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EVALUATION OF DAPTOMYCIN FORMULATIONS TO CONTROL IMPURITIES

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ABSTRACT

Daptomycin is an antibacterial drug and is a lipopeptide antibiotic used in the treatment of systemic and life-threatening infections caused by Gram-positive organisms. It is a naturally occurring compound found in the soil saprotroph Streptomyces roseosporus. Daptomycin is commercially available in the market as a lyophilized dosage form in various geographies. Approved strengths are 350 mg and 500 mg. commercially, there is no solution form availability of Daptomycin. The reconstitution time is very long which is for about 15 minutes. The lengthier reconstitution time looks difficult during emergency time and also pack insert mentions on the foam formation during the reconstitution time. Literature suggested that the drug candidate is very unstable in the liquid dosage form.

It undergoes degradation in the presence of water upon long storage. Earlier attempts of aqueous based daptomycin formulations were not fruitful due to presence of significant amount of known and unknown impurities. However, attempts with non-aqueous formulations found satisfactory with respect to the control of impurities formation.

Keywords: Daptomycin, Ethanol, DMSO, Soyaben Oil, Propylene Glycol, PEG 400.

INTRODUCTION

The increase in infections caused by Gram-positive pathogens and the rise in antibiotic-resistant bacterial strains have prompted the need for novel antibiotics.[1,2] Recent reports indicate that more than 25% of *Staphylococcus aureus* infections in Europe are caused by methicillin-resistant *S. aureus* (MRSA), and the majority of these isolates are resistant to additional antibiotics.[3]

Daptomycin, a fermentation product produced by *Streptomyces roseosporus*, is a cyclic lipopeptide antibiotic with potent bactericidal activity against most Gram-positive organisms including multiple antibiotic-resistant and -susceptible strains[4-12].

Daptomycin is an intravenously administered cyclic lipopeptide antibacterial agent with potent bactericidal activity against a broad range of Gram-positive organisms. In 2003, daptomycin for injection received approval from the US Food and Drug Administration (FDA) for the treatment of patients with complicated skin and skin structure infections (cSSSIs); in 2006, it was approved for the treatment of patients with *Staphylococcus aureus* bacteremia, including those with right-sided infective endocarditis caused by methicillin-susceptible and methicillin-resistant isolates. Daptomycin has been used to treat patients with bacterial infections of the skin and underlying tissues as well as infections that have entered the bloodstream. Daptomycin is provided by the manufacturer as a powder that requires mixing with a liquid before injection.

Daptomycin, originally designated as LY 146032, was discovered by researchers at Eli Lilly and Company in the late 1980s. LY 146032 showed promise in phase I/II clinical trials for treatment of infection caused by Gram-positive organisms. Lilly ceased development because high-dose therapy was associated with adverse effects on skeletal muscle, including myalgia and potential myositis.

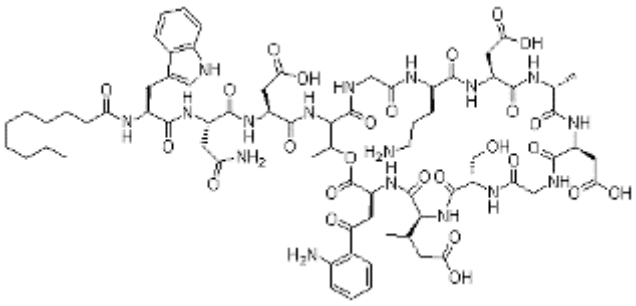
The rights to LY 146032 were acquired by Cubist Pharmaceuticals in 1997, which following U.S. Food and Drug Administration (FDA) approval in September 2003, for use in people older than 18 years, began marketing the drug under the trade name Cubicin. Cubicin is marketed in the EU and in several other countries by Novartis following its purchase of Chiron Corporation, the previous licensee[13, 14].

Daptomycin is supplied in single-use vials containing 500 mg daptomycin as a sterile, lyophilized powder. In some regions of the world, single-use vials containing 350 mg daptomycin as a sterile, lyophilized powder are also available. There is no Daptomycin

formulations available in India. In US, the product is approved in Sep 12, 2003 for 500 mg strength which is reference product. According to the reference product [CUBICIN] pack insert[16], the reconstitution time is very long which is for about 15 minutes. The lengthier reconstitution time looks difficult during emergency time and also pack insert mentions on the foam formation during the reconstitution time. There is a need to overcome both the problems.

daptomycin is approved by the DCGI for the usage of anti-emetic drug. In India, the drug product is approved as the Lyophilized powder Injection of 350 mg/ vial. Lyophilization is a time consuming, tedious and involves cumbersome procedures. Further it involves expensive technology to develop a lyophilized product. Hence, an attempt to develop a non-lyophilized drug product such as liquid formulation which would offer convenience for practitioners by avoiding the reconstitution step when preparing the drug for administration.

Daptomycin is a cyclic lipopeptide antibacterial agent derived from the fermentation of Streptomyces roseosporus. The chemical name is N-decanoyl-Ltryptophyl-D-asparaginyl-L-aspartyl-L-threonylglycyl-L-ornithyl-L-aspartyl-D-alanyl-L-aspartylglycyl-Dseryl-threo-3-methyl-L-glutamyl-3-anthraniloyl-L-alanine ε1-lactone. The chemical structure is:



The empirical formula is C72H101N17O26; the molecular weight is 1620.67.

As per the literature available, daptomycin substance undergoes severe degradation in the aqueous environment. Hence, an attempt to develop a composition focusing on adequate stability while enhancing the solubility necessary for the required therapeutic dose. Hence, an attempt is made to evaluate the simple aqueous based formulations of Daptomycin.

MATERIALS AND METHODS

Daptomycin was procured from Manus Biopharma, Gujarat. Propylene Glycol, PEG 400, Polysorbate 80, Glycerine, Soyabean oil, Ethanol and DMSO were purchased from the commercial sources. All required chemicals used were of standard grade.

Preparation of Daptomycin Formulations

Total of 3 formulations were prepared. The concentration chosen of Daptomycin is 25 mg/mL based on the solubility. Initially, the drug substance was dissolved in the solvents and added one by one excipient per composition table. Finally volume is made to solvent per composition table. pH of the formulation was adjusted to 6.5 ± 0.2

Table 01: Formulation of Daptomycin Injection.

Sl. No.	Ingredients	NADF1	NADF2	NADF3
1	Daptomycin	25 mg/mL	25 mg/mL	25 mg/mL
2	Ethanol	10 mg/mL	10 mg/mL	10 mg/mL
3	Dimethyl Sulfoxide	10 mg/mL	10 mg/mL	10 mg/mL
4	Propylene Glycol	350 mg/mL	--	--
5	PEG 400	--	350 mg/mL	--
6	Glycerine	--	--	350 mg/mL
7	Polysorbate 80	3 mg/mL	3 mg/mL	3 mg/mL
8	Citric Acid	Qs to pH	Qs to pH	Qs to pH
9	Sodium Hydroxide	Qs to pH	Qs to pH	Qs to pH
10	Soyabean oil	QS to 1 mL	QS to 1 mL	QS to 1 mL

NADF Stands for Non aqueous Daptomycin Formulations.

EVALUATION OF DAPTOMYCIN FORMULATIONS

Physical evaluation

***Description*:** This is a physical observation made by individual.

pH: pH was measured using pH meter at about 25°C temperature by diluting the one part of formulations with 10 mL of water.

Water Content: water content of all the formulations were measured using Karl-fischer USP 921 <1a>.

Light Transmission: All the formulations were tested for light transmission at 650 nm using UV spectrophotomer. The formulations were diluted in 1:10 ratio with water and then measured.

Chemical Evaluation:

Assay: HPLC method was adopted to measure the active drug content from the 3 formulations. The active obtained is expressed as percent of labelled amount of Daptomycin content. The obtained value of drug content is expected to be within limits of 90.0% to 110.0% (General compendia like USP & BP requirement)

Related Substances: % content of known and unknown impurities were determined by HPLC method.

RESULTS & DISCUSSION

The results are compiled in the table 2. A clear yellow color solution was observed in all the three formulations. pH of all 3 formulations were adjusted to 6.5 ± 0.2. Light transmission measured for the three formulations found close to 100% indicating the clear transmission of the liquid formulation when the each of the formulations were transmitted through UV spectrophotometer at 650 nm. Water content of all the three formulations were less than 1%. With respect to the chemical analysis of all the three formulations, it was observed that all the three formulations has shown satisfactory assay levels indicating the correct input of % content of daptomycin vs label claim. It also indicates that the analytical method employed for estimating the % content of Daptomycin is correct. From the related substances analysis, it was observed that all the 3 known formulations have higher amount of known and unknown impurities.

Table 2: Physical and Chemical Evaluation of Aqueous Daptomycin Formulations

Sl. No.	Formulation Codes	Description	pH	LT (in%)	Water Content	Assay (in %)	Related Substances
1	NADF1	@	6.34	98.6	0.56%	98.29%	Anhydro Daptomcyin: 0.52% Beta-isomer: 1.74% Lactone Hydrolysis: 0.38% Single Highest UNK Imp: 0.32% Total Imp: 2.88%
2	NADF2	@	6.14	98.5	0.48%	97.8%	Anhydro Daptomcyin: 0.68% Beta-isomer: 2.03% Lactone Hydrolysis: 0.45% Single Highest UNK Imp: 0.38% Total Imp: 3.58%
3	NADF3	@	6.24	99.4	0.52%	97.2%	Anhydro Daptomcyin: 0.65% Beta-isomer: 1.93% Lactone Hydrolysis: 0.55% Single Highest UNK Imp: 0.28% Total Imp: 3.72%

@: Description: A clear colorless solution. LT is Light Transmission.

CONCLUSION

Overall characterization of all the three formulations concluded that no physical description complication were observed. Analytical results of pH and light transmission test parameters were found satisfactory. pH of the formulations is adjusted towards neutral side. Chemical evaluation such as assay test parameter result was observed satisfactory wherein the level of assay in all the three formulations is around 98%.

However, with respect to impurities formation, all the known impurities such as Anydro-Daptomycin, beta-Isomer and Lactone Hydrolysis beta-isomer impurity is on higher side in all the three formulations. However, better control of Anhydro daptomycin and Lactone hydrolysis impurities were observed in non-aqueous formulations. Whereas beta-isomer impurity level in all the three formulations was on higher side. However, better control on the unknown impurity formation was also seen in non-aqueous formulations. From the above experiment, it can be concluded that daptomycin degradation in non-aqueous formulations was lesser when compared to aqueous formulations.

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