RESEARCH ARTICLE

Formulation Development and Evaluation of Freeze-dried Aviptadil Injection using Mannitol as Cryoprotectant

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ABSTRACT

Introduction: The naturally occurring human polypeptide known as vasoactive intestinal polypeptide (VIP) has a variety of physiological effects that have been well-documented, including anti-inflammatory, immune-modulatory, anti-hypertensive, enhancement of cardiac contractility, vasodilation, and fostering immune-neuroendocrine connection. Aviptadil (AVP) is the name of the vasoactive intestinal polypeptide's synthetic version.

Aims and Objectives: The main goal of this work was to create a novel, stable, lyophilized version of aviptadil injection. The stability of aviptadil is of utmost importance due to its classification as a polypeptide, recommended storage condition of -20°C, and susceptibility to degradation in aqueous solutions. To achieve this, the aviptadil injection was processed using freeze-drying technology with the addition of mannitol, serving as a bulking and cryoprotectant agent, within an aqueous solvent system. The choice of cryoprotectant and solvent system was based on factors such as the drug substance's solubility, stability, and feasibility in the manufacturing process. During the development of the formulation, the bulk solution underwent evaluation to assess the effects of process time, temperature, and compatibility with the materials it came into contact with.

Results and Discussion: The incorporation of mannitol, a sugar alcohol, led to the stability of the bulk solution for up to 24 hours before lyophilization when stored at temperatures between 2 and 8°C. Moreover, enhanced stability was observed post freeze-drying. The lyophilization process was meticulously optimized, taking into account critical quality attributes such as description, active drug content, pH of the reconstituted solution, reconstitution time, moisture content, and color absorption percentage.

The bulk solution demonstrated compatibility with various materials employed in manufacturing the drug product, such as stainless-steel vessels, polyethersulfone (PES) and polyvinylidene difluoride (PVDF) membrane filters. Notably, when the drug product bulk solution was kept refrigerated for up to 24 hours, there were no appreciable changes in the critical quality features found. The optimized freeze-dried product successfully meets the quality target product profile (QTPP)'s preset acceptance criteria.

Conclusions: The stabilization of AVP injection was successfully achieved through the implementation of the lyophilization process with mannitol as the cryoprotectant. The envisaged injectable formulation proves to be safe and showcases its economic viability, convenience, and overall safety in the preparation methods. These findings strongly support the viability of the freeze-dried formulation as a technically sound solution for ensuring the stability of aviptadil as a drug substance within the freeze-dried injectable dosage form. This formulation warrants more research due to its potential to treat patients with conditions such acute respiratory distress syndrome, acute lung injury, pulmonary fibrosis, and sarcoidosis.

Keywords: Aviptadil, Critical quality attributes Freeze dried, Cryoprotectant, Injectable,Mannitol, Vasoactive intestinal polypeptide, Lyophilization.

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INTRODUCTION

Vasoactive intestinal polypeptide (VIP), a frequently occurring, naturally occurring polypeptide in people, has a variety of physiological effects that have been well-documented, including anti-inflammatory, immune-modulator, anti-hypertensive, augmentation of cardiac contractility, vasodilation, and fostering immune-neuroendocrine connection. VIP's synthetic equivalent, aviptadil (AVP),

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increases adenosine cyclase activity, which causes smooth muscle relaxation.[1] In order to make usage of AVP in pulmonary fibrosis, acute lung injury (ALI), sarcoidosis and acute respiratory distress syndrome (ARDS), investigational new drug (IND) status and the orphan drug designation have both been granted to relief therapeutics. A neurotransmitter termed VIP, which comprises 28 amino acids and is found in the male genitourinary tract, may have a role in the control of smooth muscle activity and penile contraction. AVP appears to have a specialized role in, bronchodilation and systemic vasodilation.[2] AVP is essentially insoluble in acetonitrile but easily soluble in water and methanol. Due to its hygroscopic nature, AVP must be stored in tightly closed containers at a temperature between -20°C, away from moisture and light. AVP's calculated molecular weight is 3325.8 Daltons and its chemical formula is $C_{147}H_{238}N_{44}0_{42}S$. Figure 1 depicts the molecular structure of AVP0.[3]

MATERIALS AND METHODS

Vasoactive intestinal polypeptide (Aviptadil) was provided as gift sample by MSN Laboratories pvt. ltd. Mannitol was procured from Merck. Other excipients were of analytical grade. The proposed study's main aim is to develop a stable dosage form of aviptadil injection using cryoprotectant. Pre-formulation activity, bulk solution solubility and stability in the presence of cryoprotectant, bulk solution hold time evaluation at various temperatures prior to lyophilization, compatibility of bulk solution of drug product with various contact material, optimization of lyophilization cycle with desired water contented and other critical quality attributes (CQA) are all included in the total development work.

Selection of Ingredient in Formulation

Based on available literature and references, some commonly used excipients were evaluated for proposed injectable formulations with specific functions.

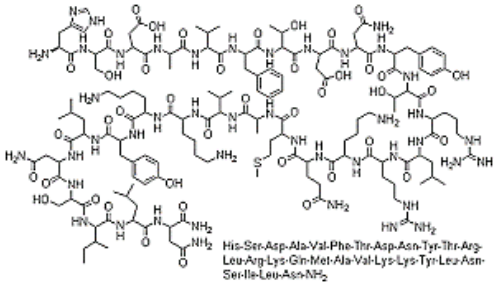

Figure 1: The synthetic form of VIP chemical structure (Aviptadil)

Table 1: Material evaluated for below developmental work

Ingredients	*Manufacturer*	*Function*
Mannitol	Merck	Cryoprotectant
Hydrochloric acid		pH adjustment
Sodium hydroxide		pH adjustment
Water*	-	Solvent

*Water will be removed after lyophilization from final drug product

In the proposed novel injectable formulation, mannitol was selected as a cryprotectant in addition to other pH modifying ingredients (hydrochloric acid and sodium hydroxide). Water work as a solvent. All the listed materials had their risk evaluated as part of the QbD process. Throughout the development study, the quantity of each ingredient was optimized. Based on research in the literature and scientific understanding, the critical material characteristics for each material were determined.[4]

Mannitol is commonly used as a cryoprotectant in peptide freeze-drying (lyophilization) technology. Mannitol is a valuable cryoprotectant in peptide freeze drying technology due to its ability to prevent ice crystal formation, preserve peptide structure, reduce oxidation, and aid in controlled drying. These properties make it an essential component in the formulation of peptide-based pharmaceuticals and biologics. It helps ensure that the peptides maintain their stability, efficacy, and quality throughout the freeze-drying process and during storage.[5]

Table 1 lists the materials used in the medicinal formulation. The product's material was chosen in accordance with global pharmacopeial standards.

Manufacturing Process Optimization

The development efforts aimed to create a new pharmaceutical formulation matching the desired quality target product profile (QTPP). Both the quantitative and qualitative compositions were established through literature review and preliminary experimental trials. This drug product was designed to meet finished product specifications and general requirements for injectable dosage forms. Process components were chosen to align with manufacturing feasibility and compatibility with the aviptadil injection's bulk solution. As part of process development, the interaction of the product solution with materials like stainless steel, glass, and filters was evaluated. Temperature sensitivity of the bulk formulation was assessed.[6] The outcomes are summarized in the results section.

Solubility study of active drug substance

The proposed drug substance (AVP) is essentially insoluble in acetonitrile but easily soluble in methanol and water. Water was chosen as the solvent system for the suggested medication formulation since drug substance is soluble in water. The drug substance's solubility in water, ethanol, and acetonitrile was assessed. AVP's solubility was assessed at a

Table 2: Solubility of Active drug substance

Drug substance (mcg/mL)	*Solvent*	*Excipient*	*Observation*	
			Initial	*After 48 hours at 2–8°C*
100	Water	None	Soluble clear solution	Soluble clear solution
100	Water	Mannitol	Soluble clear solution	Soluble clear solution
100	Ethanol	None	Soluble clear solution	Soluble clear solution
100	Acetonitrile	None	Insoluble soluble	Insoluble soluble

Table 3: Stability of AVP Injection at different temperatures when solution hold in stainless steel vessel

Test parameters	*Initial*	*8 Hours*		*24 Hours*	
		5 ± 3°C	*Room temperature*	*5 ± 3°C*	*Room temperature*
Description	Clear solution	Clear solution		Clear solution	
pH of bulk	6.6	6.2	6.7	6.4	6.7
Drug content (By % of label amount)	106	104.7	102.4	103	63.1

Values represented as mean ± SD (n = 3).

Table 4: Stability of AVP Injection at different temperature when solution hold in glass beaker

Test parameters	*Initial*	*8 Hrs*		*24 Hrs*	
		5 ± 3°C	*20–25°C*	*5 ± 3°C*	*20–25°C*
Description	Clear solution	Clear solution		Clear solution	
pH of bulk solution	6.6	6.1	6.3	6.3	6.5
Drug content (By % of label amount)	106	105.2	103.4	102	65.1

Values represented as mean ± SD (n = 3).

100 mcg/mL concentration in water with 50 mg/mL mannitol concentrations. Table 2 provides a summary of the study's findings.

Drug product solution stability and compatibility

By using a concentration of 100 mcg/mL of aviptadil in 50 mg/mL concentrations of cryoprotectant, bulk constancy was assessed. The bulk was kept in stainless steel and glass vessels at standard interior temperature (RT) and 2 to 8°C. After a defined time period, 0, 8, and 24 hours, study samples were collected and examined for CQA against 0 hours (control) sample results. Tables 3 and 4 provide a summary of the study's findings.

Aviptadil bulk solution compatibility with filter membrane

The compatibility of a filter membrane with a bulk solution is an important consideration when selecting a filtration method for various applications, such as laboratory research, pharmaceutical manufacturing, and industrial processes. The compatibility depends on factors like membrane chemical composition, the bulk solution's characteristics, and the filtration's intended purpose. The bulk solution of AVP injection was made, and various flush-out volumes were collected over time to confirm the drug solution's compatibility and make an acceptable filter membrane selection. The results are summarized in Table 5.

Development and optimization of freeze-drying cycles

When a product is lyophilized, water is taken under controlled temperature and pressure without changing its condition from solid to liquid. Pharmaceutical formulations require careful preparation before freeze-drying to manage cold stresses and achieve storage stability and visual appeal. Keeping product temperature well below the critical level during freeze-drying is crucial. Differential scanning calorimetry (DSC) is efficient for assessing this during freeze-dried product development.

Differential scanning calorimetry

In order to properly and exactly identify the critical formulation temperature, it was necessary to collect further information on the distinctive physicochemical behavior of the ingredient in the mixture throughout the freezing process. These results significantly affect the design of the freeze-drying procedure. Glass transition temperature (Tg) of the lyophilized product must be determined in this situation, hence, DSC is crucial. On the suggested formulation for recipe optimization for lyophilization, a DSC analysis was conducted. Using a DSC instrument, the proposed bulk solution sample was examined while being subjected to a series of temperatures. The detailed DSC study results are summarized in Figure 2.

Characterization of Freeze-Dried Aviptadil Injection

In the proposed formulation, mannitol was utilized as a cryoprotectant and bulking agent because it was discovered that the therapeutic substance is sensitive to temperature and significantly degrades in the presence of water or moisture. Lyophilization procedures also helped to eliminate water from the lyophilized medicinal product. It was crucial to combine the pharmacological ingredient with mannitol, as suggested, in order to achieve the intended outcome. For the purpose of assessing the degree to which a pharmacological substance forms a compound with mannitol, Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) were conducted. The result and discussion session present FTIR (Figure 3) and XRD (Figure 4).

RESULTS AND DISCUSSION

Solubility and Stability

The results are reported in Table 2, the medicinal component is nearly insoluble in acetonitrile but has aqueous and ethanol solubility. It was also noted that the medicinal material showed solubility in water when dissolved in the presence of mannitol. After 48 hours of holding of bulk in refrigerator condition, no unexpected physical changes were noticed, which is the worst-case situation for solubility assessment.[7,8]

Stability and Compatibility of AVP Bulk Formulation

The typical analytical result of drug product solution stability at different processing temperatures (5 ± 3°C and standard interior temperature (RT)) in the proposed solvent composition is shown in Table 4.

Table 5: Filter compatibility data with aviptadil injection bulk solution

S. No.	*Sample flush-out volume in mL*	*Filter Type and drug content in %*			
		Description		*%Assay*	
	Initial unfiltered bulk	CCLS*		102.4%	
	Filtered bulk solution (mL)	Polyethersulfone (PES) filter		Polyvinylidene difluoride (PVDF) filter	
		Description	Drug content (%)	Description	Drug content (%)
1	0–5	CCLS*	101.0	CCLS*	92.7
2	5–10		101.7		95.7
3	10–15		101.8		97.8
4	15–20		102.8		100.2

* Clear, colorless liquid solution; values shown as means standard deviations (n = 3).

Bulk solution stability data reveals no significant changes in pH, appearance, and content of the bulk solution up to 8 hours at both storage conditions (5 ± 3°C and standard interior temperature). However, when stored for 24 hours at ambient room temperature, the bulk solution experiences a notable reduction in active drug content compared to the 5 ± 3°C storage. This highlights the heat sensitivity of the active drug and the bulk solution's instability at room temperature within 24 hours. Consequently, it's recommended to maintain the bulk solution at 5 ± 3°C throughout the manufacturing process. Since there was no discernible difference in the results when the data was compared, it was also concluded that the bulk solution was compatible in both stainless steel and glass material.[9-11]

Compatibility of Bulk Solution with Filter Membrane

The bulk solution of AVP injection was prepared and different flush-out volumes were collected over a period and result were tabulated in Table 5.

Based on the information in Table 5, it is evident that the selected filters exhibit no noticeable physical changes or discoloration. Throughout the filtration process of the bulk solution, there was no evidence of fibre formation or filter shedding. The initial active drug content was 102.4% before filtration. After the first 5 mL bulk filtration using a PES membrane filter, the content was measured 101%. Conversely, when utilizing a PVDF filter membrane, there was an approximate 10% drug absorption within the first 5mL of bulk, and after filtering 20 mL of bulk, the content measured 100.2%. This study concludes that the PES membrane filter is more compatible, exhibiting less drug absorption compared to the PVDF filter membrane.[12-14]

Pruduct Lyophilization

Differential scanning calorimetry study

DSC stands as a robust thermal analysis method utilized for investigating the heat transfer phenomena linked with physical and chemical transformations within materials as these phenomena evolve concerning variations in temperature or time. The foundation of DSC is the measurement of the heat difference (enthalpy change) that occurs when a sample and a reference material are put through the same controlled temperature program. The DSC study was performed on drug product bulk solution.[15]

According to the DSC study shown in Figure 2, the curve exhibits a baseline shift between -35 and -30°C, signifying a "glass transition." Furthermore, a peak indicating an exothermic event caused by ice nucleation is visible between -15 and -10°C. Around 0.21°C, an endothermic peak is seen, which corresponds to an endothermic reaction brought on by "melting." The sample may have been in an amorphous form with limited crystallization as a result of quick cooling after heating, according to these findings of ice nucleation after the glass transition and subsequent melting.[16] The product's critical temperature range is thought to be between -15 and 1°C.

Lyophilization cycle optimization data

Based on DSC study and available literature, several lyophilization cycles were examined to optimise the desired cycle to obtain a consistent result by adjusting the vacuum and drying temperature. Amongst the various trials water content was observed to be diverse with different trial. With an adjusted lyophilization cycle, the water content and cake appearance were determined to be satisfactory.[18,19]

Optimized lyophilization cycle for proposed formulation

Table 6 presents the finalized optimized lyophilization cycle after various trial taken using with different value and time of freezing temperature, sublimation and secondary drying with respective vacuum.

In the optimized lyophilization cycle from Table 6, freezing was conducted at -50°C, considering the bulk solution's freezing point, scalability, and uniform freezing across all vials in the lyophilizer. Following freezing, sublimation occurred below -15°C to prevent collapse, beginning at -25°C. A stepwise sublimation process with controlled temperature increments was used for efficient and safe drying. The driving force is the temperature and vapor pressure difference between the sample's sublimation surface and the ice layer on the condenser, with a higher difference leading to faster drying. The vacuum was selected based on vapor pressure requirements; 0.12 mbar was chosen to match a -40°C ice layer, preventing melt-back during sublimation. The desorption temperature for solid

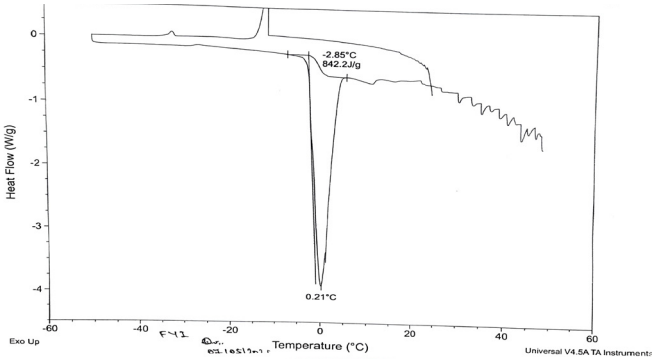

Figure 2: The DSC graph clearly shows the freezing phenomenon and collapse onset

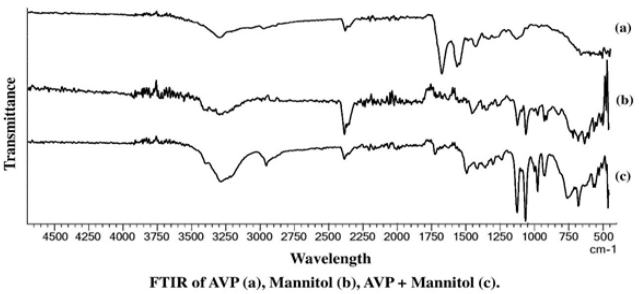

Figure 3: FTIR graph for drug substance, mannitol and freeze-dried product of aviptadil injection

Table 6: Optimized lyophilized cycle for aviptadil injection

Lyophilization steps	*Temperature (°C)*	*Ramp (minute)*	*Hold (minute)*	*Vacuum (mbar)*
Freezing	-50	60	240	Off
Primary drying	-25	30	600	0.120
	-15	30	600	0.120
Secondary drying	25	30	600	0.120

cake drying was set at 25°C. This comprehensive freeze-drying cycle effectively removed water from the drug product, resulting in a stable, lyophilized formulation with desired moisture content and consistent product quality.[17]

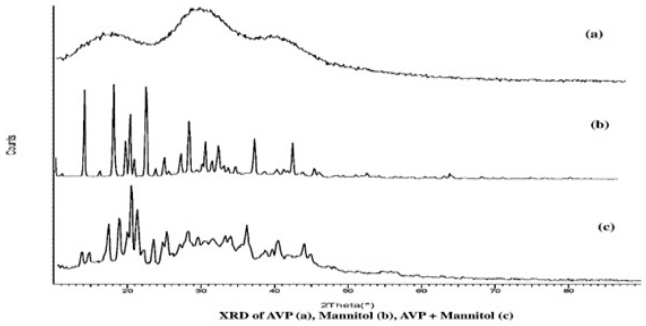

Figure 4: PXRD data for pure drug substance, mannitol and finished drug product of aviptadil injection

Additional Characterization Data for Lyophilized Drug Product

Fourier-transform infrared spectroscopy

To assess the suitability of the freeze-drying process, samples from the freeze-dried medication were subjected to FTIR analysis. FTIR spectra were collected to gain deeper insights into the complexation process between the drug substance and mannitol (Figure 3) using the individual drug material, mannitol, and the lyophilized drug product.

The O-H stretching at 3399 cm^{-1}, the C-H stretching at 2400 cm^{-1}, the C-O stretching at 1082, and at 1119 cm^{-1} were all visible in the IR spectra of mannitol. The mannitol peak was clearly seen at 3399 cm^{-1} (O-H stretching). This peak was moved to a lower frequency of 3250 cm^{-1} in the lyophilized drug sample. This observation's cause is thought to be a result of hydrogen bonds between the oxygen and hydrogen molecules in mannitol and AVP.

Powder X-ray diffraction study

When different substances are solubilized in any suitable solvent and mix and then lyophilized, few changes can affect the crystallographic characteristics of lyophilized drug. Freeze-dried drug product charged for PXRD study. PXRD spectrum Figure 4 was created utilizing mannitol, a single pharmacological ingredient, and a lyophilized product.

As shown in Figure 4, the standard drug substance appears in an amorphous state, while the standard mannitol is in its crystalline form. Fluctuations in intensity values are likely due

Table 7: Evaluation of physicochemical parameter of freeze-dried aviptadil injection

Drug Product Name: Aviptadil injection				
Sr No	*Parameter*	*Results*		
		Initial	*2–8°C*	*Room temperature*
1	Description before reconstitution	WCLC	WCLC	WCLC
2	Description after reconstitution with water	CCS	CCS	CCS
3	Reconstitution time	< 30 sec	< 30 sec	< 30 sec
4	pH of reconstituted freeze-dried cake	6.4	6.2	6.5
5	Colour absorption of reconstituted drug product solution	0.00	0.00	0.01
6	Active drug content (%)	108	104	80.4
7	Water content (%)	1.0	1.2	1.21

* Values are shown as mean SD (n = 3), with WCLC standing for white color lyophilized cack and CCS for clear colorless solution.

to the significant presence of amorphous mannitol in sample. Absence of mannitol hydrate form's distinctive peaks confirms its absence at 16.5, 17.9, 25.7, and 27.0° (2θ) in the freeze-dried, assayed samples of the final aviptadil injection product.

Characterization of developed freeze-dried product

Batch sample was characterized throughout a 6-month period while being stored at 2 to 8°C and under accelerated circumstances at room temperature. Table 7 below lists the results of physicochemical testing and stability characteristics.[19-21]

The stability data shows that the completed lyophilized medication product complies with general injectable standards and the anticipated quality profile for aviptadil injection. Rapid reconstitution, controlled moisture level, and no melt-back in the lyophilized cake are all characteristics of the product. These results attest to the effectiveness of the suggested lyophilization cycle. While samples are subjected to stability testing, the drug concentration drastically decreases from 108 to 80.7% while kept at room temperature. This demonstrates the heat sensitivity of the formulation. However, when lyophilized materials are kept at 2 to 8°C for up to 6 months, there is no discernible alteration. In light of this, it may be concluded that although the temperature sensitivity of the newly developed lyophilized medicinal product allows for extended storage at 2 to 8°C.

CONCLUSION

A new and reliable parenteral formulation of aviptadil injection was intended. The treatment of ailments such ARDS, ALI, pulmonary fibrosis, and sarcoidosis was goal of this formulation. Due to the drug's poor aqueous stability, efforts were made to enhance its stability through the process of lyophilization, with the addition of a cryoprotectant like mannitol in the formulation. The chosen cryoprotectant was found to have no adverse interactions with the drug and demonstrated compatibility with all aspects of the formulation. The bulk solution demonstrated solution stability and compatibility with various materials employed in manufacturing the drug product. Notably, when the drug product bulk solution was kept refrigerated for up to 24 hours, there were no appreciable changes in the critical quality features found. A consistent and effective product was achieved by employing a carefully optimized lyophilization cycle characterized by the appropriate water content. Further analytical characterization studies, such as FTIR and PXRD, provided insights indicating the formation of a stable complex drug product. In the presence of mannitol, this complex was essential for maintaining the stability of aviptadil in its lyophilized form. The resulting aviptadil injection formulation met all the criteria outlined in the quality target product profile. In conclusion, the developed novel freeze-dried formulation, incorporating mannitol, successfully stabilized the aviptadil drug substance in a freeze-dried dosage form.

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