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Real-Time Stability of Lyophilized Sabin Formulated Live Attenuated Bivalent Oral Polio Vaccine

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ABSTRACT

The stability of vaccines has a major impact on the success of immunization programmes worldwide. As part of its efforts to assure vaccine quality, ICH harmonized guideline for stability testing of new drug substances and product Q1A (R2) on 6th February 2003 has acknowledged the importance of clearly defining the stability characteristics of a vaccine and emphasizes the role of national regulatory authorities in overall vaccine evaluation. Therefore, this study was initiated with the aim of investigating the real-time stability of lyophilized Sabin formulated bivalent Oral Polio Vaccine (sbOPV) at room temperature. The stability of the lyophilized sbOPV was investigated through various quality attributes at room temperature for two consecutive years in our laboratory, such as physiochemical quality attributes (Colour, Appearance, Moisture content, pH and analysis of Magnesium Chloride) and biological including microbiological quality attributes (potency, identity, sterility, and kanamycin antibiotic activity). The results of the study revealed that lyophilized sbOPV is equally potent in comparison to currently used liquid sbOPV and also found stable at room temperature for two years, and also suggested that there is no need to maintain the cold chain system during transportation, and storage of lyophilized sbOPV.

KEYWORDS: Sabin formulated bivalent Oral Polio Vaccine (sbOPV), Liquid sbOPV, lyophilized sbOPV, Stability, Room temperature, Physiochemical and Biological quality attributes.

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1. INTRODUCTION

Poliomyelitis is a viral contagious disease among five-year-old children worldwide, according to the World Health Organization (WHO). There are abundant literature available, which has already been published by scientists & researchers from different countries in various national & international scientific journals and this is easily accessible through the internet. In continuation of the above, relevant information on the history, taxonomy, properties, clinical manifestation, and pathogenesis of polioviruses as well as conventional and future vaccines for poliomyelitis has been published by Singh and Kumar (2011) [I]. Researchers are still working to improve existing oral and inactivated polio vaccines in terms of stability, potency, safety, and effectiveness [II, III, IV]. In India, Liquid Sabin formulated bivalent Oral Polio Vaccine (sbOPV) including type I and III are using currently for vaccination after switch over from liquid Sabin formulated bivalent Oral Polio Vaccine (stOPV) [V].

Stability of viral vaccines is determined by the rate of loss of "integrity" of the viral active pharmaceutical ingredients & other crucial excipients during storage. For live vaccines, such as measles, mumps, rubella, canine distemper, stability is equivalent to the preservation of the vaccine titres. In 2019, Kumar & Tomar published that the lyophilized form of Sabin formulated bivalent Oral Polio Vaccine (sbOPV) was developed from the liquid form of sbOPV and studied its quality attributes (potency, sterility, identity, pH, & kanamycin antibiotic activity) on a monthly interval basis after storage at room temperature. On the basis of the results, the developed lyophilized form of sbOPV was found

stable for two years at room temperature after comparison with the liquid form of sbOPV [VI]. In continuation of this, the authors decided to extend the stability study of developed lyophilized sbOPV up to two years at room temperature and investigated the physiochemical and biological quality attributes to establish the real-time stability of the lyophilized form of sbOPV at room temperature. The lyophilized sbOPV may be used as an alternative form of the vaccine without maintaining the cold chain system during its storage, and transportation.

2. MATERIALS AND METHODS

2.1 Selection criteria and sample collection of liquid sbOPV batches

In present study, only three consecutive batches were included on the basis of exclusion and inclusion criteria and given batch code as described in table 1.

(a) Exclusion criteria: Expiry date of these batches has not been passed and storage conditions of these batches were satisfied as per GMP guidelines.

(b) Inclusion criteria: These batches were qualified the acceptance criteria of the quality attributes such as potency of type I (not < $10^{6.00}$), potency of type III (not <$10^{5.80}$), Sterility (Sterile product), pH (6.50 to 6.80) and kanamycin antibiotics (15µg per dose) according to Indian Pharmacopeia 2014 [VII].

Table 1: Summary of three qualified sbOPV batches with their code

S. No.	Batch Code of both forms of sbOPV	Selection criteria	
		Exclusion	Inclusion
A	**Liquid**		
A.1.	lsbopv - 01	Passes	Passes
A.2.	lsbopv - 02	Passes	Passes
A.3.	lsbopv - 03	Passes	Passes
B	**Lyophilized**		
B.1.	LSBOPV- 01	*N.A.	*N.A.
B.2.	LSBOPV- 02	*N.A.	*N.A.
B.3.	LSBOPV- 03	*N.A.	*N.A.

*N.A. - Exclusion and inclusion criteria of lyophilized form of sbOPV were not established at this stage.

2.2 Lyophilization process for freeze-drying of sbOPV

Vaccine vials of each liquid sbOPV batch (batch code from lsbopv – 01 to lsbopv– 03) were used to prepare lyophilized sbOPV batch (batch code from LSBOPV-01 to LSBOPV-03), respectively. Lyophilization of all three liquid sbOPV batches were performed as previously described method of Kumar & Tomar (2019) [VI]. Finally, samples of the lyophilized sbOPV were stored in new, transparent, air-tight, and pre-sterilized eppendorf tube (Capacity 2ml) at room temperature.

2.3 Testing of quality attributes of liquid and lyophilized sbOPV for real-time stability

Collected samples were stored at different temperature till completion of the real-time stability study; liquid form of sbOPV at -20^0C ±2^0C and lyophilized form of sbOPV at room temperature. These samples from both the forms of sbOPV vials were used to investigate their physiochemical and biological quality attributes at three months interval during the first year, followed by six months interval of second year as per ICH harmonized guidelines, 2003 [VIII]. The outcome of both lyophilized sbOPV and liquid sbOPV quality attributes were compared to each other for impact of the real-time stability study.

2.3.1 Physiochemical quality attributes

2.3.1.1 Colour

Colour of the lyophilized & liquid sbOPV samples was inspected through necked eye by three experienced and qualified technical officer. Finally, the result was noted and recorded in the table individually.

2.3.1.2 Appearance

Appearance of the lyophilized & liquid sbOPV samples was inspected through necked eye by three experienced and qualified technical officer. Finally, the result was noted and recorded in the table individually.

2.3.1.3 Determination of moisture content

Each batch of lyophilized sbOPV was used to determine the residual moisture content. The percent of moisture content in the lyophilized form of sbOPV was determined by Karl Fischer Titration and it was carried out as previously described by Shin *et al*. (2018) [IX].

2.3.1.4 Determination of pH

Liquid sbOPV was used in the as such form. But twenty doses of lyophilized sbOPV were prepared and reconstituted in 2 ml pre-sterile distilled water under aseptic conditions and kept at room temperature for 10 to 15 seconds to dissolve the vaccine completely. Each batch of liquid and lyophilized sbOPV was used to determine the pH by digital pH method [VI]. Standard pH buffer solutions were used to

calibrate the pH meter before check the vaccine's pH. Finally, the pH of both vaccines was measured and recorded as per standard procedure.

2.3.1.5 Analysis of magnesium chloride

Each batch of lyophilized and liquid sbOPV were used to estimate the Magnesium Chloride by titan yellow method, as previously described by Muller *et al.* (1994) [X] and the same was adopted for analysis of magnesium chloride in developed liquid rabies vaccine by Jagannathan *et al.* (2011) [XI]. In Brief, 100µl of vaccine sample was diluted to 500µl with sterile distilled water and protein gets precipitated by adding 200µl of 5% tri-chloro acetic acid. Then centrifugation is carried out at 2500rpm for 15minutes and 250µl of supernatant was collected. Then 100µl of gumghatti, 100µl of titan yellow and 100µl of 4N NaOH were added. Then the colour development was read at 520nm spectrophotometrically. Simultaneously blank was set with 250 µl of sterile distilled water and followed the same procedure.

2.3.2 Biological quality attributes

2.3.2.1 Potency

Every batch of liquid and lyophilized sbOPV was determined for its potency in single-dose containing type I and type III individually by cell culture technique [VI].

2.3.2.2 Identity

Every batch of liquid and lyophilized sbOPV was determined for its identity of type I and type III by neutralization method, individually [VI]. The test was performed separately as a confirmatory test to find out the impact of lyophilization process of sbOPV for making lyophilized sbOPV and the results of the lyophilized vaccine were also compared with liquid sbOPV.

2.3.2.3 Sterility

Lyophilized sbOPV was inspected through necked eye by three experienced and qualified technical officer. Finally, the result was noted and recorded in the table individually. The sbOPV samples were prepared batch-wise individually and tested for sterility after reconstitute in 2 ml pre-sterile distilled water under aseptic conditions and kept at room temperature for ten to fifteen seconds to dissolve the vaccine completely. Sterility test was carried out with samples of liquid and lyophilized sbOPV on Fluid Thioglycolate Broth (FTB) and Soya-bean Casein Digest (SCD) broth by membrane filtration and direct inoculation methods [VI].

2.3.2.4 Kanamycin antibiotic activity

The stock solution of lyophilized sbOPV was prepared by dissolving the dried powder of twenty doses in 1 ml pre-sterile distilled water and prepared the soak discs (size 5 mm diameter) after drying thirty minutes at room temperature. Each disc was containing antibiotic quantity equal to one dose of liquid sbOPV i.e. 50µl/disc. Finally, kanamycin activity in liquid and lyophilized sbOPV was determined against gram-positive and gram-negative bacteria by disc-diffusion method [XII].

2.4 Statistical Analysis

Test of each quality attribute was performed in triplicate and test-wise mean value was calculated. Finally, the average value of each quality attribute was recorded batch-wise of liquid sbOPV and lyophilized sbOPV in the respective table.

3. RESULT AND DISCUSSION

Bharat Immunologicals and Biologicals Corporation Limited (BIBCOL), is a vaccine production industry. It has well established Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) facility for industrial production and quality control testing of Oral polio Vaccine (OPV) since 1989 and the facility was approved by National Regulatory Authorities; Central Drugs Standard Control Organization (CDSCO) belongs to central authority and State License Authority (SLA) is a state authority. Currently the industry is producing liquid sbOPV including type I and III in GMP facility followed by testing in the GLP approved quality control laboratory after switching from tOPV to bOPV in April 2016 [V]. Therefore current study was initially designed and performed at our organization with an aim to eliminate the cold chain system during transportation and storage of the vaccine and also to improve the shelf life of the vaccine. In 2019, with the efforts of the research team, the company succeeded in development of lyophilized sbOPV form the liquid form and preliminary stability of the developed form was studied only for three month [VI]. The real-time stability of the developed lyophilized sbOPV form has extended for two consecutive years according to ICH guidelines for new drug substances and product Q1A (R2) on 6th February 2003.

In present study, real-time stability of liquid sbOPV (batch code from lsbopv – 01 to 03) and lyophilized sbOPV (batch code from LSBOPV- 01 to 03) was conducted and detail of these batches was summarized in table 1. Physiochemical and biological quality attributes of samples from liquid and lyophilized sbOPV forms were performed for find out the real-time stability of lyophilized sbOPV form at room temperature for two consecutive years and obtained results were compared with results of liquid sbOPV form. As per ICH harmonized guidelines (2003), all quality attributes of the both forms of sbOPV sample vials were investigated at three months interval during the first year, followed by six months interval of second year.

Out of thirteen quality attributes, a total of five physiochemical and eight biological quality attributes were studied at 0, 90th, 180th, 270th, 360th, 540th, and 720th day in stored liquid and lyophilized samples of sbOPV vials from the batches such as colour and appearance of the vaccine form through necked eye, determination of residual moisture content by Karl Fischer titration method, determination of pH by digital pH meter, & estimation of magnesium chloride in percentage by titan yellow method and potency of type I & III by cell culture technique, identity of type I &

III by neutralization method, Sterility by direct inoculation & membrane filtration method, and kanamycin activity against gram-positive & gram-negative bacteria by disc-diffusion method; respectively. Results of each quality attribute of both forms of sbOPV were recorded after calculation of mean value of triplicate test and the mean value of each test for the both forms was summarized in table 2 for real-time stability of liquid sbOPV stored at -20^{0}C temperature and table 3 for real-time stability of lyophilized sbOPV stored at room temperature.

3.1 Physiochemical quality attributes

A total five physiochemical quality attributes of liquid and lyophilized sbOPV were found within specification and qualified criteria throughout two years duration of the study. In liquid sbOPV form, the color and appearance were found as per the specification; pale yellow and clear liquid suspension, respectively. In lyophilized sbOPV form, the color and appearance were found as per the specification; White and powder, respectively.

Analysis of moisture content was not performed in case of the liquid vaccine form, but it was performed with lyophilized vaccine form and determined less than 1% as per the qualifying criteria ranging from 0.61 to 0.63%. pH of liquid and lyophilized form of vaccine was determined in between 6.60 to 6.62 and found to be within specification ranging from 6.50 to 6.80. Percentage quantity of Magnesium Chloride (1 Molar) was analyzed 20.31% during the entire study in the both forms of the vaccine, that was within acceptable range of the specification i.e. 20.30% ±0.1%.

3.2 Biological quality attributes

Potency, and identity of both forms of the vaccine were passed the acceptable criteria during the two years study period viz. $10^{6.00}$ (type I) & $10^{5.80}$ (type III) for potency, and antibodies neutralized of type I & type III for identity.

Sterility of both forms of the vaccine by direct inoculation & membrane filtration method was performed in Fluid Thioglycolate Broth (FTB) and Soya-bean Casein Digest (SCD) and found no growth of bacteria & fungi after 14 days of incubation of 37^{0}C for bacterial growth and 25^{0}C for fungal growth within two year duration of the study.

Kanamycin activity of both form of the vaccine was determined against gram-positive & gram-negative bacteria and efficacy of the kanamycin was found 24.50mm against gram-positive bacteria and 25.50mm against gram-negative bacteria, Kanamycin activity against the bacteria was remain same during the period of two year study.

3.3 Comparison of Liquid and lyophilized sbOPV

All the thirteen quality attributes of both form of sbOPV vaccine including physiochemical & biological were studied to determine real-time stability lyophilized sbOPV form during the two years and the obtained results of every batch were compared one-by-one quality attribute with the results of liquid sbOPV form. After the comparison, it was found that there is no difference in physiochemical & biological quality attributes of both vaccine forms within the two years except difference in colour and appearance, it happened due to liquid and solid powder form of the vaccine & moisture content test did not performed in the liquid vaccine form as it is not recommended in liquid vaccine form.

Table 2: Real-time stability of liquid sbOPV at -20^{0}C temperature

S. No.	Quality attributes	Specification	Results recorded of liquid sbOPV samples on the day						
			0	**90th**	**180th**	**270th**	**360th**	**540th**	**720th**
A	**Physiochemical**								
A 1	Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
A 2	Appearance	Clear liquid suspension	Clear liquid suspension	Clear liquid suspension	Clear liquid suspension	Clear liquid suspension	Clear liquid suspension	Clear liquid suspension	Clear liquid suspension
A 3	Moisture content by Karl Fischer Titration	Less than 1%	NR*	NR*	NR*	NR*	NR*	NR*	NR*
A 4	Determination of pH by digital pH meter	Ranging from 6.50 to 6.80	6.60	6.60	6.61	6.61	6.61	6.62	6.62
A 5	Analysis of Magnesium Chloride (1 Molar) by titan yellow method	20.30% ±0.1%	20.31%	20.31%	20.31%	20.31%	20.31%	20.31%	20.31%
B	**Biological**								
B 1	Potency of type I by cell culture technique	not < $10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$
B 2	Potency of type III by cell culture technique	not <$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$
B 3	Identity of type I by neutralization method	type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I
B 4	Identity of type III by neutralization method	type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III
B 5	Sterility by direct inoculation method	No growth In TSB & FTM	Pass	Pass	Pass	Pass	Pass	Pass	Pass
B 6	Sterility by membrane filtration method	No growth In TSB & FTM	Pass	Pass	Pass	Pass	Pass	Pass	Pass
B 7	Kanamycin activity against gram-positive bacteria by Disc-diffusion method	15µg/dose & ZoI (24.00mm) of the standard	24.50mm	24.50mm	24.50mm	24.50mm	24.50mm	24.50mm	24.50mm
B 8	Kanamycin activity against gram-negative bacteria by Disc-diffusion method	15µg/dose & ZoI (25.00mm) of the standard	25.50mm	25.50mm	25.50mm	25.50mm	25.50mm	25.50mm	25.50mm

Abbreviations: NR* - Not Recommended; ZoI - Zone of Inhibition

Table 3: Real-time stability of lyophilized sbOPV at room temperature

S. No.	Quality attributes	Specification	Results recorded of lyophilized sbOPV samples on the day						
			0	**90th**	**180th**	**270th**	**360th**	**540th**	**720th**
A	**Physiochemical**								
A 1	Colour	White	White	White	White	White	White	White	White
A 2	Appearance	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder

A 3	Moisture content by Karl Fischer Titration	Less than 1%	0.61 %	0.61 %	0.62 %	0.62 %	0.62 %	0.63 %	0.63 %
A 4	Determination of pH by digital pH meter	Ranging from 6.50 to 6.80	6.60	6.60	6.61	6.61	6.61	6.62	6.62
A 5	Analysis of Magnesium Chloride (1 Molar) by titan yellow method	20.30% ±0.1%	20.31%	20.31%	20.31%	20.31%	20.31%	20.31%	20.31%
B	**Biological**								
B 1	Potency of type I by cell culture technique	not < $10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$	$10^{6.00}$
B 2	Potency of type III by cell culture technique	not <$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$	$10^{5.80}$
B 3	Identity of type I by neutralization method	type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I	Pass for type I
B 4	Identity of type III by neutralization method	type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III	Pass for type III
B 5	Sterility by direct inoculation method	No growth In TSB & FTM	Pass	Pass	Pass	Pass	Pass	Pass	Pass
B 6	Sterility by membrane filtration method	No growth In TSB & FTM	Pass	Pass	Pass	Pass	Pass	Pass	Pass
B 7	Kanamycin activity against gram-positive bacteria by Disc-diffusion method	15µg/dose & ZoI (24.00mm) of the standard	24.50mm	24.50mm	24.50mm	24.50mm	24.50mm	24.50mm	24.50mm
B 8	Kanamycin activity against gram-negative bacteria by Disc-diffusion method	15µg/dose & ZoI (25.00mm) of the standard	25.50mm	25.50mm	25.50mm	25.50mm	25.50mm	25.50mm	25.50mm

Abbreviations: ZoI - Zone of Inhibition

A majority of human vaccines are temperature sensitive. The reliance of polio vaccine on the cold chain maintenance prevents exposure to ambient temperatures and also to freezing that can lead to failure of vaccination campaigns. Based on the successful studies and improving methods to generate thermostabile vaccines, which can reduce the number of deaths caused by vaccine-preventable diseases and cut down on the expenditure of the cold-chain system during the transportation [XI]. By keeping in view of the necessity, and with the adoption of same principle to avoid cold chain requirements of viral vaccines especially the rotavirus vaccine, Rotarix vaccine has been developed and manufactured by Glaxo Smith Kline Biologicals Rixensart, Belgium [XIII] for human use as potent & safe live oral rotavirus vaccine and well-established as a vial of lyophilized vaccine to be reconstituted with a liquid diluent in a prefilled oral applicator [XIV, XV].

In our present study, the same ideology has been applied for liquid bivalent oral polio vaccine which is being manufactured in our laboratory to make lyophilized bOPV in order to avoid cold chain necessities. As per the previous study done from our laboratory, only five batches of lyophilized bOPV were studied with quality attributes (Potency, Identity, Sterility, pH, & Kanamycin antibiotic activity) on monthly intervals and found stable at room temperature for three months which qualifies the acceptance criteria as per Indian Pharmacopeia (2014). Current study was designed to perform two consecutive years of real-time stability studies for lyophilized bOPV batches as per ICH harmonized guidelines (2003). Result of the study proved that all three lyophilized sbOPV batches were found stable after evaluation of physiochemical & biological quality attributes and also fulfills the acceptance criteria of Indian Pharmacopeia (2014) [VII] & ICH harmonized guidelines (2003) [VIII].

To achieve the successful development of lyophilized sbOPV, the product was undergone analysis of five physiochemical and eight biological quality attributes same as liquid sbOPV with its active and excipients ingredients, and subsequent investigations of stability study was also established that the newly developed vaccine is found stable up to six months at room temperature. The stability of the lyophilized sbOPV would be further analyzed to establish suitability & palatability of reconstituting solution including its ingredients, protective immunity, and safety of the lyophilized Sabin strains formulated live attenuated bivalent Oral Polio Vaccine (sbOPV) at room temperature for at least two years..

4. CONCLUSION

The objective based real-time stability studies successfully performed for lyophilized sbOPV and analyzed for physiochemical & biological quality attributes according to

Indian Pharmacopeia (2014) and ICH harmonized guidelines. Our study conclusions and achievements successfully determined and strongly recommended that the lyophilized bOPV would not necessitate any cold chain maintenance systems and vaccine vial monitor (VVM) labels during storage, and transportation.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ABBREVIATIONS

sbOPV-Sabin formulated bivalent Oral Polio Vaccine, stOPV-Sabin formulated trivalent Oral Polio Vaccine, OPV-Oral Polio Vaccine, WHO-World Health Organization, IPV-Inactivate Polio Vaccine, VVM-Vaccine Vial Monitor GPEI-Global Polio Eradication, ICH – International Conference Harmonization

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