EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 11
218-226
Year: 2019

DEVELOPMENT OF POTENT AND STABLE LYOPHILIZED SABIN FORMULATED LIVE ATTENUATED BIVALENT ORAL POLIO VACCINE

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Article Received on 09/08/2019 **Article Revised on 30/08/2019** **Article Accepted on 20/09/2019**

ABSTRACT
Current study was performed with aim to develop new lyophilized Sabin formulated bivalent Oral Polio Vaccine for oral administration purpose by using as such liquid form of available Sabin formulated bivalent Oral Polio Vaccine and also investigated various quality attributed in our laboratory such as potency, stability, identity, sterility, pH, and kanamycin antibiotic activity at room temperature for three months. The result of the investigations revealed that the newly developed vaccine is equally potent in comparison to currently using liquid Sabin formulated bivalent Oral Polio Vaccine and also found stable at room temperature for three months. The study may help to eliminate the cold chain system during transportation, storage and vaccination and also useful for its shelf life.

KEYWORDS: Liquid sbOPV, Lyophilization, Lyophilized sbOPV, Quality attributes, Potency, Stability.

1. INTRODUCTION

According to World Health Organization (WHO), poliomyelitis is one of the most viral contagious diseases among five year age of children worldwide. There is an abundant literature available on the internet. In 2011, Singh and Kumar summarized relevant information related to history, taxonomy, properties, clinical manifestation, and pathogenesis of polioviruses as well as conventional and future vaccines.[1] Currently, two types of polio vaccines are available ready to use for pediatric purposes through different administration routes like Oral Polio Vaccine (OPV) for oral administration and Inactivated Polio Vaccine (IPV) for injectable administration. Kumar and Tomar (2019) discussed in detail of these two vaccines with their vaccination schedules and administration routes.[2] In India, liquid Sabin formulated bivalent Oral Polio Vaccine (sbOPV) is using in national immunization program and pulse polio vaccination after switch over from stOPV from April 2016.[3] Nowadays combination vaccine schedule of Sabin formulated bOPV and IPV is using in India as recommended by WHO and it has been cleared that Sabin vaccine strains are safe, potent, effective and cost-effective.

In past decades, researchers were made various attempts to develop more potent, safe and effective polio vaccines but such an attempt is still in progress with different quality parameters. Out of all required parameters, the present study is concern about the cold chain system and its maintenance. Because, maintenance of the cold chain system for liquid sbOPV is the most crucial parameter and sensitive issue throughout its production process, storage and transportation and also concern about the vaccine's self-life. As recommended by WHO, the least sensitive Vaccine Vial Monitor (VVM) is using for continuous monitoring of the vaccine quality from its production to vaccination. Therefore there is need new form of as such sbOPV, which may equally potent and stable as current liquid sbOPV. The first attempt was initiated to develop lyophilized sbOPV without cold chain system from currently available as such liquid form of sbOPV with cold chain system. The newly developed lyophilized sbOPV may useful to eliminate cold chain system and without VVM labels during storage and transportation and also to increase vaccine shelf life.

2. MATERIALS AND METHODS

2.1. Selection criteria of liquid sbOPV batches

All available batches were screened with aim to cover one time produced liquid bOPV batches and only five batches of sbOPV were selected for the study. These batches with codes were arranged in a serial order to ease understanding, used and further their documentation.

All five batches were taking care of following exclusion criteria; Expiry date of these batches has not been passed. Storage conditions of these batches were satisfied as per GMP guidelines and the selected batches are summarized in table 1. 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.[4]

Table 1: Detail of studies sbOPV batches with batch-wise code.

S. No.	Batch Code of sbOPV	
	Liquid	Lyophilized
1	lsbopv - 01	LSBOPV- 01
2	lsbopv - 02	LSBOPV- 02
3	lsbopv - 03	LSBOPV- 03
4	lsbopv - 04	LSBOPV- 04
5	lsbopv - 05	LSBOPV- 05

2.2. Preparation of lyophilized sbOPV from liquid sbOPV

2.2.1. Collection of liquid sbOPV samples

The sample vials of all five liquid bOPV batches were collected after consideration of exclusion criteria. These vials were divided into two parts; one part labeled with batch code from lsbopv - 01 to lsbopv – 05 for further laboratory investigations and second part labeled with LBOPV- 01 to LBOPV- 05 for lyophilization & its quality testing purpose and stored in -20^{0}C ±2^{0}C temperature. In the present study, all collected vaccine vials were used for the preparation of lyophilized sbOPV and its quality testing as well as the samples of the same batches were also used to perform the quality tests of sbOPV. The outcome of both lyophilized sbOPV and liquid sbOPV quality tests were compared to each other.

2.2.2. Lyophilization process for freeze-drying of sbOPV

Vaccine vials of each liquid sbOPV batch with marked code from lsbopv – 01 to lsbopv – 05 were used to prepare lyophilized sbOPV batch with marked code from LSBOPV- 01 to LSBOPV- 05, respectively. Lyophilization of liquid sbOPV was done according to the previously described method[5] with minor modifications. In brief, equal volume 1 ml of the liquid vaccine was transferred in a 1.5 ml microcentrifuge tube under aseptic conditions in laminar airflow and the tubes were kept for pre-dried at -80^{0}C temperature for overnight to accelerate further lyophilization process. Complete lyophilization of sbOPV was processed in grade B area. Before starting the process, the lid of all microcentrifuge tubes was porous with the help of a sterile stainless needle. Lyophilization was performed in three steps in the present study. In step 1, the tubes were loaded into a lyophilizer for freeze-drying (Mcflow Engineering, New Delhi, India) at a shelf temperature of -55^{0}C, and subsequently frozen to -55^{0}C by reducing the shelf temperature at a rate of 1^{0}C/minute with decreasing the chamber pressure up to 0.01 mbar at -55^{0}C ±2^{0}C temperature for one hour. In step 2, the shelf temperature was further increased at a rate of 0.2^{0}C/minute to -50^{0}C with increasing the chamber pressure up to 0.045 mbar followed by drying for three hours. Thereafter, the shelf temperature was increasing up to 25^{0}C at the rate 0.2^{0}C as well as decreasing the chamber pressure up to 0.01 mbar for drying purpose. At the end of the process in step 3, the microcentrifuge tube was closed under vacuum, and then sealed with parafilm. The lyophilized vaccine tubes were kept at +4^{0}C for further experimental purpose and analysis of results.

2.2.3. Determination of residual moisture content in lyophilized sbOPV

Percent moisture content in the lyophilized formulation of sbOPV was determined by Karl Fischer Titration and it was carried out as previously described by Shin *et al.* (2018).[6] Residual moisture content in each lyophilized sample of the vaccine was continuously titrated up to reach an endpoint and results of the experiments were automatically generate. Each sample of the vaccine was measured in triplicate.

2.3. Testing of quality attributes of lyophilized sbOPV

2.3.1. Potency by cell culture technique

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.[7] The reference antisera standard of both types and HEp-2 (Cincinnati) cells were kindly provided by Central Drug Laboratory, Kasauli, Himachal Pradesh, India. Liquid sbOPV is used as such. But lyophilized sbOPV was prepared for potency test after reconstitute twenty doses in 2ml pre-sterile Minimum Essential Medium (MEM) with 5% Foetal Bovine Serum (FBS) under aseptic conditions and kept at room temperature for ten to fifteen seconds to dissolve the vaccine completely.

Confluent monolayer of HEp2 cell was grown in MEM with 10% FBS in 25cm^2 tissue culture flask using standard cell culture techniques[8] and the viable cell count was performed using Neubauer's haemocytometer and trypan blue.[9] HEp2 cell concentration per well of microtitre plate was adjusted up to 10,000 cells/0.1ml by adding MEM with 5% FBS.

Each batch of both vaccine samples and reference antisera standard (Type I & III) were dilution serially in MEM with 2%FBS ranging from $10^{-3.0}$, $10^{-3.5}$,$10^{-4.0}$,$10^{-4.5}$, $10^{-5.0}$, $10^{-5.5}$, $10^{-6.0}$, $10^{-6.5}10^{-7.0}$ and $10^{-7.5}$. 0.05ml volume of each the prepared dilution was dispensed into each of

8 wells of flat-bottomed microtitre plate with lid, starting from higher dilution to lower dilution. The plates were incubated at 35.5^{0}C ($\pm$0.5^{0}C) for three hours adding and vortex mixing of antisera. The incubation was required type-specific antiserum to neutralize of the other types of viral antigens. 0.1 ml of HEp2 cell suspension (10,000 cells/0.1ml concentration) in MEM with 5% FBS was added to all the wells. The plate was sealed and incubated at 35.5^{0}C ($\pm$0.5^{0}C) in a carbon dioxide incubator. The plates were read microscopically on daily basis after 3rd, 5th and 7th day using an inverted microscope for cytopathic effect (CPE), wherein infected cells rounded up, showed shrinkage and marked nuclear pyknosis, became refractile, degenerated and fell off the surface.[10] Kaerber's formula was used to obtain titre per dose (0.1 ml) of type I and type III of both vaccines.[11] Positive and negative controls for the potency test were performed individually and the result of the test was valid when positive and negative control of each experiment performed normally. Therefore the experiment with each sample of both vaccines was performed in triplicate.

2.3.2. Identity by neutralization method

In the present study, the procedure of the identity test of the vaccines was the same as described for the potency test. The vaccine is containing the two types of poliovirus; titration of the individual serotypes is undertaken separately, using mixtures of appropriate type-specific antiserum to neutralize each of the other types present. Therefore, 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.

2.3.3. Sterility by direct inoculation method and membrane filtration method

Sterility test was carried out with samples of both liquid and lyophilized sbOPV on Nutrient Agar Medium (NAM), Fluid Thioglycollate Broth (FTB) and Soya-bean Casein Digest (SCD) broth by membrane filtration and direct inoculation.[12] Lyophilized sbOPV samples were prepared 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. Positive and negative controls were also performed individually and the experiment with each sample of both vaccines was performed in triplicate.

2.3.3.1. Direct inoculation

For direct inoculation, one dose (100µl) of liquid and reconstituted lyophilized sbOPV was transferred through streaking on pre-prepared Nutrient Agar Medium (NAM) Petri-plates and was also transferred in FTB and SCD broth medium in McCartney bottles under laminar airflow. Finally, all inoculated plates and bottles were incubated at 22^{0}C $\pm$2^{0}C for fungal growth and 35^{0}C $\pm$2^{0}C for bacterial growth for 14 days. After the incubation period, all plates and bottles were examined and results were recorded for bacterial and fungal growth.

2.3.3.2.Membrane filtration

For membrane filtration, minimum 100 doses (five vaccine vials) of liquid and reconstituted lyophilized sbOPV was used to check sterility After this, each filter was cut into three pieces with the help of sterile stainless steel scissor and the piece was inoculated individually in FTB, SCD and NAM under laminar airflow. Finally, all inoculated plates and bottles were incubated at 22^{0}C $\pm$2^{0}C for fungal growth and 35^{0}C $\pm$2^{0}C for bacterial growth for 14 days. After the incubation period, all plates and bottles were examined and results were recorded for bacterial and fungal growth.

2.3.4. Determination of pH by digital pH meter

pH of liquid and lyophilized sbOPV was determined by standard method by using digital pH meter. Liquid sbOPV was used in the as such form. But twenty doses of lyophilized sbOPV was 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. 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.

2.3.5. Kanamycin activity by Disc-diffusion method

The disc-diffusion method is a standard method, which was already used for the quantitative evaluation of kanamycin antibiotic activity in a single dose of oral polio vaccine.[13] Based on the present study, 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 and compared by the method.

In disc-diffusion method, Petri plate with equally distributed 20ml of nutrient agar medium was used and 1.0X10^6 cells/ml of bacterial strains; *Bacillus Subtilis* MTCC 441 (Gram-positive bacteria) and *E. coli*, laboratory isolate (Gram-negative) was used for uniform growth on the medium surface. Pre-prepared each disc containing 50µl/disc of lyophilized sbOPV was aseptically transferred on the Petri plates containing nutrient agar medium with bacteria and incubated ay 37^{0}C$\pm$1 for twenty-four hours. After incubation, kanamycin activity in liquid and lyophilized sbOPV was observed and recorded to measure the diameter of the inhibition zone in millimeters.

2.3.6. Stability studies of Lyophilized sbOPV

All the lyophilized sbOPV batches from LBOPV- 01 to LBOPV- 05 were carried out for their stability in terms of potency, identity, sterility, pH, and kanamycin

antibiotic activity at room temperature on monthly interval up to three months at the time interval of 30days, 60 days and 90days. The same procedures were followed to check the potency, identity, sterility, pH, and kanamycin antibiotic activity in the studied batches, as already described above.

RESULT AND DISCUSSION

Polio vaccines whether its IPV or OPV are temperature sensitive. The dependence of current vaccines on the cold chain, which prevents exposure to ambient temperature and also to freezing, presents many obstacles that can lead to failure of polio vaccination. In spite of this, OPV is still used as a cost-effective vaccine for polio vaccination in various developing countries instead of IPV.[3] Immunization with OPV induces superior intestinal immunity compared with IPV and thus has the potential to better prevent transmission of wild viruses as well as OPV has more advantages than IPV such as well-established technology worldwide, oral administration route, comparatively easy to train the personnel's, etc. But IPV has a major advantage over the OPV that polioviruses are inactivated and there is no possibility of Vaccine-associated paralytic polio (VAPP) and Vaccine derived polioviruses (VDPP) cases. Therefore we are in the opinion that the advantage of IPV is directly related to the form of the vaccine and the lyophilization process may play an important role in the vaccine. For these reasons, we used current sbOPV as such a vaccine for the development of the lyophilized vaccine and stored at $+4^0$C. Critical quality attributes of the vaccine were checked and compared with exiting sbOPV such as potency, identity, sterility, pH, and kanamycin antibiotic activity.

Currently, sbOPV is still using for polio vaccination alone and/or before, after or in-between combination of IPV in many countries; like vaccination schedule of polio vaccine in India. As recommended by WHO, the Indian child is vaccinated with IPV in between sbOPV schedule at 3rd month of the age.[3] Bharat Immunologicals and Biologicals Corporation Limited (BIBCOL), Bulandshahr has actively engaged in Oral polio Vaccine (OPV) production since 1989 and presently we are producing liquid sbOPV including type I and III in Good Manufacturing Practices (GMP) approved production facility and Good Laboratory Practices (GLP) approved quality control testing facility after switching from tOPV to bOPV in April 2016.[3] Therefore the present study is designed and performed at our organization with the purpose to eliminate the cold chain system during transportation and storage of the vaccine and to improve the shelf life of the vaccine. With these objects and the basis of the literature survey, we conducted the research and the lyophilization process was performed after the formulation of liquid sbOPV to develop freeze-dried powder form of the vaccine as lyophilized sbOPV. A total of five batches of liquid sbOPV were studied and the same batches were used to prepare lyophilized sbOPV as mentioned in table 1 with the code, respectively and results of the lyophilized vaccine were compared batch-wise with the liquid vaccine and acceptance criteria of current Indian Pharmacopeia.

3.1. Lyophilized sbOPV quality

The lyophilization process was done to prepare freeze powder form from all five batches of liquid sbOPV. In this study, we used as such liquid form of sbOPV according to recommendation of Indian Pharmacopia 2014 formulation in GMP facility at our OPV site and contains poliovirus type I, poliovirus type III, $MgCl_2.H_2O$, Tween - 80, Kanamycin antibiotic and Water For Injection. After numerous attempts, the above-described lyophilization process with its conditions used in three steps were able to develop successfully the lyophilized vaccine. Finally, the powder form of the vaccine was achieved through the lyophilization process. Residual moisture content in batch-wise sample of the lyophilized vaccine was determined less than 1% ranging from 0.77 to 0.89%. Because, small amounts of residual moisture can cause the lyophilate to shrink, collapse, and cake up. Resultant of the product may not be dissolved completely to make the clear solution of the vaccine. The range of residual moisture content was found suitable to dissolve in water for injection and Eagle Minimum Essential Medium (EMEM). Therefore cell culture tests (Potency & Identity) of the lyophilized vaccine were performed only after reconstitute in EMEM with 5% FBS and Sterility, pH & kanamycin activity were performed only after reconstitute in pre-sterile distilled water. In both cases, the reconstitutes were kept at room temperature for two to three minutes to dissolve the vaccine completely before use for laboratory quality investigations.

3.2. Potency of liquid sbOPV and lyophilized sbOPV

All the five batches of liquid sbOPV vials with code LSBOPV -01 to 05 were used to prepared lyophilized sbOPV and then potency was checked of each lyophilized batch for type I and III as well as liquid vaccine batches with code lsbopv 01 to 05. Type-specific potency in flat bottom microtitre plate was observed based on the presence or absence of cytopathic effect (CPE) on the 7th day of incubation under an inverted microscope at 10X and 40X magnification and microscopic images were captured as shown in Fig. 1. Based on CPE, results of the potency tests were recorded and batch-wise summarized in table 2 and compare to each other. Out of the five batches, potency of type I and type III of LSBOPV-02, 03 & 05 of lyophilized vaccine as found the same as the liquid batches (lsbopv-02, 03 & 05). Simultaneously LSBOPV-01 & 04 have difference 0.01 $\log_{10}$ $CCID_{50}$ but under the acceptance criteria of potency type I (not $<10^{6.00}$) and type III (not $<10^{5.80}$).

Table 2: Comparison of Potency results of liquid and lyophilized sbOPV samples.

S. No.	Sample batch code	Type of Poliovirus	Potency titer (in $CCID_{50}$)				
			01	02	03	04	05
1	LSBOPV	I	$10^{6.13}$	$10^{6.09}$	$10^{6.11}$	$10^{6.14}$	$10^{6.16}$
		III	$10^{5.94}$	$10^{5.90}$	$10^{5.91}$	$10^{5.94}$	$10^{5.99}$
2	lsbopv	I	$10^{6.14}$	$10^{6.09}$	$10^{6.11}$	$10^{6.13}$	$10^{6.16}$
		III	$10^{5.95}$	$10^{5.90}$	$10^{5.91}$	$10^{5.93}$	$10^{5.99}$

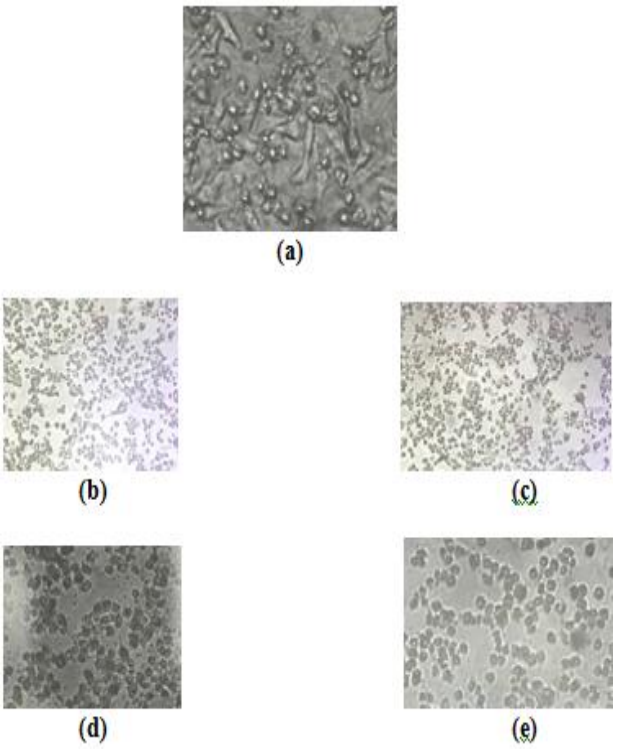

Figure 1: Flat bottom 96 well microtitre plates of both vaccines for potency observed 100% cytopathic effect (CPE) under inverted microscope on 07th day. A: Monolayer of HEp2 cells in 25cm^2 tissues culture flask at 40X magnification after 48 hours. B: CPE of lyophilized sbOPV at 10X magnification. C: CPE of liquid sbOPV at 10X magnification. D: CPE of lyophilized sbOPV at 40X magnification. E: CPE of liquid sbOPV at 40X magnification.

3.3. Identity of liquid sbOPV and lyophilized sbOPV

Neutralization assay was used to identify the presence of type I & III polioviruses by using type-specific antiserum in all batches of liquid and lyophilized sbOPV. The test was performed for both types individually in microtitre plate and results of the identity test were compiled and showing for type I & III of each batch of both vaccines. Type-specific antiserum reference standard was also used in positive control well, therefore neutralization of type I & III poliovirus antigen was observed due to the presence of type-specific antiserum. But neutralization of the antigen and antibody did not due to absence of the antiserum. Finally, all five batches of lyophilized sbOPV have the same pattern of type I & III as indicated in liquid sbOPV batches. The test was proved that there is no effect of lyophilization on type I & III of sbOPV.

Table 3: Identity test of liquid and lyophilized sbOPV samples by neutralization assay.

S. No.	Sample Batch Code	Identity		
		Type I	Type III	
1	LSBOPV	01	+	+
2		02	+	+
3		03	+	+
4		04	+	+
5		05	+	+
6	Lsbopv	01	+	+
7		02	+	+
8		03	+	+
9		04	+	+
10		05	+	+
11	Negative control	-	-	
12	Positive control	+	+	

(+) for presence of neutralization
(-) for absent of neutralization

3.4. Sterility of liquid sbOPV and lyophilized sbOPV

Samples of each batch of liquid sbOPV and lyophilized sbOPV were checked for its sterility and the test was carried out on Nutrient Agar Medium (NAM), Fluid Thioglycollate Broth (FTB) and Soya-bean Casein Digest (SCD) broth by using membrane filtration and direct inoculation methods. Culture media were pre-tested by growth-promoting test and qualified to support microbial growth. All inoculated media plates and McCartney bottles were examined daily for microbial growth up to fourteen days. The plates and bottles of both vaccines and positive control of each experiment were observed and found no microbial growth except negative control. All experiments were performed in triplicate and results were found the same as described in table 4.

Table 4: Results of sterility test of liquid and lyophilized sbOPV samples after 14 days.

S. No	Sample batch code	Result							
		Control		Direct inoculation			Membrane filtration		
		Positive	Negative	NAM	FTB	SCD	NAM	FTB	SCD
1	LSBOPV- 01	+ + ++	----	----	----	----	----	----	----
2	LSBOPV- 02	+ + ++	----	----	----	----	----	----	----
3	LSBOPV- 03	+ + ++	----	----	----	----	----	----	----
4	LSBOPV- 04	+ + ++	----	----	----	----	----	----	----
5	LSBOPV- 05	+ + ++	----	----	----	----	----	----	----
6	lsbopv- 01	+ + ++	----	----	----	----	----	----	----
7	lsbopv- 02	+ + ++	----	----	----	----	----	----	----
8	lsbopv- 03	+ + ++	----	----	----	----	----	----	----
9	lsbopv- 04	+ + ++	----	----	----	----	----	----	----
10	lsbopv- 05	+ + ++	----	----	----	----	----	----	----

Note:

(a) Sample batch code LSBOPV is used for lyophilized sbOPV
(b) Sample batch code lsbopv is used for liquid sbOPV
(c) Microbial growth: 25 % for +, 50% for + +, 75% for +++, and 100% for ++++
(d) No growth of microbes: ----

3.5. Determination of pH

First of all, the digital pH meter was calibrated with a standard solution of pH 4.0 and 9.0 and then used to check the pH of each batch of liquid and lyophilized sbOPV. Results of both were showing in Fig. 2 and found a batch-wise difference only lesser 0.01 pH in lyophilized vaccine as compare to the liquid vaccine except batch 1 with code LSBOPV-01 and lsbopv-01. Based on previous reports, It has been well-established that lyophilized vaccine was dissolved with different reconstitute solutions as well as researchers were considering pH of the solutions as crucial quality attribute to develop clear and stable human vaccine candidate for oral administration routes as oral rotavirus vaccine (16). Present study was performed to investigate the pH as one of the laboratory quality attributes; therefore developed the lyophilized powder of sbOPV was reconstituted in pre-sterile distilled water for determination of pH of the vaccine. The obtained pH of the studied batches was found within acceptance criteria for pH (6.50 to 6.80) of Indian Pharmacopeia 2014. The current study proved that there is an adverse effect on pH after freezing drying process to develop lyophilized from liquid sbOPV.

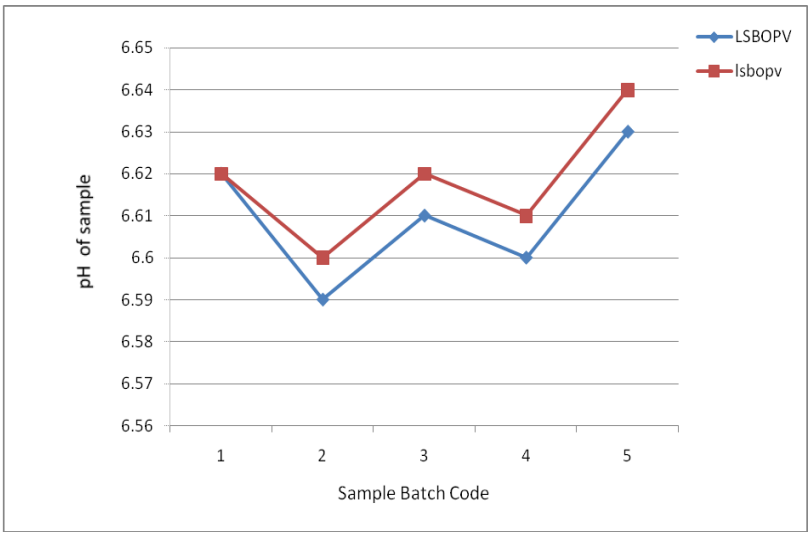

Figure 2: Results of pH of liquid and lyophilized sbOPV samples.

3.6. Kanamycin antibiotic activity

Kanamycin antibiotic is an important excipient in the formulation of sbOPV vaccine and was used to prevent contamination. Therefore it has its importance and also plays as a key quality attribute of the formulations of the vaccine. Kanamycin activity in a single dose of tOPV reported and obtained results were compared with standard kanamycin antibiotic.[13] In the present study, we also checked the activity of kanamycin antibiotic in a single dose of lyophilized sbOPV against *B. subtilis* (Gram-positive bacteria) and *E. Coli* (Gram-negative bacteria) and the results were compared with single dose of liquid sbOPV & equal single dose quantity of standard kanamycin. The antibiotic was found active as zone of inhibition; 24.00mm in standard kanamycin, 24.50mm in lyophilized sbOPV & liquid sbOPV against gram-

positive bacteria and 25.00mm in standard kanamycin, 25.50mm in lyophilized sbOPV & liquid sbOPV against gram-negative bacteria. A comparison of the results is graphically presented in a single dose of both vaccines as showing in Fig. 3.

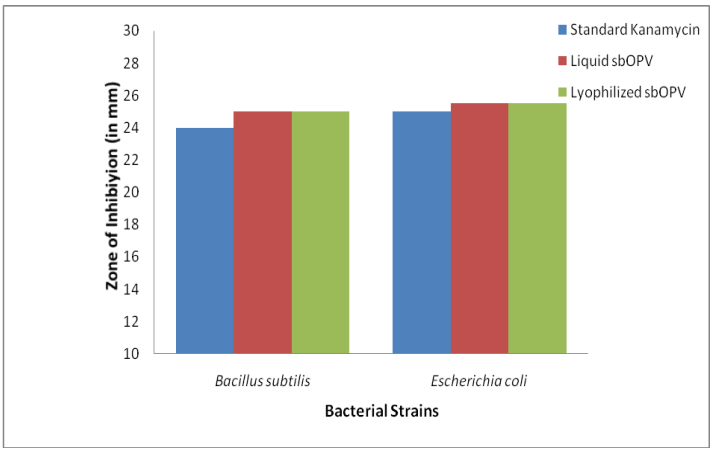

Figure 3: Kanamycin sensitivity in a single dose of liquid and lyophilized sbOPV samples.

3.7. Stability studies

Being as a preliminary stage of the study, only five lyophilized sbOPV batches from LBOPV- 01 to LBOPV- 05 were included in the stability study for three months at the time interval of 30days, 60days and 90days at room temperature and the same quality attributes were evaluated in monthly interval such as potency, identity, sterility, pH, and kanamycin antibiotic activity. The results of all quality attributes were found under the acceptance criteria for up to three months as described at point number 2.1 & in Indian Pharmacopeia 2014 and the result obtained during stability studies, thus indicating stability of newly freeze-dried sbOPV. Based on the present finding, the newly developed lyophilized sbOPV is no more required cold chain system after the lyophilization step during the production stage and without VVM labels during storage & transportation.

The Global Polio Eradication Initiative (GPEI) has seen significant progress since it began in 1988, largely due to the worldwide use of oral poliovirus vaccine (OPV). To achieve polio eradication, the global cessation of OPV is necessary because OPV contains live attenuated poliovirus, which in rare circumstances could re-gain wild poliovirus (WPV) characteristics with potential to establish transmission. In continuation of the above, IPV was developed through introducing lyophilization of polioviruses and it is administrated through dermal injectable route. Lyophilizing of vaccine is challenging in IPV. Since in some studies IPV have obtain stable with preservation of all three serotypes. After April 2016, tOPV was switch on bOPV.[3] Therefore present investigations were initiated to find out the effect of freeze-drying on pre-qualified Sabin strains formulated live attenuated bivalent Oral Polio Vaccine; abbreviated as sbOPV.

A majority of human vaccines are temperature sensitive. The dependence of polio vaccines on the cold chain, which prevents exposure to ambient temperature and also to freezing, presents many obstacles that can lead to failure of vaccination campaigns. Pharmaceutical Commerce reported that $12.5 billion was spent on cold-chain logistics, of which $9.1 billion was for cold-chain transportation and $3.4 billion was for specialized packaging and instrumentation. Thus, improving methods to generate thermostabilized vaccines can reduce the number of deaths caused by vaccine-preventable diseases, and cut down on the expenditure used for cold-chain transport.[6] However, the focus in vaccine developed has been on optimization of the immunological properties, while stability issues are minimally addressed most vaccines are insufficiently stable to allow them to be purified, transported and stored at unrefrigerated conditions. OPV creates challenges in maintaining the cold chain for vaccine storage and transportation for distribution purpose. The temperature sensitivity of bOPV remains a significant hurdle during the immunization campaign, OPV can be stored up to two years at optimal temperature (-20^{o}C to +4^{o}C) outside this range drastically reduce vaccine potency.[15] thus there have been major efforts to improve the stability of OPV and to eliminate the need for the cold chain process during distribution and storage such as the use of an artificial hydrated silica exterior on virion[16], and lyophilization.[17,18] Based on previous success of rotavirus vaccine, Rotarix vaccine has already been developed for human use as 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.[12-14] The Live oral rotavirus vaccine namely Rotarix (Trade Name), which is Manufacturing by Glaxo Smith Kline Biologicals Rixensart, Belgium.[19] Current efforts were made to develop dry powder form using as such liquid sbOPV through the freeze-drying process as lyophilized sbOPV. The study was performed of lyophilized sbOPV after storage at +4^{0}C and the quality attributed to liquid and lyophilized sbOPV were found similar in terms of the quality.

To develop the freeze-dried powder polio vaccine without requirement of cold chain maintenance during production, storage and transportation, the current study established with no difference in quality attributes of lyophilized sbOPV after comparison of result one to each other. Besides this, The newly developed lyophilized vaccine is also found stable for at least three months at room temperature. The study may also support to extend the shelf life span of liquid sbOPV vaccine because the vaccine will be used to produce lyophilized sbOPV before the date of expiry of the liquid polio vaccine and the lyophilized vaccine is considered as new product & it has its extended expiry date.

4. CONCLUSION

To achieve the successfully developed lyophilized sbOPV with quality attributes from as such form of liquid sbOPV with its active and excipients ingredients, further investigations of stability study was also established that the newly developed vaccine is stable up to three months at room temperature. In future, investigations will be required in direction of its stability at room temperature for at least two years, 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) and the investigations are still in progress to accomplish and to develop the vaccine successfully.

5. ACKNOWLEDGMENT

This work was carried out at Bharat Immunologicals and Biologicals Corporation Limited (BIBCOL), Chola, Bulandshahr, Uttar Pradesh, India and supported by our organization. The authors are grateful to Mr. S. K. Tyagi (Vice President and Head of Department), Quality Assurance and Training, for his motivational suggestions and approval to conduct the study in our organization.

6. AUTHOR CONTRIBUTIONS

Dr Amit Kumar designed the experiments, analyzed the data & finalized the manuscript after checking and Mr. Vaibhav Tomar carried out the experiments & wrote the paper. The authors read and approved the final manuscript.

7. COMPLIANCE WITH ETHICAL STANDARDS

7.1. Conflict of interest

The authors declare that they have no conflict of interests.

7.2. Animal and Human Rights Statement

This article does not contain any studies with human or animal subjects performed by any of the authors.

8. 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 Initiative, WPV-Wild Poliovirus, CPE-Cytopathic Effect, EMEM-Eagle Minimum Essential Medium, GMP- Good Manufacturing Practices, GLP-Good Laboratory Practices, VAPP-Vaccine-associated paralytic polio, VDPP-Vaccine derived polioviruses, NAM-Nutrient Agar Medium.

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