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ORIGINAL RESEARCH ARTICLE

Study of the Stability of Sandoz Rituximab Biosimilar Rixathon®/ Riximyo® When Subjected for up to 21 Days to Ambient Storage

Roman Borišek[1] · André Mischo[2] · Ida Šmid[1]

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Abstract

Aim The purpose of this study was to evaluate the extended physicochemical and biological stability of Sandoz Rixathon®/ Riximyo® (SDZ-RTX) after exposure to out-of-fridge (OOF) conditions.

Materials and Methods The impact of the short-term temperature excursion on stability parameters of SDZ-RTX was simulated by subsequently exposing the three batches of SDZ-RTX (100 and 500 mg) to OOF conditions, (I) 25 ± 2 °C/60 ± 5% relative humidity (RH) and (II) 30 ± 2 °C/65 ± 5% RH, for up to 21 days after more than the claimed 36-month shelf-life storage in long-term conditions (5 ± 3 °C). Analytical methods used included the cation exchange chromatography (CEX), size exclusion chromatography (SEC), and non-reducing capillary electrophoresis-sodium dodecyl sulfate (nrCE-SDS), as well as biological activity by complement-dependent cytotoxicity (CDC)-bioactivity as well as further methods, for example, related to identity and pharmacopoeia test methods.

Results No notable changes were observed across all batches with respect to identity (charge and primary structure), pharmaceutical tests (clarity, visible and subvisible particles analytics, container appearance, degree of coloration, pH, osmolality, extractable volume, and container closure integrity testing), protein content by UV and microbiological parameters (sterility and bacterial endotoxins) under both OOF conditions. Only minor changes were observed for parameters evaluated via SEC, CEX, and nrCE-SDS. For potency (CDC-bioactivity) only one of the batches showed a relevant change. Even for these stability-indicating test methods, all analyzed parameters complied with the shelf-life specifications.

Conclusion SDZ-RTX is safe for use even under worst-case conditions, for example, after subjecting it for up to 21 days at OOF conditions (25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/65 ± 5% RH) after the batches had reached an age that was already beyond the claimed shelf-life.

Key Points

Findings of this study support single-time out-of-fridge temperature excursion in an unopened vial of Sandoz rituximab (SDZ-RTX) stored in the original outer box.

SDZ-RTX samples with the actual age even beyond the claimed shelf-life were shown as safe and fit for use even under worst-case conditions.

✉ Roman Borišek
roman.borisek@novartis.com

[1] Lek Pharmaceuticals d.d., Kolodvorska 27, 1234 Menges, Slovenia

[2] Novartis AG, Biochemiestraße 10, 6336 Langkampfen, Austria

1 Introduction

Rituximab (RTX) is a genetically engineered chimeric mouse anti-human monoclonal antibody (mAb)-targeting CD20 antigen. CD20 is a transmembrane protein that is thought to play a role in B-cell proliferation, activation and differentiation, and signal transduction [1]. RTX is widely utilized in the treatment of several B cell-derived hematological malignancies [2, 3]. In oncology, RTX is administered for the treatment of non-Hodgkin's lymphoma, diffuse large B-cell lymphoma (DLBCL), and chronic lymphocytic leukemia (CLL). Additionally, RTX is indicated for the treatment of non-oncology indications such as autoimmune diseases [4, 5]. RTX was the first CD20 mAb approved by the US Food and Drug Administration (FDA) for the treatment of relapsed or refractory, CD20-positive, B-cell, low-grade, or follicular non-Hodgkin's lymphoma in 1997 [6]. RTX mediates anti-tumor effects by a variety of mechanisms including induction of

apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and direct induction of cellular apoptosis [7, 8]. Extensive stability testing is required for biopharmaceuticals such as RTX by national health authorities to demonstrate that the product quality is not compromised by standard transport and storage conditions and that product specifications are met throughout the declared shelf-life [9, 10]. Many stress factors are routinely encountered during the preparation, purification, shipping, or storage of protein products and especially mAbs. For RTX and further mAb-based therapeutics, several studies have evaluated various stability aspects, for example, in-use, out of fridge, or opened vial stability [11–13].

Sandoz biosimilar of RTX (SDZ-RTX), marketed worldwide under the trade name Rixathon® or Riximyo®, is available as a concentrate solution for infusion as a single-dose vial containing either 100 mg or 500 mg of active pharmaceutical ingredient at a concentration of 10 mg/mL [14]. As defined in the product's Summary of Product Characteristics, SDZ-RTX should be stored in a refrigerator (2–8 °C) and kept in the original outer carton to protect from light exposure. However, based on the newly available stability data presented in this publication, some markets (e.g., the European Union [EU]) have already approved single-time temperature excursions outside 2–8 °C [14].

In addition, none of the RTX manufacturers in the EU have claimed a permitted temperature excursion above 8 °C (MabThera®, Ruxience®, and Truxima®) [4, 15, 16]. On the contrary, for any short-term temperature excursion outside the intended storage conditions, it is recommended to discard SDZ-RTX unless the excursion is permitted according to the provided patient information leaflet (PIL). Of note, this is the case for the EU market alone at present. However, in other markets where out-of-fridge (OOF) excursion is not permitted according to the enclosed PIL, evaluation of short-term temperature excursion on the stability and quality of SDZ-RTX is required. The study, which is the basis for the defined OOF period in the PIL, was thus designed to evaluate the extended physicochemical and biological stability of Rixathon®/Riximyo® after exposure to OOF conditions.

2 Material and Methods

2.1 Study Samples and Storage Conditions

The OOF stability study was performed with three SDZ-RTX batches of both strengths, one batch of 100 mg (10 mL vials) and two batches of 500 mg (50 mL vials). SDZ-RTX batches beyond approved shelf-life of 36 months (i.e., batch age of 39–43 months) were selected to account for the worst-case scenario where SDZ-RTX within but at the end of the approved shelf-life is subjected to the patient. Thus, the OOF stability study was performed with SDZ-RTX material, which had slightly higher starting levels of product-related impurities (e.g., degradation/aggregation products, charge variants) at the start of OOF study as a result of storage at intended conditions for at least the entire shelf-life period of 36 months. Therefore, subjecting and evaluating aged material against specifications after OOF exposure is considered a worst-case scenario for the assessment of product quality. The study was designed to simulate temperature excursion outside the intended storage conditions of 2–8 °C, with a duration of up to 21 days. Each SDZ-RTX batch was divided into three parts: the first part was subjected to OOF condition (I), 25 ± 2 °C/60 ± 5% relative humidity (RH; climatic zone II) and the second part to OOF condition (II), 30 ± 2 °C/65 ± 5% RH (climatic zone IVa). The last, third part, was not exposed to the OOF conditions and thus served as a reference point to provide additional insight for assessing the actual change in each tested quality attribute as a result of the OOF exposure.

SDZ-RTX samples were transferred into stability chambers with temperature conditions 25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/65 ± 5% RH in reverse order; samples with OOF durations of 21, 14, and 7 days were transferred from the intended conditions (2–8 °C) into stability chambers to OOF conditions I or II on Day 0, 7, and 14, respectively, whereas the reference samples were maintained in intended storage conditions (2–8 °C). On Day 21, all samples including reference samples were pulled from storage conditions and further subjected to analyses, thus allowing direct head-to-head comparison, and reducing the potential variability of the analytical method. State-of-the-art analytical methods were employed to assess the impact of conditions on identity, purity, potency (CDC-bioactivity) as well as physicochemical and microbiological properties of SDZ-RTX.

2.2 Analysis of the Impact of Temperature Excursion on Quality of Sandoz Rituximab (SDZ-RTX)

To assess the impact of the short-term temperature excursion outside the intended storage conditions of 2–8 °C, extensive analytical testing for identity, purity, and potency of SDZ-RTX was evaluated with the below-mentioned analytical methods. Methods were selected to cover for critical quality attributes identified based on SDX-RTX product development. For example, no method for detection of higher order structure was employed, as circular dichroism development data indicated no relevant change of SDZ-RTX when subjected to a long-term stability study including 2–8 °C, 25

°C, and even 40 °C storage conditions (data not shown). The specification limit(s) for the methods included in this study are set based on extensive SDZ-RTX development knowledge as an essential part of the SDZ-RTX control strategy to ensure safety and efficacy of the product.

2.2.1 Cation Exchange Chromatography

Cation exchange chromatography (CEX) is highly selective to product-related variants of RTX, being able to separate the RTX main compound from acidic and basic variants [17, 18]. Different charged variants of SDZ-RTX were separated using CEX due to differential electrostatic interaction with the charged moieties of the stationary phase. Samples were treated with carboxypeptidase B (CPB), which can specifically remove C-terminal basic amino acid residues [19]. The chromatographic analysis was performed using a weak CEX resin of linear sodium chloride gradient and an ultraviolet (UV) detection at 280 nm. CEX was utilized to analyze purity as well as to confirm the identity SDZ-RTX batches after OOF exposure.

2.2.2 Size Exclusion Chromatography

SEC was used to assess the formation of high- and low-molecular-weight variants. SDZ-RTX species of different sizes (e.g., high-molecular-weight variants, monomers, and fragments) are separated by SE-HPLC under native conditions [20]. Chromatographic separation was achieved using a column, and UV detection was carried out at 210 nm on an HPLC chromatographic system.

2.2.3 Non-reducing Capillary Electrophoresis-Sodium Dodecyl Sulphate

CE-SDS, in contrast to SEC, is more reliable for the quantification of low-molecular-weight species in denaturing conditions and is a key quality control (QC) method for size-variant quantification of antibodies and related products. Non-reduced CE-SDS (nrCE-SDS) was used to assess the number of intact species (main peak) and low-molecular-weight species resulting from partial reduction of fragmentation products [21].

The analysis was performed on a CE system. Samples were denatured, and any free sulfhydryl group from the cysteine side chain was alkylated with iodoacetamide (IAM) to prevent disulfide shuffling before analysis. Samples were pressure-injected, and the separation was performed with a voltage of 15 kV, and UV detection was carried out at 214 nm.

2.2.4 Liquid Chromatography-Ultraviolet Peptide Mapping

The confirmation of the primary structure is the cornerstone in the verification of the identity of RTX [18]. With the peptide mapping method, a protein is investigated on the level of its peptides. The identity of SDZ-RTX was evaluated by LC-UV peptide mapping. For this, SDX-RTX was denatured and reduced with dithiothreitol (DTT), alkylated with IAM solution, and enzymatically digested by protease lysyl endopeptidase C (Lys-C). Chromatographic analysis was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection at 214 nm. The chromatogram of SDZ-RTX sample was compared to the typical peak pattern of a reference material.

2.2.5 Biological Activity by Complement-Dependent Cytotoxicity-Bioactivity

Complement activation, by the fragment crystallizable (Fc) portion of the antibody leading to cell lysis (CDC), is an important postulated mechanism of action of monoclonal anti-CD20 antibody [22]. To determine the CDC activity of SDZ-RTX, Raji B cells expressing CD20 on their surface were incubated with SDZ-RTX and a fixed concentration of rabbit complement. Later, the degree of cell death was determined by a cell viability assay. Cell viability was assessed by measuring ATP concentration via the luciferin-luciferase system [23].

2.2.6 Physicochemical Properties of SDZ-RTX

The content of SDZ-RTX was measured with UV/visible absorbance spectrometry. Formation of stability-related visible and subvisible particles after short-term temperature excursion was assessed in accordance with requirements stated in Ph Eur 2.9.20, United States Pharmacopeia (USP) <790> and Japanese Pharmacopeia (JP) 6.06 (visible particles) and in accordance with Ph Eur 2.9.19, USP <788> and JP 6.07 (subvisible particles) [24–26].

Clarity of solution of SDZ-RTX was assessed in accordance with Ph Eur 2.2.1 with an instrumental method with ratio turbidimetry. The impact on the integrity of the primary packaging material was evaluated with the container closure integrity testing (CCIT); this testing was performed with the dye ingress method in a dye bath applying vacuum and overpressure with the addition of color indicator methylene blue. Subsequently, potential ingression of the color indicator into SDZ-RTX due to an unsatisfactorily tight container is evaluated through change in the color of solution with visual inspection. CCIT was performed in the worst-case condition only (30 ± 2 °C/65 ± 5% RH).

The degree of coloration of SDZ-RTX was determined in accordance with Ph Eur 2.2.2 (Method I) in the worst-case condition only (30 ± 2 °C/65 ± 5% RH). pH of SDZ-RTX was determined by potentiometry according to the requirements stated in Ph Eur 2.2.3, USP <791> and JP 2.54 in the worst-case condition only (30 ± 2 °C/65 ± 5% RH) [24–26].

Osmolality of SDZ-RTX, which is determined by the measurement of the depression of the freezing point, was performed in accordance with requirements in Ph. Eur. 2.2.35 and USP <785> in the worst-case condition only (30 ± 2 °C/65 ± 5% RH). Extractable volume of SDZ-RTX was measured in accordance with Ph Eur 2.9.17, USP <1> and JP 6.05 in the worst-case condition only (30 ± 2 °C/65 ± 5% RH). The appearance of SDZ-RTX container was visually evaluated in the worst-case condition only (30 ± 2 °C/65 ± 5% RH) [24–26].

2.2.7 Microbiological Tests

Sterility of solution was performed by means of the membrane filtration method according to requirements defined in Ph. Eur. 2.6.1, USP <71> and JP 4.06. Also, the test for bacterial endotoxins (BETs) was used to detect or quantify endotoxins from Gram-negative bacteria using amoebocyte lysate from the horseshoe crab (LAL method). The procedure was performed in accordance with Ph. Eur. 2.6.14, USP <85> and JP 4.01. Both sterility and BET were assessed in the worst-case condition only (30 ± 2 °C/65 ± 5% RH) [24–26].

3 Results

In this study, no notable changes were observed in the purity of SDZ-RTX for the defined OOF conditions I and II, assessed using CEX, SEC, and non-reducing capillary electrophoresis sodium dodecyl sulfate (nrCE-SDS) methods (Table 1).

3.1 Purity by Cation Exchange Chromatography

After 21 days of OOF study, a decrease in percentage of the main peak (Fig. 1a), contaminant change in percentage of sum of acidic peaks (increase, see Fig. 1b), and sum of basic peaks (decrease, see Fig. 1c) were observed in all three SDZ-RTX batches. The rate of change is more pronounced at the higher tested temperature. However, all results comply with the shelf-life specification limits that are defined for SDZ-RTX. Representative CEX chromatogram overlay is presented in Fig. 2, which shows no unexpected novel variants had formed.

3.2 Purity by Size Exclusion Chromatography

After 21 days of temperature excursion, the analysis of SDZ-RTX by SEC revealed a decrease in purity of up to 0.4% (Fig. 3a). For the sum of high-molecular-weight variants, no clear common trend could be determined as the results fluctuated by maximally 0.1% (Fig. 3b). The rate of decrease in purity is slightly more pronounced at the higher tested temperature; however, all results are well within the shelf-life specification limits. All results comply with the shelf-life specification limits that are defined for

Table 1 Changes in SDZ-RTX after 21 days of out-of-fridge study

OOF duration	Δ after 21 days at 25 ± 2 °C/60 ± 5% RH			Δ after 21 days at 30 ± 2 °C/65 ± 5% RH		
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Purity by cation exchange chromatography						
Main peak (0K)	− 0.5%	− 0.6%	− 0.2%	− 1.8%	− 1.8%	− 1.4%
SAP	+ 1.7%	+ 1.6%	+ 1.7%	+ 3.9%	+ 3.8%	+ 3.8%
SBP	− 1.1%	− 1.0%	− 1.3%	− 2.1%	− 2.1%	− 2.4%
Purity by size exclusion chromatography						
Purity	− 0.1%	− 0.2%	− 0.2%	− 0.4%	− 0.4%	− 0.4%
Sum of HMWs	0.0%	+ 0.1%	0.0%	+ 0.1%	+ 0.1%	0.0%
Purity by non-reducing capillary electrophoresis-sodium dodecyl sulfate						
Purity	− 0.3%	− 0.7%	− 0.5%	− 0.9%	− 0.7%	− 0.5%
Potency						
CDC-bioactivity	− 2%	+ 3%	− 7%	+ 1%	− 2%	− 21%

CDC complement-dependent cytotoxicity, *HMW* high molecular weight, *OOF* out-of-fridge, *SAP* sum of acidic peaks, *SBP* sum of basic peaks, *RH* relative humidity, *SDZ-RTX* Sandoz rituximab

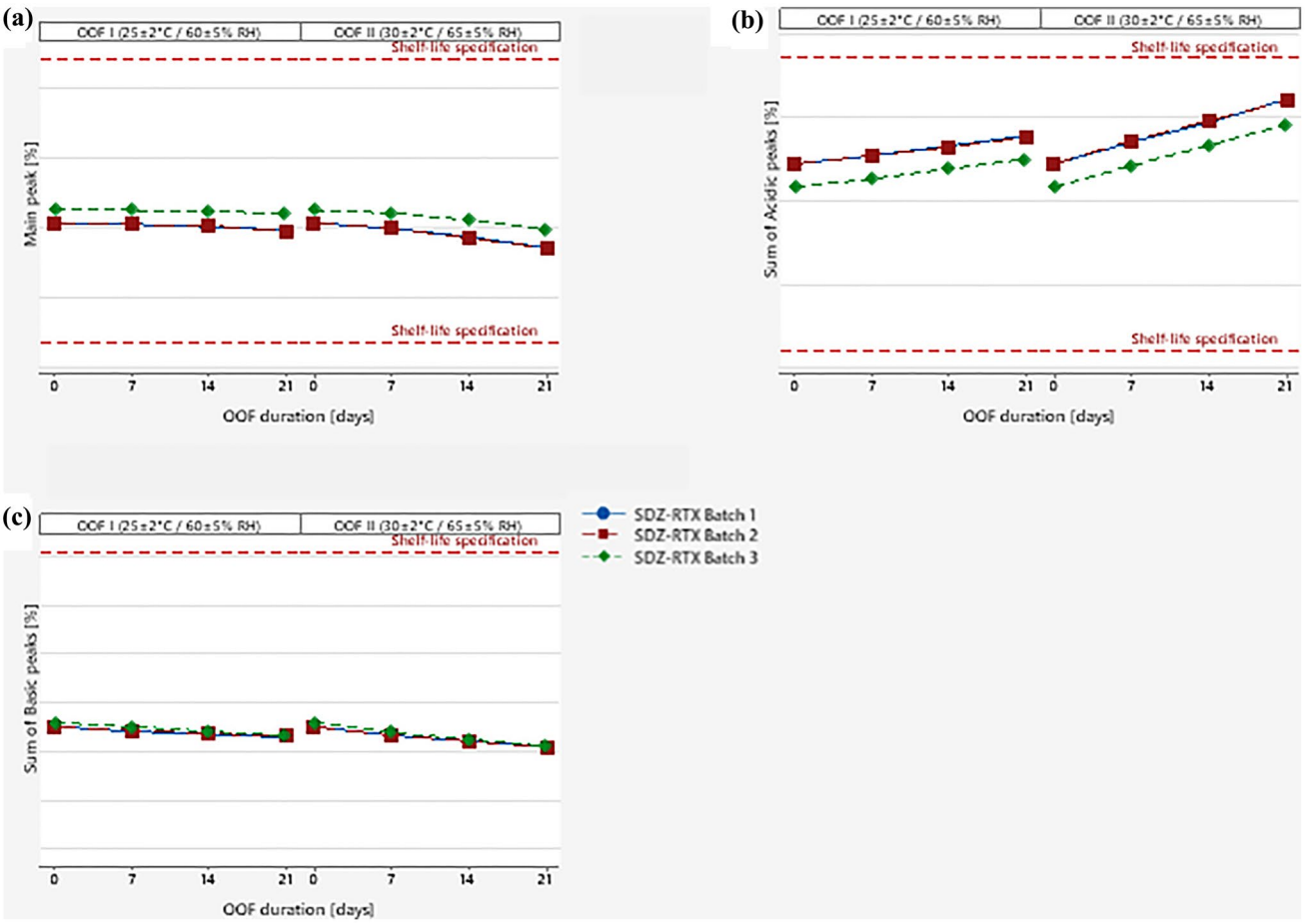

Fig. 1 Throughout 21 days of storage at 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/65 ± 5% RH, a decrease in the main peak (**a**), increase in the sum of acidic peaks (**b**), decrease in the sum of basic peaks (**c**) was observed for all three SDZ-RTX batches. *RH* relative humidity, *SDZ-RTX* Sandoz rituximab

SDZ-RTX. Figure 4a shows no unexpected novel variants had formed.

3.3 Purity by Non-reducing CE-SDS

After 21 days of the temperature excursion study, the analysis of SDZ-RTX by non-reducing CE-SDS revealed, as already observed with the SEC analysis, a decrease in purity of up to 0.9% (Fig. 5). However, all results comply with the shelf-life specification limits that are defined for SDZ-RTX. The rate of decrease in purity is slightly more pronounced at a higher tested temperature; however, all results are well within the shelf-life specification limits. Figure 4b shows no unexpected novel variants had formed.

3.4 Potency by CDC-Bioactivity

For potency that was determined with CDC-bioactivity, no common trend in stability behavior could be determined for all three tested SDZ-RTX batches; at OOF condition I (25 ± 2 °C/60 ± 5% RH), changes within the range of method variability can be seen for all three tested batches. At OOF condition II (30 ± 2 °C/65 ± 5% RH), Batch 1 and Batch 2 do not show a relevant trend; Batch 3 has a decreasing trend, which might be still due to bioassay result fluctuations (see the fluctuations Batch 1 shows at OOF II condition). Hence, no overall conclusions on the trend for both OOF that were tested can be determined for CDC-bioactivity. Importantly, all results comply with the shelf-life specification limits that are defined for SDZ-RTX (Fig. 6).

3.5 Physicochemical Properties of SDZ-RTX

No notable changes were observed across all batches in clarity (6 NTU [nephelometric turbidity unit] in all batches), presence of visible and subvisible particles, container appearance, degree of coloration, pH, osmolality, extractable volume, container closure integrity testing, and

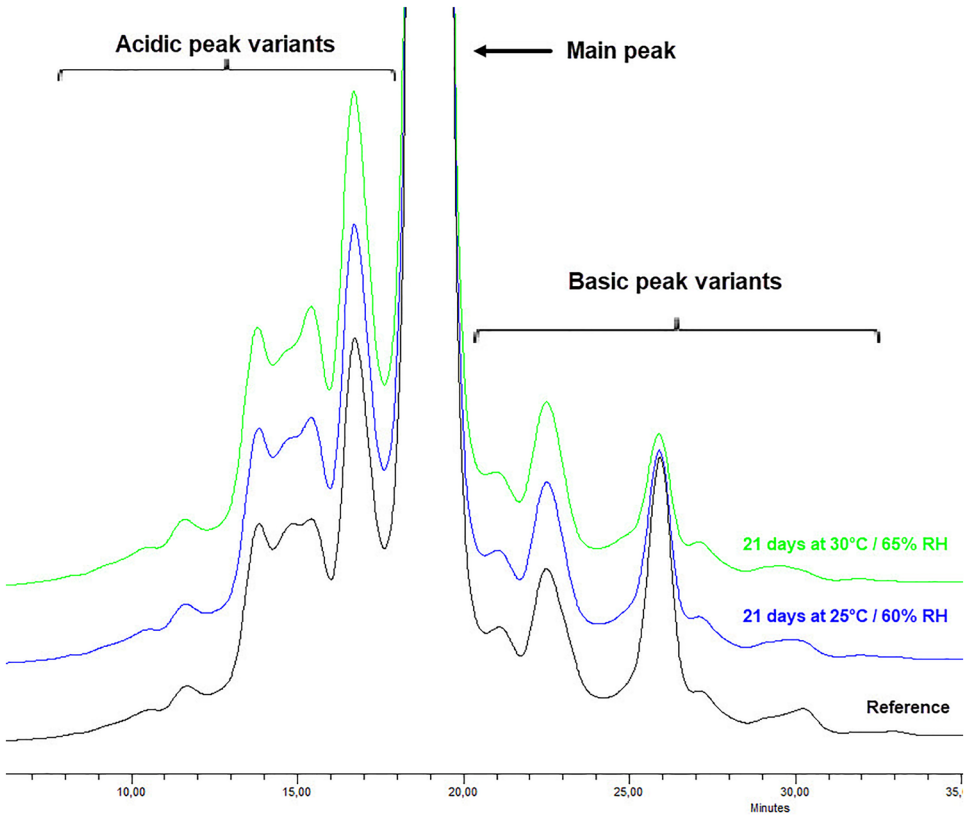

Fig. 2 Representative chromatogram overlay of SDZ-RTX measured by CEX comparing reference sample (black line, not exposed to OOF conditions) with sample stored for 21 days at 25 ± 2 °C/60 ± 5% RH (blue line) and 30 ± 2 °C/65 ± 5% RH (green line). *CEX* cation exchange chromatography, *OOF* out-of-fridge, *RH* relative humidity, *SDZ-RTX* Sandoz rituximab

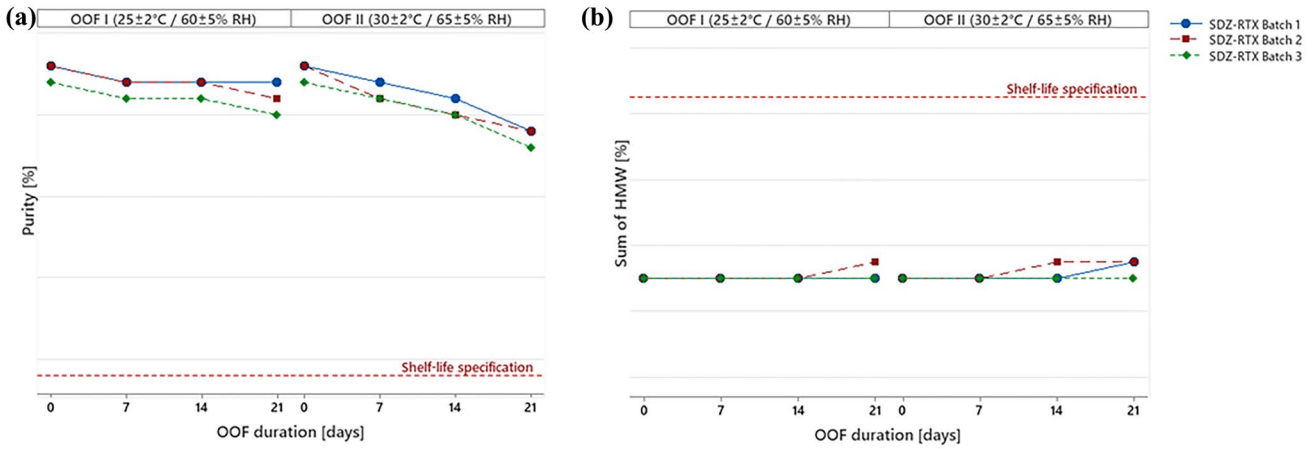

Fig. 3 Throughout 21 days of storage at 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/65 ± 5% RH, measurement of purity by SEC revealed slight decrease in purity (**a**), no clear common trend could be identified for the sum of HMW species (**b**) for all three SDZ-RTX batches. *HMW* high molecular weight, *SDZ-RTX* Sandoz rituximab, *SEC* size exclusion chromatography

protein content by UV (see Online Supplementary Material (OSM) Tables 1, 2, and 3).

3.6 Microbiological Tests

No notable changes were observed. All three batches meet the specification limits (OSM Tables 1 and 2).

3.7 Identity Tests

Identity was confirmed via CEX and LC-UV peptide mapping for all SDZ-RTX batches at both OOF conditions (OSM Tables 1 and 2).

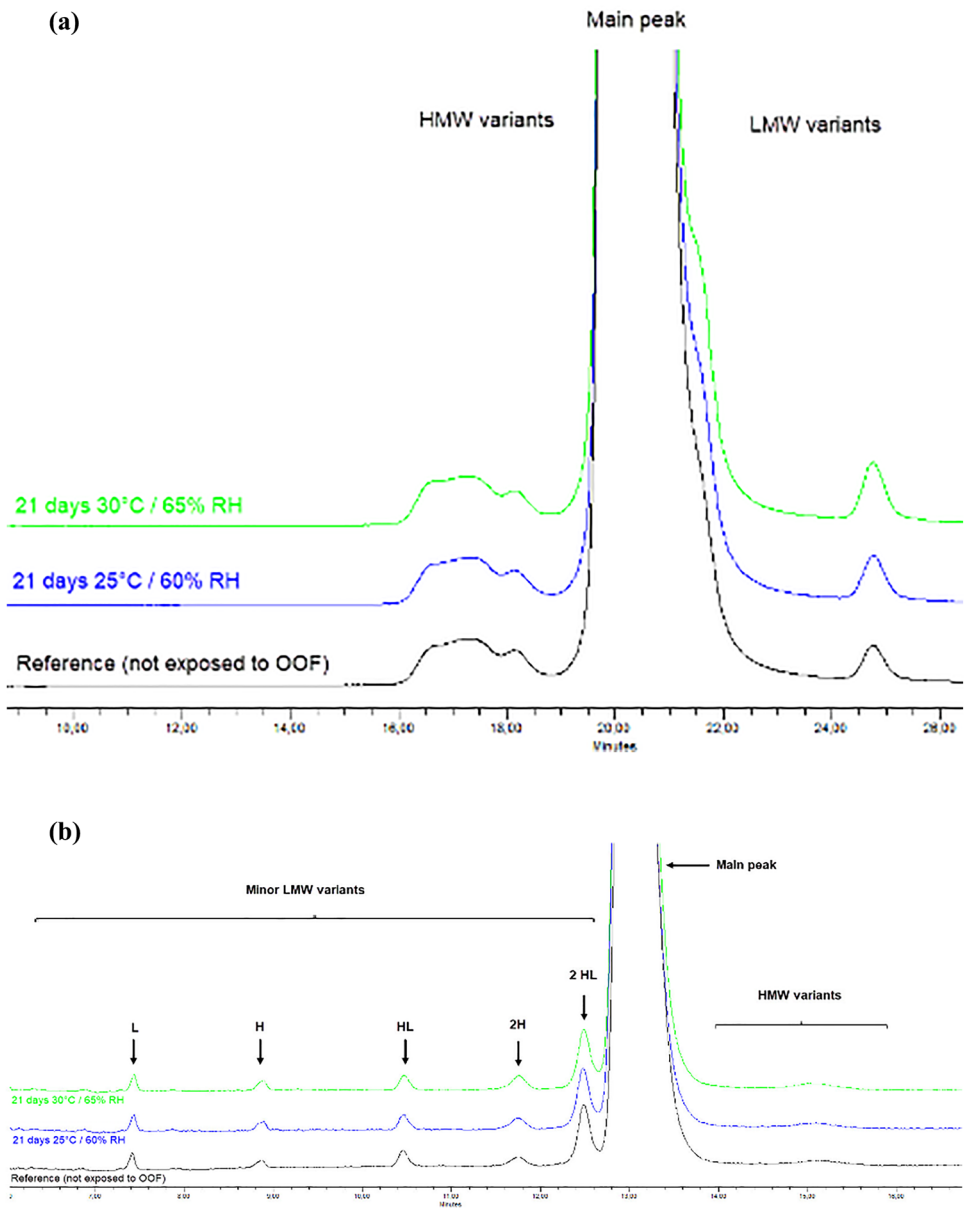

Fig. 4 Storage of SDZ-RTX for 21 days at 25 ± 2 °C/60 ± 5% RH (blue line) and 30 ± 2 °C/65 ± 5% RH (green line) does not cause significant increases in the abundance of the HMW variants compared to the sample that was not exposed to temperature excursion (reference sample—black line). However, a slight increase in LMW species is observed as a decrease in purity up to 0.4% and 0.9% measured by SEC and nrCE-SDS, respectively. (**a**) Representative overlay of the SEC chromatograms of SDZ-RTX; (**b**) representative overlay of the non-reducing SDS gel capillary electrophoresis (CE-SDS) electropherogram. The main peak, the 2HL fragment (two heavy chains and one light chain), and additional minor LMW and HMW variants are indicated on the figure: 2H (two heavy chains), HL (heavy and light chain), H (heavy chain), L (light chain). *LMW* low molecular weight, *HMW* high molecular weight, *SDZ-RTX* Sandoz rituximab, *SEC* size exclusion chromatography

4 Discussion

The purpose of this study was to evaluate the extended physicochemical and biological stability of SDZ-RTX biosimilar product after exposure to OOF conditions. ICH Q5C states the product shelf-life must be backed by real-time data, that is, spanning (at least) the intended shelf-life [10]. This study used a worst-case approach as even aged SDZ-RTX material beyond the end of shelf-life was used to then subsequently apply OOF conditions I and II and check whether the analytical results are still within the specification limits (which would typically only apply for up to the claimed shelf-life of 36 months). These data are not to be used to allow use of SDZ-RTX after the PIL stated shelf-life.

Of the many employed methods, only a few analytical methods revealed slight changes (see above). Changes in purity of SDZ-RTX, assessed using CEX, SEC, and nrCE-SDS methods, were more pronounced at condition 30 °C/65% RH, which is fully expected, given the higher temperatures can influence the physicochemical stability of monoclonal antibodies [27].

All quality attributes assessed in this study comply with the shelf-life specifications defined for SDZ-RTX. Overall, the study suggests SDZ-RTX remains safe and of appropriate quality after 21 days of OOF exposure up to 30 ± 2 °C/65 ± 5% RH, even in the batches that significantly exceeded the shelf-life by up to 7 months. While RTX potency relies on multiple modes of action in addition to CDC, CDC activity

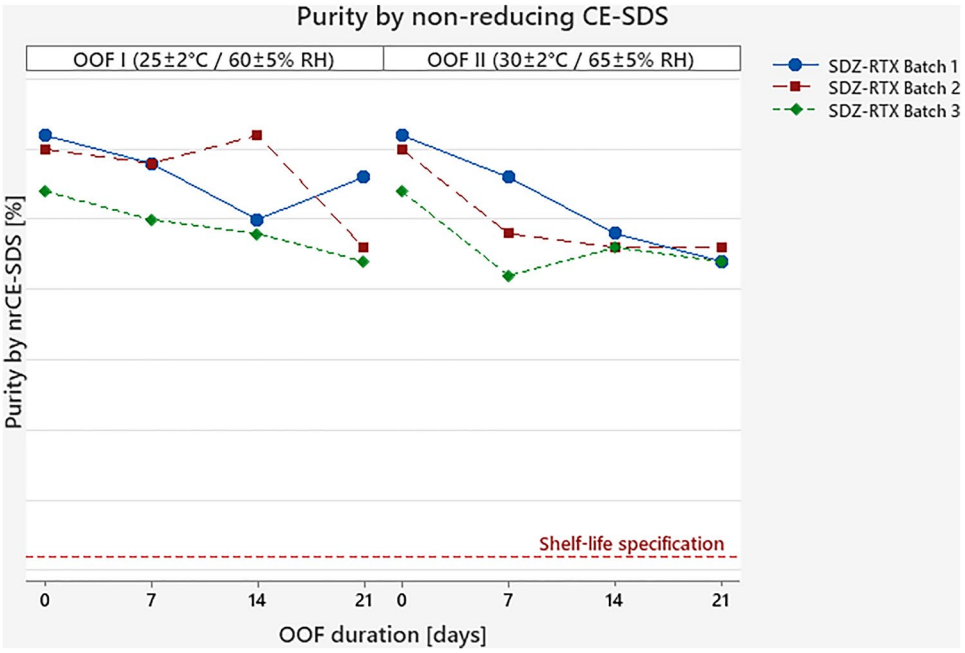

Fig. 5 Assessment of purity by non-reducing capillary electrophoresis sodium dodecyl sulfate method

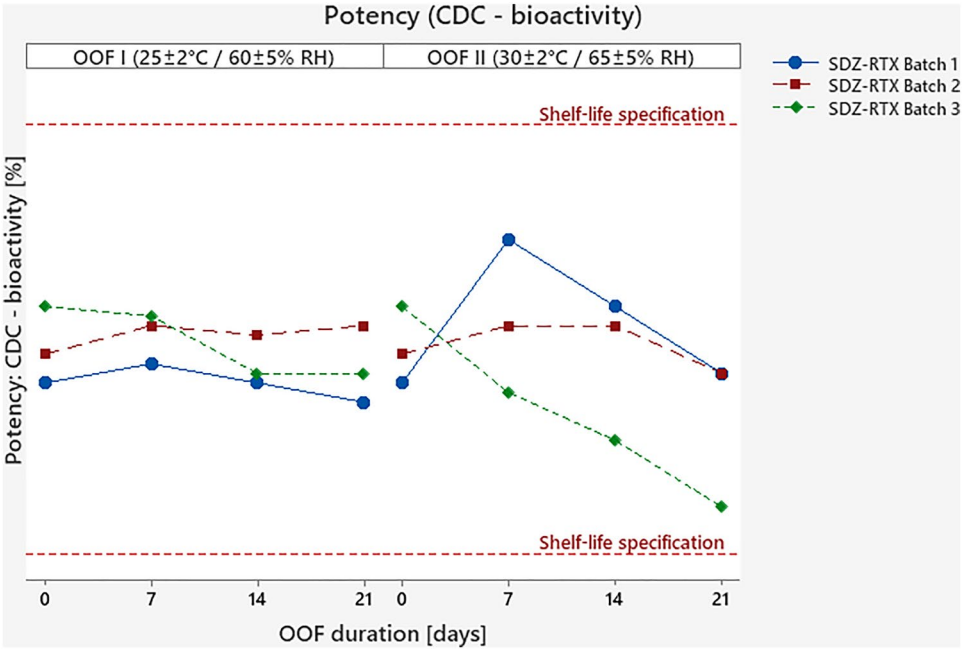

Fig. 6 Potency of SDZ-RTX measured by CDC-bioactivity of the temperature excursion study during 21 days of storage at 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/65 ± 5% RH. *CDC* complement-dependent lysis, *RH* relative humidity, *SDZ-RTX* Sandoz rituximab

is ideal for functional comparison as it requires both F_{ab} and F_c integrity and is independent of additional variables such as effector cell activity [18]. CDC analysis demonstrated no fully conclusive stability trend in potency for all three SDZ-RTX batches; a decrease was seen in one out of three SDZ-RTX batches for one OOF condition. Importantly, the biological quality was confirmed for SDZ-RTX batches as all results from CDC-bioactivity complied with the shelf-life specification throughout the entire course of the OOF stability study.

The PIL of a drug product contains instructions of the intended storage conditions. Exceedance of these conditions can have a significant impact on the quality of the product and can thus have potential safety and/or efficacy implications [28]. Therefore, the PIL must be strictly adhered to. An acceptable extended quality window of a drug for OOF conditions that can be reflected in the PIL can prevent unnecessary product wastage and healthcare financial burden. Simultaneously, this can address potential patients' and

caregivers' concerns regarding the quality of the drug after short-term OOF temperature excursions.

The findings of this study support an allowable single-time OOF temperature excursion, which applies for an unopened vial of SDZ-RTX stored in the original outer box. However, these study results must not be used to justify any temperature excursions or deviations, which may occur during shipping, storage, or administration of SDZ-RTX. The time and temperature defined for in-use after preparation for usage in hospital must be adhered to at all times. SDZ-RTX must always be handled according to the respective patient leaflet provided with the drug.

5 Conclusions

In conclusion, the quality of SDZ-RTX subjected up to 30 ± 2 °C/65 ± 5% RH for 21 days beyond the shelf-life was confirmed. The study results show that SDZ-RTX is safe and fit for use after short-term temperature excursions/OOF conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40268-022-00393-4.

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Declarations

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Conflict of interest All authors are employees of Novartis Pharma AG.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material The data collected during the study will be made available from the corresponding author on reasonable request.

Code availability Not applicable.

Author contributions The authors confirm contribution to the paper as follows: manuscript draft preparation and coordination of the OOF study execution, RB; manuscript draft preparation, AM; manuscript draft preparation and quality oversight, IŠ. All authors reviewed the results and approved the final version of the manuscript.

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