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RESEARCH ARTICLE

EXPLORING POSSIBILITY OF USING RECONSTITUTED BRITISH REFERENCE PREPARATION BEYOND ITS RECOMMENDED SHELFLIFE FOR STUDYING MOLECULAR SIZE DISTRIBUTION IN HUMAN NORMAL INTRAVENOUS IMMUNOGLOBULIN

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ABSTRACT

The objective of the present study was to explore possibility of using reconstituted British Reference Standard (BRP) (Catalogue No. Y0000488) beyond its recommended shelf life for studying molecular size distribution in human normal intravenous immunoglobulin so as to reduce wastage, expenses and minimize the dependency on such BRP. Though the initial HPLC test results revealed that BRP after reconstitution retains required relative retention time of dimer and monomer up to 309 and 374 days when stored at 2-8ºC and -10ºC respectively whereas leaflet of instruction for BRP recommends its use, after reconstitution, maximum up to 14 days when storing at 4ºC but without freezing. Present study suggests that BRP of molecular size after reconstitution and stored properly may be used beyond the recommended shelf life of 14 days and till the required relative retention time of dimer and monomer is retained by BRP.

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INTRODUCTION

Human normal intravenous immunoglobulin is one of the biological drugs which is complex protein in nature and their quality is assured by comparing with suitable reference standards. It is used intravenously in the patient suffering from diseases like Primary immunodeficiency, Kawasaki syndrome, Idiopathic thrombocytopenic purpura, Bone marrow transplantation, Chronic B-cell lymphocytic leukaemia and paediatric HIV infection etc. The shelf life of the drug is around 2 years when stored at 2-8ºC. At present day of science, comparison of the test results with globally accepted reference standard is required worldwide. Without use of proper reference standards the quality of biological drugs cannot be assured. It is like a spinal cord of the biological and other allied sciences because it is a pre-characterized substance which is used as a measurement base for test drugs. Expiry dates are not assigned to biological reference standards / preparation, but the long-term stability is predicted on the basis of real time and accelerated temperature data (WHO TRS 932, 2006).

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In contrast, information's available on the stability of the reconstituted reference standards is provided to the users. However, this type of information is limited because the conditions of reconstitution and storage generally cannot be extensively studied during collaborative studies. In view of this users are encouraged to send to WHO or the custodian laboratory, accounts of their experience in the use of the reference standard under routine laboratory conditions (WHO TRS 932, 2006). Accordingly, present study has been carried out to explore possibility of using reconstituted BRP beyond its shelf life for studying molecular size distribution in human normal intravenous immunoglobulin.

MATERIALS AND METHODS

A commercial available BRP [Immunoglobulin (molecular size) (Catalogue number Y0000488, Batch Number1.1 and ID 002VH8)] mentioned in the official monograph of the European Pharmacopoeia (EP), 2014 was used for the study. This was prepared through the collaborative study by European Directorate for the Quality of Medicines & HealthCare (EDQM, France) (Sandberg *et al*., 2006). It was reconstituted with 5 ml sterile de-ionized water and 28 aliquots of 60 µl were prepared under sterile condition. 18 aliquots

were stored at 2 -8ºC for 2, 3, 7, 9, 14, 16, 28, 29, 36, 52, 57, 83, 85, 120, 232, 239, 281 and 309 days and other 10 aliquots frozen at -10ºC for 2, 3, 7, 16, 28, 36, 83, 120, 336 and 374 days. Each aliquot was diluted with 0.9 percent w/v solution of sodium chloride to obtain a concentration 0.4 percent w/v followed by filtration through low protein 0.45µ syringe filter in the screw top vial (12 x 32 mm with cap and pre slit / silicon septa). To know the retention time of the dimer and monomer the sample preparation was run through 300 mm x 7.8 mm hydrophilic silica packed stainless steel size exclusion column protected by the guard size exclusion column (Waters) attached with the High Performance Liquid Chromatography (HPLC) work station of Alliance of M/s Waters. The mobile phase was used as per requirement of IP 2014 and molecular size distribution of all 28 samples was determined by dual λ detector at 280 nm wavelength with flow rate of 0.5ml/minute as per procedure mentioned in IP 2014.

RESULTS

The mean data of 18 different observations of reconstituted BRP stored at 2-8^{o}C for 2 to 319 days revealed Retention Time (RT) 17.30 of monomer, 14.92 of dimer and relative retention of 0.85 (up to 3 days) and 0.86 (from 3rd day onwards) which are statistically significant (Table 1).

Table 1. Retention Time (RT) of dimer and monomer in aliquots of reference standard stored at 2 – 8ºC

Statistical parameters	RT		Relative retention time of dimer and monomer
	Dimer	Monomer	
Mean (µ)	14.92956	17.30978	0.858889
SD (σ)	0.214883	0.254011	0.003143
CV	0.014393	0.014674	0.003659
%CV	1.439314	1.467445	0.365903

Table 2. Retention Time (RT) of dimer and monomer in aliquots of reference standard stored under frozen condition at -10ºC

Statistical parameters	RT		Relative retention time of dimer and monomer
	Dimer	Monomer	
Mean (µ)	15.0126	17.412	0.858
SD (σ)	0.320872	0.369136	0.004
CV	0.021374	0.0212	0.004662
%CV	2.137353	2.120011	0.4662

Similarly, the mean data of 10 different observations of reconstituted BRP and stored at -10^{o}C for 2- 375 days revealed Retention Time (RT) 17.41 of monomer, 15.01 of dimer and relative retention time of 0.85 (up to 3 days) and 0.86 (from 3rd day onwards) which are also statistically significant (Table 2). There was no degradation, fragmentation and aggregation observed during both the studies carried out at 2-8^{o}C and -10^{o}C.

DISCUSSION

Guidelines to study and predicts the shelf life of new biological drugs are available from World Health Organization (WHO, 2006), The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 2011) and European Medicines Agency (EMA, 2007).

Shelf life of reference standards are usually predicted through collaboration studies however it is not mentioned in the leaflet of the reference standard because of their prolong shelf life. The reference standard / preparation, in general, are required to be used judiciously. But assigning shelf life to reconstituted reference standard / preparation is difficult task because the conditions of reconstitution and storage generally cannot be extensively studied during collaborative studies. Though the guidance has been provided on maximum shelf life for sterile products for human use after first opening or following reconstitution (EMA, 1998), therefore, the present study was carried out to find out possibility of using reconstituted BRP beyond 14 days of its recommended shelf life. The results of molecular size distribution of the present study shows that commercial available BRP after reconstitution has been found to retain the required relative retention time of dimer and monomer for 309 days and 374 days when stored at 2-8^{o}C and even under frozen condition (-10^{o}C), respectively. Similar studies carried out on effect of storage temperature on human serum immunoglobulin showed that there was significant degradation of immunoglobulin at +37ºC greater after 12 months. However, there was no significant change in the same material stored at +4ºC or -20ºC (Rowe *et al*., 1970). In our opinion the reconstituted and properly aliquoted BRP stored at 2-8^{o}C or frozen may be used regularly till relative required retention time of dimer and monomer is retained by the BRP for its judiciously use and avoid wastage.

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