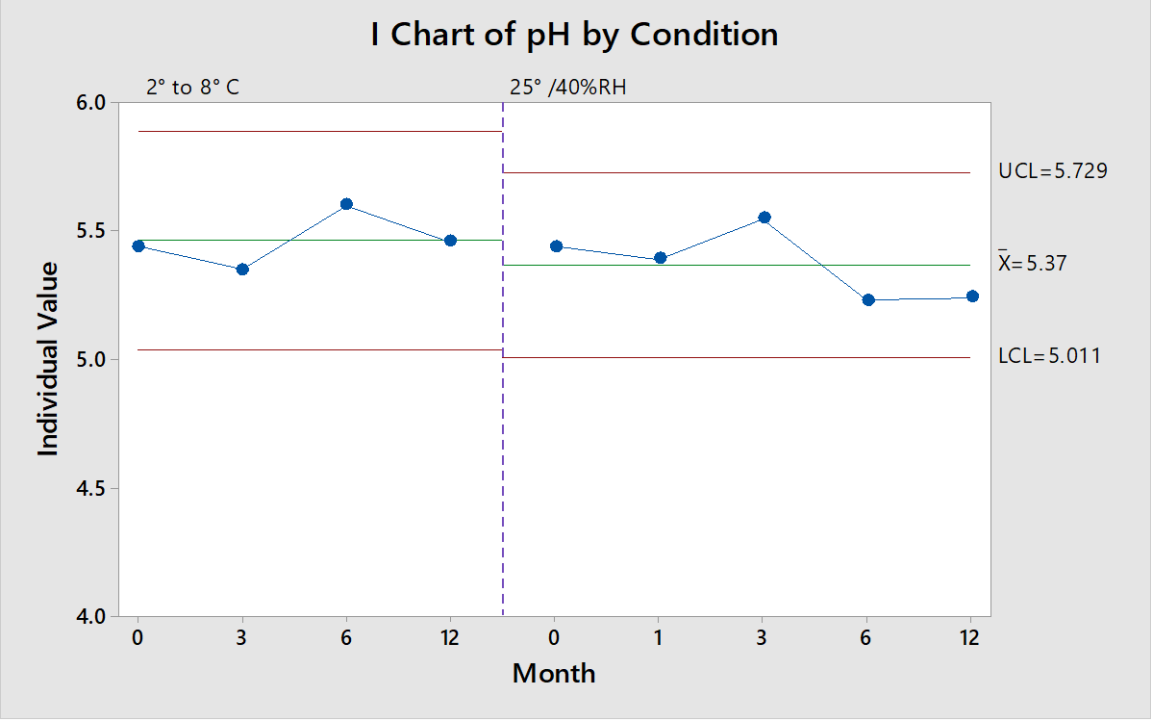

Figure 4.43: pH of Angiotensin-II formulation at different stability conditions and time points

Table 4.33: Stability of final formulation in infusion bags at initial stage and after 12M at different temperature conditions

Condition	Months	Assay (%)	pH	Osmolality (mOsm/kg)	PMT		% Transmittance at 650 nm	Absorbance at 420 nm	Sterility
					≥10µm	≥25µm			
Specification		90-110	4-6	250-350	6000	600	>95	<1%	No evidence of microbial growth should be found
Initial	0	102.12±1.87	5.44±0.09	285±2.08	20.20±0.98	0.67±0.01	99.951±0.02	0.00±0.00	Complies
5 ± 3 ºC	1	102.04±1.14	5.35±0.07	284±1.87	33.30±1.05	3.33±0.01	99.241±0.03	0.02±0.00	
5 ± 3 ºC	3	100.45±1.03	5.60±0.08	285±1.67	67.67±1.65	0.33±0.09	99.875±0.03	0.01±0.00	
5 ± 3 ºC	6	100.14±1.09	5.46±0.09	284±1.54	39.33±1.72	0.67±0.08	99.888±0.04	0.0±0.00	
5 ± 3 ºC	12	99.87±1.06	5.43±0.07	285±1.71	80.65±1.87	10.87±0.00	98.881±0.04	0.01±0.00	
25°C/60%RH	1	102.51±1.08	5.39±0.8	282±1.75	6.67±0.13	0.67±0.00	99.779±0.02	0.023±0.00	
25°C/60%RH	3	100.54±1.65	5.55±0.09	284±1.91	33.33±1.31	0.0±0.00	99.961±0.03	0.01±0.00	
25°C/40%RH	6	97.72±1.42	5.23±0.11	286±1.62	67..67±1.76	0.67±0.76	98.889±0.03	0.0±0.00	
25°C/40%RH	12	95.76±1.04	5.32±0.08	288±1.87	76.98±1.76	2.09±0.62	99.870±0.02	0.0±0.00	

Note:

- Each mL contains Angiotensin-II USP 0.01 mg/mL, Sodium Chloride 9 mg, Mannitol 1 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.
- Extrapolation and stability study report generated for 5 ± 3°C & 25°C/40%RH.
- Container content @ initial, 3M, 6M & 12 M & 12M done and found NLT 100mL

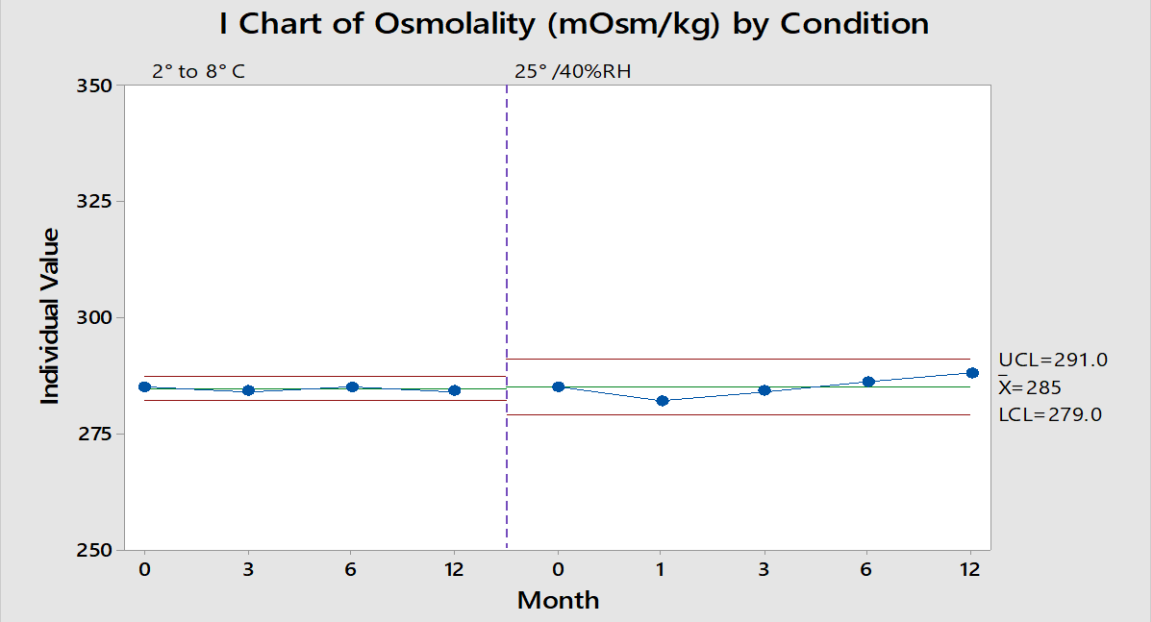

Figure 4.44: Osmolarity of Angiotensin-II formulation at different stability conditions and time points: Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

To validate the final composition, a duplicate batch with same composition was prepared and stability of second batch was initiated as performed for batch 1. The stability data of second batch of angiotensin II RTI formulation is tabulated in Table 4.34 and depicted in figure 4.45 – 4.47.

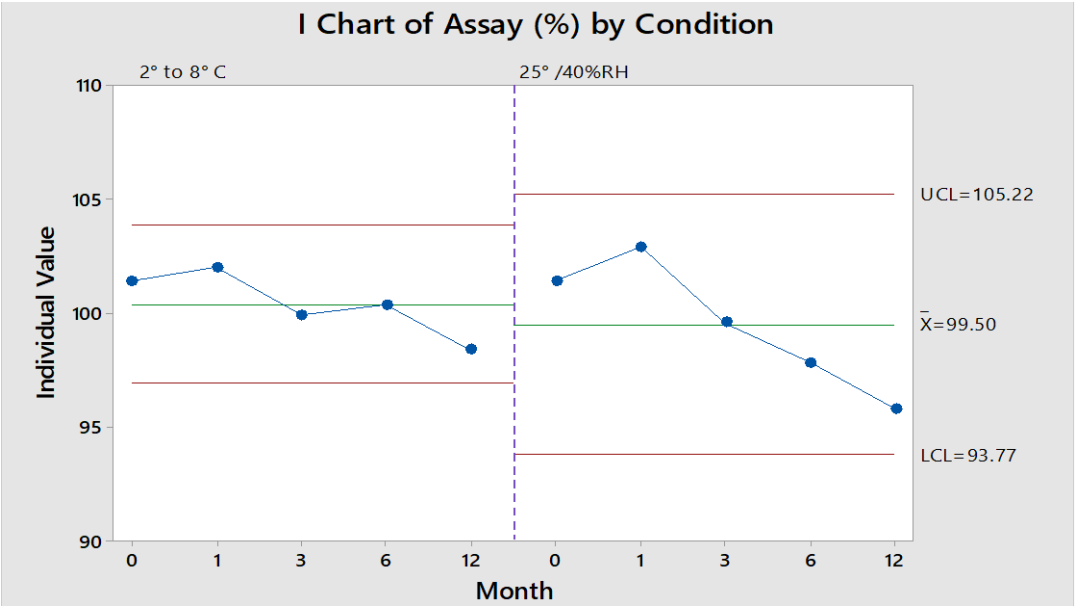

Figure 4.45: Assay of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

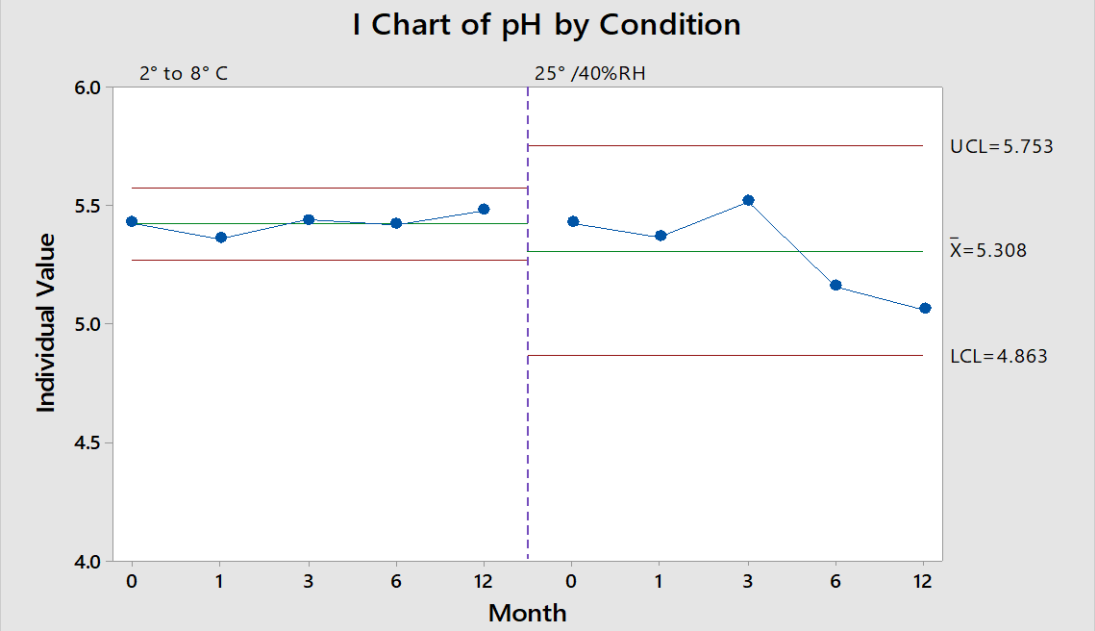

Figure 4.46: pH of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.

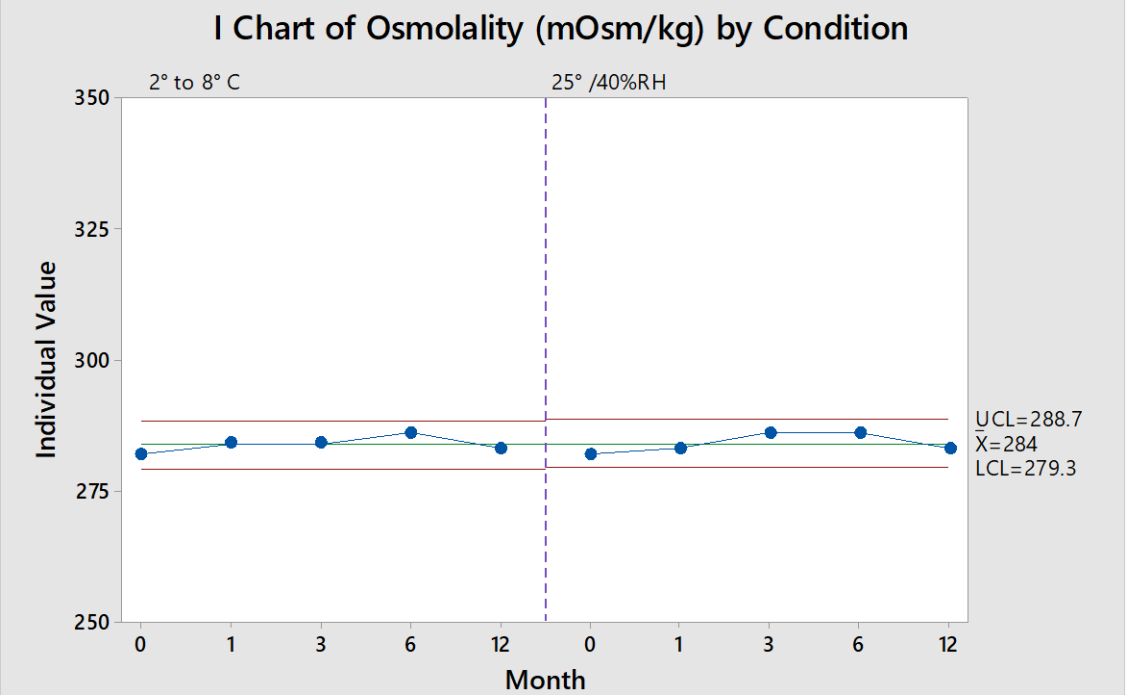

Figure 4.47: Osmolality of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL and LCL.

Table 4.34: Stability of final formulation in infusion bags at initial stage and after 12M at different temperature conditions (Batch 2)

Condition	M	Assay	pH	Osmolality	PMT		% Transmittance at 650 nm	Absorbance at 420 nm	Sterility
					≥10µm	≥25µm			
Specification		90-110	4-6	250-350	6000	600	>95	<1%	No evidence of microbial growth should be found
Initial	0	101.41±1.07	5.43±0.08	282±2.61	293.33±1.08	6.67±0.07	99.92±0.01	0.001±0.00	Complies
5 ± 3 ºC	1	102.01±1.32	5.36±0.09	284±1.89	213.33±1.34	46.67±1.07	99.83±0.02	0.001±0.00	
5 ± 3 ºC	3	99.89±1.15	5.44±0.07	284±2.76	563.33±1.65	6.67±0.09	99.96±0.03	0.001±0.00	
5 ± 3 ºC	6	100.37±1.87	5.42±0.05	286±2.15	80.65±1.08	40.76±1.87	99.78±0.02	0.003±0.00	
5 ± 3 ºC	12	98.37±1.96	5.48±0.07	283±2.76	80.4±1.87	40.87±1.69	99.78±0.04	0.003±0.00	
25°C/60%RH	1	102.91±1.34	5.37±0.06	283±2.17	993.33±1.54	93.33±0.54	99.94±0.02	0.001±0.00	
25°C/60%RH	3	99.56±1.35	5.52±0.08	286±2.64	336.67±1.76	31.67±0.074	99.73±0.03	0.002±0.00	
25°C/40%RH	6	97.8±1.56	5.16±0.06	286±2.87	186.67±1.83	46.67±0.97	99.87±0.04	0.003±0.00	
25°C/40%RH	12	95.8±1.71	5.06±0.09	283±1.93	176.67±1.54	36.67±0.87	99.07±0.02	0.003±0.00	

Note:

- Stability data for final formulation. Each mL contains Angiotensin-II USP 0.01 mg/mL, Sodium Chloride 9 mg, Mannitol 1 mg, HCl/NaOH q.s. to pH 5.5, WFI q.s.
- Extrapolation and stability study report generated for 5 ± 3°C & 25°C/40%RH.
- Container content @ initial, 3M, 6M & 12 M & 12M done and found NLT 100mL

The percentage of assay of batch 2 changed from 101.411±1.07 to 98.37±1.96 after 12 months at 5±3 °C, while it was 95.80±1.71 after 12 months at 25°C/40%RH. angiotensin exhibited no significant changes at 5±3 °C, whereas changes in assay were noted at 25°C/40%RH. Other tested parameters of RTI formulation remained nearly same from their initial values. Additionally, there were no noteworthy differences in the initial baseline measurements of pH, osmolality, PMT, percentage absorbance, percentage transmittance, and sterility when compared to the values recorded throughout the various stability conditions. Thus, the data gathered from the stability testing strongly indicated that the RTI formulations of oxytocin stored at the cooler temperature of 5±3 °C exhibited a significantly higher degree of stability over time.

Stability Study report at 5 ± 3°C & 25°C/40%RH for Final formulation as per ICH Q1E

The stability study of angiotensin RTI formulation 0.01 mg/mL was done at 5 ± 3 °C and 25°C/40%RH, as per ICH Q1E [19]. A risk assessment was performed in order to identify CQAs of drug product. Drug product quality attributes were assessed for likely impact on product safety and efficacy. Variation in drug product quality attributes like Assay of Angiotensin II, pH and Osmolality can impact product safety and efficacy and hence, were categorized as "CQAs" and were monitored in development batches. The stability data was processed with statistical tool (Minitab, version 21) to find out an appropriate statistical model and to extrapolate the shelf-life of drug product while considering all major CQAs of formulation. The Angiotensin II assay remained same during stability at 5 ± 3°C, while found significantly decreased at 25°C/40%RH. The extrapolation and stability study report were generated for both conditions i.e. 5±3°C & 25°C/40%RH. The summary of shelf life is tabulated in Table 4.35 & 4.36, graphs (Figures 4.48 to 4.55) and calculation are represented in Annexure 1. CQAs of Formulations are the same as discussed in the case of Oxytocin.

4.3.3.6.1 Stability Study: Assay vs Months at 25 °C and 40 % RH

4.3.3.6.2.1 Stability Study: Assay (%) versus Month, Batch No

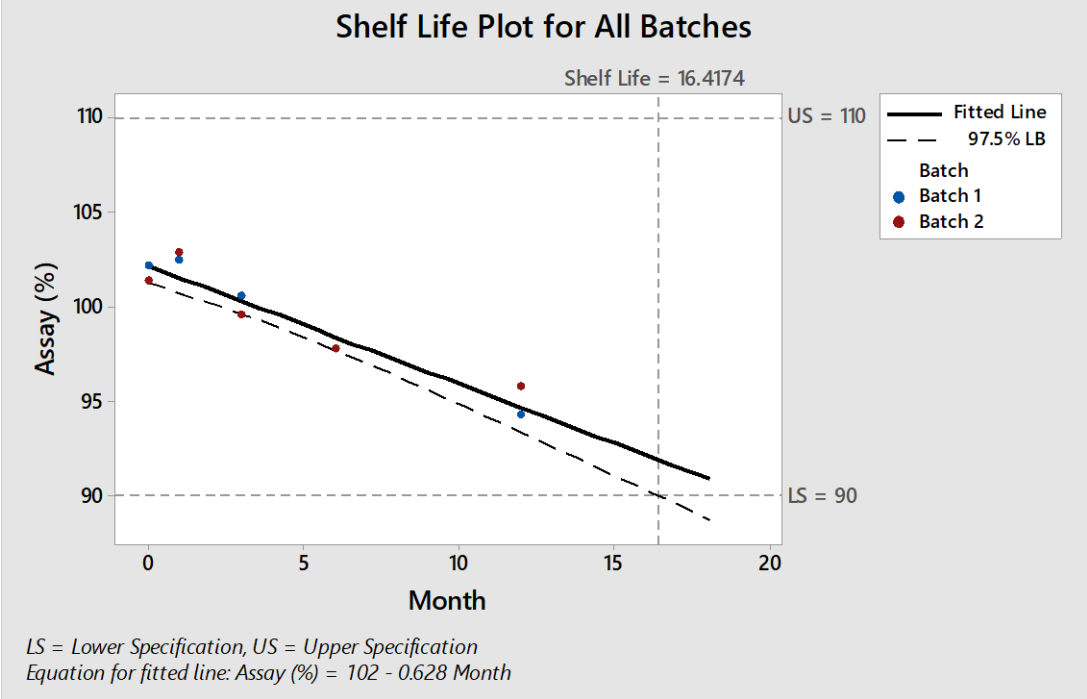

Figure 4.48: Shelf-Life Plot for Batch 1 & 2

4.3.3.6.2.2 Stability Study: pH versus Month, Batch No

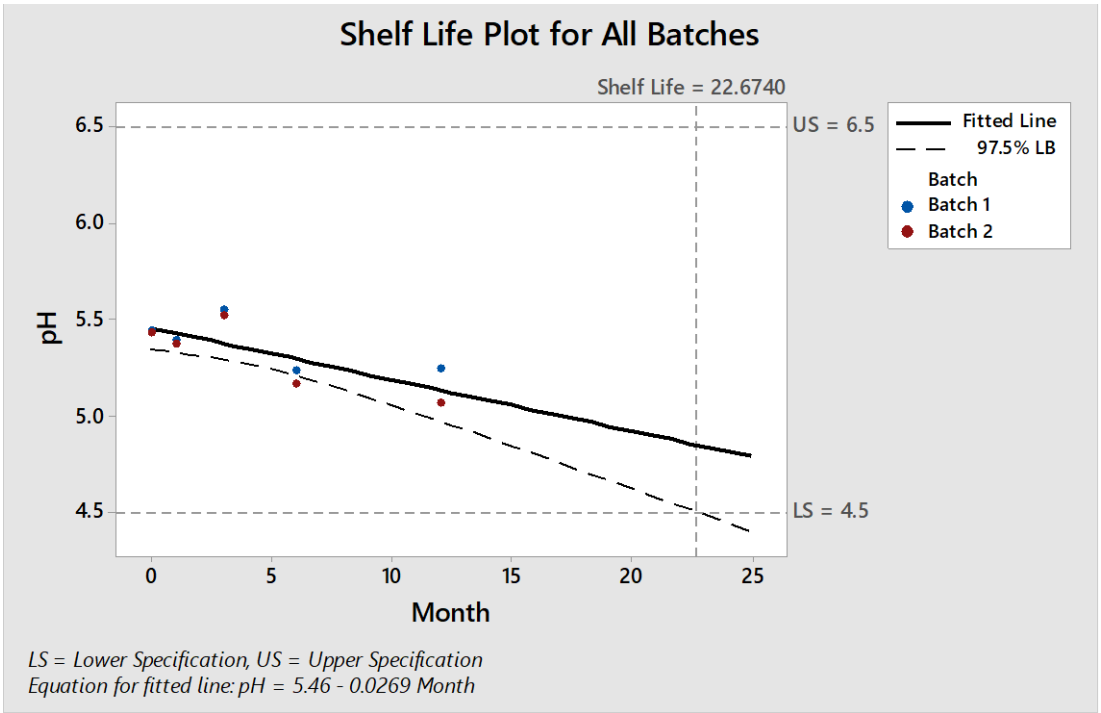

Figure 4.49: Shelf-Life Plot for All Batches

4.3.3.6.2.3 Stability Study: Osmolality (mOsm/kg) versus Month, Batch No

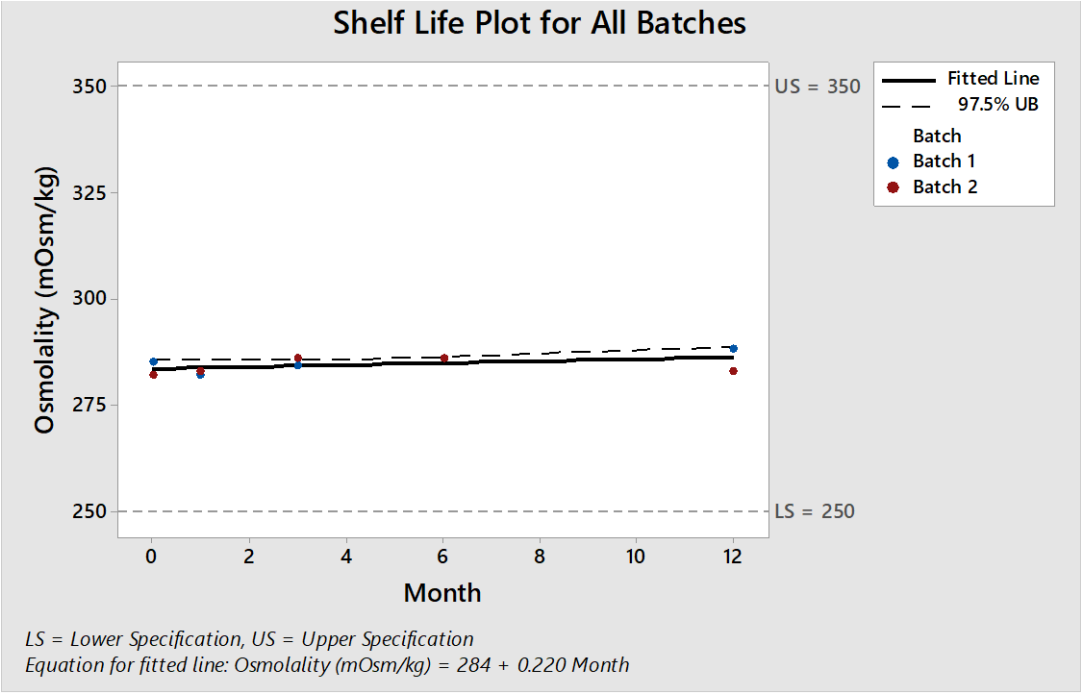

Figure 4.50: Shelf-Life Plot for All Batches

4.3.3.6.2 Stability Study Report of Angiotensin – II RTI at 5 ± 3 °C

4.3.3.6.2.1 Stability Study: Assay (%) versus Month, Batch No

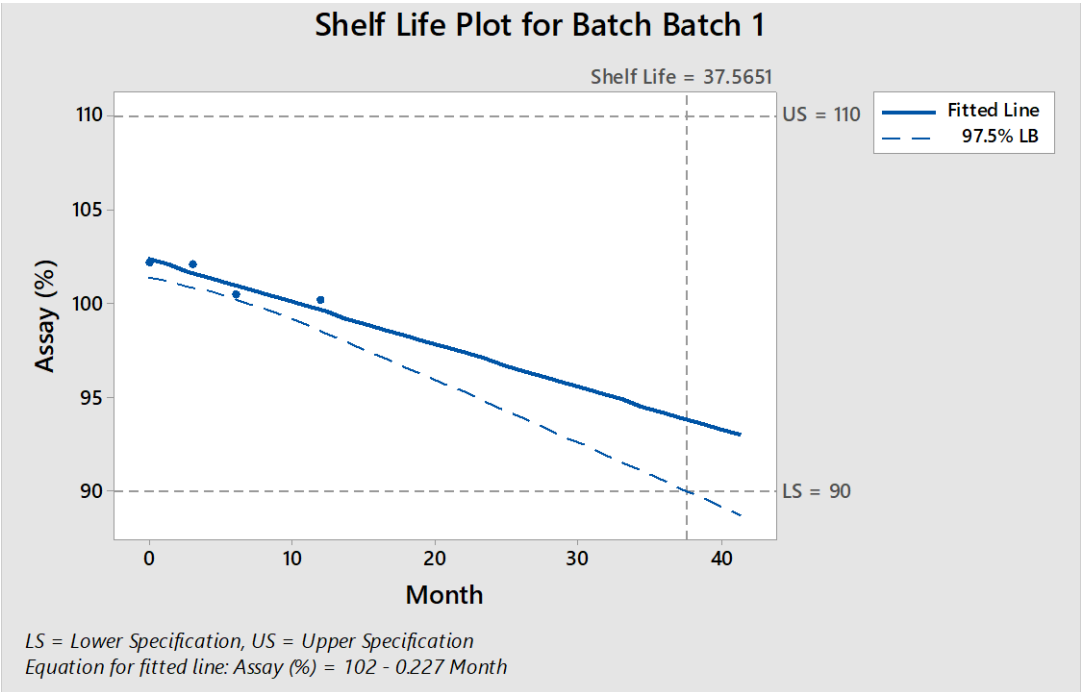

Figure 4.51: Shelf-Life Plot for Batch 1

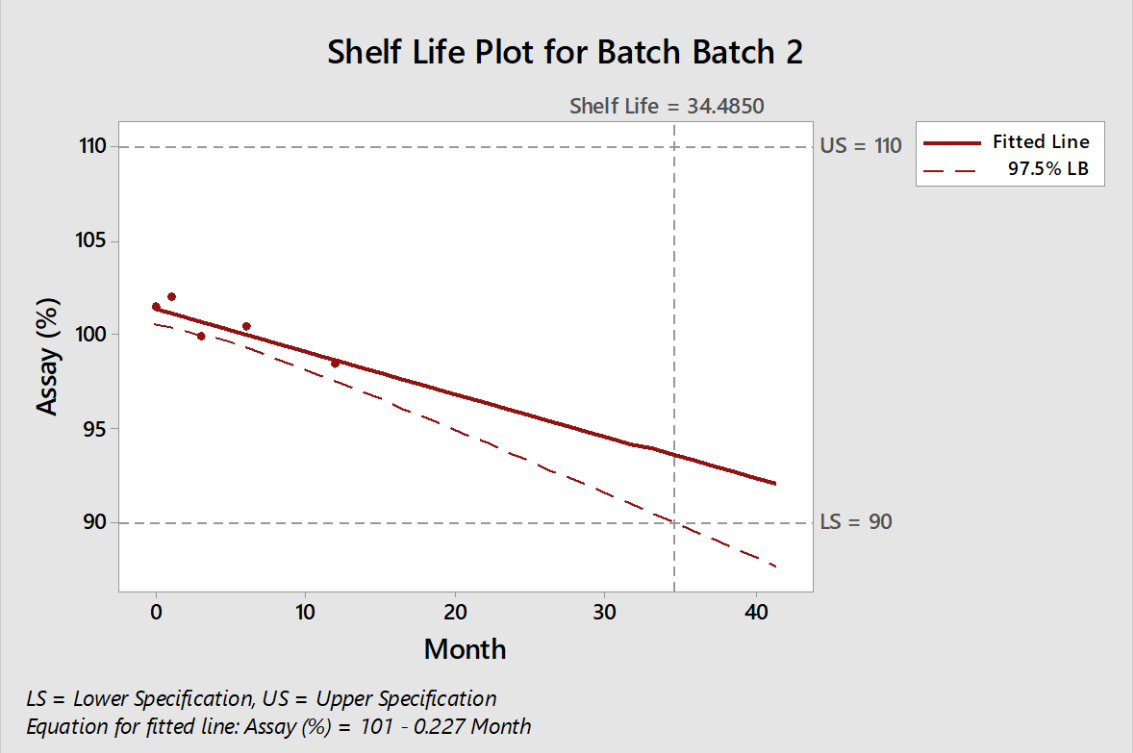

Figure 4.52: Shelf-Life Plot for Batch 2

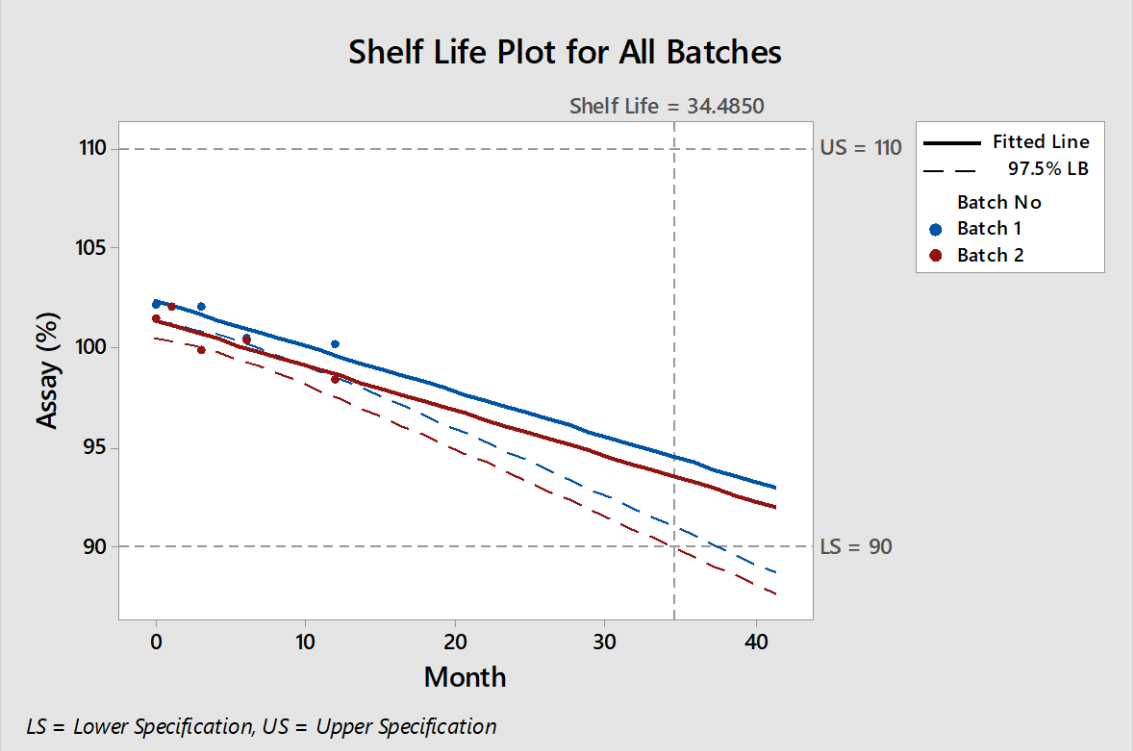

Figure 4.53: Shelf-Life Plot for Batch Shelf-Life Plot for All Batches

4.3.3.6.2.2 Stability Study: pH versus Month, Batch No

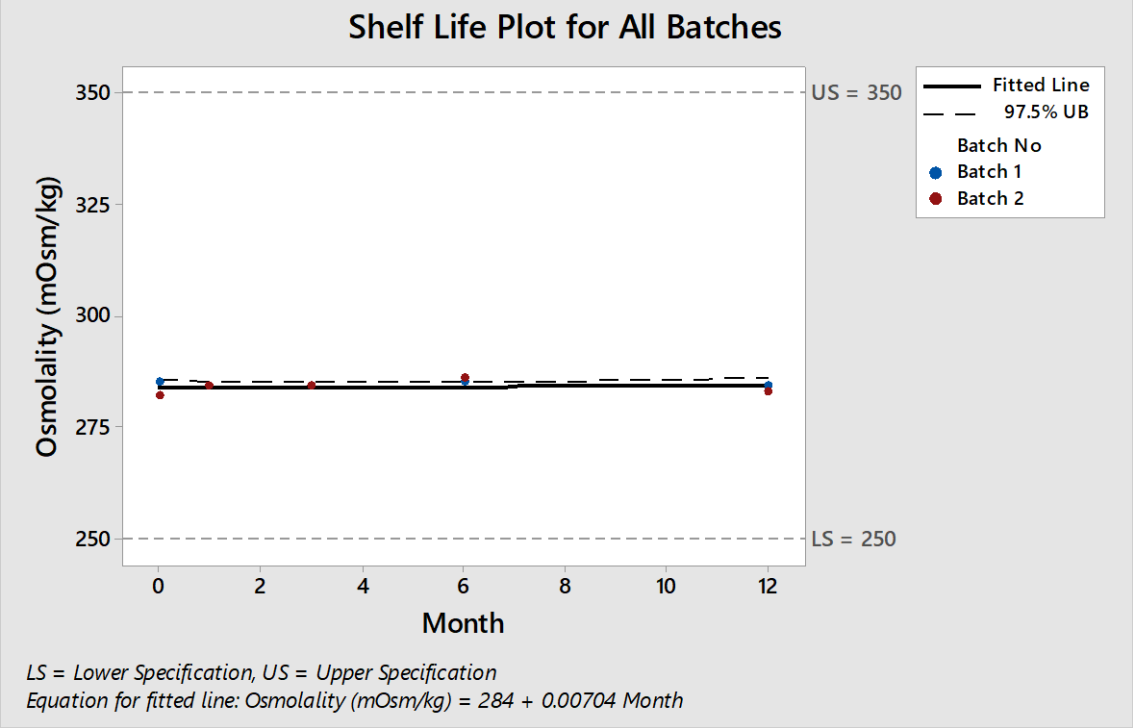

Figure 4.54: Shelf-Life Plot for Batch Shelf-Life Plot for All Batches

4.3.3.6.2.3 Stability Study: Osmolality (mOsm/kg) versus Month, Batch No

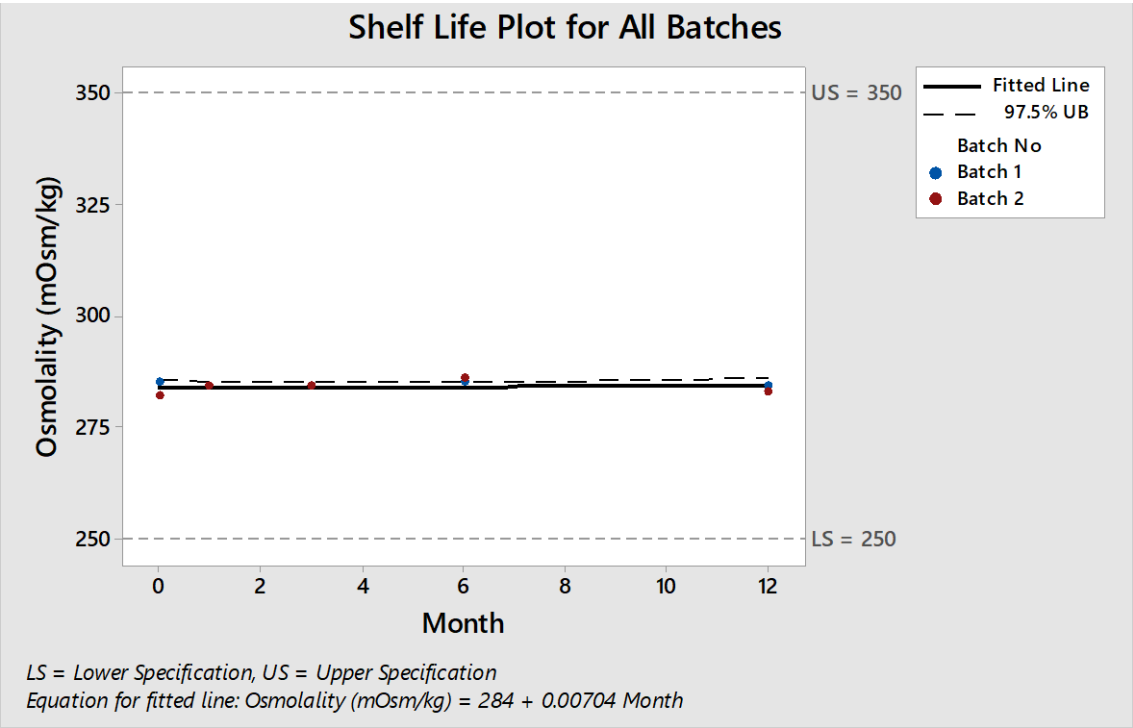

Figure 4.55: Shelf-Life Plot for Batch Shelf-Life Plot for All Batches

Table 4.35: Shelf-life estimation of developmental batches of Angiotensin II RTI @ 25°C/40%RH

<table>
<tr><th rowspan="2">CQAs</th><th rowspan="2">Proposed shelf-life specification</th><th colspan="3">Result</th><th colspan="3">Inference</th><th rowspan="2">Selected model</th><th rowspan="2">Shelf life indicating?</th></tr>
<tr><th>Time, p-value</th><th>Batch & Time interaction, p value</th><th>Intercept, p value</th><th>Effect of Time</th><th>Slope (Batch & Time interaction) Poolability</th><th>Intercept Poolability</th></tr>
<tr><td>Assay</td><td>90-110</td><td>0.000</td><td>0.271</td><td>0.908</td><td><0.05; Significant</td><td>>0.25 Poolable</td><td>>0.25 Poolable</td><td><table><tr><th>Batch</th><th>Intercept</th><th>Slope</th><th>Shelf life</th></tr><tr><td>1</td><td>102.223</td><td>- 0.6279</td><td>16.41</td></tr><tr><td>2</td><td>102.223</td><td>- 0.6279</td><td>16.41</td></tr><tr><td colspan="3">Over all</td><td>16.41</td></tr></table></td><td>Yes</td></tr>
<tr><td>Osmolality (mOsm/kg)</td><td>250-350</td><td>0.150</td><td>0.258</td><td>0.430</td><td>>0.05; Not Significant</td><td>>0.25 Poolable</td><td>>0.25 Poolable</td><td><table><tr><th>Batch</th><th>Intercept</th><th>Slope</th><th>Shelf life</th></tr><tr><td>1</td><td>283.532</td><td>0.22</td><td rowspan="3">*The mean response slope is not significantly larger than zero</td></tr><tr><td>2</td><td>283.532</td><td>0.22</td></tr><tr><td colspan="3">Over all</td></tr></table></td><td>NO</td></tr>
<tr><td>pH</td><td>4.5-6.5</td><td>0.018</td><td>0.425</td><td>0.408</td><td><0.05; Significant</td><td>>0.25 Poolable</td><td>>0.25 Poolable</td><td><table><tr><th>Batch</th><th>Intercept</th><th>Slope</th><th>Shelf life</th></tr><tr><td>1</td><td>5.4572</td><td>-0.0268</td><td rowspan="2">22.67</td></tr><tr><td>1</td><td>5.4572</td><td>-0.0268</td></tr><tr><td>Over all</td><td></td><td></td><td>22.67</td></tr></table></td><td>Yes</td></tr>
<tr><td colspan="8">Over all shelf life</td><td>16.41 M</td><td></td></tr>
<tr><td colspan="10">Data of particulate matter were observed well within the specification limit for all the time points hence not considered for extrapolation</td></tr>
</table>

Table 4.36: Shelf-life estimation of developmental batches of Angiotensin II RTI @ 5 ± 3°C

CQAs	Proposed shelf-life specification	Result			Inference			Selected model				Shelf life indicating?
		Time, p-value	Batch & Time interaction, p value	Intercept, p value	Effect of Time	Slope (Batch & Time interaction) Poolability	Intercept Poolability					
Assay	90-110	0.007	0.444	0.060	>0.05; non-Significant	>0.25 Poolable	<0.25 Not-Poolable	**Batch**	**Intercept**	**Slope**	**Shelf life**	Yes
								1	102.382	- 0.2275	37.56	
								2	102.411	- 0.2275	34.485	
								Over all			34.485	
Osmolality (mOsm/kg)	250-350	0.948	0.639	0.447	>0.05; Not Significant	>0.25 Poolable	>0.25 Poolable	**Batch**	**Intercept**	**Slope**	**Shelf life**	NO
								1	284.077	0.0070	*The mean response slope is not significantly larger than zero	
								2	284.077	0.0070		
								Over all				
pH	4.5-6.5	0.347	0.97	0.555	>0.05; non-Significant	>0.25 Poolable	>0.25 Poolable	**Batch**	**Intercept**	**Slope**	**Shelf life**	Yes
								1	5.4115	0.006542	*The mean response slope is not significantly larger than zero	
								2	5.4115	0.006542		
								Over all				
Over all shelf life								34.485 M				
Data of particulate matter were observed well within the specification limit for all the time points hence not considered for extrapolation												

4.3.3.7 Stability Report at 5 ± 3 °C & 25°C/40%RH

In light of the stability data predictions as prescribed by ICH Q1E, the overall shelf life was calculated to be approximately 16.41 months when stored at 25°C with 40% relative humidity, and an impressive 34.485 months when kept at a controlled temperature of 5±3°C. The extended shelf life at the lower temperature is attributed to the managed degradation of the assay, which helps maintain its integrity over time. Hence, it can be confidently concluded that even under the most challenging conditions, the stability of any angiotensin II RTI formulation is expected to last beyond 15 months at 25°C/40% RH and reach up to 36 months at the more favourable storage condition of 5±3°C, according to the proposed specifications.

4.4 *In vitro* Biological Reactivity of Final RTI formulations of Oxytocin, Vasopressin and Angiotensin-II by Agarose Diffusion Assay

The purpose of this test is to evaluate the biological reactivity of mammalian cell cultures after they come into contact with elastomeric plastics and a variety of other polymeric materials that may have either direct or indirect exposure to patients. This includes the assessment of specific extracts that are prepared from the materials under examination. There are three distinct tests involved in this evaluation:

1. Systemic Injection Test: (This test is designed to assess the systemic biological responses in animal subjects following the injection of a single dose of specific extracts derived from the plastic or polymer sample);

2. Intracutaneous Test: (This test focuses on determining the local biological responses of animals by administering a single-dose injection of specific extracts made from the sample beneath the skin); and

3. Implantation Test: (This test is conducted to investigate the reaction of living tissue to the plastic or polymer by directly implanting the specimen being tested into the animal tissue, allowing for observation of the tissue's response).

Materials: NCTC clone 292 (L-929) ATCC (Sigma Aldrich)

Reference: USP general chapter 87

Methodology: A uniform monolayer of cells was meticulously cultivated in sterile petri dishes. After a designated incubation period, the spent culture media from the experimental plate was

carefully aspirated and replaced with a fresh serum-supplemented culture media that contained agarose. Samples from the various formulations were then withdrawn from their protective bags. These samples were strategically placed in direct contact with the surface of the solidified agarose (with each sample tested in duplicate) and subsequently incubated for a full 24 hours at a controlled temperature of 37°C within an atmosphere of 5% CO2. Each formulation sample was cultured in proximity to both positive and negative control samples. After the incubation period, the samples were examined under a microscope using an appropriate staining technique involving 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide to assess cell viability. The reactivity grades used for the evaluation process are systematically detailed in Table 4.37. The resulting formulations were visually represented in Figure 4.56

Figure 4.56: Final all three Ready to use formulations

Table 4.37: Reactivity grades for agarose diffusion test

Grade	Reactivity	Description of reactivity zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extended 0.5 to 1.0 cm beyond specimen
4	Severe	Zone extended > 1.0 cm beyond specimen

Acceptance criteria

1. The sample shall meet requirement of test if reactivity to sample is not greater than grade 2 (mild reactive).
2. The reactivity of negative control should always be grade 0 (non-reactive).
3. The reactivity of positive control should be at least grade 3 in (moderate reactive)
4. The reactivity and grade for each sample are to be assigned as description of reactivity zone mentioned in table 4.37.

- **Results and Discussion of biological reactivity study:**

The outcomes of *In vitro* reactivity study are listed in Table 4.38.

Table 4.38: Outcomes of Biological reactivity study

Sample tested	Lot #	Sample description	Microscopy		MTT Dye assessment	
			Reactivity	Scale	Reactivity	Grade
Positive control	F0D014	Positive bioreaction USP Reference standard	Moderate	3	Moderate	3
Negative Control	HOF041	High density polyethylene USP Reference standard	None	0	None	0
Oxytocin (0.02 Units/mL)	FP004	25°C/40%RH-6M	None	0	None	0
Oxytocin (0.08Units/mL)	FP005	25°C/40%RH-6M	None	0	None	0
Vasopressin (1.887 µg/mL)	FP006	25°C/40%RH-6M	None	0	None	0
Angiotensin (10 mcg/mL)	FP007	25°C/40%RH-6M	None	0	None	0

All the tested RTI formulation samples have shown grade ‘0’ in microscopic evaluation showing no *In vitro* reactivity. Similarly, in MTT test, all the tested samples have shown no *In vitro* reactivity. This indicates that selected packaging materials are biocompatible and that the formulation can be used for further *In vivo* evaluation.

4.5 Hemolytic Toxicity Study

Whole human blood samples were collected from a healthy person (with the kind permission) and heparinized in HiAnticlot blood collection vials (Himedia, India). Subsequently, the red blood cells (RBCs) suspension was centrifuged (Navyug Udyog, Haryana, India) and re-suspended in normal saline. Two milliliter of the RBCs suspension was separately dispersed in normal saline solution producing no hemolysis (served as control) and in distilled water considered as producing 100% hemolysis [23, 24]. 2 mL of RBC suspension was mixed separately with Oxytocin-RTU, Vasopressin-RTU, and Angiotensin-RTU. After centrifugation, supernatants were taken and diluted with an equal volume of normal saline and absorbance was taken at 540 nm (spectrophotometrically; Evolution 201, Thermofisher) against supernatant of normal saline diluted similarly as blank. The percent hemolysis was thus determined for each sample by taking absorbance of water as 100% hemolytic sample.

The degree of hemolysis can be determined by below Equation:

$$\text{Hemolysis}(\%) = \frac{Abs - Abs0}{Abs100 - Abs0} \times 100$$

Where Abs, Abs0 and Abs100 are the absorbance of samples, a solution of 0% hemolysis and a solution of 100% hemolysis, respectively.

Results and discussion of i*n vitro* hemolysis study

The hemolytic potential of Ready-to-Use (RTU) formulations of three key peptide hormones Oxytocin, Vasopressin, and Angiotensin-II was investigated. The results indicated minimal to no hemolytic toxicity across all tested conditions. RTU formulations of Oxytocin, Vasopressin, and Angiotensin-II exhibited negligible hemolytic activity, with mean hemolysis percentages of 0.21±0.09%, 0.29±0.10%, and 0.41±0.12% respectively (Figure 1). All RTU formulations were engineered to be isotonic, meaning their osmotic pressure was meticulously balanced to match that of human blood plasma [25]. This precise formulation prevents the osmotic stress that was observed in the plain drug solutions. The excipients used in these RTU formulations were carefully selected for their biological compatibility and were present at concentrations considered safe and non-toxic, ensuring that the final products had no adverse effects on the structural integrity of red blood cell membranes. This confirms the safety of the RTU formulations for clinical intravenous administration.

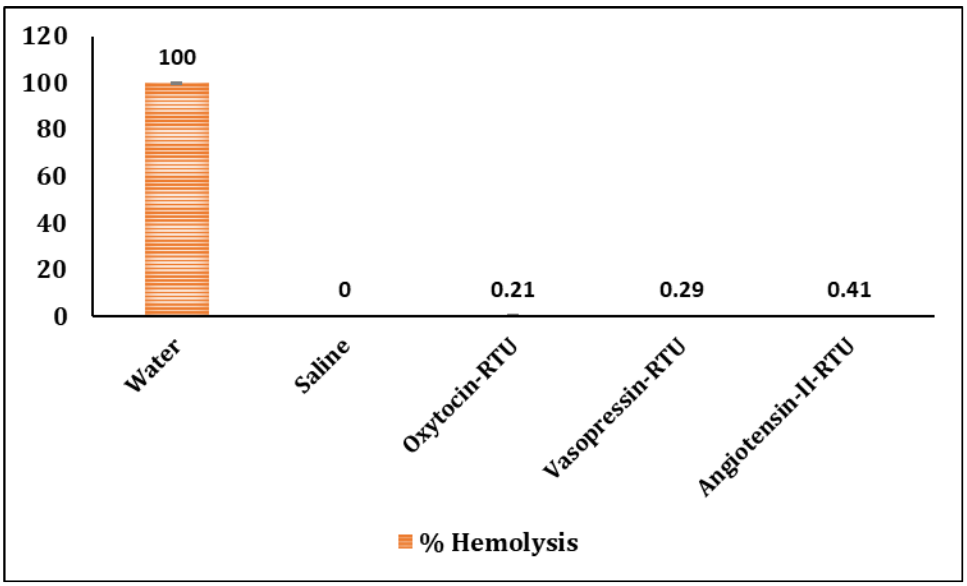

Figure 4.57: % Hemolysis for RTI formulation of Oxytocin, Vasopressin, Angiotensin-II

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