LC–MS/MS Analysis on Infusion Bags and Filled Syringes of Decitabine: New Data on Physicochemical Stability of an Unstable Molecule

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ABSTRACT: Many anticancer drugs are reported to have low physicochemical stability after dilution; therefore, producers impose short times from reconstitution, dilution, and the end of administration. The precariousness of cancer patients’ health in real-life experience within cancer hospitals often forces delays in the drug administration with respect to the standard treatment schedule timing, because of acute toxicities or the need to postpone a control analysis before administration. The public health costs for discarded anticancer drugs due to administration interruptions can be avoided, thanks to independent analytical studies, which integrate the producer’s data reported in the technical sheet, referring to the real conditions of preparation in a sterile atmosphere under a cabin in a laboratory dedicated to handling cytotoxic drugs in controlled conditions of temperature, pressure, and particulate contamination. Decitabine is apparently an unstable molecule, whose reported stability is only 3 h at 2−8 °C when diluted, while the mother solution must be immediately used or, otherwise, discarded. This study has investigated the physicochemical stability of decitabine both in diluted infusion bags and in sterile water reconstituted syringes at 4 °C for 0, 24, 48, and 72 h. In all performed studies, the stability-indicating method involves, for the first time, the use of liquid chromatography−tandem mass spectrometry analysis. Unexpectedly, both diluted and reconstituted solutions of decitabine are more stable than previously reported data, with a 48 h-long physicochemical stability at 2−8 °C and protected from light.

1. INTRODUCTION

Oncologic and onco-hematologic diseases represent a great burden on worldwide health systems, involving, at different levels, patients and caregivers in their multiple paths of care, physicians that must choose the right therapy for the right patient for the best possible quality of life, different professional operators involved in each passage of care and, finally, the whole hospital organization in which the patients are confident in.

Target therapies, immunotherapy, early access programs to innovative medicines, investigational medicinal drugs (IMPs) within clinical trials are only the last conquests of hospital pharmacists’ management upon pharmaceutical market.

The precariousness of cancer patients’ health often provokes delays in the drug administration with respect to the standard treatment schedule timing, because of acute toxicities or the need to postpone a control analysis before administration. Sometimes, the physician’s warning to postpone therapies is transmitted to hospital pharmacies after preparation; for this reason, it is important to know the real stability of the drugs diluted in infusion bags, syringes, and elastomeric devices according to the producer’s indication reported in the technical sheet. Many drugs are reported as not having sufficient physicochemical stability after dilution and producers impose short times from reconstitution, dilution, and the end of administration. Decitabine (Dacogen) is an anticancer drug belonging to the nucleic acid synthesis inhibitors (cytidine analogue) class and is indicated in clinics for myelodysplastic syndromes and acute myeloid leukemia at a dose of 20 mg/m² of body surface for 5 consecutive days every four weeks. No reduction or adjustment dose is prescribed in the case of toxicities, but temporary or definitive suspension of the treatment. Decitabine has very low nominal stability according to its technical sheet: the reconstituted powder with water for injectables at 5 mg/mL must be furtherly diluted within 15 min or, otherwise, must be discarded, while the diluted preparations in cold sodium chloride 0.9% at 0.15−1 mg/mL concentrations are stable for 3 h if stored at 2−8 °C.† After this
reconstitution/dilution, and other peaks in the chromatograms were not reported.

2.5. Sample Preparation. Decitabine was recovered from commercially available Dacogen 50 mg (Janssen-Cilag). The powder in its commercial vial was reconstituted at 5 mg/mL with cold water for injectables. After reconstitution, the decitabine solution for the infusion bag was then diluted in 100 mL cold sodium chloride solution of 0.9%.

Water and normal saline solution were refrigerated for one night at 2–8 °C before use.

The manipulation and preparation of the mother solutions were performed in accordance with international standard guidelines on injectables by nurses with expertise in handling chemotherapeutics within U.Ma.C.A. Laboratory in IRCCS IstitutoTumori “Giovanni Paolo II” in Bari.

The samples for analysis were collected and analyzed by Biofordrug operators.

The collection was carried out directly from the terminal part of the infusion bag in a tube and then transferred into vials for LC–MS/MS analysis. The collected solutions were diluted in water for the LC–MS/MS analysis.

All chemicals and reagents had the highest purity. All solvents were ultra performance liquid chromatography grade quality, and all chemicals were purchased from Honeywell Riedel-de-Haën.

3. RESULTS AND DISCUSSION

After the routine calibration curves with the standard solutions, in the analysis samples, the concentrations were 4.81 mg/mL (instead of theoretical 5 mg/mL) and 0.334 mg/mL (instead of theoretical 0.37 mg/mL) for the reconstituted and diluted solutions, respectively. The infusion bag and the syringe have been stored at 2–8 °C for 0, 24, and 48 h and then at 72 h, and

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determinations are reported in Table 1. The diagram of the calibration curve for the standard analysis solutions is reported in Figure 1.

Moreover, in Figure 3, the chromatograms of decitabine reconstituted solution after 0, 24, and 48 h in refrigerated storage conditions are reported chronologically.

Analogous stability has been reported also for the diluted decitabine solution in the NaCl infusion bag and the relative chromatograms are reported in Figure 4. In this study, the experimental method involved LC−MS/MS analysis. Decitabine standard solutions were prepared and used to implement the method and the calibration curve. The samples were taken all along the studied period and submitted to LC−MS/MS analysis allowing an unambiguous assessment of drug purity, stability, and compatibility. The establishment of drug purity and stability was conducted by comparing the LC−MS/MS results with those deduced from the standard solutions.

In particular, to appreciate its physicochemical stability, the quantitative evaluation was carried on by comparing the areas of the decitabine peak at different analysis times and the peak of reference solutions. Final quantitative results are reported in terms of “average concentration” and “% concentration” of samples considering the starting concentration measured at \( t = 0 \) and calculated after 24 and 48 h. Moreover, particular attention was given to the monitoring of color and clarity of the solution in the device and in the taken samples. The mixture was transparent.

The slight difference between theoretical concentration data of the reconstituted (4.81 mg/mL instead of 5 mg/mL) and diluted (0.334 instead of 0.37 mg/mL) solutions of analysis was probably due to expanded volume solution in the vial during reconstitution and to overfill volume in the NaCl 0.9% infusion bag, as declared by the producer, respectively.

The same LC−MS/MS analysis after 48 h has been performed for both examined solutions. At 72 h from preparation, the peak area is halved confirming the degradation of the compound (Figure 5).

The average mass spectrum as acquired under positive ion ESI exhibited one major ion at \( m/z \) 251.15 (Figure 4). The protonated molecule \( m/z \) 229 was mostly used as the precursor ion in previously published methods. In our study, the average mass spectrum as acquired under positive ion ESI exhibited one major ion at \( m/z \) 251.15 (Figure 4), and decitabine formed predominantly sodium adducts. Moreover, sodium adducts are still predominant at the highest formic acid concentrations and other protonated ions in Q1 have been not observed. These ions correspond to \((M−Na^+).\) Decitabine sodium adducts were observed, with formic acid as eluent. MRM spectrum of the decitabine at \( m/z \) 251.15 exhibited a base fragment ion at \( m/z \) 135.00, corresponding to the sodium adducts formed by glycosidic cleavage of decitabine sodium

Table 1. Operating Conditions and Determinations for LC−MS Analysis of Reconstituted and Diluted Decitabine Solutions

| Analysis parameters | Reconstituted solution | Diluted solution |
|---------------------|------------------------|-----------------|
| Concentration       | 4.81 mg/mL (syringe)   | 0.334 mg/mL (infusion bag) |
| Diluent             | Water for injectables  | Normal saline   |
| Storage conditions  | 2−8 °C                 | 2−8 °C          |
| Determinations      | 0 h 24 h 48 h          | 0 h 24 h 48 h   |
| Average concentration | 4.81 ± 0.02 4.77 ± 0.1 4.81 ± 0.05 | 0.334 ± 0.01 0.327 ± 0.1 0.343 ± 0.2 |
| % Average concentration change | −0.8 0 | −2.1 +2.7 |

Figure 1. Decitabine calibration curve with standard samples.

Figure 2. Representative chromatograms obtained by LC−MS/MS analysis for standard decitabine samples.

Figure 3. Representative chromatograms of decitabine reconstituted solution after 0, 24, and 48 h in refrigerated storage conditions.
adducts. Therefore, the ion transition of $m/z$ 251.15−135.00 was selected for monitoring decitabine (Figure 6).

3.1. Experimental Methods. 3.1.1. Linearity. The correlation coefficient of $R^2 = 0.9977$ proved linearity over the concentration range. The equation of the calibration curve is $y = 265.571x + 199.692$.

3.1.2. Accuracy. Accuracy was evaluated at two different concentration levels (1 and 2.5 mg/mL) and with 9-fold injection. The accuracy was 97.8 ± 0.1% for 1 mg/mL and 98.3 ± 0.1% for 2.5 mg/mL of decitabine solutions.

3.1.3. Intra and Interday Precision. The intraday precision of the assay method was evaluated by carrying out 9 independent assays of decitabine solution prepared with cold ($4\,^\circ\text{C}$) buffer solution pH = 7.4 at two concentration levels (1 and 2.5 mg/mL).

For interday precision at each concentration level, a single injection of diluted decitabine solution and cold ($4\,^\circ\text{C}$) buffer solution pH = 7.4 was assayed daily for three consecutive days. The % relative standard deviation of the intra and interday assays were 0.5% for 1 mg/mL and 0.7% for 2.5 mg/mL decitabine, respectively.

The limit of detection and the limit of quantification were calculated by the equation LOD = 3.3 SD/s and LOQ = 10.

Figure 3. Chromatograms of the reconstituted solution samples after 0, 24, and 48 h (from left to right), respectively, of refrigerated storage conditions.

Figure 4. Chromatograms of the diluted solution samples after 0, 24, and 48 h (from left to right), respectively, in refrigerated storage conditions.

Figure 5. The chromatograms of the diluted solution samples after 72 h of refrigerated storage conditions. The reduction of peak area confirms the degradation of the compound.

Figure 6. Average mass spectrums of 5-methyl-2′-deoxycytidine (1 mg/mL) and its fragmentation, sodium adduct molecular ion.
4. CONCLUSIONS

In conclusion, both mother and diluted decitabine solutions have been demonstrated to be stable at 2–8 °C for 48 h long. The study of percentage variation of concentration has been employed to appreciate the stability of decitabine during the period of observation (24 and 48 h) at 2–8 °C, and as reported in Table 1, variability of concentration is acceptable (<5%).

The importance of these data is reflected in the activity of cancer hospitals, where the precariousness of patients’ health, the frequent emergency management of adverse events during the administration of chemotherapeutics, and the occasional need to postpone treatments, can also put in trouble different health professionals’ organization, hospital pharmacists in particular to prepare a new therapy.3

The higher stability of decitabine in refrigerated conditions that we have demonstrated integrates both the 15 min and the 3 h stability of the reconstituted and diluted decitabine reported in the technical sheet while using the frequent dosage range in clinics (32–40 mg, corresponding to 0.30–0.37 mg/mL). The 48 h stability is an acceptable time for all clinical occurrences that can provoke the postponement of therapy infusion.5,7

This result also translates into an economic advantage for the public health system, avoiding any drug discard and excessive consumption of drug commercial vials, besides permitting a redistribution of economic forces in other important sections of the hospital assistance.8

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Notes

The authors declare no competing financial interest.

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