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Physicochemical Stability of Reconstituted Decitabine (Dacogen®) Solutions and Ready-to-Administer Infusion Bags when Stored Refrigerated or Frozen

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Abstract

Background: Profound knowledge about the physicochemical stability is necessary in order to determine the “beyond-use-dates” of ready-to-administer preparations after reconstitution and dilution. This is especially true for unstable azanucleoside drugs like decitabine. The aim of this study was to determine the physicochemical stability of decitabine after reconstitution and dilution of Dacogen® 50 mg lyophilized powder. Decitabine concentration of Dacogen® powder reconstituted with cold water for injections (5 mg/mL) has been determined after storage in the original vials under refrigeration for 48 hours and in single use syringes in a freezer at –25 °C for 28 days. Concentration of diluted decitabine infusion solutions (0.5 mg/mL) prepared in prefilled 0.9 % NaCl polyolefine (PO) infusion bags has been determined after storage under refrigeration (2–8 °C) for 48 hours.

Methods: To determine the stability of frozen Dacogen® solutions the powder was reconstituted with 10 mL of cold (2–8 °C) sterile water for injections, transferred into 1 mL plastic polycarbonate (PC)/polypropylene (PP) syringes and stored at –25 °C. Decitabine concentrations were determined on day 0, 22 and 28 after thawing at room temperature immediately before assaying. In parallel, pH-values were determined.

To determine the stability of reconstituted Dacogen® 50 mg powder in the original glass vials, three Dacogen® 50 mg vials were aseptically reconstituted with 10 mL of cold sterile water. The reconstituted solutions were stored under refrigeration and decitabine

concentrations were determined at 0, 3, 7, 12 and 24 hours after reconstitution. The pH-values were determined at 0, 7 and 24 hours.

Diluted Dacogen® test solutions were aseptically prepared by adding 2 mL of the reconstituted Dacogen® solution from each of the three vials to 18 mL cold (2–8 °C) 0.9 % NaCl solution in prefilled multi-layer PO infusion bags of the nominal value 50 mL. Test solutions of the nominal concentration 0.5 mg/mL were stored under refrigeration.

Decitabine concentrations were determined at 0, 5, 8, 12, 24 and 48 hours after preparation. The pH-values were determined at 0, 8, 24 and 48 hours. Each sample was assayed by a validated stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay with photodiode array detection.

Results: When test solutions of reconstituted Dacogen® solution were stored frozen at –25 °C, decitabine concentrations decreased less than 2% and no degradation products were detected in the HPLC chromatograms over the storage period of 28 days.

In reconstituted test solutions in glass vials and in diluted test solutions in infusion bags stored under refrigeration decitabine concentrations remained above 90 % of the initial concentration for 12 hours and 24 hours, respectively. Several peaks of degradation products were observed which explicitly increased over time.

In all test solutions the pH-values amounted to pH 7 and remained unchanged. No particulate matter and no colour changes were observed over the test period.

Conclusions: Reconstituted decitabine solution (Dacogen® 50 mg powder) stored in 1 mL PC/PP syringes and frozen at –25 °C is physicochemically stable for at least 28 days. Decitabine solution in glass vials after reconstitution with cold sterile water for injections and in PO infusion bags after dilution with cold 0.9 % NaCl solution and stored under refrigeration is physicochemically stable for approximately 12 hours and 24 hours, respectively. The results of our study facilitate the preparation of Dacogen® powder in pharmacy based centralized preparation units.

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Keywords: decitabine, physicochemical stability, reconstitution, ready-to-administer infusion bag, reversed-phase high-performance liquid chromatography (RP-HPLC)

Introduction

Dacogen® 50 mg powder for concentrate for solution for infusion containing decitabine as active pharmaceutical ingredient is indicated for the treatment of adult patients with newly diagnosed de novo or secondary acute myeloid leukaemia (AML), who are not candidates for standard induction chemotherapy [1]. Recent clinical evidence has shown it to be effective in producing remissions lasting several months or disease stabilization in a subset of this population [2].

The azanucleoside derivatives decitabine (5-aza-2'-deoxycytidine, NSC 127716) and azacytidine (5-azacytidine, NSC 102816, marketed as Vidaza®), are analogues of the natural nucleoside 2'-deoxycytidine (chemical structures compare Figure 1). Decitabine exerts its antineoplastic effects after phosphorylation and direct incorporation into DNA and inhibition of DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. Aberrant DNA hypermethylation in the promoter regions of certain genes, such as tumor-suppressor genes, and their consequent silencing is a characteristic of leukemias and myelodysplastic syndromes (MDS) [3]. Decitabine-induced hypomethylation in neoplastic cells may restore normal function to genes that are critical for the control of cellular differentiation and proliferation. In rapidly dividing cells, the cytotoxicity of decitabine may also be attributed to the formation of covalent adducts between DNA methyltransferase and decitabine incorporated into DNA [1].

Each single-use vial of Dacogen® 50 mg contains decitabine as sterile lyophilized powder. Potassium dihydrogen phosphate (E340), sodium hydroxide (E524) and hydrochloric acid for pH-adjustment (target pH=6.7 to 7.3) are used as excipients. Decitabine is sparingly soluble in water. Compatibility of the reconstituted solution with 0.9 % sodium chloride (0.9 % NaCl) or 5 % dextrose solutions for injection in infusion bags and typical infusion systems (syringe, infusion sets) is given. Control of leachables is not required. Light protection during handling and administration is not necessary [1].

According to the SmPC, last updated in 2017, 50 mg Dacogen® powder has to be reconstituted with 10 ml of sterile water for injections. The concentrated solution (5 mg/mL) must be further diluted with cold (2–8 °C) vehicle solutions within a period of 15 minutes. The diluted infusion solutions can be stored at 2–8 °C for maximum 3 hours, followed by up to 1 hour at room temperature (RT, 20–25 °C) before administration over 3 hours [4]. In the earlier SmPC version, published in 2012, cold Ringer's lactate solution was also mentioned as vehicle solution and a longer stability period was indicated for the diluted infusion solutions (compare Table 1). In the FDA approved prescribing information of Dacogen® for injection 0.9 % NaCl, 5 % dextrose or Lactated Ringer's Injection are recommended as vehicle solutions and once more different stability information is indicated (compare Table 1) [5]. According to the manufacturer, decitabine exhibits 2 % loss in 15 min and 5–6 % loss in 45 minutes in reconstituted solutions (5 mg/mL) when stored at RT [6]. Decitabine losses in diluted infusion solutions amount to 8–9 % over a 3 hour period when stored at RT and about 2 % over a period of 7 hours when stored under refrigeration [6]. In the latter case during subsequent administration at RT

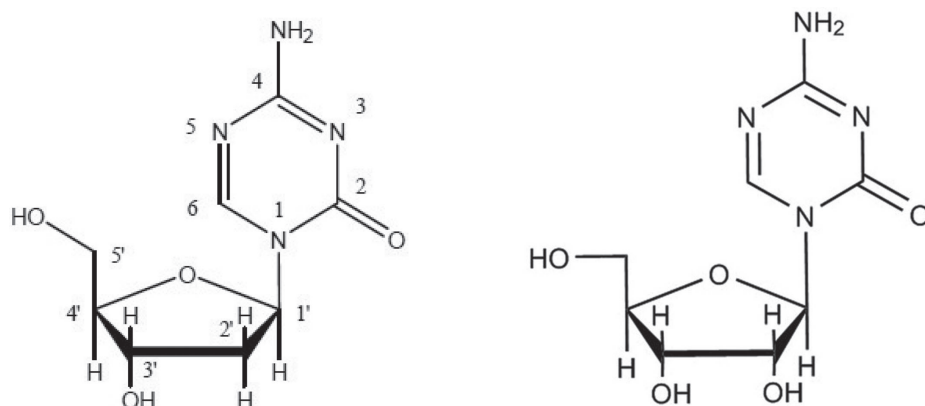


Figure 1: Chemical structure of decitabine (NSC 127716, 5-aza-2'-deoxycytidine) (left) and azacytidine (NSC 102816, 5-azacytidine) (right).

Table 1: Stability information of Dacogen® powder after reconstitution and dilution.

Reference	Reconstituent	Vehicle solution	Decitabine concentration in diluted solutions	Stability of reconstituted Dacogen® solution	Stability of diluted Dacogen® solution
SmPC (EMA, 2017)	10 mL of sterile water for injections	Cold (2–8 °C) 0.9 % NaCl or 5 % dextrose solutions	0.1–1.0 mg/mL	15 min	Max 3 hours at 2–8 °C, followed by up to 1 hour at RT (20–25 °C) before administration
SmPC (EMA, 2012)	10 mL of sterile water for injections	Cold (2–8 °C) 0.9 % NaCl, 5 % dextrose or Ringer's lactate solutions	0.1–1.0 mg/mL	15 min	Max 7 hours at 2–8 °C, followed by up to 2 hours at RT (20–25 °C) before administration
Prescribing information (FDA, 2006)	10 mL of Sterile Water for Injection (USP)	Cold (2–8 °C) 0.9 % NaCl, 5 % dextrose or lactated Ringer's injection	0.1–1.0 mg/mL	15 min	Max 7 hours at 2–8 °C (36°F - 46°F), until administration
Fernández-Ginés et al. (2017)	10 mL of sterile water for injections	Not applicable	Not applicable	7 hours at (4 ± 2 °C)	Not applicable
Patel (2006)	10 mL of Sterile Water for Injection (USP)	0.9 % NaCl, 5 % dextrose and lactated Ringer's solution at RT	0.1 and 1.0 mg/mL	1 hour at RT	3 hours at RT
		Cold (2–8 °C) 0.9 % NaCl, 5 % dextrose and lactated Ringer's solution	0.1 and 1.0 mg/mL		10 hours (7 hours refrigerated and 3 hours at RT)

over 3 hours the decitabine concentration remained at or above 92% [6].

By a literature research two poster presentations regarding the stability of Dacogen® preparations were identified [7, 8]. Patel studied the stability of reconstituted Dacogen® powder stored at RT and diluted solutions (0.1 and 1.0 mg/mL) using either vehicle solutions stored at RT or prechilled (2–8 °C) [8]. More recently the stability of reconstituted Dacogen® powder stored refrigerated in polyethylene (PE) syringes and determined by a nuclear magnetic resonance (¹H-NMR) spectroscopy assay was presented [7]. The currently available stability information regarding Dacogen® preparations is compiled in Table 1.

The storage periods of maximum 15 minutes and 4 hours indicated in the SmPC by the manufacturer obstruct the centralized aseptic preparation of ready-to-administer Dacogen® containing parenteral solutions. Extended in-use-stability data could improve the feasibility and efficiency of pharmacy based aseptic preparation. The purpose of this study is to determine the physicochemical stability of Dacogen® powder for concentrate for solution for infusion after reconstitution in the original vials and in single-use plastic syringes as primary containers. Furthermore, the impact of using prechilled water for injections for reconstitution and storage of reconstituted Dacogen® solution at decreased

temperatures (– 25 °C) over a period of 28 days has been investigated. Diluted infusion solutions has been prepared in prefilled 0.9 % sodium chloride infusion bags, stored under refrigeration (2–8 °C) and physicochemical stability determined over a period of 48 hours.

Chemical stability of decitabine was determined with a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay based on the known method published by Yuan et al. [9]. Physicochemical stability was determined by visual inspection as well as pH measurement of the products.

Materials and methods

Stability of frozen Dacogen® test solutions

One vial of commercially available Dacogen® 50 mg powder for concentrate for solution for infusion, Janssen-Cilag GmbH, Neuss, DE (lot-no.: GEZTJ00 (30th April 2019)) was reconstituted with 10 mL of cold (2–8 °C) sterile water for injections (lot-no.: 20170117–01, in-house production, UM Mainz, DE) using cannulas (BD Microlance^{TM3}) and 10 mL 3-piece plastic syringes (BD PlastipakTM 10 mL Luer-LokTM syringe). The barrel and the plunger of the used 10 mL syringes are made of polypropylene (PP) and siliconized

[10]. Nine test solutions were prepared by withdrawing 1 mL aliquots of the reconstituted solution with cannulas into 1 mL 3-piece plastic syringes (BD 1 mL syringe Luer-Lok™ Tip). The barrel of the 1 mL Luer-Lock syringes is made of polycarbonate (PC) and the plunger is made of PP [10]. The syringes were capped with Luer lock combi-stoppers made of PE (lot-no. 17D01A8171, B. Braun Melsungen AG, Melsungen, DE). Six syringes were immediately frozen and stored at -25°C . From the remaining three syringes 100 μL aliquots were withdrawn and diluted with 900 μL cold water HPLC grade (lot-no.: 7T012578, AppliChem GmbH, Darmstadt, DE) and assayed immediately. On day 22 and 28 of storage three syringes each were thawed at RT (25°C) for 5 minutes and diluted aliquots were assayed immediately. The pH-values were determined in each test solution prepared on day 0 or thawed on day 22 and 28.

Stability of reconstituted and diluted Dacogen® solutions

Preparation of test solutions

In order to determine the stability of reconstituted Dacogen® 50 mg powder in the original glass vials, three Dacogen® 50 mg vials (lot-no.: GEZTJ00 (30th April 2019)) were aseptically reconstituted with 10 mL of cold ($2-8^{\circ}\text{C}$) sterile water for injections (lot-no.: 20170117-01) using cannulas (BD Microlance™3) and 10 mL 3-piece plastic syringes (BD Plastipak™ 10 mL Luer-Lok™ syringe). The reconstituted solutions were stored light protected under refrigeration ($2-8^{\circ}\text{C}$). Diluted Dacogen® test solutions were aseptically prepared by adding 2 mL of the reconstituted Dacogen® solution from each of the three vials to 18 mL cold ($2-8^{\circ}\text{C}$) 0.9% NaCl solution in prefilled multi-layer polyolefine (PO) infusion bags of the nominal value 50 mL (lot-no.: 13KMS171, Isotonische Kochsalzlösung freeflex®, Fresenius SE & Co, Bad Homburg von der Höhe, DE). Prior to the addition, superfluous vehicle solution was withdrawn and discarded. The diluted test solutions in infusion bags of the nominal concentration 0.5 mg/mL were stored under refrigeration ($2-8^{\circ}\text{C}$) and light protection.

Sample preparation

Samples of the reconstituted solutions were taken immediately after reconstitution and after 3, 7, 12 (day 0) and 24 hours (day 1). At each predetermined interval 0.2 mL

aliquots were withdrawn from each vial in triplicate using cannulas and 1 mL 3-piece plastic syringes (BD 1 mL syringe Luer-Lok™ Tip). Between sampling intervals the vial stoppers were covered with a plastic film, consisting of paraffin wax and PO (ParafilmM®, Bemis Company, Neenah, Wisconsin, US). The aliquot syringes were capped with Luer lock combi-stoppers, immediately frozen and stored at -25°C . On the date of analysis each sample was thawed at RT (25°C) for 5 minutes. Aliquots of 100 μL reconstituted Dacogen® solution (5 mg/mL decitabine) were withdrawn and mixed with 900 μL cold ($2-8^{\circ}\text{C}$) water HPLC grade (lot-no.: 7T012578, AppliChem GmbH, Darmstadt, DE) to achieve a concentration of 0.5 mg/mL decitabine (one dilution step) and directly assayed. At hour 0, 7 and 24 one additional sample was withdrawn and the pH-value determined.

Samples of the diluted test solutions were taken immediately after preparation and after 5, 8, 12 (day 0), 24 (day 1) and 48 hours (day 2). At each predetermined interval 1 mL aliquots were withdrawn in triplicate from each bag using cannulas and 1 mL 3-piece plastic syringes. Samples were immediately frozen, stored and thawed prior to analysis in the same manner as samples of reconstituted solutions. 10 μL aliquots were immediately assayed without further dilution step. After 0, 8, 24 and 48 hours one additional sample was withdrawn and the pH-values were determined.

Each sample was assayed once by a validated stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay with photodiode array detection (PDA) to analyze the concentration and purity of decitabine. Whenever samples were taken the vials and syringes were visually checked for colour changes or particulate matter.

Determination of the chemical stability

HPLC Assay

The HPLC system consisted of a Waters Alliance 2695 pump and a Waters photodiode array detector 996 (Waters, Eschborn, DE). The Waters Empower Pro, Empower Build 1154 Software, version 5.00.00.00 (Waters, Eschborn, DE) was used for data evaluation.

The separation was performed with a C18 column (Kromasil C18, 250 mm x 4.6 mm, 5 μm , lot-no.: 27161926/27161920/27142515, MZ-Analysentechnik GmbH, Mainz, DE), which was maintained at 25°C . The mobile phase consisted of 98% 0.01 molar buffer solution

pH = 6.0 (dipotassium hydrogen phosphate (lot-no.: 0000715183, Applichem, Darmstadt, DE) water HPLC grade (lot-no.: 7T012578, AppliChem GmbH, Darmstadt, DE) and hydrochloric acid 1 mol/L Titrisol® (lot-no.: HC562719, Merck, Darmstadt, DE)) and 2% acetonitrile optigrade (lot-no.: SZBG279A, Sigma-Aldrich, Steinheim, DE). The overall run time was 13 minutes with a flow rate of 1.8 mL/min. 10 μ L samples were injected by an autosampler. The flushing solution consisted of 95 % water HPLC grade and 5 % acetonitrile optigrade.

The detection wave length was set at 240 nm. The identity of the decitabine peak was confirmed by concentration dependent peak area and PDA-chromatograms (Figure 2).

Validation of the RP-HPLC assay

The method was validated on the basis of the ICH Q2 (R1) Guidelines [11] for stability studies and followed as far as technically feasible.

Suitability

Suitability of the HPLC method was proven by analyzing “heat” degraded samples of reconstituted Dacogen® 50 mg powder for concentrate for solution for infusion solutions. The reconstituted solution with nominal concentration of 5 mg/mL was diluted with water HPLC grade

to achieve the concentration of 1 mg/mL decitabine and stored at RT over 30 hours.

Linearity

In order to study the linearity of the assay standard concentrations ($n = 7$) were prepared by diluting reconstituted Dacogen® solution with cold (2–8 °C) water HPLC grade to achieve the following concentrations: 0.75 mg/mL, 0.6 mg/mL, 0.55 mg/mL, 0.5 mg/mL, 0.45 mg/mL, 0.4 mg/mL, 0.25 mg/mL.

Three test solutions were prepared per concentration. Each test solutions was immediately frozen at –25 °C, thawed at RT (25 °C) and directly assayed. Aliquots of the calibration standards were injected once. The line of best fit was constructed by analyzing plots of peak area versus the nominal decitabine concentrations.

Accuracy

Accuracy was evaluated with two different concentration levels (100 %: 1 mg/mL and 80%: 0.8 mg/mL) and 12-fold injection.

1 mg and 0.8 mg decitabine (5-aza-2'-deoxycytidine ≥ 97 %) from Sigma-Aldrich Chemie GmbH, Munich, DE (lot-no.: SLBS4457/SLBQ1508V) were exactly weight with a Sartorius MSA2.7S-OCE-DM Cubis Ultra Micro Balance (Sartorius Lab Instruments, Göttingen, DE) and dissolved in 1 mL cold (2–8 °C) buffer solution pH = 7.4 (potassium dihydrogen phosphate R, lot-no.: 0000550126,

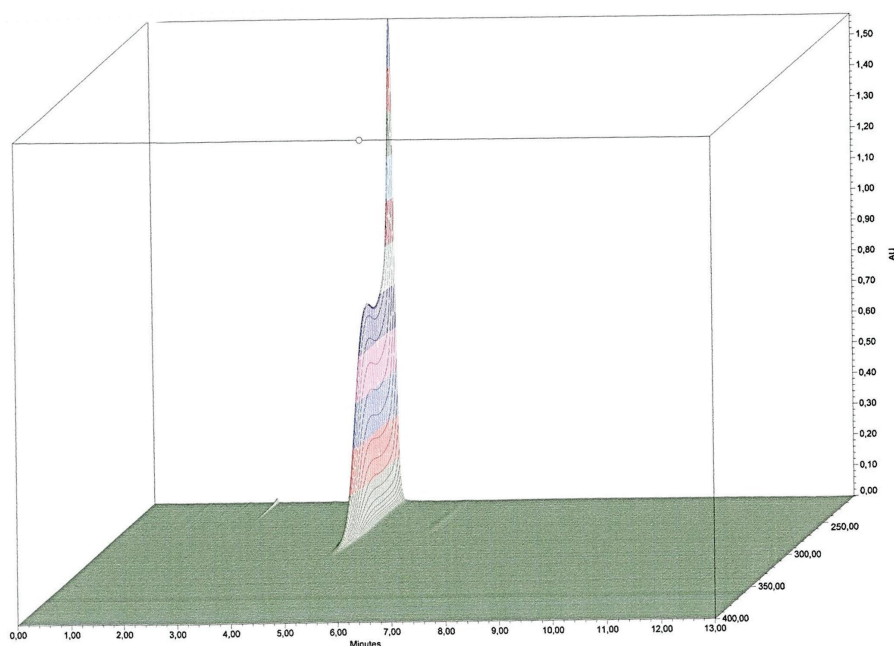


Figure 2: PDA chromatogram of a freshly prepared 0.5 mg/mL decitabine solution (Dacogen®).

Applichem, Darmstadt, DE and sodium hydroxide solution 1 mol/L Titrisol®, lot-no.: HC681977, Darmstadt, DE).

Intra- and Inter-day precision

The intra-day precision of the assay method was evaluated by carrying out 12 independent assays of decitabine solution prepared with cold (2–8 °C) buffer solution pH = 7.4 at two concentration levels (1 mg/mL and 0.8 mg/mL).

For inter-day precision at each concentration level a single injection of diluted decitabine solution and cold (2–8 °C) buffer solution pH = 7.4 was assayed daily on three consecutive days.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection and the limit of quantification were calculated by the equation $LOD = 3.3 SD/s$ and $LOQ = 10 SD/s$, respectively. In the equation s represents the slope of the calibration curve and SD the standard deviation of the peak area.

Determination of the physicochemical stability

pH-values were measured once in undiluted aliquots taken from the test solutions at the predetermined intervals using a pH 210 Microprocessor pH-meter (Hanna Instruments, Kehl am Rhein, DE) with a InLab Ultra-Micro pH-glass electrode (Mettler Toledo Inlab Ultra-micro pH, Gießen, DE). The instrument was calibrated with Hamilton Duracal buffer pH 4.01 ± 0.01 and pH 7.01 ± 0.01 .

Whenever samples were taken the vials were visually checked for colour changes and particulate matter.

Results

RP-HPLC assay

In the present study a modified HPLC assay based on a known method was implemented and validated by using the medicinal product Dacogen® and decitabine powder.

Suitability

Temperature and pH dependent decomposition instability of azanucleoside drug substances such as azacytidine and decitabine are obvious. Forced degradation of decitabine was therefore limited to “heat” degradation. Storage of the reconstituted Dacogen® powder diluted with water HPLC grade at RT for 30 hours caused significant degradation. The resulting peaks of degradation products did not interfere with the decitabine parent peak (retention time 4.5 minutes) (compare Figure 3).

Linearity

The correlation coefficient of $R^2 = 0.9989$ proved linearity over the concentration range. The equation of the calibration curve was $y = 9.04e + 006x - 5.75e + 004$.

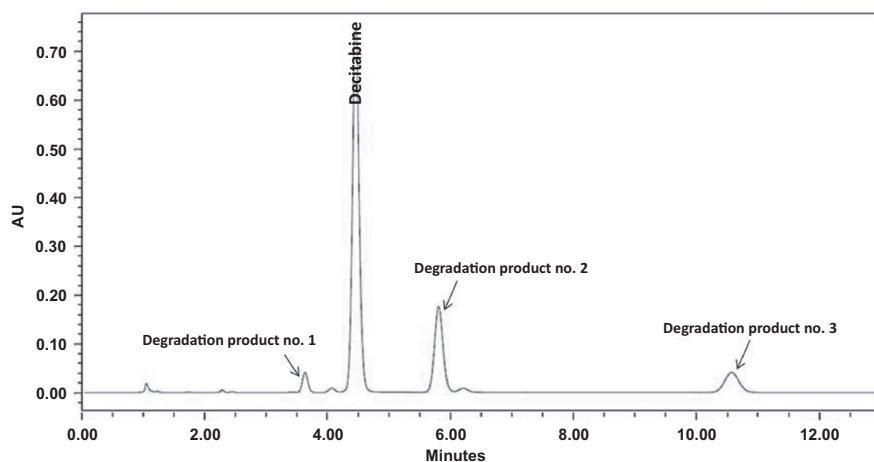


Figure 3: Representative HPLC-chromatogram of decitabine solution (Dacogen®) 1 mg/mL after a 30 hours storage-period stored at RT (25 °C).

Accuracy

The mean recovery was $99\% \pm 4.3\%$ ($n=12$).

The accuracy was $98.5\% \pm 2.1\%$ for 1 mg/mL and $99.4\% \pm 2.6\%$ for 0.8 mg/mL decitabine solutions.

Intra- and Inter-day precision

The % RSD (Relative Standard Deviation) of the intra- and inter-day assays were 6.05% for 1 mg/mL and 4.90% for 0.8 mg/mL as well as 6.18% for 1 mg/mL and 5.74% for 0.8 mg/mL decitabine.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and the LOQ amounted to 0.029 mg/mL and 0.089 mg/mL of decitabine, respectively.

Chemical stability of decitabine solutions

When test solutions of reconstituted Dacogen® solution were stored frozen at -25°C no degradation products

were detected in the HPLC chromatograms over the storage period of 28 days. In addition the concentrations of decitabine decreased less than 2% over the test period. Measured concentrations of decitabine in the test solutions are presented in Table 2.

In samples taken from the reconstituted Dacogen® solutions 5 mg/mL in glass vials stored under refrigeration ($2-8^\circ\text{C}$) several peaks of degradation products were observed. These peaks matched with the peaks spotted during degradation at RT (see Figure 3) and increased over the storage period of 24 hours.

The same degradation peaks were observed in samples taken from diluted Dacogen® solutions in PO infusion bags stored under refrigeration. The peaks increased over the storage period of 48 hours. Especially the degradation products eluted with the retention time 3.6, 5.8 and 10.6 min (degradation product no. 1 to 3) explicitly increased over time (see Figures 4 and 5). Measured concentrations of decitabine in the various test solutions are presented in Tables 3, 4 and in Figures 6 and 7.

In reconstituted test solutions in glass vials or in diluted test solutions in infusion bags stored under

Table 2: Stability and pH-values of decitabine containing solutions (Dacogen®) in plastic syringes stored frozen (-25°C) over a period of 28 days. Concentration expressed as mean $\pm$ RSD of single assays of three test solutions ($n=3$). pH-values expressed as mean $\pm$ SD of single measurement of three test solutions ($n=3$).

Primary package of test solutions	Drug concentration [mg/mL]		% Initial concentration remaining $\pm$ relative SD	
	Nominal	Actual Day 0	Day 22	Day 28
Plastic syringe (PC/PP)	5.0	4.9 ± 0.4	98.4 ± 0.4	98.8 ± 0.5
pH-value (mean $\pm$ SD)		7.06 ± 0.1	7.08 ± 0.1	7.06 ± 0.1

Note: Drug concentrations in samples taken at time zero were designated as 100 %.

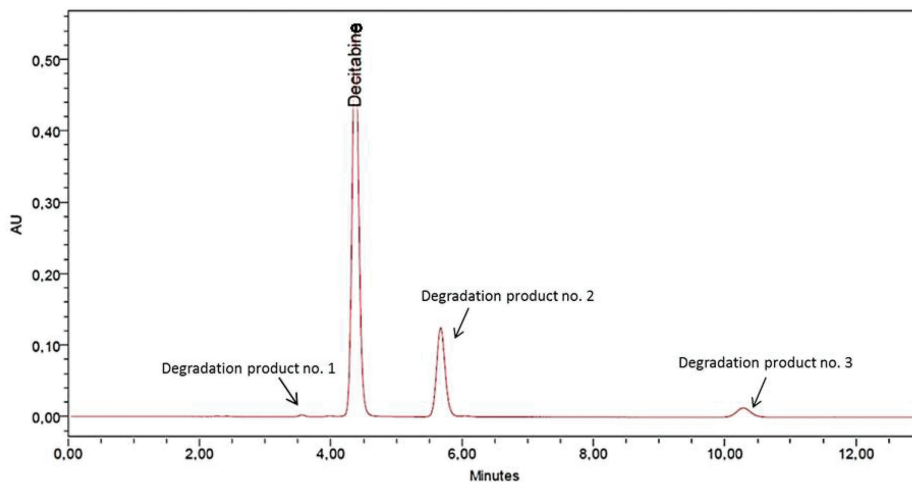


Figure 4: Representative HPLC-chromatogram of reconstituted decitabine solution (Dacogen®) 5mg/mL in glass vials after a 24 hours storage-period stored under refrigeration ($2-8^\circ\text{C}$).

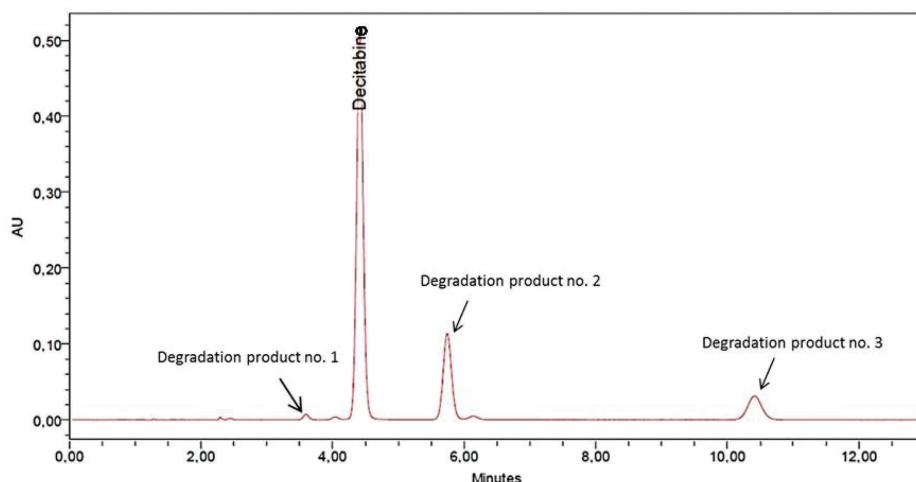


Figure 5: Representative HPLC-chromatogram of diluted decitabine solution (Dacogen®) 0.5mg/mL in PO infusion bags after a 48 hours storage-period stored under refrigeration (2–8°C).

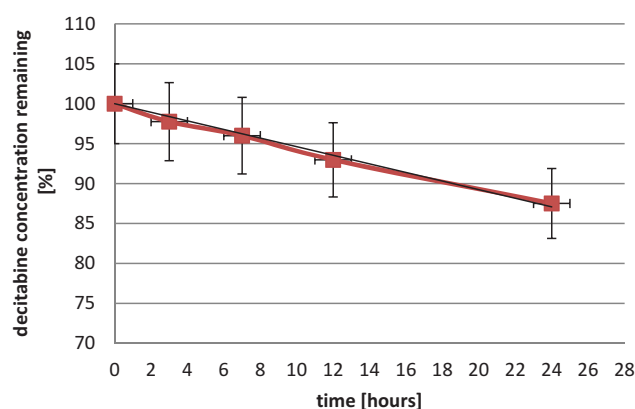


Figure 6: Remaining decitabine concentration and 95% to 105% confidence interval in reconstituted Dacogen® solution in glass vials stored under refrigeration (2–8°C) over a period of 24 hours (n = 9). Calculated t_{95} - and t_{90} -value amount to 9.3 and 18.6 hours according to the line of best fit, respectively.

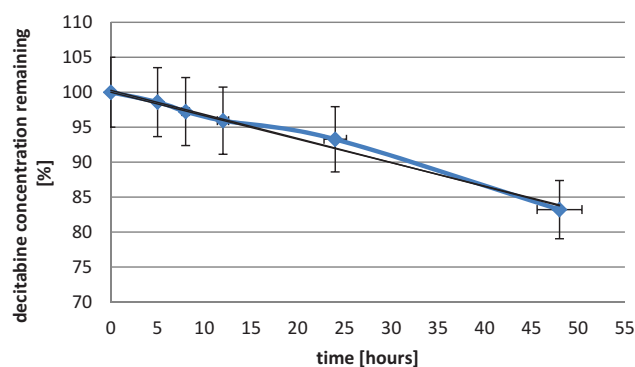


Figure 7: Remaining decitabine concentration and 95% to 105% confidence interval in diluted Dacogen® solution in PO infusion bags stored under refrigeration (2–8°C) over a period of 48 hours (n = 9). Calculated t_{95} - and t_{90} -value amount to 15 and 30 hours according to the line of best fit, respectively.

refrigeration the measured decitabine concentrations remained above 90% of the initial concentration for 12 hours or 24 hours (Tables 3 and 4). Calculated t_{90} values of decitabine amounted to about 19 hours and 30 hours for reconstituted and in diluted test solutions, respectively.

Physicochemical stability of decitabine solutions

In none of the test solutions particulate matter or colour changes were observed over the test period. The mean pH-values amounted to about pH 7 in concentrated and diluted solutions thereby matching the target range of 6.7 to 7.3. The decomposition rate is known to be lowest at pH 7.0 [12]. The pH slightly varied in the reconstituted test solutions (Tables 2 and 3) and in the ready-to-administer preparations stored in PO infusion bags as primary containers (Table 4) but remained in fact unchanged over the observation test period.

Discussion

Stability of azanucleoside drugs

The chemical instability of azanucleoside drugs like decitabine (5-aza-2'-deoxycytidine) and azacytidine (5-azacytidine) is well-known [12, 13]. Medicinal products containing these active substances therefore have to be handled with considerable care in the clinic, primarily in pharmacy-based centralized preparation units. The main concern is

Table 3: Stability and pH-values of reconstituted decitabine (Dacogen®) solution in glass vials stored under refrigeration (2–8 °C) over a period of 24 hours. Concentration expressed as mean $\pm$ RSD of a single assay of three samples of each test solution ($n=3$). pH-values expressed as mean $\pm$ SD of single measurement of three test solutions ($n=3$).

Test solution	Drug concentration [mg/mL]		% Initial concentration remaining $\pm$ relative SD			
	Nominal	Actual 0 hour	3 hours	7 hours	12 hours	24 hours
Glass vial 1	5.0	4.8 $\pm$ 0.9	96.9 $\pm$ 0.5	95.4 $\pm$ 0.6	91.9 $\pm$ 0.2	86.0 $\pm$ 0.1
Glass vial 2		4.8 $\pm$ 0.6	99.2 $\pm$ 0.8	95.6 $\pm$ 0.3	92.7 $\pm$ 0.5	88.3 $\pm$ 0.1
Glass vial 3		4.8 $\pm$ 0.3	97.2 $\pm$ 2.4	97.0 $\pm$ 0.3	94.3 $\pm$ 0.4	88.3 $\pm$ 0.2
pH-value (mean $\pm$ SD)		7.06 $\pm$ 0.2	n. d.	7.05 $\pm$ 0.1	n. d.	7.05 $\pm$ 0.0

Note: Drug concentrations in samples taken at time zero were designated as 100 %.

Table 4: Stability and pH-values of diluted decitabine (Dacogen®) containing solution in PO infusion bags over a period of 48 hours, stored under refrigeration (2–8 °C). Concentration expressed as mean $\pm$ RSD of a single assay of three samples of each test solution ($n=3$). pH-values expressed as mean $\pm$ SD of single measurement of three test solutions ($n=3$).

Test solution	Drug concentration [mg/mL]		% Initial concentration remaining $\pm$ relative SD				
	Nominal	Actual 0 hour	5 hours	8 hours	12 hours	24 hours	48 hours
Infusion bag 1	0.5	0.45 $\pm$ 1.7	100.7 $\pm$ 2.0	97.9 $\pm$ 2.0	99.3.0 $\pm$ 0.7	96.2 $\pm$ 0.1	87.1 $\pm$ 1.5
Infusion bag 2		0.48 $\pm$ 0.3	97.6 $\pm$ 0.1	96.9 $\pm$ 0.3	95.0 $\pm$ 0.2	91.8 $\pm$ 0.1	80.9 $\pm$ 0.3
Infusion bag 3		0.49 $\pm$ 0.4	97.5 $\pm$ 0.5	96.8 $\pm$ 0.1	93.6 $\pm$ 0.2	91.8 $\pm$ 0.2	81.6 $\pm$ 0.3
pH-values (mean $\pm$ SD)		6.93 $\pm$ 0.1	n. d.	6.95 $\pm$ 0.0	n. d.	6.95 $\pm$ 0.2	6.9 $\pm$ 0.1

Note: Drug concentrations in samples taken at time zero were designated as 100 %.

the rapid loss of activity and diminishing clinical efficacy. Increasing toxicity is of less concern as toxicity of the degradation products is not expected [1, 14].

Lin et al. investigated the chemical decomposition of decitabine [12] under various conditions. In alkaline solutions, decitabine undergoes a reversible hydrolytic reaction to form *N*-(formylamidino)-*N'*- β -D-2-deoxyribofuranosylurea, which by the irreversible loss of the formyl group, results 1- β -D-2'-deoxyribofuranosyl-3-guanylyurea. The opening of the triazine ring is facilitated by a hydroxyl ion nucleophilic attack. In neutral solutions additional degradation products got obvious, which were not identified [12]. Decitabine degradation highly depends on temperature and pH-value. The compound is most stable in neutral solution and when stored at low temperature. When Stresemann et al. studied the stability of decitabine powder in neutral aqueous solutions (0.45 mg/mL) with a capillary electrophoresis assay and UV detection at 254 nm, the half-times (t_{50}) were 7 days at 4 °C, 4 days at 20 °C and 21 hours when stored at 37 °C [13].

The commercially available Dacogen® 50 mg lyophilized powder is formulated with potassium dihydrogen phosphate (E340) and sodium hydroxide to guarantee an optimal pH-value after reconstitution and dilution [4, 5]. Furthermore according to the SmPC

reconstituted Dacogen® solution should be immediately diluted with prechilled (2–8 °C) vehicle solutions in order to increase the stability. However the stability is only indicated up to 15 minutes for the reconstituted Dacogen® powder and 4 hours for the diluted Dacogen® solution [4]. The study design was primarily based on this information about the instability of the medicinal product.

Preparation of test solutions and samples

According to the EPAR, Dacogen® 50 mg lyophilized powder should be reconstituted with 10 mL of sterile water for injections. There is no information about the physico-chemical stability of the reconstituted solution, if prechilled sterile water for injections is used for reconstitution and the solution is immediately stored in the refrigerator. According to the SmPC of Vidaza® 25 mg/mL powder for suspension for injection containing 5-azacytidine as active pharmaceutical ingredient, cold water for injections should be used for reconstitution [15]. Consequentially we decided to dissolve the Dacogen® 50 mg lyophilized powder with prechilled sterile water for injections.

In the EPAR and US SmPC it is also given that the vehicle solutions (0.9 % NaCl, 5 % dextrose (or Lactated Ringer's injection)) should be prechilled. In our study prechilled 0.9 % NaCl solution was chosen as vehicle solution because of the most appropriate properties. 5 % dextrose and Ringer's lactate infusion solution are less considerable in practice and the latter one is less suitable because of the ion content. Because of the known accelerated decomposition of decitabine at RT, the test solutions were only stored refrigerated.

To prove the accuracy of the HPLC assay, we also used decitabine powder which was dissolved in prechilled phosphate buffer solution pH=7.4. This favourable solvent and refrigeration were used throughout our experiments. The retained decomposition of decitabine dissolved in the phosphate buffer solution pH 7.4 was shown by Lin et al. [12]. Furthermore water HPLC grade for diluting Dacogen® solutions was refrigerated to reduce the degradation process of decitabine test solutions during sample preparation and assaying.

To determine the stability of the Dacogen® solutions samples were withdrawn at predetermined intervals and immediately frozen and stored at -25 °C. Freezing samples at -25 °C to disrupt the decomposition of decitabine in Dacogen® solutions refers to the proven stability of frozen decitabine solution at -70 °C and in liquid nitrogen [12, 13]. Consequently the number of Dacogen® vials to be reconstituted for the stability study could be minimized. We also have to admit that the restricted number of test solutions limits our study results. The reason is the high price of the Dacogen® medicinal product and limited financial resources associated with this investigator initiated study.

HPLC assay

In the present study a modified HPLC assay based on a known HPLC-method was implemented and validated. According to the HPLC method of Yuan et al. [9] the Kromasil C18 250 mm×4.6 mm, 5 µm column was used. The mobile phase equally consisted of phosphate buffer solution pH=6.0, but was completed with 2 % acetonitrile in order to decrease the retention time of decitabine. For the same purpose, a flow rate of 1.8 mL/min was chosen. The overall run time should be shortened as much as possible to inhibit the degradation process during assaying. Because of the short in-use-stability of 15 minutes indicated in the SmPC [4], the overall run time of 13 minutes with a retention time of 4.5 minutes was a critical point of stability testing of the Dacogen®

solutions. The high flow rate affected the column in different degrees depending on the batch related specification of the Kromasil column. To minimize the damage of the column, it is recommended to increase a low initial flowrate of 0.5 mL/min stepwise to 1.8 mL/min during equilibration. To minimize the strain on the performance of the column, post hoc the decision was made to dilute the samples to a concentration of 0.5 mg/mL decitabine. Subsequently the concentration differed from the concentrations used during the validation tests. However reproducibility is obvious by the minimum RSD of the concentrations determined.

The detection wavelength of 240 nm was chosen as a compromise of optimum absorption of decitabine at 254 nm and the absorption of potential degradation products at 220 nm. In our studies the degradation product no. 2 with a retention time of 5.8 minutes (see Figures 3, 4 and 5) most likely corresponds to the ring open form of decitabine which is chromophoric at 254 nm. The degradation product no. 1 presumably corresponds to 1-β-D-2'-deoxyribofuranosyl-3-guanylylurea, which is chromophoric at 220 nm.

The method revealed to be stability-indicating. In the resulting chromatograms, the sharp peak of decitabine (R_t about 4.5 min) was clearly separated from the degradation products. The degradation pathway and the highly temperature- and pH-dependent decomposition of decitabine are already described elsewhere [12, 13, 16]. Because of the rapid degradation of decitabine, suitability of the HPLC assay was only investigated by gently "heat" degraded samples. Furthermore the pH-value of the samples is predetermined by the formulation of Dacogen®.

The validation of the HPLC assay was originally planned by dissolving decitabine powder with cold buffer solution pH=7.4 and without freezing the samples. However, assaying of frozen Dacogen® solution revealed to be more favourable and convenient. Therefore suitability and linearity of the HPLC assay were proved with frozen samples of Dacogen® solutions. Each sample was assayed immediately after thawing. SDs amounting to 5 % when testing the accuracy are probably caused by individual weighing of 1 mg decitabine samples and rapid decomposition of the substance.

Physicochemical Stability of reconstituted Dacogen® solution

Measured decitabine concentrations in reconstituted Dacogen® solution stored frozen at -25 °C in single-use

syringes decreased only slightly and varied within the accuracy limits of the assay and dilution steps. Physicochemical stability is given over a storage period of 28 days. For azacytidine suspension increased stability was also shown when stored at -20°C compared to refrigeration [17].

In reconstituted Dacogen® solutions prepared with prechilled water and stored refrigerated in glass vials, measured decitabine concentrations fell below the 90 % limit after 24 hours. Having notice of this fact, we stopped the experiment after 24 hours. The calculated t_{95} - and t_{90} -value amounts to 9.3 and 18.6 hours according to the line of best fit and taking the initially measured concentration as 100 %. When t_{90} was calculated by taking the nominal decitabine concentration as 100 %, the interval came to 16 hours. According to these results, the reconstituted Dacogen® solutions can be declared as physicochemical stable over the storage period of 12 hours. During the studies of Patel [8] reconstitution of Dacogen® powder with non-prechilled water for injections resulted in 5 % degradation after 60 minutes. The reconstitution of Dacogen® with prechilled water for injections is obviously related to increased stability and highly recommended.

As indicated in the EPAR, the pH-values of reconstituted Dacogen® solution amount to 6.7–7.3. In our study the measured pH-values uniformly amounted to pH=7 and remained unchanged over the observed test period. This was also the case for reconstituted Dacogen® solutions when stored frozen. Changes of the pH-value caused by degradation products were not expected because of phosphate buffer present in the Dacogen® formulation.

Physicochemical Stability of diluted Dacogen® solution

Diluted Dacogen® solutions in prefilled 0.9 % NaCl solution in PO infusion bags stored under refrigeration revealed to be physicochemical stable over a storage period of 24 hours (93 % remaining decitabine concentration). The pH-values remained unchanged. The calculated t_{95} - and t_{90} -value amount to 15 and 30 hours when taking the initially measured concentration as 100 %, respectively. Taking the nominal concentration as 100 %, the t_{90} came to 26 hours. These results correspond to the findings of Patel [8]. In his studies, independent of the initial decitabine concentration (0.1 and 1.0 mg/mL) and the type of vehicle solution (0.9 % NaCl, 5 %

dextrose, lactated Ringer's solution) the remaining decitabine concentration amounted to 92 % after 10 hours of refrigerated storage [8].

Different stability of reconstituted and diluted Dacogen® solutions

While for the diluted Dacogen® infusion solutions (0.5 mg/mL) a t_{90} of 30 hours was calculated, the t_{90} of reconstituted Dacogen® solution (5 mg/mL) amounted to only 19 hours. Of note, the SmPC recommends to use the diluted solution within 4 hours and reconstituted solution within 15 minutes [4]. The same phenomenon was reported by Patel, i. e. 10 hours physicochemical stability for diluted solutions and 1 hour for the reconstituted solution [8]. However, there was no difference in stability when Patel tested diluted Dacogen® solutions of the concentration 1 mg/mL and 0.1 mg/mL. Therefore we studied only the mean concentration of 0.5 mg/mL decitabine recommended in the SmPC for diluted solutions (1 mg/mL = upper limit, 0.1 mg/mL = lower limit). The different stability of the reconstituted Dacogen® solutions and the tenfold diluted infusion solutions can be explained by higher concentrations of degradation inducing hydroxyl and hydrogen ions deriving from the phosphate buffer in the reconstituted solution.

Practical aspects

According to our results remaining reconstituted Dacogen® solutions can be transferred into single-use 1 mL syringes as primary packaging, stored frozen at -25°C up to 28 days and further used. It is more easy and logical to freeze Dacogen® glass vial when the residual volume is greater than 1 mL. However, if the volume in the vial is greater than the needed volume for the next preparation, the usability of the residual thawed volume is questionable. There are no published data about the stability of re-frozen and re-thawed Dacogen® solution. A favourable solution is to freeze maximum 1 mL volumes in order to reduce the wasting. After thawing at RT the reconstituted solutions must be diluted immediately. Because of the rapid degradation of decitabine the storage of Dacogen® solutions at RT is not recommended and was not in the scope of our stability study. As decitabine is not photosensitive, light protection during storage is not mandatory, but light protected storage was guaranteed in the freezer and refrigerator. In general, besides the

physicochemical stability, microbiological stability is a crucial factor in assigning extended beyond-use-dates. In the case of Dacogen® preparations this is only relevant for the frozen reconstituted solutions because of the limited physicochemical stability under refrigeration. Anyway handling of intravenous preparations has to be done under strict aseptic conditions. Material and treatment expenses can be reduced because remaining reconstituted solutions can be stored frozen and are not to be discarded.

Conclusion

Decitabine solution (Dacogen® 50 mg powder for concentrate for solution for infusion) is physicochemically stable in glass vials after reconstitution with cold (2–8 °C) sterile water for injections and in PO infusion bags after dilution with cold 0.9 % NaCl solution for 12 hours and 24 hours when stored under refrigeration (2–8 °C), respectively. Reconstituted decitabine solution stored in PC/PP syringes of the nominal volume 1 mL and frozen at –25 °C is physicochemically stable for at least 28 days. The residual Dacogen® solution in glass vials can be transferred into 1 mL single-use syringes, stored frozen and further used after thawing at RT. The results of our study facilitate the preparation of Dacogen® lyophilized powder in pharmacy based centralized preparation units.

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