Physicochemical stability of lurbinectedin reconstituted at 500 μg/mL and diluted at 15, 30, and 70 μg/mL in 0.9% sodium chloride and 5% dextrose

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Abstract

Background: Lurbinectedin (ZEPZELCA) is a cytotoxic, with alkylating properties. The commercially available pharmaceutical form is a glass vial containing 4 mg of lyophilized powder of lurbinectedin. The recommended dose of lurbinectedin is 3.2 mg/m². For a 1.7-m² body surface area, the average dosage is 5.44 mg. The manufacturer indicates a 30-hour stability for the reconstituted solution which cannot give the possibility to re-use the vial for the following days resulting in an average loss of 3600 € per preparation.

Objectives: The first objective was to study the stability of 500 μg/mL reconstituted lurbinectedin in water for injection after storage at 2 to 8°C. The second objective was to study the stability of 15 to 70 μg/mL diluted lurbinectedin in 0.9% NaCl or dextrose 5% in water stored at 2 to 8°C.

Methods: Three solutions in glass tubes for each condition (reconstituted 15 and 70 μg/mL solutions) were prepared. Due to the high cost, only 1 infusion bag at 30 μg/mL in polyolefin container was prepared. At each time of the analysis, 3 samples for each vial were analyzed by high-performance liquid chromatography coupled to a photodiode array detector. Physical stability was evaluated by visual and subvisual inspection (spectrophotometry).

Results: The analytical method was validated according to International Conference on Harmonisation guidelines Q2 (R1). The 500 μg/mL lurbinectedin reconstituted solution retained more than 95% of the initial concentration after 14 days as the 15 to 70 μg/mL diluted solutions. No interaction between the polyolefin infusion bag and lurbinectedin was observed. No physical changes were observed both visually and subvisually.

Conclusion: The reconstituted solution and the diluted lurbinectedin solutions were physically and chemically stable for 14 days when stored at 2 to 8°C protected from light. This new data makes it possible to re-use the reconstituted vial and to store a preparation in case of cancellation of an administration.

Keywords: HPLC, lurbinectedin, stability

1. Introduction

Lurbinectedin (ZEPZELCA) is a cytotoxic, with alkylating properties. Lurbinectedin binds guanine residues on DNA. Adduct formation leads to a cascade of events, preventing transcription as well as DNA repair pathways, leading to cell cycle disruption and even cell death. Lurbinectedin is indicated for the treatment of adult patients with metastatic small cell lung cancer with disease progression during or after platinum-based chemotherapy.[1,2] Lurbinectedin as trabectedin is an analogue of a marine compound isolated from the clubbed tunicate Ecteinascidia turbinata where a hydrogen atom has been replaced by a methoxy group. Lurbinectedin and trabectedin have a close chemical structure, the tetrahydroisoquinoline ring of trabectedin being replaced by a tetrahydro-β-carboline ring in lurbinectedin (Fig. 1).

The recommended dose of lurbinectedin is 3.2 mg/m² by intravenous infusion over 6 minutes, every 21 days until disease progression or unacceptable toxicity.

The commercially available pharmaceutical form is a glass vial containing 4 mg of lyophilized powder of lurbinectedin with 800 mg sucrose, 22.1 mg lactic acid, and 5.1 mg sodium hydroxide. As recommended by the manufacturer, lurbinectedin lyophilized powder must be reconstituted with 8 mL of sterile water for injection (WFI) to obtain a 500 μg/mL solution.[3] For administration through a central venous line, the appropriate amount of reconstituted solution from the vial is added to an infusion container containing at least 100 mL of diluent (0.9% sodium chloride injection [0.9% NaCl] or dextrose 5% in water [D5W] injection). For peripheral venous administration, the infusion volume must be at least 250 mL of 0.9% NaCl or D5W. The reconstituted or diluted solution can be stored for 30 hours at room temperature unprotected from light or in...
the refrigerator (2–8°C). In France, 1 vial of lurbinectedin 4 mg costs around 5600 €. The average body surface area being 1.7 m², the average dosage is 5.44 mg. The preparation requires 2 vials of 4 mg. The reconstituted solution with a stability of only 30 hours cannot be used for a preparation the next day or the following days, resulting in an average loss of 3600 € per preparation. If the infusion is cancelled, the financial loss is 11,250 €.

Lurbinectedin is commercialized since 2020 in the United States of America. To the best of our knowledge, no stability studies have been published for lurbinectedin. Lipp and Gfröer demonstrated a 21-day stability at a temperature between 2 and 8°C for 50 mg/mL trabectedin solutions.[4]

The first objective of this work was to study the stability of lurbinectedin 500 mg/mL in WFI between 2 and 8°C for further preparation. The second objective was to study the stability of diluted lurbinectedin in 0.9% NaCl or D5W in order to be able to maintain an annulled or staggered infusion.

2. Materials and methods

2.1. Chemical and reagents

Ammonium acetate (Merck; batch: A1426116916) and acetonitrile for HPLC isocratic grade (Carbo Erba; batch: 17231482) were used for the mobile phase. Hydrochloric acid 1 M (VWR Chemicals; batch: 190403C001), sodium hydroxide 1 M (VWR Chemicals; batch: 190423C004), and hydrogen peroxide 30% (Merck; batch: K48743810713) were used for forced degradation. Water for chromatography was obtained from a reverse osmosis system (Millipore Iberica, Madrid, Spain). Lurbinectedin 4 mg, powder for injection, for intravenous use (Pharma Mar, batch 20111, 20132, 20133, and 20154), 0.9% NaCl 250 mL glass vial (Chaix et du Marais, Lavoisier, batch: 9F582) or DW5 250 mL glass vial (Chaix et du Marais, Lavoisier, batch: 9F591) and water for injection 500 mL (Chaix et du Marais, Lavoisier, batch: 0F564) were used for test solutions, forced degradation, and the validation of the analytical method. 5-Hydroxymethylfurfural (Fisher scientific; batch: A0406699) was used for the specificity of the method. Infusion bag: 50 mL 0.9% NaCl (Easyflex; batch: 18K15H), 50 mL D5W (Easyflex; batch: 19J31E).

2.2. Preparation of test solutions

All manipulations were performed under a biological safety cabinet. As recommended by the highlight of prescribing information, lurbinectedin was reconstituted with 8 mL of WFI to obtain a 0.5 mg/mL solution (500 μg/mL). For cost reasons, the rests of reconstituted solutions from the centralized cancer chemotherapy preparation unit were recovered to perform our stability study. The choice of concentrations was based on the smallest dose in the largest volume for the least concentrated solution: body surface area of 1.5 m² × 3.2 mg = 4.8 mg in 250 mL giving a concentration of 19.2 μg/mL (rounded to 15 μg/mL for ease of measurement) and on the largest dose in the smallest volume for the most concentrated solution: body surface area of 2.2 m² × 3.2 mg = 7.04 mg in 100 mL or a concentration of 70.4 μg/mL (rounded to 70 μg/mL for ease of measurement). All 15 and 70 μg/mL solutions were stored in closed glass tubes, stored between 4 and 8°C protected from light. Lurbinectedin was also diluted in a 50 mL bag of 0.9% NaCl or D5W at a concentration of 30 μg/mL. In this condition, only 1 infusion bag was prepared for cost reasons. This bag brings us closer to the real conditions, in order to verify a potential phenomenon of lurbinectedin absorption into the polyolefin infusion bag.

2.3. Dilutions for analysis by HPLC

Direct injection for 15 and 30 μg/mL solutions. For the 70 μg/mL solution: 1/2 dilution with the mobile phase to obtain a concentration of 35 μg/mL. For the 500-μg/mL solutions: 1/20 dilution with the mobile phase to obtain a concentration of 25 μg/mL.

2.4. HPLC Assay

Lurbinectedin solutions were analyzed by a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) assay with photodiode array detection adapted from Lipp et al. The HPLC system consisted of an ELITE LaChromVWR/Hitachi plus autosampler, a VWR photodiode array 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 LiChrospher 100 RP-18, LiChroCART 125-4, 12.5 cm
length, and 5 μm particle size (Analytical Chromatography, Merck).

The mobile phase consisted of 0.02 mol/L buffer ammonium acetate (Merck; batch: A1426116916) and acetonitrile (Carlo Erba, batch 17J231482), 50/50 (v/v). The pH of the mobile phase was 7.4. The flow rate was set at 1.5 mL/minutes, with an injection volume of 20 μL. The detection wavelength was set at 284 nm. The temperature of the injector was set at 4 °C and the temperature of the column oven at 30 °C. The calibration curve was constructed from plots of peak area versus concentration. Linearity of the method was evaluated with 5 concentrations (10, 20, 30, 40, and 50 μg/mL) of lurbinectedin diluted in WFI. The intra-day reproducibility was evaluated as recommended by the International Conference on Harmonisation Q2 (R1),[5] using 3 determinations for each concentration at 10, 30, and 50 μg/mL. For interday precision, 3 determinations for each concentration at 10, 30, and 50 μg/mL of lurbinectedin solutions were assayed daily on 3 different days. The evaluation of the stability in the autosampler was performed. Solutions of lurbinectedin diluted in ultrapure water at a concentration of 50 μg/mL were stored in the automatic injector at 7 °C. Chemical stability in the autosampler was evaluated over a 24-hour period.

The photodiode array detector allows the evaluation of the UV spectrum of the chromatographic column effluent every 0.4 seconds. The wavelength of the analysis spectrum ranges from 190 to 400 nm and the analysis is carried out at a wavelength of 284 nm.

The stability-indicating capability was evaluated by analyzing forced degraded lurbinectedin solutions. Acidic condition: 1 mL of a 200 μg/mL lurbinectedin solution was diluted with 1 mL HCl 1 M, stored at 20 to 25 °C for 24 hours, neutralized by 1 mL of NaOH 1 M and diluted with 1 mL of ultrapure water to obtain a theoretical concentration of 50 μg/mL. Alkaline condition: 1 mL of a lurbinectedin 200 μg/mL solution was diluted with 1 mL NaOH 0.01 M, stored at 20 to 25 °C for 20 minutes, neutralized by 1 mL of HCl 0.01 M and diluted with 1 mL of ultrapure water to obtain a theoretical concentration of 50 μg/mL. Oxidative degradation: 1 mL of a lurbinectedin 200 μg/mL was diluted with H₂O₂ 0.3% 1 mL, stored at 50 °C for 1 hour and diluted with 2 mL of ultrapure water to obtain a theoretical concentration of 50 μg/mL. Heat degradation: a solution of 50 μg/mL of lurbinectedin was exposed to a temperature of 60 °C for 72 hours. The solution was analyzed directly without dilution. Photolytic degradation: 50 μg/mL lurbinectedin solution was exposed to a UV source of 254 nm for 24 hours. The solution was analyzed directly without dilution.

To detect a phenomenon of absorption a single bag of a 30 μg/mL lurbinectedin solution diluted either in 0.9% NaCl or in D5W was carried out in real conditions.

The specificity of our method was evaluated with the analysis of 0.9% NaCl, D5W, and WFI solutions. A 100 μg/mL solution of 5-hydroxymethylfurfural, the main degradation product of dextrose was also analyzed as it has a maximum absorbance of 284 nm.

2.5. Determination of physical stability

Physical stability was defined as the absence of particulate formation, haze, color change, and gas evolution. The samples were visually inspected against a white/black background with unaided eye at each analysis time. The subvisual aspect was assessed by using a Safas Monaco UV m² spectrophotometer. The absorbance was measured at 350, 410, and 550 nm as recommended by the guideline for conducting stability studies of anticancer drugs (A European consensus conference).[6]

3. Results

3.1. HPLC assay

The calibration curve was linear, the correlation coefficient was 0.9997. The equation for the calibration curve was $y = 91928.32x - 40679.53$. The intra-day precision expressed as relative standard deviation (RSD) was 1.66% at 10 μg/mL.
1.84% at 30 μg/mL, and 1.50% at 50 μg/mL. The interday precision expressed as RSD was 2.41% at 10 μg/mL, 1.44% at 30 μg/mL, and 0.95% at 50 μg/mL.

For the evaluation of stability in the autosampler, solutions were stable, with a degradation rate less than 1%.

A sample chromatogram of lurbinectedin without degradation is presented in Figure 2, with a retention time of 9.0 minutes.

The specificity of our method was evaluated with 0.9% NaCl and WFI samples, these 2 samples show no observable peaks. On the other hand with the D5W, we observed a peak, with a
retention time of 0.8 minutes. The injection of a 100 μg/mL 5-HMF solution is shown in Figure 3 with a peak at the same retention time.

The stability-indicating capability of the assay was tested under various conditions of forced degradation. The set of lurbinectedin forced degradation chromatograms is shown in Figure 4. In alkaline condition the solution of lurbinectedin could not be analyzed because of the immediate appearance of a precipitate. A total of 8 peaks of degradation products were observed under all degradation methods. The first peak is the main one observed, it was found with acid degradation, oxidative, and in the presence of heat. The mass balance and retention of degradation products relative to the lurbinectedin peak undegraded are presented in Table 1. After forced degradation, the extent of degradation was 15% under acidic conditions, 11% under oxidative conditions, 15% with heating, and 24% under photolytic conditions. The various conditions tested allowed good separation and detection of degradation products. Only peak n°9, observed during heat degradation with a relative retention time of 1.13, is not correctly separated from the lurbinectedin peak.

The mass balance for acid, oxidative, and thermal degradation allows the entire surface of the initial peaks to be recovered. However for photolytic degradation, a 24% degradation of the lurbinectedin leads to a 21% decrease of the total peak area.

### 3.2. Chemical stability of solutions

The percentage of lurbinectedin solution reconstituted in WFI at 500 μg/mL and diluted in 0.9% NaCl or D5W at 15 and 70 μg/mL after storage at 4 to 8°C for various time points is shown in Table 2.

### Table 1

| Peaks (n°) | Retention times (minutes) | Relative retention | Without stressed degradation | Acid degradation | Oxydative degradation | Heat degradation | Photolytic degradation |
|-----------|---------------------------|--------------------|-------------------------------|-----------------|-----------------------|-------------------|------------------------|
| Lurbinectedin | 8.61 | 1 | 2,991,450 | 2,702,110 | 2,653,946 | 2,534,793 | 2,279,509 |
| 1 | 0.62 | 0.07 | 203,377 | 32,979 | 51,787 | – | – |
| 2 | 0.74 | 0.09 | 113,179 | 55,249 | 47,514 | – | 78,461 |
| 3 | 1.1 | 0.13 | – | – | – | – | 29,648 |
| 4 | 1.26 | 0.15 | – | 24,906 | 284,364 | – | 38,055 |
| 5 | 2.32 | 0.27 | – | 65,541 | – | – | – |
| 6 | 2.91 | 0.34 | – | 28,710 | – | – | – |
| 8 | 4.89 | 0.57 | 32,356 | – | – | – | – |
| 9 | 9.75 | 1.13 | – | – | 81,710 | – | – |
| Total mass balance | 2,991,450 | 3,051,022 | 2,861,331 | 3,000,168 | 2,425,673 |
| % Degradation | 10% | 11% | 15% | 24% |

### Table 2

| Solvent and concentration | Time; % of initial concentration (mean ± SD) |
|---------------------------|---------------------------------------------|
| WFI 500 μg/mL | T0 D | T3 D | T7 D | T14 D |
| 1 | 100.00% ± 0.97% | 99.14% ± 2.98% | 99.71% ± 1.06% | 95.68% ± 2.16% |
| 2 | 100.00% ± 0.78% | ND ± – | 96.58% ± 2.06% | 97.12% ± 0.29% |
| 3 | 100.00% ± 4.21% | 100.63% ± 6.01% | 101.19% ± 0.45% | 98.29% ± 0.36% |
| D5W 15 μg/mL | 1 | 100.00% ± 1.78% | 98.24% ± 0.48% | 98.69% ± 0.48% | 94.67% ± 2.43% |
| 2 | 100.00% ± 1.66% | ND ± – | 95.85% ± 0.81% | 94.75% ± 0.21% |
| 3 | 100.00% ± 4.26% | 101.04% ± 2.15% | 99.46% ± 3.11% | 95.81% ± 2.36% |
| 0.9% NaCl 70 μg/mL | 1 | 100.00% ± 0.37% | 98.92% ± 1.02% | 97.56% ± 1.16% | 97.31% ± 0.28% |
| 2 | 100.00% ± 0.92% | ND ± – | 98.88% ± 0.68% | 98.97% ± 0.36% |
| 3 | 100.00% ± 0.76% | 99.66% ± 0.12% | 99.13% ± 0.46% | 98.62% ± 1.69% |
| 0.9% NaCl 15 μg/mL | 1 | 100.00% ± 1.32% | 98.69% ± 0.80% | 95.54% ± 1.21% | 95.15% ± 1.25% |
| 2 | 100.00% ± 1.46% | ND ± – | 96.13% ± 2.04% | 95.45% ± 2.23% |
| 3 | 100.00% ± 1.01% | 93.01% ± 2.21% | 93.39% ± 0.92% | 92.54% ± 0.55% |
| 0.9% NaCl 15 μg/mL | 1 | 100.00% ± 0.76% | 98.73% ± 1.82% | 97.69% ± 0.42% | 96.37% ± 0.91% |
| 2 | 100.00% ± 0.2% | ND ± – | 99.37% ± 0.19% | 98.99% ± 1.6% |
| 3 | 100.00% ± 0.08% | 99.92% ± 0.2% | 99.68% ± 1.15% | 98.38% ± 0.18% |

D = days; n = 3 by sample; ND = not determined.
Table 2. After 14 days, lurbinectedin solutions reconstituted in WFI retained more than 95% of the initial concentration. After 14 days, solutions of lurbinectedin diluted in 0.9% NaCl or D5W at 15 μg/mL retained more than 90% of the initial concentration. For concentrations of lurbinectedin at 70 μg/mL and for solutions at 30 μg/mL kept in a bag (Table 3), diluted in 0.9% NaCl or in D5W, more than 95% of initial concentration was demonstrated. Peak n°9 found during the forced degradation by heat was detected on the reconstituted solution from the first day, and then no variation of its concentration during the stability study was observed.

3.3. Physical stability of solutions

Lurbinectedin solutions were clear after the preparation. The solutions were limpid with no precipitation or gas formation during the study. For the subvisual analysis: no major changes were observed at 350, 410, and 550 nm for solutions diluted in 0.9% NaCl or in D5W.

4. Discussion

Lurbinectedin solutions were analyzed by a stability-indicating reversed-phase high-performance liquid chromatography, a method adapted from the methods of Lipp et al and Zangarini et al.[4,7] The assay wavelength has been modified (initial value: 225 nm) for detection at 284 nm. However, this wavelength is at the maximum absorbance of the main dextrose degradation product, 5-HMF. The analysis of the specificity of our method allowed to prove the absence of interaction between 5-HMF and lurbinectedin. As shown in Figure 3, the 2 peaks are clearly separated. On the other hand, in dextrose, the analysis of the degradation products n° 1, 2, 3, 4 appearing during the forced degradation process is not possible, because these degradation products have the same retention time as 5-HMF. During our study, the purity of the 5-HMF peak on day 0 and on day 14 was identical.

The stability-indicating capacity of this method has been proved with forced degradation of lurbinectedin solutions in extreme conditions (acidic, oxidative, photolytic, and heat conditions). The extend of degradation was about 20%, the limit recommended by several guidelines.[6,8] For photolytic degradation, the percentage of degradation obtained was 24%. The mass balance in comparison with the undegraded lurbinectedin solution is not optimal. That can be explained by the loss of chromophores after the exposure under UV light.

The small volume of solution available did not allow us to carry out pH measurements and subvisual analysis by spectrophotometry. As recommended by the guideline for conducting stability studies of anticancer drugs (A European consensus conference), the reconstituted and diluted lurbinectedin solutions were stored in sealed glass tubes for cost reasons. The same results are obtained in the glass tubes and in the infusion bag in real conditions, proving that there is no sorption phenomenon of the lurbinectedin onto the container. Normally at least 3 batches are required but as recommended by Bardin et al[6] a single preparation in real conditions may suffice for expensive cytotoxic studies. Our method was accurate and repeatable since only 1 of our concentration measurements has a coefficient of variation greater than 5% measured on the reconstituted lurbinectedin solution, this inaccuracy can be explained by the low working volume (1 mL) and the realization of a 1/20 dilution for this analysis.

The solvent has no influence on the stability of the lurbinectedin solution at equivalent concentration. Lurbinectedin is less stable at low concentrations, less than 95% of the initial lurbinectedin concentration was found in solutions diluted to a concentration of 15 μg/mL after 14 days. For solutions at 70 μg/mL, more than 95% of the initial concentration was found after 14 days of storage. For the reconstituted solution at 50 μg/mL, Lipp et al obtained a trabectedin concentration of 99% compared to the initial concentration after 14 days. Our results are similar with the reconstituted solution of lurbinectedin with a tenfold higher concentration.

5. Conclusion

The reconstituted solution of lurbinectedin was physically and chemically stable for 14 days when stored at 2 to 8°C protected from light. This new data makes it possible to keep the reconstituted vial for use with another preparation. A 14-day stability of 15 and 70 μg/mL lurbinectedin solutions diluted in 0.9% NaCl and in D5W was also demonstrated after storage at 2 to 8°C protected from light. No interaction between the polyolefin infusion bag and lurbinectedin was observed. This new data makes it possible to preserve a preparation already prepared in case of cancellation of an infusion or a shift for the same patient.

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References

1 Farago AF, Drapkin BJ, Lopez-Vilarino de Ramos JA, et al. ATLANTIS: a phase III study of lurbinectedin/doxorubicin versus topotecan or cyclophosphamide/doxorubicin/vincristine in patients with small-cell lung cancer who have failed one prior platinum-containing line. Future Oncol Lond Engl 2019; 15:231–239.
2 Trigo J, Subbiah V, Bese B, et al. Lurbinectedin as second-line treatment for patients with small-cell lung cancer: a single-arm, open-label, phase 2 basket trial. Lancet Oncol 2020b; 21:645–654.
3 ZEPZELCA™ (lurbinectedin) for injection, for intravenous use. Summary of Product Characteristics. Updated June 2020. PharmaMar.
Lipp H-P, Gfröer W. Physikalisch-chemische Stabilität von rekonstituierten Trabectedin-haltigen Lösungen. Weitergehende Überlegungen zur Haltbarkeit und Aufbrauchfrist. Krankenhauspharmazie 2017; 38:423–429.

5 International Conference on Harmonisation: Validation of Analytical Procedures: Text and Methodology Q2 (R1). Guideline 2005. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed: September 2020.

6 Bardin C, Astier A, Vulto A, et al. Guidelines for the practical stability studies of anticancer drugs: a European consensus conference. Ann Pharm Fr 2011; 69:221–231.

7 Zangarini M, Ceriani I, Sala F, et al. Quantification of trabectedin in human plasma: validation of a high-performance liquid chromatography-mass spectrometry method and its application in a clinical pharmacokinetic study. J Pharm Biomed Anal 2014; 95:107–112.

8 Methodological Guidelines for Stability Studies of Hospital Pharmaceutical Preparations [Internet]. [cited Oct 2020]. Available at: https://www.gerpac.eu/IMG/pdf/guide_stabilite_anglais.pdf.