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Physicochemical stability of azacitidine suspensions at 25 mg/mL in polypropylene syringes stored under different conditions of storage

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Abstract

Objectives: Azacitidine is a pyrimidine nucleoside analogue whose stability is temperature dependent. Numerous publications have studied the stability of this drug with discordant results. The purpose of this work is to study the stability of azacitidine suspensions under different conditions to allow preparation in advance: vials stored at room temperature or between 2 and 8 °C, reconstituted with refrigerated water for injection (WFI) or frozen/thawed WFI, azacitidine suspensions stored at room temperature, 2–8 °C or at –20 °C. The feasibility of a vented ChemoClave® Spike vial was also tested to reconstitute and collect azacitidine to aid the preparation stage.

Methods: The stability study was performed by HPLC coupled to a photodiode array detector. The method was validated according to ICH Q2(R1). Two syringes were prepared for each analysis condition and two samples were realised for each syringe at each time of the analysis. For a storage at 2–8 °C, analyses were performed for up to 168 h. The stability was studied after 2 h at room temperature. For frozen storage, the stability was studied after 28 days.

Results: Azacitidine 25 mg/mL suspensions stored between 2 and 8 °C, prepared with refrigerated WFI or frozen/thawed WFI, retained more than 90% of the initial concentration for 96 h and then for 2 h at room temperature. Prepared with frozen/thawed WFI, azacitidine 25 mg/mL suspensions stored at –20 °C for 28 days and then 72 h between 2 and 8 °C after thawing, retained more than 90% of the initial concentration. When using a Spike system compared to using a needle for reconstitution and collection of the suspension, the results obtained by HPLC showed a decrease of 1.47% in the concentration of azacitidine. The comparisons of the volumes withdrawn after reconstitution were similar when using a Spike system or a needle.

Conclusions: Azacitidine 25 mg/mL suspensions reconstituted with refrigerated WFI were chemically stable for 4 days when stored at 2–8 °C whatever the storage of vials (refrigerator or room temperature), and 2 h at room temperature. A storage of azacitidine 25 mg/mL suspensions in syringes prepared with frozen/thawed WFI at –20 °C has been validated for up to 28 days, leading to the possibility to prepare in advance. A Spike device can be used to reconstitute and collect azacitidine.

Keywords: administration; azacitidine; stability; suspension.

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Introduction

Azacitidine is an antimetabolite pyrimidine nucleoside analogue, indicated in the treatment of haematological pathologies such as myelodysplastic syndrome and acute myeloid leukemia. The recommended dose is 75 mg/m^2 of body surface area by subcutaneous injection per day for 7 days, followed by a 21-day rest period. Azacitidine is a lyophilised powder that is reconstituted with water for injections (WFI) to obtain a suspension for injection at 25 mg/mL [1].

This cytotoxic drug is unstable in aqueous solutions. The description of the hydrolysis degradation has been realised by different research teams [2–4]. Azacitidine is degraded in two steps: the first is a rapid and reversible hydrolysis, to form N-formylribosylguanylurea (RGU-CHO). The second step is an irreversible hydrolysis of RGU-CHO to ribosylguanylurea (RGU).

As recommended by manufacturers, if azacitidine is reconstituted with WFI that has not been refrigerated, the stability has been demonstrated for 45 min at 25 °C and for 8 h between 2 and 8 °C. If the reconstitution is performed with refrigerated WFI, the stability can be extended to 22 h between 2 and 8 °C [5]. The short stability of azacitidine requires to mobilize pharmaceutical personnel for the preparation of syringes on weekends. Duriez et al. have demonstrated a stability of 8 days for frozen suspensions (–20 °C) of azacitidine at 25 mg/mL reconstituted with refrigerated WFI [6]. Under the same conditions, Balouzet et al. have concluded to a stability of 30 days and Walker et al. of 23 days [2, 7]. Regarding storage between 2 and 8 °C, different stability data are demonstrated: 2 days of stability for azacitidine suspensions by Légeron et al. to 5 days by Vieillard et al. [8, 9]. The temperature is a factor which affects the hydrolysis of azacitidine and, consequently, the stability of this molecule [10].

In daily practice of a central cytotoxic preparation unit, an isolator can be used and the preparation requires a sterilization step of the products, bringing heat. Savry et al. compared the temperature of WFI stored between 2 and 8 °C, after the complete decontamination procedure in isolators, in several containers: 50 mL glass vial, 250 mL glass vial, 20 mL plastic ampoule [11]. The mean ± standard deviation (SD) temperatures were 16.6 ± 0.7, 11.9 ± 0.3, and 10.3 ± 0.2 °C for the 20 mL ampoules, 50 mL bottles, and 250 mL bottles, respectively. The temperature is too warm for maximum azacitidine stability. They have concluded that "*a better way to reconstitute a 100 mL vial of azacitidine is to freeze a 20 mL plastic ampoule containing sterile WFI, thaw it for 30 min, agitate the ampoule for 30 s, and remove the 4 mL needed for reconstitution.* ». However, in this study, the impact of the use of thawed WFI on the stability of azacitidine compared to refrigerated WFI already partially warmed was not studied.

The objective of this work was to study the physicochemical stability of a new generic of azacitidine, from Viatris company (ex Mylan) considering the working conditions in a centralized cytotoxic preparation unit. The impact of the temperature of WFI for the reconstitution and the condition of storage of azacitidine vials were studied.

Azacitidine vials were reconstituted either with refrigerated WFI or WFI initially frozen, placed in the refrigerator the day before, for partial thawing: on the day of the manipulation, the bag is taken out of the refrigerator. The study was carried out under two storage conditions for azacitidine vials: room temperature or 2–8 °C. To simulate the worst condition of storage, a stability study was performed on azacitidine suspensions which were prepared with vials stored at room temperature, reconstituted with refrigerated WFI, stored 24 h between 2 and 8 °C and kept at room temperature up to 2 h. Another study was carried out with storage of the syringes at –20 °C for 28 days then an evaluation of the stability after thawing for 4 days.

The use of a Spike device (ICU Medical) has been studied to replace a needle and to facilitate the preparation of azacitidine suspensions.

Materials and methods

Chemicals and reagents

Di-sodium hydrogen phosphate Na_2HPO_4 (VWR Chemicals, batch: AM1616521035), sodium dihydrogen phosphate NaH_2PO_4 anhydrous (VWR Chemicals, batch: 17D104101), acetonitrile gradient grade for HPLC (VWR Chemicals, batch: 21C092035), water for chromatography produced by a reverse osmosis system (Millipore Iberica, Madrid, Spain) were used for the mobile phase. The pH was adjusted at 6.5 by sodium hydroxide 1 M (VWR chemicals, batch: 210305C001). Hydrochloric acid 1 M (VWR chemicals, batch: 201124C003) and sodium hydroxide 1 M (VWR chemicals, batch: 210305C001) were used for the forced degradation study. Azacitidine 100 mg, powder for injectable suspension (Mylan, batch 1: 7U10030A and batch 2: 7U10010A), WFI 250 mL glass vial (Lavoisier, batch: 1F516), WFI 500 mL (Macoflex, Batch 21A07A) ChemoClave® vented vial Spike (ICU Medical), a female luer-lock to female luer-lock connector in polycarbonate (Vygon) and polypropylene syringes 5 mL (Medicina, batch: 20,120,511) were used for test solutions, the validation of the analytical method, the forced degradation, and the stability studies.

HPLC assay

Azacitidine suspensions were analyzed by a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method with photodiode array detection adapted from Duriez et al. [6] The HPLC system consisted of an ELITE LaChrom VWR/Hitachi plus autosampler, a VWR photodiode array (DAD) detector L-2455 and a VWR L-2130 HPLC-pump. Data were acquired and integrated by using EZChrom Elite (VWR, Agilent). The column used was Synergi™ Fusion-RP 80 A, **150 × 4.6 mm** and 4 µm particle size (Phenomenex®), with a security Guard™ cartridge fusion-RP 4 × 3.0 mm (Phenomenex®). The mobile phase was in isocratic mode constituted by 99% of 10 mM phosphate buffer (1154.8 mg NaH_2PO_4 with 231.8 mg Na_2HPO_4 qs 1 L of ultrapure water) adjusted at pH=6.5 with NaOH 1 M and 1% of acetonitrile gradient grade for HPLC. The detection of azacitidine was performed at 240 nm. The flow rate was set at 1.0 mL/min, with an injection volume of 20 µL. The temperatures of the column and of the injector were set at 25 °C and 4 °C respectively. The method was validated as recommended by the international conference on harmonisation (ICH)

Q2 (R1) [12]. The calibration curve was constructed from plots of peak area vs. concentration. The linearity of the method was evaluated with five concentrations (80, 90, 100, 110, 120 µg/mL). A solution of azacitidine 1 mg/mL was prepared from 100 mg powder azacitidine diluted qs 100.0 mL of refrigerated WFI. This solution at 1 mg/mL was refrigerated and used to prepared standard curves by dilution with refrigerated mobile phase. The intra-day precision was evaluated using three determinations for each concentration at 80, 100 and 120 µg/mL. For interday precision, three determinations for each concentration at 80, 100 and 120 µg/mL of azacitidine were assayed daily on three different days.

The evaluation of the stability in the autosampler was not realized: each sample prepared was immediately analysed by HPLC. The stability-indicating capability was evaluated by analysing forced degraded azacitidine solutions:

- Acidic conditions: one mL of a 400 µg/mL azacitidine solution was diluted with 1 mL HCl 0.01 M, stored at 20–25 °C for 1 min, neutralized by 1 mL of NaOH 0.01 M and diluted with 1 mL of ultrapure water to obtain a theoretical concentration of 100 µg/mL.
- Alkaline degradation: one mL of a 400 µg/mL azacitidine solution was diluted with 1 mL NaOH 0.001 M, stored at 20–25 °C for 30 s, neutralized by 1 mL of HCl 0.001 M and diluted with 1 mL of ultrapure water to obtain a theoretical concentration of 100 µg/mL.
- Heat degradation: a solution of 100 µg/mL azacitidine was exposed to a temperature of 37 °C for 4 h.

The specificity of our method was evaluated with the analysis of a mannitol solution, the excipient of azacitidine Mylan® at 1 mg/mL.

Analysis conditions of the stability study

Different analysis conditions have been studied in this work (Table 1). As recommended by manufacturers, the product does not require any special storage conditions [1]. Temperature is a factor influencing the stability of azacitidine, therefore two storage conditions for the 100 mg azacitidine vials were investigated: room temperature and between 2 and 8 °C.

Preparation of test solutions

All manipulations were performed under a biological safety cabinet.

Stability study in polypropylene syringes: for the preparation of 25 mg/mL suspensions, each vial was reconstituted with 4 mL of WFI as in practice. Two types of WFI were used for the reconstitution:

- Refrigerated WFI: a vial of 500 mL WFI was placed in a refrigerator at least 24 h before syringe preparation. This vial was taken out of the refrigerator on the day of the preparations and left for 15 min on a bench at temperature to simulate a sterilization cycle in an isolator.
- Frozen/thawed WFI: a 500 mL WFI infusion bag was in a freezer. The day before preparation, this bag was placed in a refrigerator. On the morning of the preparation, part of the bag was thawed and the WFI was used for reconstitution.

For each test condition, two syringes were prepared. Several vials of 100 mg azacitidine were reconstituted and the total volume was collected in a 60 mL syringe to obtain a homogeneous suspension. This suspension was then dispensed into 5 mL syringes using a female luer-lock to female luer-lock connector. Each syringe contained 3 mL of the 25 mg/mL azacitidine suspension. These syringes were stored between 2 and 8 °C or –20 °C. Two 3 mL syringes were prepared for each analysis time and for each analysis condition.

For vials stored in the refrigerator, the total time from removal of the vial to storage of the syringe in the refrigerator or freezer should not exceed 30 min.

The impact of room temperature storage on azacitidine stability was studied. An analysis of 25 mg/mL azacitidine suspension was performed after 2 h of storage at room temperature for azacitidine syringes stored for 24 h at 2–8 °C. Syringes were prepared from vials stored at room temperature. Each vial was reconstituted with 4 mL of refrigerated WFI. The syringes were placed between 2 and 8 °C for 24 h, then left for 2 h at room temperature. As the main stability study, two syringes were performed and two samples per syringe were analysed by HPLC after the preparation, after 24 h between 2 and 8 °C and after 2 h at room temperature.

Two different batches of azacitidine vials were tested for all conditions.

Sample dilution for analysis by RP-HPLC

For each condition studied and for each time of analysis, two identical syringes for two batches of azacitidine vials were realised and two

Table 1: Conditions of analysis for the stability study of azacitidine.

Azacitidine vials stored between 2 and 8 °C		
Frozen/thawed WFI[a]	Syringes stored between 2 and 8 °C	Times of analysis: T0, 24 h, 48 h, 72 h, 96 h, 168 h
	Syringes stored at –20 °C	Times of analysis: T0 and 28 days at –20 °C and after thawing, analysis at 24 h, 48 and 72 h between 2 and 8 °C
Refrigerated WFI	Syringes stored between 2 and 8 °C	Times of analysis: T0, 24 h, 48 h, 72 h, 96 h, 168 h
Azacitidine vials stored at room temperature		
Refrigerated WFI	Syringes stored between 2 and 8 °C	Times of analysis: T0, 24 h, 48 h, 72 h, 96 h, 168 h
	Syringes stored at room temperature	Times of analysis: After 24 h between 2 and 8 °C, analysis after 2 h at room temperature
Frozen/thawed WFI[a]	Syringes stored between 2 and 8 °C	Times of analysis: T0, 24 h, 48 h, 72 h, 96 h, 168 h

[a]WFI, water for injection.

samples were prepared and analysed by RP-HPLC for each syringe. At each time of the analysis, the entire content of the azacitidine 25 mg/mL syringe to be analyzed was injected into a glass tube. The samples were diluted before analysis with refrigerated mobile phase to obtain a concentration of 100 µg/mL (middle of the standard curve): 1 mL of 25 mg/mL azacitidine suspension was diluted in a 25 mL volumetric flask to obtain a 1 mg/mL azacitidine solution. Two millilitres of 1 mg/mL azacitidine solution were diluted in a 20 mL volumetric flask to obtain a solution at 100 µg/mL, the concentration for the analysis by RP-HPLC. Samples were prepared in duplicate and analysed by RP-HPLC. This process was repeated at each time of the analysis and for each condition of analysis. Total run time was set at 10 min.

Chemical stability was defined as not less than 90% of the initial azacitidine concentration [13].

Additional studies

In usual practice, questions about the preparation of 25 mg/mL azacitidine suspensions have arisen. Additional studies were carried out to answer these questions:

- *The impact of using a ChemoClave® vented vial Spike*: to secure the preparation of azacitidine suspensions in daily practice, the use of ChemoClave® Spike has been investigated for reconstituting azacitidine vials and thus replacing the use of a needle.

With a needle, one vial of 100 mg azacitidine was reconstituted with 4 mL of refrigerated WFI. Two independent samples were prepared for analysis by HPLC. The needle was withdrawn and then a Spike system was inserted on the already reconstituted vial. Two independent samples were prepared for analysis by HPLC. The results obtained by HPLC in the two situations (needle or Spike system) were compared.

Secondly, the volumes taken from the vials by a needle or by a Spike system were compared. Three vials were reconstituted with a needle (4 mL of refrigerated WFI). All the contents of the three vials were removed with a needle. The same procedure was performed using a Spike for reconstitution and for removing the contents of the vials.

- *Measurements of the temperatures of refrigerated WFI and frozen/thawed WFI:* on three consecutive days, the temperature of refrigerated WFI glass vials was measured 15 min after removing from the refrigerator, 30 min, 45, 60, 90, 120, 150, 180, 210 and 240 min at room temperature. This period takes into account the preparation time (removal of the azacitidine vials, alcohol decontamination and reconstitution). Similar measurements were carried out on frozen WFI bags for several days. These infusion bags were placed in the refrigerator the day before the measurement. The measurements were taken 15 min after removing from the refrigerator, 30 min, 45, 60, 90, 120, 150, 180, 210 and 240 min at room temperature.

Determination of physical stability

Physical stability was defined as the absence of aggregate formation. The samples were visually inspected with unaided eye at each analysis time by two technicians [13]. The objective of this physical evaluation was to observe a potential aggregation of the suspension.

Results

Reversed phase HPLC

The calibration curve was linear, the correlation coefficient was 0.99987. The equation of the calibration curve was y=122450.62x + 216077.67. The intra-day precision expressed as relative standard deviation (RSD) was between 0.29 and 1.98%. The intermediate precision expressed as RSD was 2.50% at 80 µg/mL, 1.16% at 100 µg/mL and 1.55% at 120 µg/mL. The analytical method was specified with the absence of interference by a 1 mg/mL mannitol solution.

Stability-indicating capacity was proved by using various stressed conditions. The retention time of azacitidine was approximately 4.8 min. The chromatogram obtained without stress conditions is presented in Figure 1 and the chromatogram after heat stressed conditions is presented in Figure 2.

Similar chromatograms were obtained after acid or basic degradation. A total of two peaks of degradation products (DP) were observed under all degradation methods. These peaks appear to be RGU (DP n°1) and RGU-CHO (DP n°2) and. Similar retentions times have been observed in the publication of Balouzet et al. [2].

The mass balance was evaluated and is presented in Table 2.

Chemical stability of suspensions

HPLC assay

With 100 mg azacitidine vials stored between 2 and 8 °C, the percentages of 25 mg/mL azacitidine suspensions reconstituted with refrigerated WFI and partially thawed WFI, stored between 2 and 8 °C are shown in Table 3.

The percentages of 25 mg/mL azacitidine suspensions prepared with vials stored between 2 and 8 °C, reconstituted with frozen/thawed WFI and stored at –20 °C for 28 days then after thawing, stored between 2 and 8 °C for 72 h are shown in Table 4.

Results obtained with vials stored at room temperature are presented in Table 5.

The percentages of azacitidine suspensions prepared with refrigerated WFI after 2 h of storage at room temperature of azacitidine syringes are presented in Table 6.

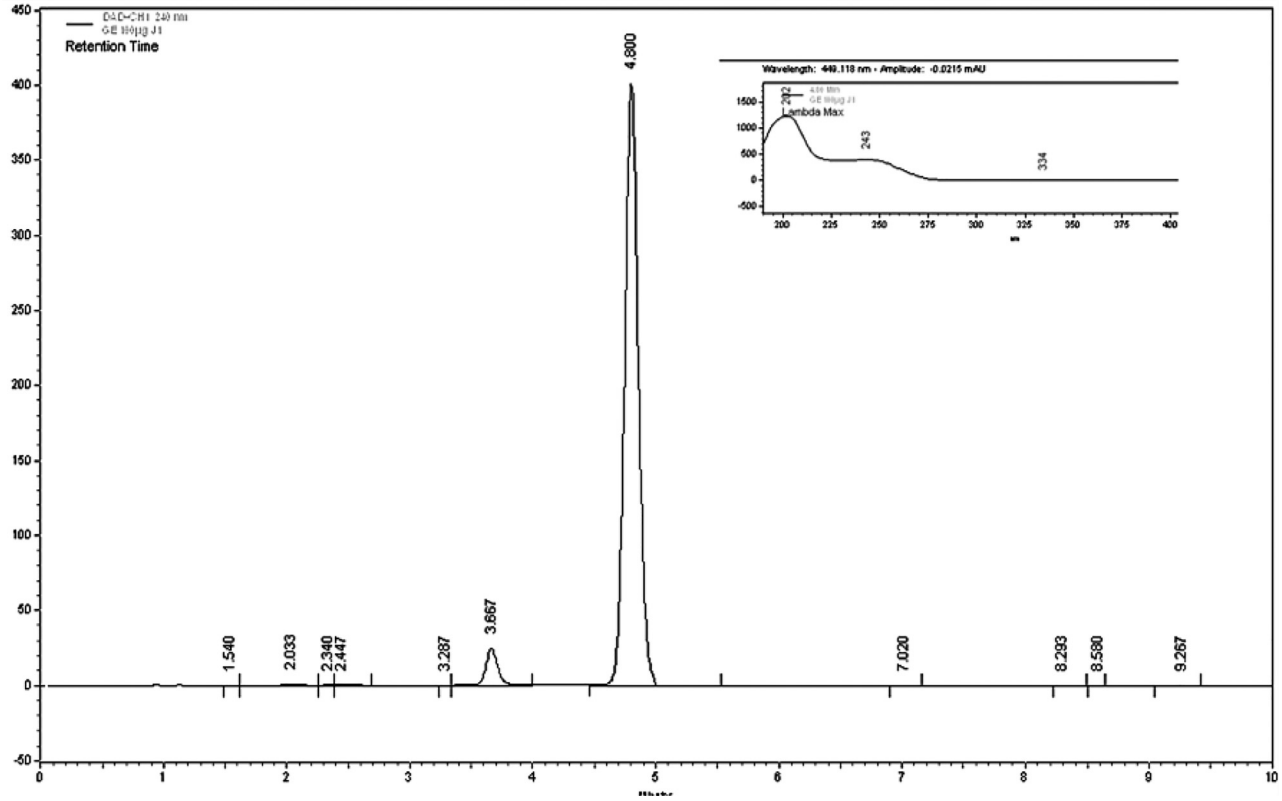

Figure 1: Chromatogram of 25 mg/mL azacitidine suspension (diluted at 100 µg/mL for HPLC analysis) and UV spectrum without stressed conditions.

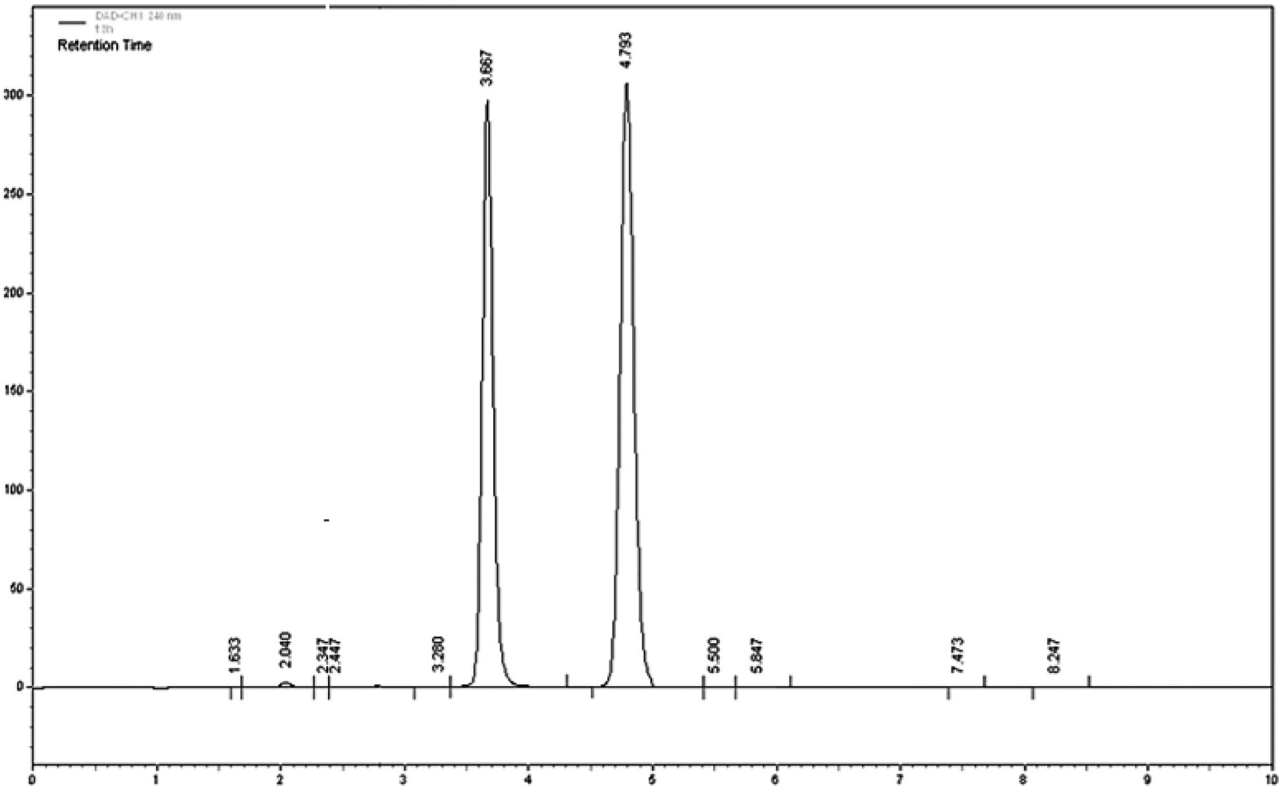

Figure 2: Chromatogram of 100 µg/mL azacitidine after heat stressed conditions (37 °C, 4 h).

Table 2: Mass balance of azacitidine suspension after various stressed degradation (expressed as peak areas).

Peaks (n°)	Retention times, min	Relative retention	Without stressed degradation	Area		
				Acid degradation HCl 0.01 M, 1 min	Alkaline degradation NaOH 0.001 M, 30 s	Heat degradation 37 °C, 4 h
Azacitidine	4.84	1.00	12,315,540	9,291,511	9,284,052	9,491,090
1	2.04	0.42	13,491	18,544	34,147	59,924
2	3.69	0.76	545,131	7,932,478	8,455,242	7,437,313
Total mass balance			12,874,162	17,242,533	17,773,441	16,988,327
% Degradation				24.6%	24.6%	22.9%

Table 3: Chemical stability of azacitidine suspensions, prepared with azacitidine **vials stored between 2–8 °C** and 25 mg/mL azacitidine syringes stored between 2–8 °C.

Batch number	Initial concentration, mg/mL		% of Initial Concentration (Mean ± RSD[a])				
	T0		24 h	48 h	72 h	96 h	168 h
Refrigerated WFI[b]							
1	24.51	100.00% ± 1.18%	99.46% ± 0.07%	96.30% ± 2.82%	95.65% ± 1.57%	92.29% ± 0.18%	91.20% ± 2.84%
			98.99% ± 1.65%	94.37% ± 1.18%	95.95% ± 0.60%	95.48% ± 1.00%	91.32% ± 0.50%
2	24.50	100.00% ± 0.22%	99.26% ± 1.98%	96.10% ± 0.64%	94.37% ± 0.65%	93.85% ± 0.06%	87.11% ± 0.05%
			97.82% ± 2.93%	94.29% ± 2.08%	95.79% ± 1.84%	95.16% ± 0.58%	87.72% ± 1.88%
Frozen/thawed WFI							
1	24.73	100.00% ± 1.40%	99.36% ± 1.01%	94.95% ± 0.49%	93.24% ± 0.57%	93.94% ± 0.33%	88.64% ± 1.52%
			98.20% ± 4.08%	96.51% ± 0.77%	94.96% ± 0.19%	94.95% ± 1.46%	88.48% ± 1.82%
2	24.35	100.00% ± 1.10%	97.94% ± 0.96%	95.61% ± 3.17%	94.84% ± 1.83%	95.26% ± 0.64%	87.53% ± 3.63%
			103.28% ± 0.94%	96.09% ± 2.24%	94.41% ± 0.30%	95.13% ± 0.44%	87.76% ± 1.41%

Drug concentrations in samples taken at time zero were designated as 100% (n=2). Samples were prepared in duplicate for each syringe. [a]RSD, relative standard deviation; [b]WFI, water for injection.

Degradation products (DPs)

The two DPs highlighted during the validation of the method were also observed during the stability study under all conditions.

The evolution of DPs during stability studies is identical when the vials are stored at room temperature or between 2 and 8 °C, if the reconstitution is made with refrigerated or frozen/thawed WFI and if the syringes are stored between 2 and 8 °C. DP n°1 expects around 1.2% of the total sum of peaks after 168 h of storage under all of these conditions. DP n°2 expects around 12% of the total sum of peaks after 168 h of storage under all of these conditions.

The use of vials stored at 2–8 °C, reconstituted with frozen/thawed WFI and storage of the suspensions at –20 °C for 28 days followed by 72 h at 2–8 °C showed a DP n°1<0.5% of the total sum of peaks after 28 days at –20 °C + 72 h between 2 and 8 °C and a DP n°2 less than 12% after 28 days at –20 °C + 72 h between 2 and 8 °C.

For a storage at room temperature of syringes for up to 2 h, DP n°1 expects around 0.25% of the total sum of peaks after 2 h at room temperature. DP n°2 expects around 12% of the total sum of peaks after 2 h at room temperature.

Physical stability of solutions

Under the different analytical conditions, the 25 mg/mL azacitidine suspensions were cloudy and homogeneous without agglomerates after reconstitution with refrigerated WFI or frozen/thawed WFI. After storage at 2–8 °C or –20 °C, stirring is necessary to homogenize the suspension.

Additional studies

The impact of using a ChemoClave® vented vial spike

The results obtained by HPLC showed a decrease of only 1.47% in the concentration of azacitidine suspensions

Table 4: Chemical stability of azacitidine suspensions, prepared with 100 mg azacitidine **vials stored between 2–8 °C** and 25 mg/mL azacitidine syringes stored for 28 days at –20 °C and then, after thawing, between 2–8 °C.

Batch number	Initial concentration (mg/mL)		% of Initial Concentration (Mean ± RSD[a])			
	T0		28 days –20 °C	28 days –20 °C + 24 h 2–8 °C	28 days –20 °C + 48 h 2–8 °C	28 days –20 °C + 72 h 2–8 °C
			Frozen/thawed WFI[b]			
1	24.10	100.00%	99.36% ± 0.69%	96.28% ± 1.03%	95.57% ± 0.64%	93.35% ± 0.60%
		±0.88%	100.21% ± 1.32%	98.12% ± 1.21%	99.90% ± 0.22%	94.69% ± 0.68%
2	24.40	100.00%	99.49% ± 1.24%	96.95% ± 0.18%	97.39% ± 0.56%	91.58% ± 0.39%
		±0.75%	98.78% ± 1.50%	98.05% ± 0.51%	97.16% ± 0.16%	92.73% ± 0.90%

Drug concentrations in samples taken at time zero were designated as 100% (n=2). Samples were prepared in duplicate for each syringe. [a]RSD, relative standard deviation; [b]WFI, water for injection.

when using a Spike system compared to using a needle. The comparison of the volumes withdrawn after reconstitution are similar when using a Spike system and a needle.

Measurements of the temperatures of refrigerated WFI and frozen/thawed WFI

Measurements of the temperatures of refrigerated WFI and frozen/thawed WFI are presented in Figure 3. The frozen/thawed WFI kept at a temperature lower than 10 °C for 90 min against less than 30 min with refrigerated WFI.

Discussion

Reversed phase HPLC

Azacitidine suspensions were analysed by a stability indicating reversed phase high-performance liquid chromatography, a method adapted from the method of Duriez et al. [6]. Regarding the mobile phase, 1% acetonitrile was added, which led to a reduction in the analysis time. The maximum of absorption of acetonitrile was obtained at 200 nm, a modification of the wavelength for analysis to 240 nm was realised. The stability indicating capacity of this method has been proved with forced degradation of azacitidine suspensions in extreme conditions (acidic, basic and heat conditions). The percentages of degradation obtained compared to the initial concentration were close to 20% in accordance with the recommendations of Bardin et al. [13]. The different stress conditions tested allowed good separation and detection of the degradation products of the azacitidine peak. In stressed conditions, the sums of the peaks areas are different than the sum obtained without stressed conditions indicating a higher coefficient of absorption of DPs.

Chemical stability of suspensions

Vials stored between 2–8 °C

25 mg/mL azacitidine suspensions stored between 2 and 8 °C, prepared with refrigerated WFI or frozen/thawed WFI retained more than 90% of the initial concentration for 96 h.

25 mg/mL azacitidine suspensions, prepared with frozen/thawed WFI, stored at –20 °C for 28 days followed by 72 h between 2 and 8 °C after thawing retained more than 90% of the initial concentration for 96 h.

Table 5: Chemical stability of azacitidine suspensions, prepared with azacitidine **vials stored at room temperature** and 25 mg/mL azacitidine syringes stored between 2 and 8 °C.

Batch number	Initial concentration, mg/mL		% of Initial concentration (mean ± RSD[a])				
	T0		24 h	48 h	72 h	96 h	168 h
			Refrigerated WFI[b]				
1	24.50	100.00% ± 0.22%	102.52% ± 0.77%	96.06% ± 2.10%	93.68% ± 0.88%	94.26% ± 0.02%	87.63% ± 1.34%
			99.59% ± 1.11%	93.47% ± 0.68%	94.57% ± 1.40%	96.21% ± 0.37%	89.00% ± 0.46%
2	24.73	100.00% ± 1.37%	98.77% ± 3.24%	95.28% ± 0.83%	94.11% ± 0.26%	94.80% ± 0.36%	88.96% ± 2.19%
			97.83% ± 1.62%	93.37% ± 3.95%	94.48% ± 0.43%	94.61% ± 0.20%	88.34% ± 3.72%
			Frozen/thawed WFI				
1	24.50	100.00% ± 1.02%	99.27% ± 1.40%	96.15% ± 0.41%	94.00% ± 0.04%	95.80% ± 0.24%	89.41% ± 1.55%
			98.64% ± 0.45%	94.81% ± 0.96%	95.85% ± 0.07%	95.90% ± 0.57%	89.18% ± 1.03%
2	23.77	100.00% ± 0.96%	98.74% ± 1.45%	93.40% ± 1.58%	96.44% ± 1.17%	96.25% ± 0.04%	89.18% ± 0.57%
			98.51% ± 0.50%	97.07% ± 0.70%	94.86% ± 0.91%	96.14% ± 0.35%	90.71% ± 2.83%

Drug concentrations in samples taken at time zero were designated as 100% (n=2). Samples were prepared in duplicate for each syringe. [a]RSD, relative standard deviation; [b]WFI, water for injection.

Table 6: Chemical stability of azacitidine suspensions, prepared with azacitidine vials stored **at room temperature** and 25 mg/mL azacitidine syringes stored for 24 h between 2 and 8 °C and then at room temperature.

Batch number	Initial concentration, mg/mL		% of Initial concentration (mean ± RSD[a])	
	T0		24 h, 2–8 °C	24 h, 2–8 °C + 2 h, 20–25 °C
		Refrigerated WFI[b]		
1	23.84	100.00% ± 0.13%	101.32% ± 0.82%	99.09% ± 0.41%
			101.98% ± 0.00%	100.22% ± 0.93%
2	23.97	100.00% ± 0.42%	99.33% ± 0.33%	97.03% ± 1.28%
			100.42% ± 0.92%	98.30% ± 0.65%

Drug concentrations in samples taken at time zero were designated as 100% (n=2). Samples were prepared in duplicate for each syringe. [a]RSD, relative standard deviation; [b]WFI, water for injection.

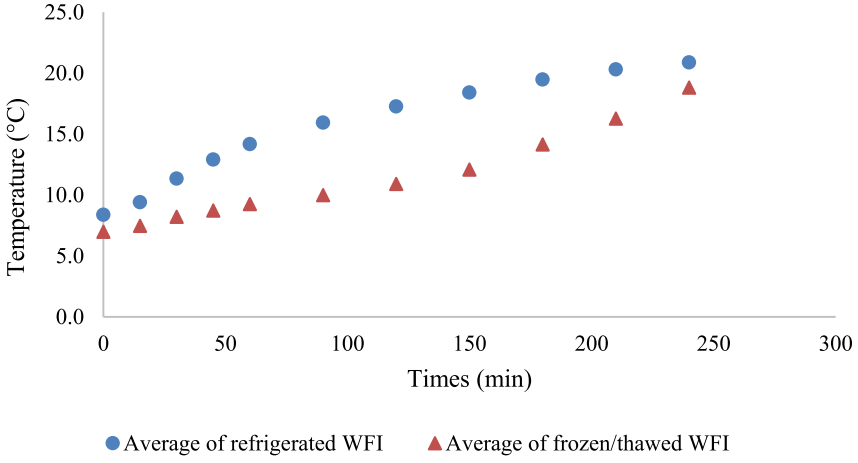

Figure 3: Evolution of temperatures of refrigerated WFI and frozen/thawed WFI.

Vials stored at room temperature

25 mg/mL azacitidine suspensions stored between 2 and 8 °C, prepared with refrigerated WFI or frozen/thawed WFI retained more than 90% of the initial concentration for 96 h. After 24 h between 2 and 8 °C, 25 mg/mL azacitidine suspensions retained more than 90% of the initial concentration for 2 h at room temperature.

In view of the results, when the vials were stored at room temperature, and the reconstitution was performed by using refrigerated WFI, the azacitidine suspensions retain around 94% of the initial concentration at T96h. After 24 h at 2–8 °C, the suspensions retain nearly 99% of the initial concentration and at T24h 2–8 °C + 2 h at room temperature, the suspension retains at least 97% of the initial concentration. Storage for 2 h at room temperature results in a loss of about 2%. We can therefore extrapolate that the stability of azacitidine suspensions, produced from vials stored at room temperature, reconstituted from refrigerated WFI, is 4 days at 2–8 °C, followed by 2 h at room temperature. This stability data makes it possible to limit wastage and to respond, in daily practice, if an azacitidine syringe is taken out of the refrigerator and left for 2 h at room temperature before administration.

When the syringes are stored at 2–8 °C, the use of vials stored at 2–8 °C and reconstitution with frozen/thawed WFI does not improve the stability compared to the manufacturer recommendations with a storage of the vials at room temperature and the use of refrigerated WFI. Under these conditions, azacitidine suspensions at 25 mg/mL are stable for up to 4 days, but unstable after 7 days stored between 2 and 8 °C.

Storage at −20 °C

25 mg/mL azacitidine suspensions, prepared with frozen/thawed WFI and stored 28 days at −20 °C retained more than 90% of the initial concentration. After thawing 30 min at room temperature, 25 mg/mL azacitidine suspensions retained more than 90% of the initial concentration for 72 h between 2 and 8 °C. These stability data allow advance preparation at standard doses, thus reducing the waiting time for patients.

The use of frozen/thawed WFI did not improve the stability of the azacitidine suspensions despite the cooler temperature for a longer period compared to refrigerated WFI. Its interest is to allow reconstitution of a larger number of vials with WFI keeping a cold temperature longer.

Additional studies

The use of a Spike device has been demonstrated to reconstitute and to collect the azacitidine suspension. This system had no impact on the volume collected compared to the use of a needle and did not lead to a decrease in the concentration of the suspension collected. The use of this device allows to shorten the preparation time of azacitidine syringes and to secure the reconstitution of vials.

Conclusions

Azacitidine 25 mg/mL suspensions reconstituted with refrigerated WFI were chemically stable for 4 days when stored at 2–8 °C whatever the storage of vials used for the manufacturing (refrigerator or room temperature), and 2 h at room temperature. A storage of azacitidine suspensions prepared with frozen/thawed WFI at −20 °C of the syringes has been validated for up to 28 days, leading to the possibility to prepare in advance. **The use of frozen WFI does not significantly improve the stability of the azacitidine suspension.** A Spike device can be used to reconstitute and collect azacitidine. **This study did not evaluate the microbiological stability of stored azacitidine preparations.**

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