International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.7, pp 570-586, 2017

Formulation, Development, and Characterization of Lyophilized Para-Amino Salicylate Sodium Injection for an Effective Treatment for Multi-Drug Resistance-Tuberculosis

Mukhopadhyay Sayantan[1]*, Shivanand[1]

[1]Division of Pharmaceutical Sciences S.G.R.R.I.T.S, Patel Nagar Dehradun Uttarakhand 248001, India.

Abstract : The purpose of this study was to the development and manufacture of stable lyophilized injection formulation of Para-Amino Salicylate sodium, a drug that used in the treatment of multi-drug resistance tuberculosis. The drug was unstable in the aqueous solution and it shows high impurity level. Lyophilization technique in the pharmaceutical industries used in the formulation of thermolabile and moisture sensitive drug. For the formulation purpose, initial drug-excipients compatibility study was conducted and on the basis of percentage impurity level, present excipients were selected. Drug- Excipients compatibility study result shows that sodium metabisulphite increases the percentage of impurity level found that 0.12%, 0.15%, 0.06%, 0.09%, where the composition of API with sodium sulphite and other excipients reduces the impurities level. For the development of an effective formulation that provides stable, less impure and improved physicochemical characteristic of the model, several formulation methodologies were adopted.Compatibility study with reconstitution fluids was conducted for formulation (F-I, & F-II), the result shows that reconstitution with 0.9% Sodium chloride& 5% Dextrose injections, the reconstituted solution becomes hypertonic, where water for injection gives an isotonic solution. Accelerated stability perusal result for related substance during time period of initial to 6th month stability result indicates formulation (F-I, 0.05%, 0.13%), (F-II, 0.08%, 0.16%), and assay percentage (F-I, 102.14%, 98.61%), (F-II, 100.49%, 96.27%). Related substance (impurity) and Assay result indicate that formulation F-I shows less impurity level and high assay percentage as compared to formulation F-II. Accelerated stability 6th-month result indicates that Para-Amino Salicylate Sodium lyophilized injection found to be physically and chemically stable.
Keywords : Lyophilized, Para-Amino Salicylate sodium, Thermolabile, Hypertonic, Isotonic.

Introduction

Tuberculosis is one of the major health problems worldwide. According to the World Health Organisation (WHO) approximately death 5,000 people per day ubiquitously, the world due to tuberculosis& it is a single treatable infectious disease. In 2008 approximately death due to tuberculosis is 1.8 million that is reported by The World Health Organisation (WHO) [1, 2]. Causing agent of tuberculosis is Mycobacterium tuberculosis, which is airborne.During the active stage, TB is highly contagious disease and it can be generating by inhaling the airborne particles of M. tuberculosis[3, 4].

Para-aminosalicylic acid is a second line drug effectively used in the treatment of multi-drug resistance tuberculosis. It is aclinically introduced in the 1940sand used in the treatment of tuberculosis into the 1960s.

Para-aminosalicylic acid is a bacteriostatic chemotherapeutic agent used in the treatment of all types of tuberculosis, in extrapulmonary and pulmonary. Chemically it is (4-Amino-2-hydroxybenzoic acid).Para-aminosalicylic acid is a white or almost white crystalline powder[5]. A daily dose of para-aminosalicylic acid is 10-15g for adult and children and older than 14 years, recommended by the German Bundesanzeiger and the guidelines of the German central committee. The parenteral dosage of para-aminosalicylic acid is administered by intravenously with 500 ml of reconstituted sterile water for injections.Para-aminosalicylic acid is get degraded due to the presence of impurity m-aminophenol, and it contain high moisture content about 16-18%[6,7].

The active pharmaceutical ingredient that is thermolabile and moisture sensitive in nature generally degraded in atmospheric condition and thus have reduced stability and self-life. Lyophilization is the most common technique for the manufacturing of parenteral pharmaceutical product when the product is unstable in aqueous solution. It helps in improving the stability and self-life of a pharmaceutical product. Lyophilization technique mainly involved in the removal of moisture content from the pharmaceutical product. In this process, thefirst Solvent is in a frozen state and then water is removed by the process of sublimation[8, 9]. The Lyophilization technique mainly involved, freezing formation of ice crystal, primary drying removal of unbound water content from the drug product by the process of sublimation, and secondary drying removal of bound water which present in a very less amount of moisture content in the primary drying and make the final product stable[10, 11].

Experimental

Materials

Sodium Aminosalicylate dehydrate was procured from Rusan Pharma Ltd. All other chemicals and reagents used were analytical grades such as Sodium Chloride, Disodium Edetate, Sodium Sulphite, Sodium Metabisulphite, Sodium Hydroxide and sterile water for injection.

Methods

Preformulation study regarding formulation development

Drug- Excipients compatibility study[12, 13, 14]

Compatibility study carried out for the selection of suitable excipients for the development of stable and robust lyophilized formulation. Compatibility study of Para-Aminosalicylate sodium drug substances with various excipients was carried out. To separately prepare a solution of each excipient with API based on the suitable ratio, each excipient plays an important role in the formulation. Each separately prepared solution to be filled in a separate glass vial and charged in the stability chamber at condition 40°±2°C/75%RH±5%RH and 25°±2°C/60%RH±5%RH for the time period of 15days and 1month respectively. Analysis result for related substances during compatibility studies are recorded.

Order of addition drug-excipient study

From the various literature study, it is clear that thePara-Amino Salicylate Sodium shows more stable impurity profile in basic pH. In order to establish the desired pH, order of addition of excipients and drug, the following of experiments were performed.

Method. A

Direct addition of all excipients in water for injection Followed by Para-Amino Salicylate Sodium& pH adjustment with 1M NaOH Solution

- Step 1. Weighed quantity of Sodium sulphitewas dissolved into 70 % of water for injection with continuous stirring.
- Step 2. Weighed quantity of Disodium EDTA was dissolved, to the solution of step 1. with continuous stirring.
- Step 3. Weighed quantity of Sodium chloride was dissolved, to the solution of step 2. with continuous stirring and check the solution pH.

- Step 4. Weighed quantity of Para-Amino Salicylate Sodium was dissolved, to the solution of step 3. with continuous stirring.
- Step 5. Adjust the pH of the solution to 6.5±0.1 with 1M NaOH solution at step 4.
- Step 6. Finally make up the volume with WFI, with continuous stirring to get a uniform solution.

Method. B

Adjust the pH of the solution before addition of Disodium EDTA &Para-Amino Salicylate Sodium Followed by pH adjustment with 1M NaOH Solution

- Step 1. Weighed quantity of Sodium sulphitewas dissolved into 70 % of water for injection with continuous stirring.
- Step 2. Adjust pH of the solution to 6.5 - 7.0 with 1M NaOH solution at step 1. with continuous stirring.
- Step 3. Weighed quantity of Disodium EDTA was dissolved, to the solution of step 2. with continuous stirring and check the solution pH.
- Step 4. Weighed quantity of Para-Amino Salicylate Sodium was dissolved, to the solution of step 3. with continuous stirring.
- Step 5. Weighed quantity of Sodium chloride was dissolved, to the solution of step 4. with continuous stirring.
- Step 6. Adjust the pH of the solution to 6.5±0.1 with 1M NaOH solution at step 5. with continuous stirring.
- Step 7. Finally make up the volume with WFI, with continuous stirring to get a uniform solution.

Method. C

Addition of Sodium Hydroxide followed by addition of Disodium EDTA, Sodium sulphite & Para-Amino Salicylate Sodium

- Step 1. Weighed quantity of Sodium Hydroxide was dissolved into 70 % of water for injection with continuous stirring.
- Step 2. Weighed quantity of Disodium EDTA was dissolved, to the solution of step 1. with continuous stirring.
- Step 3. Weighed quantity of Sodium sulphitewas dissolved, to the solution of step 2. with continuous stirring and check the solution pH.
- Step 4. Weighed quantity of Para-Amino Salicylate Sodium was dissolved, to the solution of step 3. with continuous stirring.
- Step 5.Weighed quantity of Sodium chloride was dissolved, to the solution of step 4. with continuous stirring.
- Step 6. If required, adjust the pH of the solution to 6.5±0.1 with 1M NaOH solution at step 5. with continuous stirring.
- Step 7.finally make up the volume with WFI, with continuous stirring to get a uniform solution.

Formulation development

PreparationPara-Amino Salicylate Sodium solution

From the aboveperformed Order of addition experiment Method, C. was selected for the development of the lyophilized product. Preparation of the bulk solution.

- About 70% of the required batch quantity of Water for Injection (WFI) is taken in a jacketed stainless-steel formulation vessel and bring the temperature down to 40°C, by Nitrogen purging for removing of dissolved oxygen.
- Step 1. Weighed quantity of Sodium Hydroxide was dissolved into 70 % of water for injection with continuous stirring.
- Step 2. Weighed quantity of Disodium EDTA was dissolved, to the solution of step 1. with continuous stirring.
- Step 3. Weighed quantity of Sodium sulphitewas dissolved, to the solution of step 2. with continuous stirring.

- Step 4. Weighed quantity of Para-Amino Salicylate Sodium was dissolved, to the solution of step 3. with continuous stirring.
- Step 5. Weighed quantity of Sodium chloride was dissolved, to the solution of step 4. with continuous stirring.
- Step 6. Adjust the pH of the solution to 6.5±0.1 with 1M NaOH solution at step 5. with continuous stirring.
- Step 7. finally make up the volume with WFI, with continuous stirring to get a uniform solution.
- After volume make-up, the bulk solution is filtered through 5µ, 2µ& 0.2µ positively charged retentive filters into a pre-autoclaved and pre-cooled jacketed vessel and solution filled into aglass vial and loaded into lyophilization chamber.

Optimization of Lyophilization cycle time[15, 16, 17, 18]

Differential Scanning Calorimetry (DSC)

In order to understand the enthalpic phase transition behavior of the formulation components during lyophilization, the behavior of the formulation in solution was studied by Differential Scanning Calorimetry (DSC). Identification of the eutectic temperature and crystallization behavior of the formulation constituents was particularly important because dramatic and rapid changes in physical properties of these could affect lyophilization and the quality of the lyophilized product.

Freezing

Freezing is a critical step in the lyophilization that will affect the quality of product and also influence the primary and secondary drying process.

Primary Drying Step

After freezing next step in the lyophilization process is primary drying. During the primary drying,the product temperature must set below the product collapse temperature and glass transition temperature. To achieve the primary drying pressure and shelf temperature are required to be controlled. To ensure completion of primary drying, before going to secondary drying, to avoid any product melt back. A gas bleed system in the instrument maintained the desired pressure during the cycle.

Secondary Drying Step

Secondary drying involves desorption of adsorbed water from the product. In secondary drying, very less amount of bound water present in the formulation. Moisture content present in the secondary drying which affects the product stability and self-life of thedrug product. Secondary drying is the slower process as compared to primary drying. Desired moisture content at the end of secondary drying to ensure residual moisture content below 4%.

Compatibility study with reconstitution fluids

Para-Aminosalicylate sodium Lyophilized Powder for Infusion were reconstituted with 500 ml of sodium chloride injection IP 0.9 % w/v & 500 ml of Dextrose injection IP 5 % w/v. The reconstituted solutions were kept at 25°±2°C/60%RH±5%RH& stability was evaluated at Initial & after 24 hrs.

Accelerated stability studies

The stability studies were performed as per ICH guidelines Q1A (R2). The accelerated stability studies were performed at the temperature of 40°C±2°C/75%RH±5%RH for a time period of 6month. Various evaluation parameter wasanalyzed during stability studies such as Description, Reconstitution time, Clarity of solution, pH, Particulate Matter, Related Substances (Impurities) By HPLC, Water Karl Fischer method, BET, Assay of Para-Amino Salicylate Sodium (By HPLC).

Results and Discussion

Drug- Excipients compatibility study

Drug- Excipients compatibility studywas performed at condition 40°C±2°C/75%RH±5%RH and 25°C±2°C/60%RH±5%RH for the time period of 15days and 1month. The result shows that there are no physical & chemical changes or interaction between drug and excipients were observed. But, from the above observation, it is clear that sodium metabisulphite increases the percentage of impurity found that 0.12%, 0.15%, 0.06%, 0.09% where the limitation is NMT 0.10% (m-aminophenol related substances). However, the composition of API with sodium sulphite reduces the impurities. Based on these studies, the excipients which are compatible with Sodium Aminosalicylate Dihydrate are evaluated for the formulation development. Result obtained during compatibility studies were mention on Table 1. Graphical representation of compatibility studies was shown in figure 1.

Table 1.Observed data during drug- excipients compatibility study

Related Substances (In % w/w m-aminophenol NMT 0.10%)						
S. No	**Composition details** Drug & Excipient	**Initial Observation**	**40°C/ 75%RH /15days**	**40°C/ 75%RH /1M**	**25°C/ 60%RH /15days**	**25°C/ 60%RH /1M**
1.	Para-Amino Salicylate Sodium	Found to be a clear colorless Solution	0.08	0.09	0.06	0.06
2.	Para-Amino Salicylate Sodium + Sodium Chloride	Found to be a clear colorless Solution	0.07	0.08	0.05	0.06
3	Para-Amino Salicylate Sodium + Disodium Edetate	Found to be a clear colorless Solution	0.06	0.06	0.04	0.05
4	Para-Amino Salicylate Sodium + Sodium Sulphite	Found to be a clear colorless Solution	0.05	0.06	0.03	0.04
5	Para-Amino Salicylate Sodium + Sodium Hydroxide	Found to be a clear colorless Solution	0.05	0.05	0.03	0.04
6	Sodium Para-Amino Salicylate Sodium Metabisulphite	Found to be a clear colorless Solution	0.12	0.15	0.06	0.09

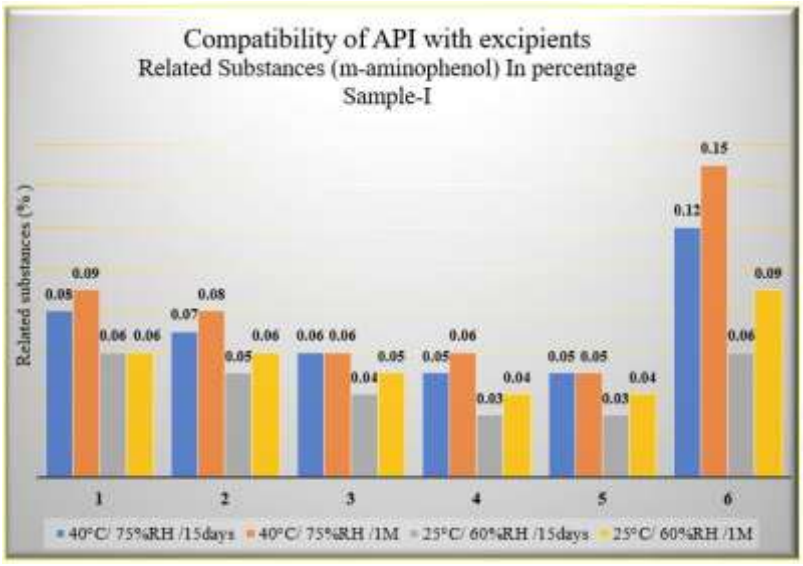

Figure 1.Graphical representation of drug- excipients compatibility study

Order of addition drug-excipient study

Result obtained for an order of addition drug with excipients it shown in Table 2. Since the processes, Method A & B, required 45 minutes & 20 minutes respectively for complete dissolution of Disodium EDTA,

where the Method C is required only 8minutes for complete dissolution of Disodium EDTA and it also shows less impurity profile. Best on these result Method C selected for formulation development. Graphical representation of the order of addition studies was shown in figure 2.

Table 2. observation data obtained during order of addition study

S. No	Method No.	pH (Before drug addition step)	Time required for complete dissolution of Disodium EDTA	Related Substances (m-Aminophenol: NMT 0.2%)
1.	A	5.5	45 minutes	0.21
2.	B	6.4	20 Minutes	0.12
3.	C	6.7	8 minutes	0.04

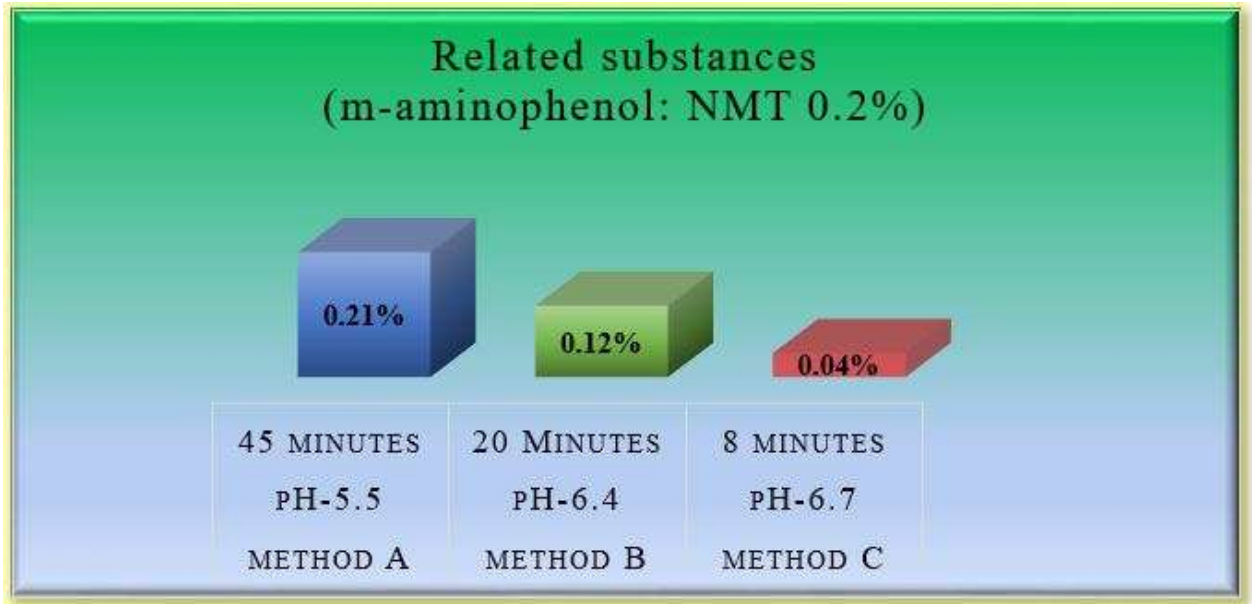

Figure 2. Graphical representation of the order of addition studies

Selection of excipients

Based on the compatibility and order of addition result following excipients selected for development of the lyophilized product. Two trial batch (F-I, F-II) were selected for the development of lyophilized product both the batch having the same quantity and same excipient. Drug and excipient quantity were shown in Table 3.

Table 3. Composition table of drug &excipients

S No.	Name of Ingredients	Function	Quantity per bottle	Formulation	
				F-I (100 bottles)	F-II (100 bottles)
1	Para-Amino Salicylate Sodium	API	13.49 grams/bottle	1.349kg	1.349kg
2	Sodium Chloride	Isotonic	0.65 grams	0.065kg	0.065kg
3	Di-sodium edetate	Antioxidant	0.05 grams	0.005kg	0.005kg
4	Sodium sulphite	Antioxidant	0.25 grams	0.025kg	0.025kg
5	Sodium Hydroxide	pH adjusts	Q. S	Q. S	Q. S
6	WFI	Diluent	Q. S	Q. S	Q. S

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) graph shows the thermal behaviour of Para-Amino Salicylate Sodiumformulation in solution was followed during both freezing and heating ramps in the temperature range of -40°C and -20°C. DSC thermograms, obtained upon freezing the Para-Aminosalicylate sodiumformulation exhibited two events between -22.32ºC to -5.01°C, i.e. from -22.32ºC to -9.26ºC, A broad endotherm peak at -15.79°C and from-7.31°C to -5.01°C, A sharp exotherm with a peak at -6.18ºC. Differential scanning calorimetry graph is shown in the figure 3., and their observation of differential scanning calorimetry thermogram mentioned in table 4.

Table 4. Observation of differential scanning calorimetry thermogram

Type of analysis	Temperature (°C)	Description of events
Differential Scanning Calorimetry	From -22.32ºC to -5.01°C	Para-Aminosalicylate sodium formulation exhibited two events.
	From -22.32ºC to -9.26°C	A broad endotherm peak at -15.79°C.
	From-7.31°C to -5.01°C	A sharp exotherm with a peak at -6.18ºC.

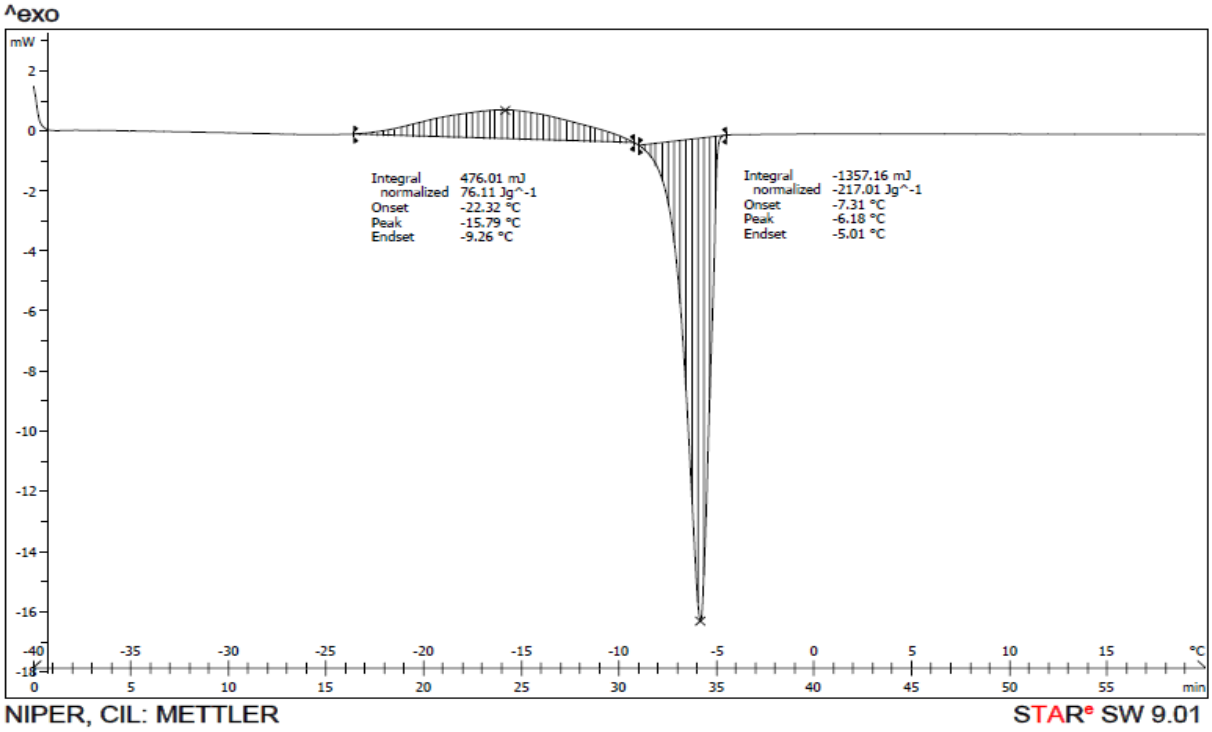

Figure 3. Differential Scanning Calorimetry (DSC)

Lyophilized cycle development

Freezing

In this cycle time take for the complete freezing of para Aminosalicylate sodium solution was found to be 13.03 hour at the particular temperature -40 ºC.

Primary drying

In the primary drying For Para-Amino Salicylate Sodiumformulation, the shelf temperature was raised slowly to -40°C to facilitate removal of water and majority of the primary drying was then conducted at -25°C. Additional primary drying was then carried out at -20°C, -10°C and 0°C to ensure completion of primary drying, while keeping the vacuum pressure (mbar 0.050). Total time required for the completion of primary drying was found to be 24hours.

Secondary drying

For Para-Amino Salicylate Sodiumformulation, secondary drying of the product was carried out by increasing the shelf temperature from 0°C to +25°C, while keeping chamber pressure at 0.050, 0.030 millibar. Further drying was performed by increasing shelf temperature from +35°C, +45°C and +50°C keeping chamber pressure at 0.020, 0.010, 0.010 millibar to ensure low moisture content. Total time required for the completion of secondary drying was found to be 33hours. The length of the cycle was titrated by analyzing the product for residual moisture content at the end of secondary drying to ensure residual moisture content below 4%. Total time required for complete lyophilized cycle development was found to be 71hours.Optimized lyophilized cycle time was given in Table. 5.

Table 5. Optimized lyophilized cycle

Step	Stage	Temperature (°C)	Ramp Time (min)	Drying Time (min)	Vacuum (mBar)
i	Freezing	-40	120	540	-------
		-40	2	120	-------
ii	Primary Drying	-25	60	300	0.050
		-20	60	300	0.050
		-10	60	300	0.050
		0	60	300	0.050
iii	Secondary Drying	25	60	450	0.030
		35	30	450	0.020
		45	30	450	0.010
		50	30	480	0.010

Compatibility study with reconstitution fluids

The reconstituted solutions were found to be a clear solution. Result for compatibility studies with reconstitution fluid was shown in table 6. Hence, the product is stable up to 24 hrs. in 0.9 % Sodium chloride, 5 % Dextrose injections& in water for injection. There are no significance changes were found in impurity profile up to 24 hours in all type of reconstituted solutions. But, after reconstitution with 0.9% Sodium chloride& 5% Dextrose injections, the reconstituted solution becomes hypertonic which is generally not recommended to be injected into the body at large volumes. The reconstitution with water for injection gives an isotonic solution. Water for injection is thus preferably recommended for reconstitution of Para-Aminosalicylate sodium lyophilized powder for infusion. Graphical representation of compatibility studies with reconstitution solution was shown in figure 4. and figure 5.

Table 6. Observed data during compatibility study with reconstitution fluids

Batch No.	Reconstitution Fluid	Color of solution	Initial	After 24 Hrs	
			% Impurity		Osmolarity
			m-Aminophenol NMT 0.2%	m-Aminophenol NMT 0.2%	Blood ranges between 285 - 310 (mOsmol/L)
F-I	Sodium Chloride Injection IP 0.9 % w/v	Found to be a clear Solution	0.10	0.12	620
	Dextrose Injection IP 5% w/v	Found to be a clear Solution	0.09	0.10	582
	Water for Injection	Found to be a clear Solution	0.10	0.11	300

F-II	Sodium Chloride Injection IP 0.9 % w/v	Found to be a clear Solution	0.10	0.11	640
	Dextrose Injection IP 5 % w/v	Found to be a clear Solution	0.12	0.13	578
	Water for Injection	Found to be a clear Solution	0.09	0.10	295

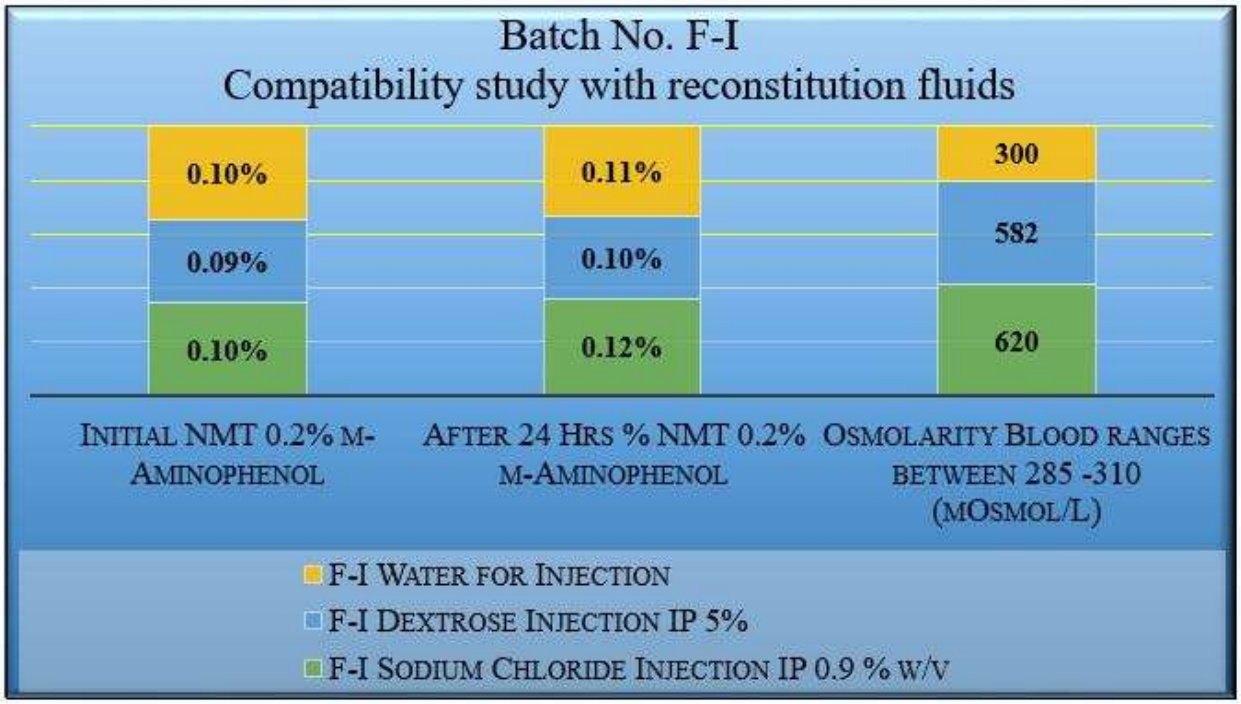

Figure 4. Graphical representation of compatibility studies with reconstitution fluids Batch No. F-I

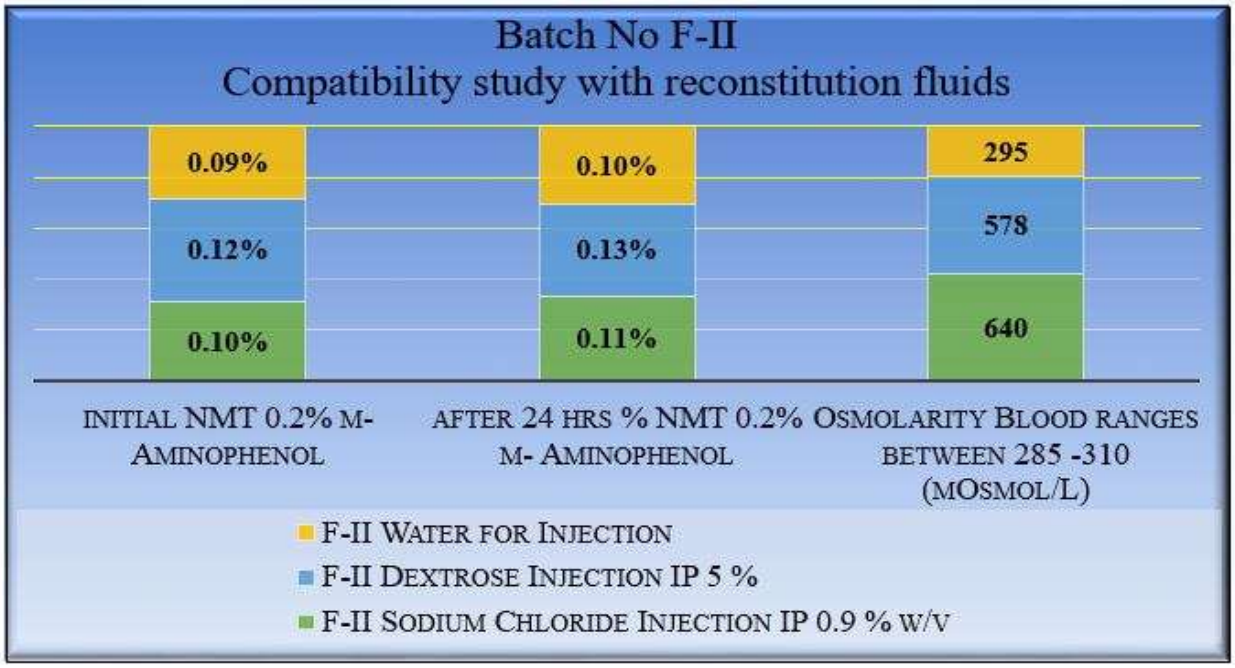

Figure 5. Graphical representation of compatibility studies with reconstitution fluids Batch No. F-II

Accelerated stability studies

Accelerated stability studies result for description test for Batch No. F-I, F-II lyophilized product initial to 6thmonth accelerated stability studies result found to be within the observation limit. Result for description testmentions the table

Table 7.Accelerated stability result for description initial to 6th-month study

Test- Description		**Accelerated Condition(40°C±2°C/75%RH±5%RH)**			
Batch No.	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	Lyophilized powder white to pale yellow color.	Lyophilized powder founds in white color.	Lyophilized powder founds in white color.	Lyophilized powder founds in white color.	Lyophilized powder founds in white color.
F-II	Lyophilized powder white to pale yellow color.	Lyophilized powder founds in white color.	Lyophilized powder founds in white color.	Lyophilized powder founds in white color.	Lyophilized powder founds in white color.

Reconstitution time

Reconstitution time for lyophilized product initial and 6month accelerated stability studies for both the Batch F-I, F-II result found within the observation range, where the limit is NMT 5minutes in 500ml water for injection. Result for Batch F-I, F-II were shown in table 8. Graphical representation of reconstitution time was shown in figure 6.

Table 8. Result for reconstitution time initial and 6month accelerated stability studies

Test- Reconstitution time		**Mean ± standard deviation (n=3)**			
		Accelerated Condition (40°C±2°C/75%RH±5%RH)			
Batch No.	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	NMT 5 minutes in 500 ml of water for injection.	51.5±1.290 Sec	52.5±1.290 Sec	55.5±1.290 Sec	54.5±1.290 Sec
F-II	NMT 5 minutes in 500 ml of water for injection.	52.5±1.290 Sec	51.5±1.290 Sec	54.5±1.290 Sec	55.5±1.290 Sec

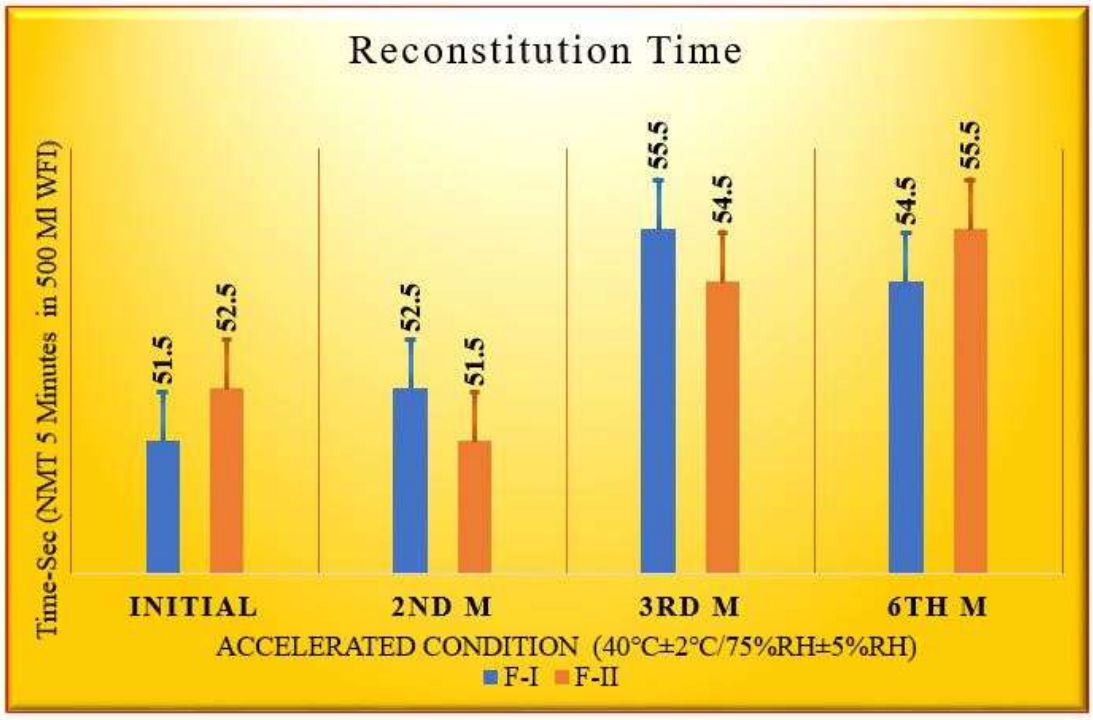

Figure 6.Graphical representation of reconstitution time Batch No. F-I, F-II

Clarity of solution

Clarity of solution test for lyophilized product initial and 6month accelerated stability studies for both the Batch F-I, F-II result a clear solution was found and the result shown in the table.

Table 9: Result for clarity test initial to 6th month accelerated stability

Test- Clarity of solution		**Accelerated Condition(40°C±2°C/75%RH±5%RH)**			
Batch No	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	The solution should be clear.	A clear solution was found.	A clear solution was found.	A clear solution was found.	A clear solution was found.
F-II	The solution should be clear	A clear solution was found.	A clear solution was found.	A clear solution was found.	A clear solution was found.

pH

pH test for lyophilized product initial and 6month accelerated stability studies for both the Batch No. F-I, F-II result found to be within the observation range and pH range is from 6.5 to 8.5 and the result shown intable 10. Graphical representation of pH was shown in figure 7.

Table 10. Accelerated stability result for pH

Test- pH		Mean ± standard deviation (n=3)			
		Accelerated Condition (40°C±2℃/75%RH±5%RH)			
Batch No	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	From 6.5 to 8.5	7.05±0.129	7.35±0.129	7.85±0.129	8.15±0.129
F-II	From 6.5 to 8.5	6.75±0.129	7.25±0.129	7.75±0.129	7.95±0.129

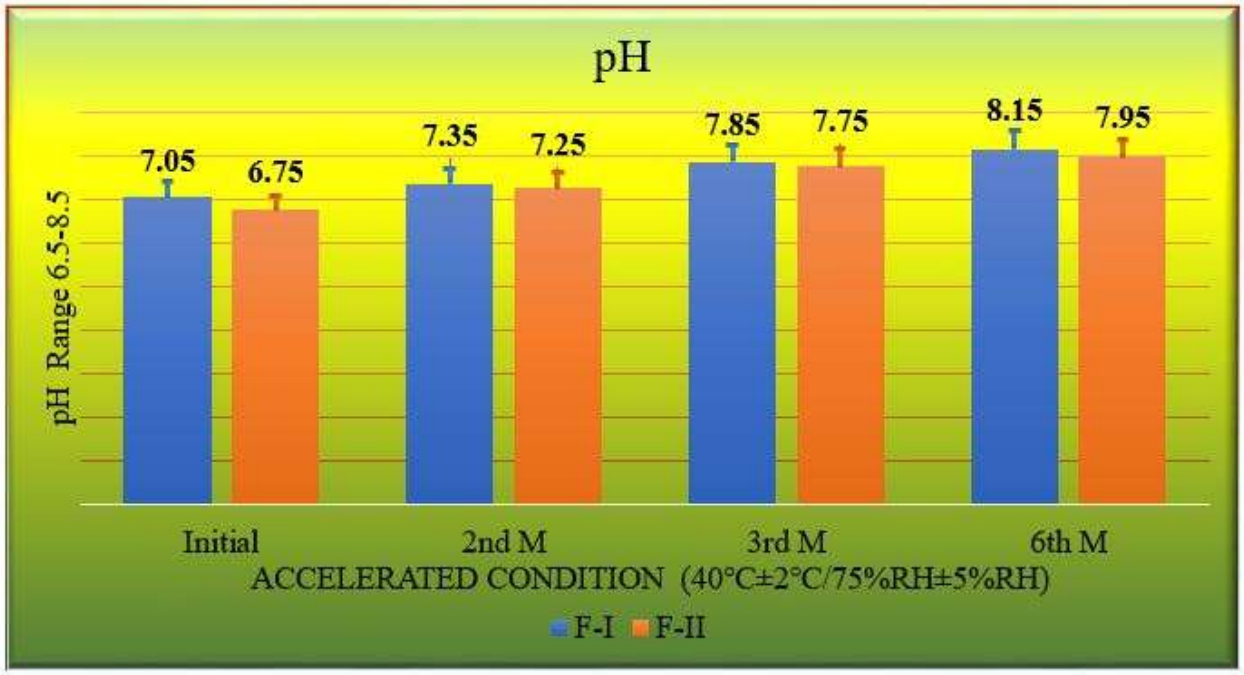

Figure 7. Graphical representation of pH initial to 6th-month study Batch No. F-I, F-II

Particulate matter

Particulate matter test for lyophilized product initial to 6thmonth accelerated stability studies for both the Batch F-I, F-II result for visible particles & invisible particle found to be within the observation range and the for particulate matter shown intable 11.An invisible particle which observed during the stability study does not affect the stability of thelyophilized product.

Table 11. Accelerated stability result for particulate matter

Test- Particulate Matter			Accelerated Condition (40°C±2℃/75%RH±5%RH)			
Batch No	**Method**	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	Visible particles	Visible particles should be absent.	No, any visible particles observed.	No, any visible particles observed.	No, any visible particles observed.	No, any visible particles observed.
	Invisible particle	Particles ≥ 10 µm NMT 25 per ml	04 Particles per ml	07 particles per ml	07 particles per ml	11 particles per ml
		Particles ≥ 25 µm NMT 3 per ml	00 Particles per ml	00 particles per ml	00 particles per ml	01 particles per ml

F-II	Visible particles	Visible particles should be absent.	No any visible particles observed.	No any visible particles observed.	No any visible particles observed.	No any visible particles observed.
	Invisible particles	Particles ≥ 10 µm NMT 25 per ml	05 particles per ml	09 particles per ml	15 particles per ml	17 particles per ml
		Particles ≥ 25 µm NMT 3 per ml	00 particles per ml	00 particles per ml	00 particles per ml	00 particles per ml

Related substances (impurity)

Related substances (impurity) for lyophilized product initial and 6month accelerated stability studies for both the Batch F-I, F-II result found to be within the observation limit. Impurity obtained stability study initial month(F-I, 0.08%, F-II, 0.05%) 6th month (F-I, 0.16%, F-II, 0.13%). Where the limit is NMT 0.2%m-aminophenol, NMT 0.2%. Result for related substance was shown intable 12. Related substances that found in the lyophilized product doesn't affect the stability of the lyophilized product. Graphical representation for related substance both Batch F-I, F-II was shown in figure 8.& figure 9.

Table 12. Result obtained during stability study for related substances (impurity)

Accelerated Condition(40°C±2°C/75%RH±5%RH)						
Batch No	**Test**	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	Related Substances (Impurities) By HPLC	m-Aminophenol: NMT 0.2%	0.05%	0.08%	0.10%	0.13%
		Individual unidentified impurity: NMT 0.2%	0.01%	0.05%	0.09%	0.12%
		Total unidentified impurities: NMT 0.5%	0.04%	0.08%	0.10%	0.13%
F-II	Related Substances (Impurities) By HPLC	m-Aminophenol: NMT 0.2%	0.08%	0.11%	0.14%	0.16%
		Individual unidentified impurity: NMT 0.2%	0.01%	0.07%	0.13%	0.14%
		Total unidentified impurities: NMT 0.5%	0.03%	0.07%	0.08%	0.10%

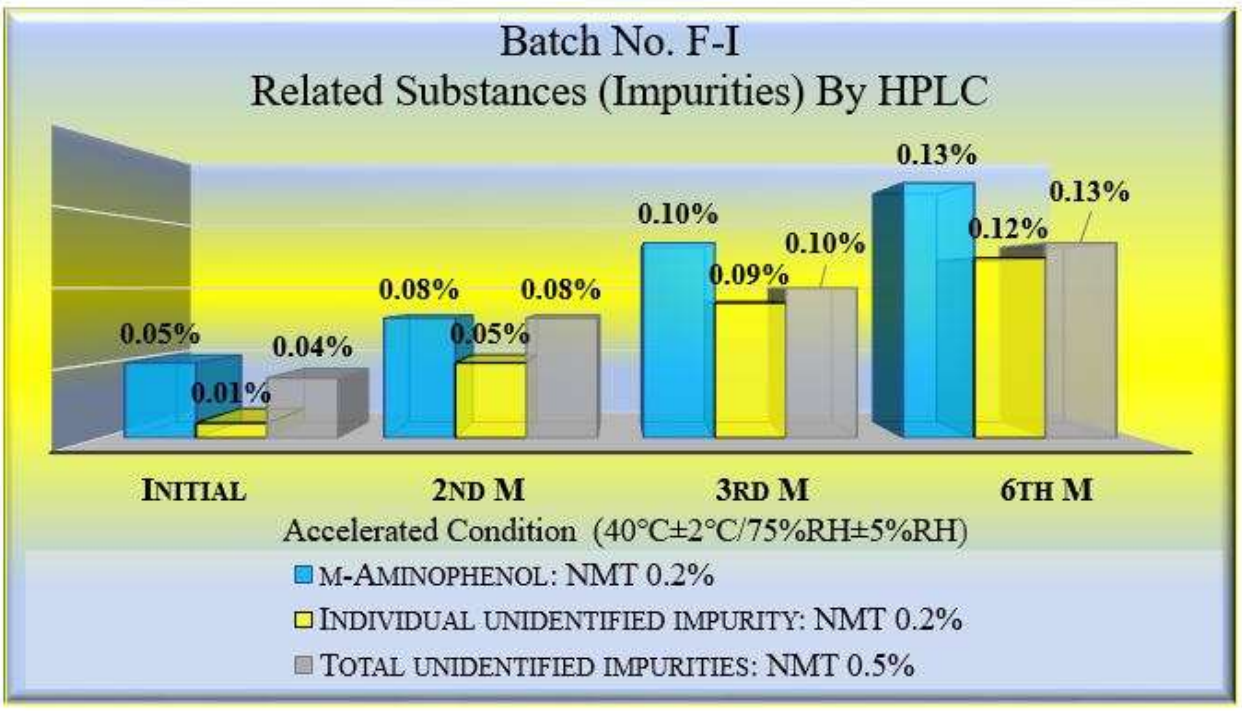

Figure 8.Graphical representation for therelated substance of Batch No. F-I shows impurity profile obtained during accelerated stability study.

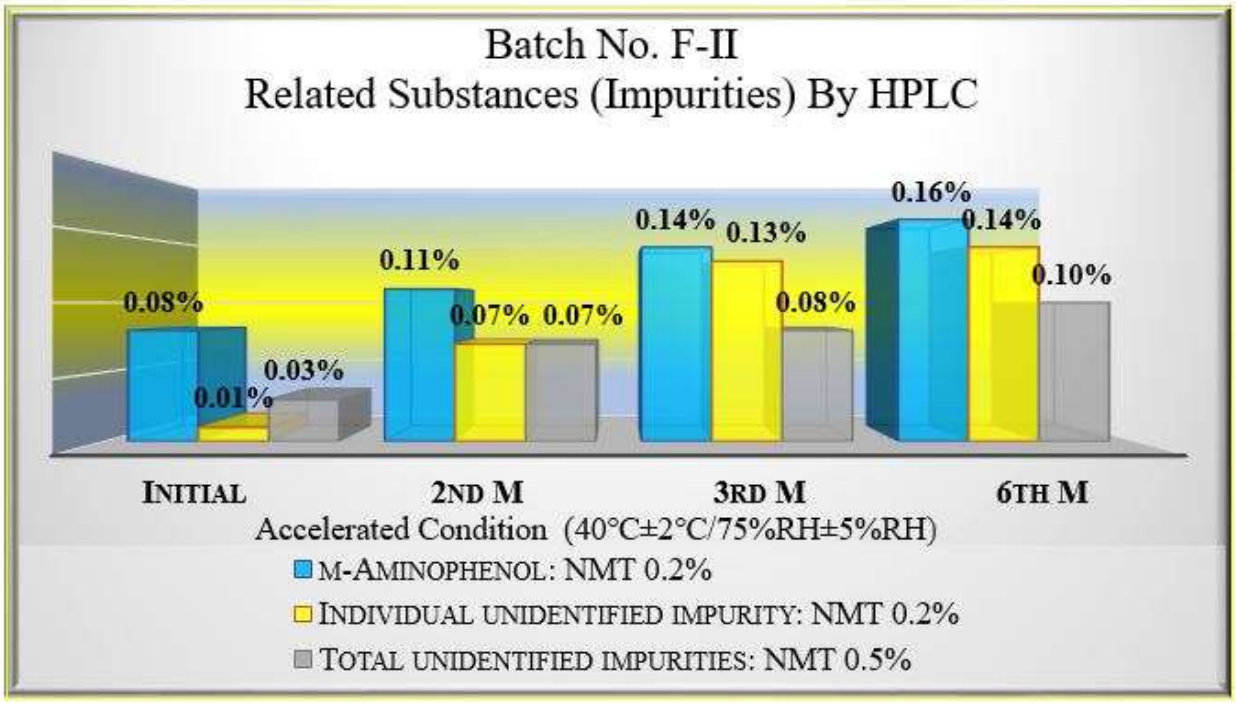

Figure 9. Graphical representation for related substance for Batch No. F-II showsimpurity profile obtained during accelerated stability study.

Determination of water content

Water content result for lyophilized product initial and 6month accelerated stability studies for both the Batch F-I, F-II result shows that initial (F-I 1.5%, F-II 1.4%) 6month (F-I 2.7%, F-II 2.4%) respectively. Result found within the limit NMT 4.0%. observed data during stability test shown in table 13. Graphical representation for water content both Batch F-I, F-II was shown in figure 10.

Table 13. Observed data for water content by Karl-Fisher method during stability study

Accelerated Condition(40°C±2°C/75%RH±5%RH)						
Batch No	**Test**	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	Water K. Fischer method	NMT 4.0%	1.5 %	1.8%	2.0%	2.7 %
F-II	Water K. Fischer method	NMT 4.0%	1.4%	1.6%	2.2%	2.4%

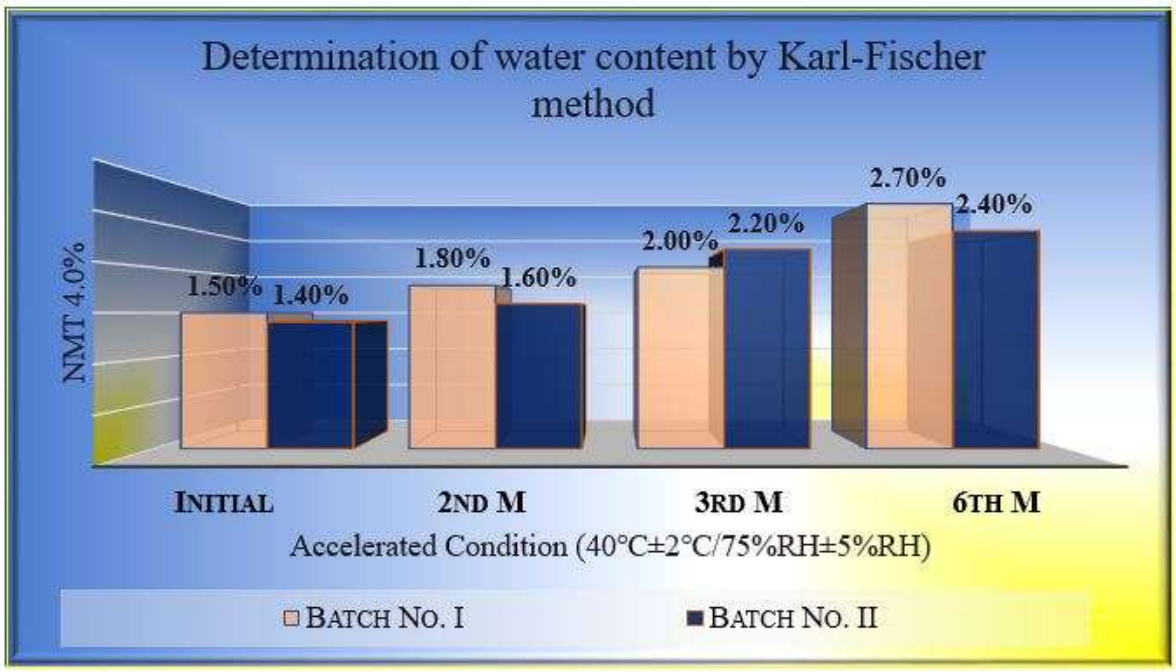

Figure 10. Graphical representation of water content observed during stability study for Batch No. F-I, F-II.

Bacterial endotoxins test

Bacterial endotoxins test for lyophilized product initial and 6month accelerated stability studies for the Batch No. F-I, F-II result shows that it found to be less than 0.024 EU/mg. result for bacterial endotoxins tests shown intable 14.

Table 14. Bacterial endotoxins result obtained during accelerated stability study

Accelerated Condition (40°C±2°C/75%RH±5%RH)						
Batch No	**Test**	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	BET	NMT 0.024EU/mg anhydrous sodium Aminosalicylate	Found less than 0.024 EU/mg	Found less than 0.024 EU/mg	Found less than 0.024 EU/mg	Found less than 0.024 EU/mg
F-II	BET	NMT 0.024EU/mg anhydrous sodium Aminosalicylate	Found less than 0.024 EU/mg	Found less than 0.024 EU/mg	Found less than 0.024 EU/mg	Found less than 0.024 EU/mg

Assay

Assay of Para-Amino Salicylate Sodium(By HPLC) for lyophilized product initial and 6month accelerated stability studies for the Batch No. F-I, F-II result shows that reduced in the assay percentage when compared with initial (F-I 102.14%, 13.7789 g, F-II 100.49%, 13.5565g) and 6month (F-I 98.61%, 13.3021g, F-II 96.27%, 12.9879g) stability study. Assay % obtained during stability study found within the observation limit from 95% to 105% and 12.8155g to 14.1645g/bottle. Result for assay observed during stability study shown in Table 15. Graphical representation for assay% Batch No. F-I, F-II was shown in figure 11.

Table 15. Assay result obtained during accelerated stability study

Accelerated Condition (40°C±2°C/75%RH±5%RH)						
Batch No	**Test**	**Observation**	**Initial**	**2nd M**	**3rd M**	**6th M**
F-I	Assay of Aminosalicylate sodium by HPLC	From 12.8155 to 14.1645 g/bottle, from 95 % to 105 %	13.7789g 102.14%	13.6196g 100.96%	13.3516g 98.97%	13.3021g 98.61%
F-II	Assay of Aminosalicylate sodium by HPLC	From 12.8155 to 14.1645 g/bottle, from 95 % to 105 %	13.5565g 100.49%	13.4865g 99.97%	13.1875g 97.75%	12.9879g96.27%

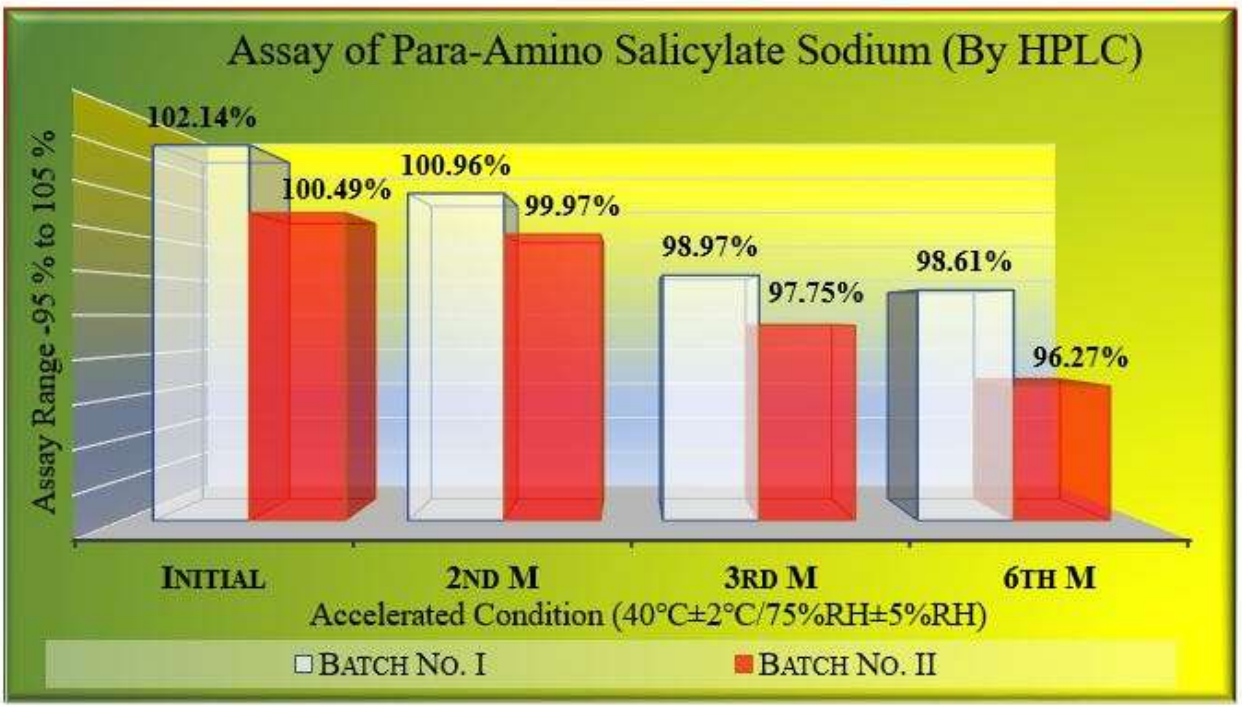

Figure 11. Graphical representation of Assay percentage obtained during accelerated stability study Batch No. F-I, F-II.

Conclusion

The final cause of research work in the field of pharmaceuticals is to develop such a product which is highly influenced and secure. Para-Amino Salicylate Sodium which is used in the treatment of multi-drug resistance tuberculosis shows unstable nature and high level of impurities in aqueous solution. Here lyophilization technique is introduced to increase self-life and stability of drug that is thermolabile and moisture sensitive in nature and generally degraded in atmospheric condition. Study of the effect of excipients over

impurity level confirms that sodium metabisulphite increases the percentage of impurity, whereas sodium sulphite and other excipients reduce it significantly. Optimized lyophilized cycle time was found to be 71hours. Results of accelerated stability study provide conclusive evidence that lyophilization technique is beneficial for drugs which are unstable and shows a high impurity level in an aqueous solution. Thus, from a currentstudy, it can be concluded that lyophilized injection of Para-Amino Salicylate sodium is more physically & chemically stable and provide better therapeutic benefit in MDR-TB.

Reference

1. Sarkar S and Suresh M.R., An overview of Tuberculosis Chemotherapy-a literature review, J Pharm Pharmaceut Sci., 2011, 14, 148-161.
2. Goletti D, Weissman D, Jackson RW, Graham NM, Vlahov D, Klein RS, Munsiff SS, Ortona L, Cauda R and Fauci AS., Effect of Mycobacterium tuberculosis on HIV replication. Role of immune activation, J. Immunol.,1996, 157, 1271-1278.
3. Mariani F, Goletti D, Ciaramella A, Martino A, Colizzi V andFraziano M., Macrophage response to Mycobacterium tuberculosisduring HIV infection relationships between macrophage activation and apoptosis, Curr Mol Med.,2001, 1, 209-216.
4. Strohmeier GR and Fenton JM., Roles of lipoarabinomannan in the pathogenesis of tuberculosis, Microbes Infect.,1999, 1, 709-717.
5. Momekov G, Momekova D, Stavrakov G, Voynikov Y and Peikov P., Para-aminosalicylic acid biopharmaceutical pharmacological and clinical features and resurgence as an antituberculous agent, Pharmacia., 2015, 62, 25-51.
6. Schaberg T, Forssbohm M, Hauer B, Kirsten D, Kropp R and Loddenkemper R., Richtlinien zurmedikamentosen Behandlung der Tuberkulose im Erwachsenen- und Kindesalter, Pneumol-ogie.,2001,55, 494-511.
7. Rengarajan J, Sassetti CM, Naroditskaya V, Sloutsky A, Bloom BR and Rubin EJ., The folate pathway is a target for resistance to the drug para-aminosalicylic acid (Para-Amino Salicylate Sodium) in myco-bacteria, Mol Microbiol.,2004,53, 275-82.
8. Khairnar S, Kini R, Harwalkar M, Salunkhe K and Chaudhari SR., A review on freeze drying process of pharmaceuticals, Int. J. Res. Pharm. Sci., 2013, 4, 76-94.
9. Vishwasrao SS., A review on rational design to optimize stable lyophilized parenteral products, Int. J. Pharm. Sci. Rev. Res.,2014, 29, 169-178.
10. Rautela S, Badola A, Verma R, Baluni S and Kothiyal P., A review: the lyophilization process for the development of unstable drug, Eur. J. Biomed. Pharm. Sci., 2015, 2, 1137-1151.
11. Jennings TA., Lyophilization: introduction and basic principles. Colorado, Interpharm Press., 1999, 4-12.
12. Patel RP, Patel KP, Modi KA and Pathak CJ., Formulation, development and evaluation of injectable formulation of Aspirin, Drugs Ther. Stud., 2013, 3, 6-12.
13. Bouma M and Nuijen B., Pharmaceutical development of a parenteral lyophilized formulation of the antimetastatic ruthenium complex NAMI-A, Int J Pharm.,2002,248, 247-59.
14. Venna DP, Allam SN and Phanindra., Enhancing stability of anti Ulser drug through lyophilization technique, IJSRP., 2013, 3, 1-13.
15. Randolph, James A, Searles, John F, Carpenter and Theodore W., The ice nucleation temperature determines the primary drying rate of lyophilization for sample frozen on a temperature-controlled shelf, J Pharm Sci., 2001, 90, 860-71.
16. Xiang J, Hey JM, Liedtke V and Wang DQ., Investigation of freeze-drying sublimation rates using a freeze-drying microbalance technique, Int J Pharm., 2004, 279, 95-105.
17. Abdelwahed W, Degobert G, Stainmesse S and Fessi H., Freeze-drying of nanoparticles: Formulation process and storage considerations, Int J Pharm., 2005, 309, 178-88.
18. Wallen AJ, Van Ocker SH, Sinacola JR and Phillips BR., The effect of loading process on product collapse during large-scale lyophilization, J Pharm sci., 2009, 3, 997-1004.

*****