Original Article

Shelf-life estimation of recombinant *hepatitis B* vaccine using R software in comparison with WHO manual protocol

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ABSTRACT

Introduction: Pharmaceutical companies as well as food and cosmetics manufacturers are legally required to provide a shelf-life label on their products packaging as part of their stability study report. There are different recommended software like R software package and SAS which can perform as shelf-life estimating tools for analyzing the data achieved by the stability testing of drugs and vaccines. **Methods:** Recombinant hepatitis B surface antigen vaccine (Pasteur Institute of Iran) was used as a sample for the entire quality control assays according to Pharmacopeia and NIH (National Institute of Health) procedures. For the stability study, full test examinations were done and R software package was applied to estimate the shelf-life. **Results:** The results of R software indicated a variety of statistical information which makes the data interpretation more intelligible and apprehensible. **Conclusion:** Based on our results and experience, the best way to obtain a shelf-life or the expiration date is to calculate it manually; however, using software such as R can increase the accuracy of the results.

KEYWORDS: ICH, FDA, Hepatitis B vaccine, shelf-life, expiration date.

INTRODUCTION

In addition to food and cosmetics manufacturers, pharmaceutical companies are also legally required to provide a shelf-life label on their product packaging [1]. For pharmaceutical products, the minimum requirements in this regard have been specified by the International Conference of Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use [2]. The ICH guidelines which were first introduced in 2003 were consequently adopted by various regulatory agencies throughout the world[3]. Historically, some manufacturers used protocols consisted of manual estimation of the expiry date. However in 1987, US Food and Drug Administration (FDA) provided a program named SAS (Statistical Analysis Systems Institute, Cary, NC, USA) for establishment of product shelf-life, using methods which had subsequently been described by ICH Q1[4]. One major concern in recombinant vaccines and drugs production is the stability profile of the finished product over time due to the effects of environmental factors, such as temperature, humidity and light [5]. In order to determine the rates of chemical and physical reactions and their relationships with the environmental factors, accelerated, intermediate and long-term stability studies are required [6-8]. The protocols for the design and analysis of such stability studies are outlined in ICH Q1E (Evaluation of Stability Data) [9, 10]. The stability studies are often based on potency assays that are used to characterize the stability of a product under the storage temperature. In addition, the guidelines indicate that at least three batches of a drug product should be tested in order to allow for some estimation of batch-to-batch variability [1, 2, 6, 8]. Hence, batch variation testing is needed to be performed using appropriate statistical tests. In these situations, the analysis of covariance (ANOVA) is commonly employed where time is treated as a covariate, for testing the statistical differences of both the slopes and the intercepts of the regression lines between the batches. A significance level of 0.25 used in the pooling test is adopted for this program, owing to the expected low power of the design and the limited sample size in formal stability studies. Thus, if batch-to-batch variation is small ($P > 0.25$), drug stability data from several batches should be pooled in order to obtain a unified estimated of the shelf life for all the batches [11]. Different software like R software package (The R Project for Statistical Computing <https://www.r-project.org/>) and SAS are recommended as a shelf-life estimating tools for analyzing the data achieved by the stability testing of drugs and vaccines [12, 13, 4]. In this study, we attempted to define the shelf-life of a recombinant hepatitis B vaccine produced by Pasteur Institute of Iran, using shelf-life estimating R software package in order

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to determine the expiry date (which marks the end of the shelf-life on the label) and compared the result with a previously calculated manual method.

MATERIALS and METHODS

Recombinant hepatitis B Vaccine (Pasteur Institute of Iran) was a solution at pH 7.2, containing recombinant hepatitis B surface antigen, aluminum adjuvant and phosphate buffer. HBsAg quantity kit (Pasto Kit®, Pasteur Institute of Iran) were used in ELISA to determine the concentration of hepatitis B antigen. The entire quality control assay was performed according to Pharmacopeia and NIH procedures. For the stability study, full test examination was done. R software package version i386 3.2.2 was applied to estimate the shelf-life (or the expiry date) and manual shelf-life calculations were done according to WHO (World Health Organization) guidelines.

Keeping vaccines at refrigerated storage temperatures to ensure their effectiveness is a universal challenge. The WHO recommends storing nearly all vaccines at 2–8 °C [14, 15]. To meet these temperature requirements, as well as the requirement of 2 to 3 years of storage shelf-life, stabilization studies have been included as part of vaccine development processes for a long time. Extreme heat can adversely impact all vaccine products to some degree and freezing can also damage many vaccines significantly, especially those containing aluminum adjuvants [5]. Based on ICH guidelines, we used results data achieved by three continuous batches of recombinant hepatitis B vaccine in this stability study [16]. We entered this data in R software according to the program manual and analyzed the output result as described in the result section.

RESULTS

design tree for hepatitis B vaccine shelf-life data evaluation

This step involved the building of a "Decision tree for drug stability data evaluation", based on Appendix-A of "Evaluation of stability data ICHQ1E" [17]. According to this appendix, our vaccine should have been stored in refrigerator (2 to 8 **°C**) during the stability study examination time because this temperature was its routine keeping temperature.

The design of the stability study for the finished product of hepatitis B vaccine

According to ICH Topic Q1 (R2) "Stability testing of new drug substances and products", for long-term stability studies, repeating time of a testing must be well-designed to prepare the acceptable stability profile of a drug or vaccine. For instance, for a drug with a re-test period of at least 12 months, the repetition time in the long term storage condition is normally every 3 months in the first year and each 6 months during the second year and yearly after the second year. Minimum long-term testing is at least 12 months.

The evaluation

One approach for analyzing the data on a quantitative attribute that is expected to change with time is to determine the time at which 95% of one-sided confidence limit for the mean curve intersects the acceptance criterion. If the results show that the batch-to-batch variability is small, the data then can be estimated together. For this reason, appropriate statistical tests must be used such as significance of rejection of more than 0.25 to the regression lines slopes and zero time intercepts for the each batch tested. To combine several batches which show less than 0.25 variability, the result should be analyzed for each batch, separately.

analyzing the data with R software (R i386 3.2.2)

Our data set included the assay data of three batches of a produced hepatitis B vaccine which were assayed at 0, 3, 6, 9, 12, 18 and 24-month time intervals. The data are shown in Table 1.

Table 1. Data indicating batch, time and assay percentage.

Data Editor

	batch	time	assay (%)
1	1	0	100
2	2	0	99
3	3	0	100
4	1	3	99
5	2	3	99
6	3	3	100
7	1	6	98
8	2	6	97
9	3	6	99
10	1	9	98
11	2	9	97
12	3	9	96
13	1	12	97
14	2	12	96
15	3	12	99
16	1	18	97
17	2	18	96
18	3	18	97
19	1	24	96
20	2	24	96
21	3	24	96
22			

Data analysis for multiple batches

The "Statistical analysis menu" is divided into two procedures, namely "Data analysis for a single batch" and "Data analysis for multiple batches". Statistical analysis of data for the three batches of hepatitis B vaccines were done by ANOVA while the changes between the three batches should not be less than 0.25 which would indicate significant changes among the three vaccine batches. After inputting the data in R software, the basic information regarding the assay such as the assay percentage of a drug product at time T were entered. In this step, options for editing or entering the data were provided. The assay for hepatitis B vaccine as a finished product with the upper and lower acceptance criteria of the label claim was modified as depicted in Fig. 1.

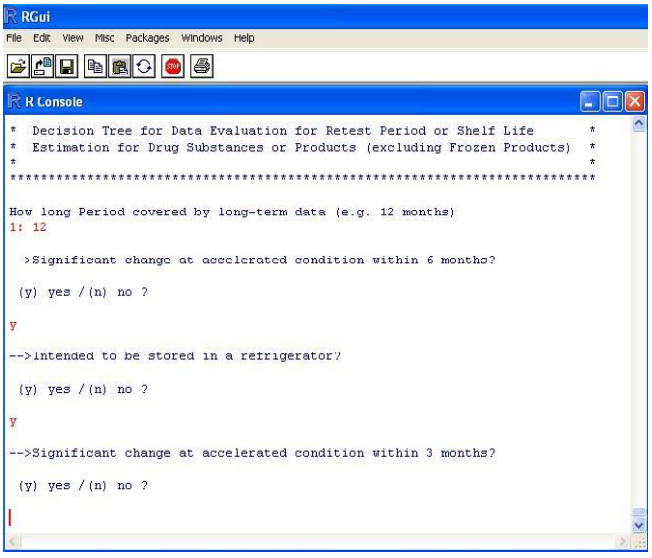

Fig. 1. Decision tree for data evaluation according to CHQ1E -Appendix A.

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Data analysis

Finally, the shelf-life was estimated. The output included ANOVA results, linear regression model and ANOVA data as depicted in Fig. 2.

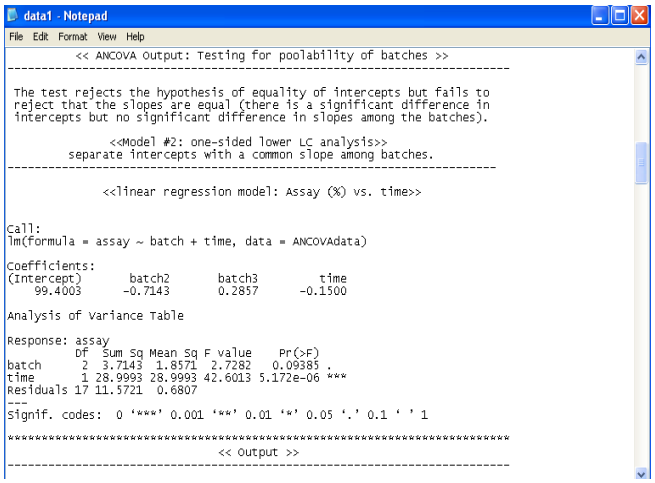

Fig. 2. Data analyzed by the software and displayed.

The observed and calculated concentrations, residuals, shelf-life and a graphic plot were extracted as shown in Fig. 3.

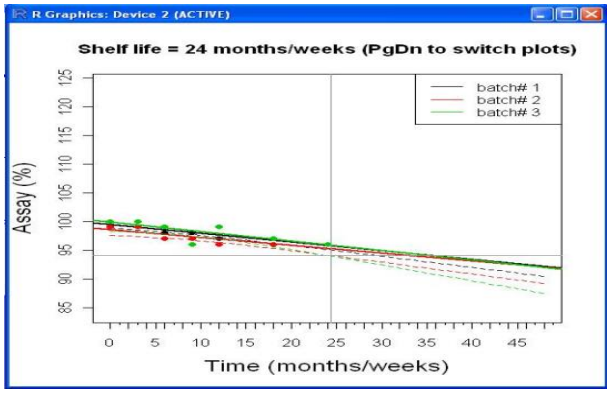

Fig. 3. Shelf-life of the vaccine estimated by R software. The expiration time is determined when according to the assays result, the confidence limit 95% (vertical level) crosses the month of experience (horizontal level) which was 24 months.

The residuals were obtained using a normalized quantile-quantile (Q-Q) plot which is a graphical technique for determining whether two data sets come from populations with a common distribution as shown in Fig. 4.

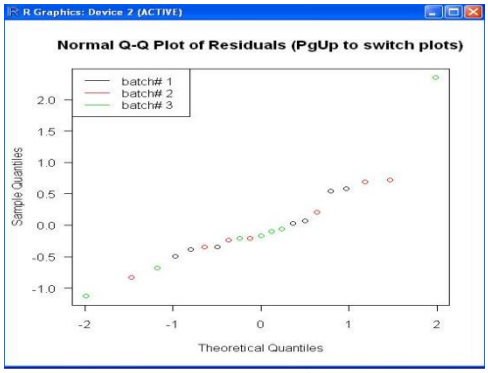

Fig. 5. Q-Q plot pertaining to three batches of hepatitis B vaccine.

DISCUSSION

The stability study is one of the major parts of any pharmaceutical or biotechnological production process. The shelf-life or the expiry date of a product is completely dependent on the result extracted from the stability study data. There are different valid methods to calculate and analyze the stability study data and some of them are recommended by WHO and FDA such as R software package and SAS. Some experts prefer to calculate the shelf-life manually according to their protocol without using any software; however, they may reduce the calculation errors by using statistical software. Our results indicated that using R software could increase the accuracy and sensitivity of the obtained results when compared to similar manual calculations. Having a user-friendly interface, is another advantage of this software which makes it easier for the operators and the experts to input their data and to estimate the shelf-life of their products while reducing the human error in this process. In addition, this software presents a variety of statistical information which makes the result more intelligible and apprehensible for the users or the regulatory authorities. Based on our experience in similar situations, we recommend obtaining a shelf-life or expiration date by manual calculation and confirming the results by using a software method, as indicated in this study in order to increase the accuracy of the estimation.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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