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The stability of topotecan (Teva) in concentrate after re-use and in dilute infusions in sodium chloride 0.9% in polyethylene bags

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Abstract

Background. The study concerned the stability of topotecan in concentrate in glass vials and diluted in polyethylene (PE) bagsstored at 15-25°C and 4-8°C for up to 31 days. ***Material and methods.*** Original vials of topotecan injection (1 mg/mL, Teva) were stored at room and refrigerator temperature and subjected to re-piercing at 1, 2, 3, 7, 9, 14, 21 and 31 days following the initial piercing. Topotecan infusions at nominal concentrations of 0.03 mg/mL were prepared in 0.9% sodium chloride (100 mL) in PE bags. The chemical stability was measured with a stability-indicating high-performance liquid chromatography (HPLC) assay. The physical stability was assessed by visual inspection in normal light. ***Results.*** The concentration of topotecan at each sampling time in the analysed solutions remained > 90% of the initial concentration, regardless of the container. No changes in colour or turbidity were observed in any of the vials and the prepared solutions. ***Conclusions.*** Topotecan, both undiluted in glass containers and diluted with NaCl 0.9% in PE bags, remains stable (< 10% degradation) for at least 31 days at room and refrigerator temperature when protected from light. *Anestezjologia i Ratownictwo 2012; 6: 268-275.*

Keywords: topotecan, stability, concentrate, infusion

Introduction

Each drug used for injection of infusion must retain its physiochemical qualities from the moment of preparation in a hospital pharmacy until the end of administration to the patient. The quality of drugs is inseparably linked with their stability during storage of originally packaged drugs as well as their stability after opening and preparing a dilution. The stability of a therapeutic substance is of particular importance for parenteral preparations, which are often administered in 24h intravenous infusions. Too fast decomposition of the drug may lead to the development of toxic compounds or compounds without therapeutic qualities, which would be particularly dangerous to patients in grave general condition. Topotecan is a semisynthetic derivative of camptothecin that is isolated from the Chinese yew tree, *Camptotheca acuminate* [1]. The molecular formula of topotecan is $C_{23}H_{23}N_3O_5$ (MW 421.5) and the structure is provided in Figure 1. The chemotherapeutic belongs to the group of cell cycle phase specific drugs. The effect mechanism is related with topoisomerase I inhibition, whose concentration in neoplastic cells is about 14-16 times higher than in normal cells [2]. Topotecan reacts with topoisomerase I and DNA, making the triple complex oftopoisomerase I-DNA-topotecan. As a result of contact between the topotecan-stabilised topoisomerase I-DNA splitting complex and a replication fork the replication becomes inhibited and the double-stranded DNA becomes irreversiblybroken [3-5]. Topotecanis applied in the following therapies: the second-line in recurrent small cell

lung cancer; the second-line in patients with ovarian cancer with diagnosed resistance, disease progression (within a short period of time following the end of the standard first-line therapy); with advanced and recurrent cervical cancer after radiotherapy [6-9].

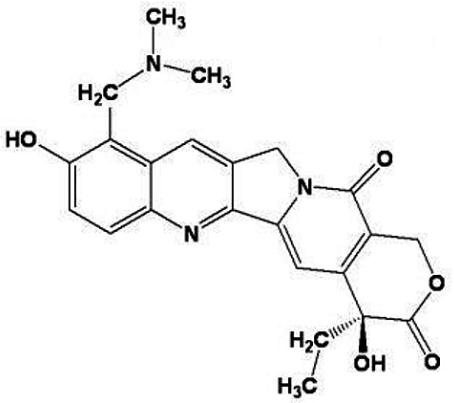


Figure 1. The molecular structure of topotecan

The purpose of this study was to determine the extended chemical and physical stability of topotecan in concentrate in glass vials and diluted in polyethylene (PE) bagsat normal in-use concentration in saline.

Material and methods

The research was done at the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań, Poland.

Reagents

Topotecan was purchased from LGC Standards (Lomianki, Poland), HPLC grade acetonitrile from Sigma-Aldrich. Metanol and *o*-phosphoric acid were from Merck. Water used in the mobile phase was deionised, distilled and filtered through a Millipore system (Direct-Q3 UV) before use. Vials containing topotecan concentrate 1 mg/1mL and 4 mg/4mL (batch number: 8190110, 1780610) were supplied by Teva Pharmaceuticals Polska, Warsaw, Poland. The 100 mL polyethylene (Viaflo®) infusion bags, containing 0.9% sodium chloride (batch number 15DH165P) were purchased from Baxter Polska, Warsaw, Poland.

Chromatographic assay method

The concentration of topotecan was carried out by means of the high pressure liquid chromatographic (HPLC) method with UV detection, which was a modified version of the method developed by T. Bansal et al. [10]. The highperformance liquid chromatographic (HPLC) system (Alliance, model 2695; Waters Association, Milford, MA, USA) was used with a DAD (Diode Array Detector) detector (model 2487; Waters Association) and a data acquisition and processing module (Empower Pro Software 1154; Waters Association). Chromatographic separations were achieved with YMC, C-18, ODS-A RP column (250 mm × 4.6 mm, 5 µm) stainless steel column. The column temperature was maintained at 35°C, and the UV–Vis detection wavelength was set at 380 nm. The samples of 10 µl were injected into the HPLC system. The total analysis time for each run was 26 min.

Mobile phase solutions

The mobile phase consisted of 100% acetonitrile (mobile phase A) and Milli-Q water, adjusted to pH 3.0 with 20% *o*-phosphoric acid (mobile phase B) at a flow rate of 1.0 ml/min. The gradient programme conditions are given in Table 1.

Table 1. Gradient elution programme

Time [min]	Solvent A [%]	Solvent B [%]
0	98.0	2.0
5.0	98.0	2.0
20.0	20.0	80.0
22.0	90.0	10.0
26.0	98.0	2.0

Standard preparation

Stock solution of topotecan was prepared from accurately weighed (24 mg) pure powder dissolved in water (10 mL). The solution was kept at 4°C. Working standard solutions were prepared by appropriate dilutions of the stock solution in 0.9% NaCl to obtain concentrations across the range of 12-40 µg/mL and 400-1300 µg/mL. Quality control (QC) samples were prepared freshly on each experiment day.

Calibration curve

The calibration curve was constructed from plots of peak area *versus* concentration. The linearity of the method was evaluated at seven topotecan concentrations varying from 12 µg/mL to 40 µg/mL (three-fold injections) and from 400 µg/mL to 1300 µg/mL (three-fold injections).

Intra- and inter-day precision and accuracy

To evaluate the intra- and inter-day accuracy and precision, the QC standard samples at three concentrations were prepared and determined by quantitating five replicates on the same day and on 3 consecutive days. Table 1 shows intra- and inter-day precision (CV%) and accuracy of this assay method. The precision of the method at each concentration was calculated as the relative standard deviation of the mean (CV) with the following equation:

CV = (SD/mean) x 100.

Accuracy was measured as the percentage difference from the theoretical calculation, according to the equation:

Bias (%) = ($concentration_{measured}$ – $concentration_{theoretical}$)/($concentration_{theoretical}$) x 100%.

Limit of detection (LOD) and lower limit of quantification (LLOQ)

The LOD was defined as the analyte concentration giving a signal to noise ratio of 3:1. The LLOQ was defined as the analyte concentration giving a signal to noise ratio of 10:1. Under the optimised conditions, the LOD and LLOQ of the topotecan concentrations were 2.7 ng/mL and 12 μg/mL respectively.

Preparation of topotecan infusion

All topotecan infusions were prepared under EU Class A conditions, in accordance with the principles of Good Pharmaceutical Manufacturing Practice. Topotecan infusions at nominal concentrations of 0.03 mg/mL, were prepared in 0.9% sodium chloride (100 mL) in PE bags. Twelve of the infusions were stored, well protected from light in green polyethene overwraps, six at room temperature (22.6 ± 1.1°C) and six at refrigerator temperature (3.6 ± 0.5°C). The samples were analysed immediately after preparation (t = 0) and at the following scheduled time intervals: 1, 2, 3, 7, 9, 14, 21, 31 days. The concentrations of topotecan in the analysed samples were calculated by means of the regression equation of the straight line y = ax + b for lower and higher concentration.

Visual inspection

Infusions were visually inspected under standard laboratory lighting against dark and light backgrounds for changes in clarity, colour, and presence of particulate matter.

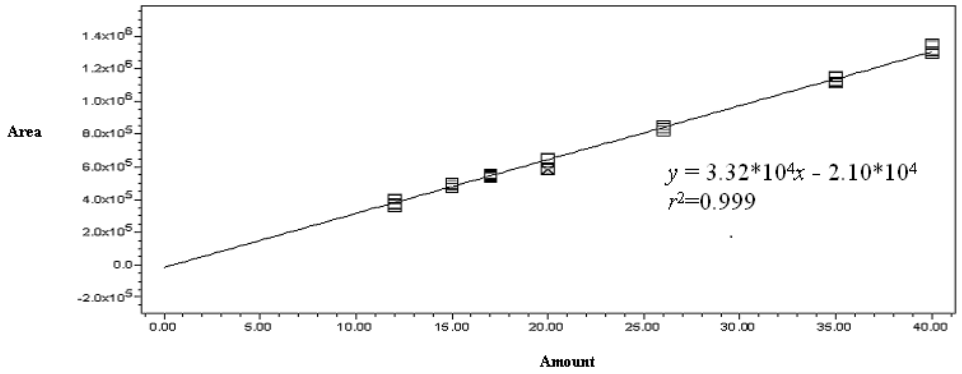
Results and discussion

The calibration curve of the peak area *versus* concentration was found to be linear over the evaluated range from of 12-40 μg/mL and 400-1300 μg/mL in 0.9% NaCl. The calibration equations were $y = 3.32*10^4x - 2.10*10^4$ and $y = 1.02*10^4x + 2.78*10^6$ for lower and higher concentration curves, respectively. The linear regression coefficients in plasma were r^2=0.999 and r^2 = 0.995 for lower and higher concentration curves, respectively. Linearity was achieved in this range (Figure 1). The intra- and inter-day precision and accuracy of the LQC (14 and 600 μg/mL), MQC (28 and 900 μg/mL), and HQC (38 and 1300 μg/mL) were well within the acceptable limit of 10% coefficient of variation (CV%). The results of intra-day precision and accuracy (n = 5) are summarised in Table 1. The intra-day precision and inter-day precision were acceptable with all CVs less than 4.2%. The intra-day accuracy and inter-day accuracy were also acceptable with the range of 90.7-103.2%. Figure 2 A, B and C show typical chromatograms obtained from a drug-free solution, concentrate and solution of topotecan in 0.9% NaCl, respectively. The chromatograms show that the separation from matrix constituents is sufficient for reliable quantitation and no endogenous components interfered with the analyte peak. Topotecan peak was detected with the retention time of 12.419 ± 0.071 min.

Table 1. Intra- and inter-day accuracy and precision of topotecan (n = 5)

Concentration (μg/mL)	Mean ± S.D. (μg/mL)	Accuracy (bias %)	Precision (CV%)
Intra-day			
14	12.81 ± 0.16	-8.5	1.3
28	26.79 ± 0.27	-4.3	1.0
38	36.85 ± 0.21	-3.0	0.6
600	584.14 ± 3.34	-2.6	0.6
900	928.60 ± 5.24	3.2	0.6
1300	1298.08 ± 4.57	-0.2	0.4
Inter-day			
14	12.70 ± 0.2	-9.3	1.3
28	26.15 ± 0.89	-6.6	3.4
38	35.86 ± 1.50	-5.6	4.2
600	587.46 ± 5.78	-2.1	0.9
900	926.12 ± 4.25	2.9	0.5
1300	1300.47 ± 5.53	0.04	0.4

A.



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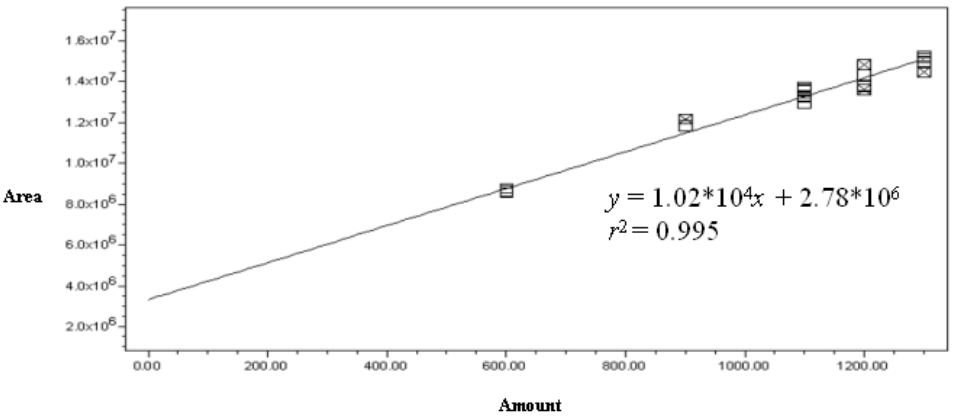
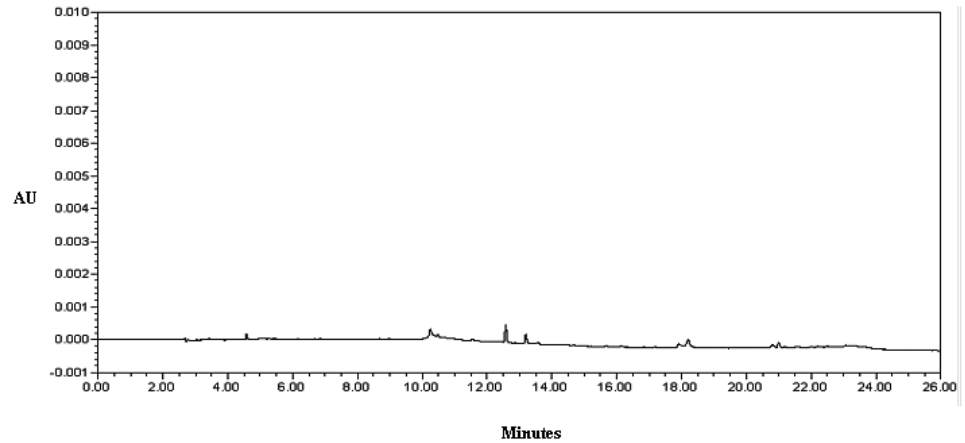


Figure 1. Calibration curves of topotecan: A within the concentration range of 12-40 μg/mL (n = 7), B within the concentration range of 400-1300 μg/mL (n = 5)

A.



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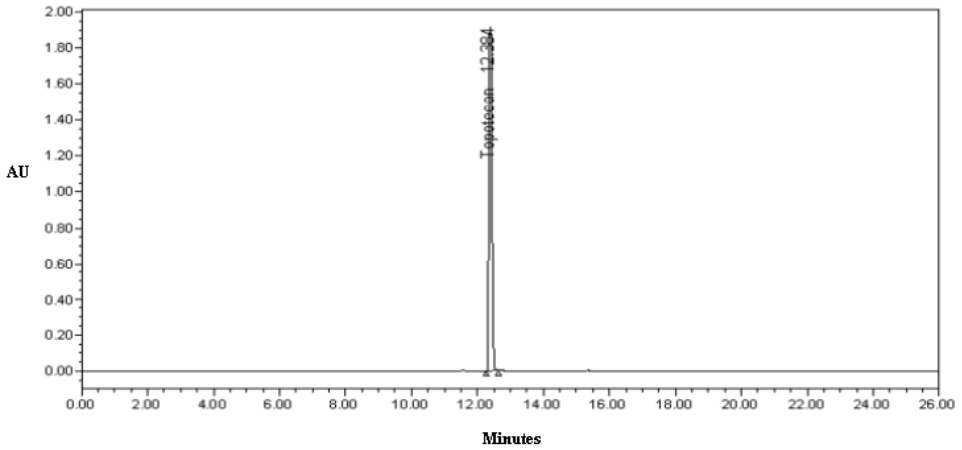

C.

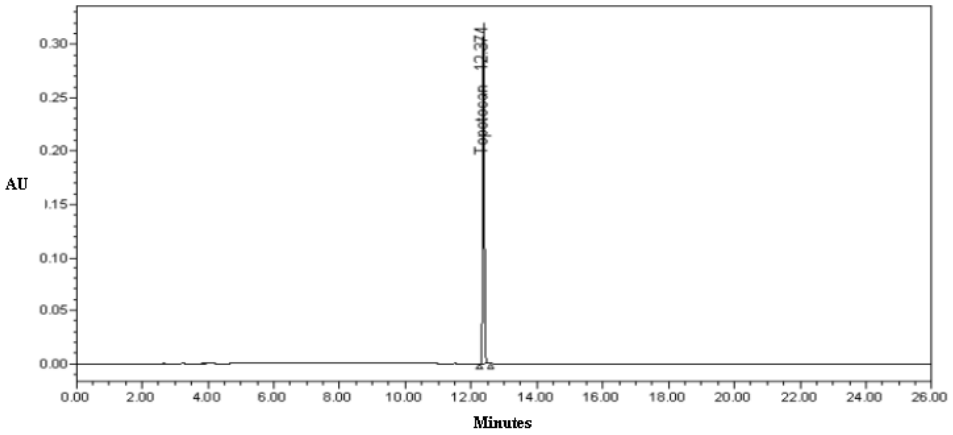

Figure 2. The chromatogram of topotecan: a drug-free solution (A), concentrate – 1138.99 μg/mL (B) and solution of topotecan in 0.9% NaCl – 33.196 μg/mL (C)

Table 2. Topotecan (%) at different sampling times in concentrate, stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for TopotecanTeva® 1 mg/1 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	9	14	21	31
2-8°C Mean SD n Visual appearance	100.0 0.0 3 pass	98.63 3.12 3 pass	97.69 2.74 3 pass	96.37 0.50 3 pass	99.85 1.99 3 pass	99.17 2.31 3 pass	107.42 2.92 3 pass	- - - pass	105.34 3.41 3 pass
15-25°C Mean SD n Visual appearance	100.0 0.0 3 pass	102.16 5.36 3 pass	101.53 2.13 3 pass	97.29 2.12 3 pass	97.68 3.79 3 pass	97.90 2.00 3 pass	111.87 2.45 3 pass	- - - pass	105.23 5.18 3 pass

Table 3. Topotecan (%) at different sampling times in sodium chloride 0.9%, stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for TopotecanTeva® 1 mg/1 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	9	14	21	31
2-8°C Mean SD n Visual appearance	100.0 0.0 3 pass	100.20 0.48 3 pass	100.13 0.46 3 pass	99.85 0.33 3 pass	100.49 0.31 3 pass	100.28 0.16 3 pass	100.32 0.79 3 pass	101.68 0.43 3 pass	100.66 0.72 3 pass
15-25°C Mean SD n Visual appearance	100.0 0.0 3 pass	99.52 1.38 3 pass	99.90 1.41 3 pass	99.18 1.75 3 pass	100.29 1.39 3 pass	100.08 2.67 3 pass	100.37 1.56 3 pass	100.08 3.39 3 pass	101.71 4.89 3 pass

Table 4. Topotecan (%) at different sampling times in concentrate stored, at refrigerator temperature (2-8°C) and room temperature (15-25°C) for TopotecanTeva® 4 mg/4 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	9	14	21	31
2-8°C Mean SD N Visual appearance	100.0 0.0 3 pass	102.27 2.04 3 pass	99.55 5.18 3 pass	97.55 0.77 3 pass	100.25 2.27 3 pass	98.34 3.07 3 pass	108.63 1.24 3 pass	103.86 1.39 3 pass	103.94 3.69 3 pass
15-25°C Mean SD n Visual appearance	100.0 0.0 3 pass	98.28 2.57 3 pass	99.55 0.88 3 pass	98.49 3.29 3 pass	94.62 2.87 3 pass	95.74 4.42 3 pass	109.51 5.56 3 pass	102.64 1.91 3 pass	103.08 5.92 3 pass

Table 5. Topotecan (%) at different sampling times in sodium chloride 0.9%, stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for TopotecanTeva® 4 mg/4 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	9	14	21	31
2-8°C Mean SD n Visual appearance	100.0 0.0 3 pass	99.92 0.51 3 pass	101.56 0.54 3 pass	99.90 0.33 3 pass	99.12 0.20 3 pass	101.41 1.23 3 pass	101.35 0.74 3 pass	103.75 0.04 3 pass	106.29 6.51 3 pass
15-25°C Mean SD n Visual appearance	100.0 0.0 3 pass	99.48 1.67 3 pass	101.12 1.61 3 pass	99.51 1.61 3 pass	99.69 2.12 3 pass	100.56 1.63 3 pass	99.71 1.77 3 pass	102.49 2.33 3 pass	100.32 1.57 3 pass

The biological activity of topotecanis conditioned by the presence of the lactone ring, which depending on the pH, becomes reversibly hydrolysed in a water solution. It is worth noting that when the pH < 4.0, the cytostatic is present only in the form containing the lactone ring, whereas when the pH > 10, there is total hydrolysis to the carboxylic form with the open ring [11].

Craig et al. analysed topotecan solutions with concentrations of 0.05 and 0.025 mg/ml in 0.9% NaCl or 5% glucose, where the former was stored without access to light at a temperature of 23-24°C for 24 hours and where the latter wasstored at a temperature of 2-8°C for 7 days. The solutions were placed in glass bottles,

polyolefin bags and PVC bags. Upon the obtained results topotecan was found to be stable regardless of the storage conditions and percentage of initial concentration in the concentration was different from the initial value by 98.8-101.0% 12].

Then the studies by Kramer and Thiesen [13] confirmed the above mentioned observations. They proved that both the concentrate (1mg/ml) and the solutions at the concentrations of 10 μg/ml, 25μg/ml and 50μg/ml stored without access to light in PVC bags and elastomeric infusion sets at temperatures of 25°C and 2-8°C were stable for 28 days at the level of > 97% in all of the test conditions [13]. This confirmed the earlier assumptions according to which the solvent, type of package and temperature do not significantly influence the drug stability. Furthermore, the stability of topotecan solutions was proved, where the drug was placed in elastomeric infusion sets stored at temperatures of 35°C for 5 days. This is extremely important in view of the fact that topotecan was administered in a thirty-minute intravenous infusion in a five-day scheme [13]. Light is a significant factor limiting the stability of topotecan. Therefore, the samples exposed to daylight were stable for as short as 17 days. A drop in the concentration of the cytostatic exposed to UV radiation was not accompanied by a rise in the pH, which proves the fact that the drug degradation was not the effect of pH-dependent hydrolysis [13].

The authors of this study analysed the drug concentrate of 1mg/ml and the volume of 1 and 4 ml after the opening of an original glass vial, which was stored at a hospital pharmacy at room temperature (22.6 ± 1.1°C) and at refrigerator temperature (3.6 ± 0.5°C) without access to light. The concentration was labelled on opening of the preparation and after 24 h, 48 h, 72 h, 7, 9, 14, 21, 31 days. After 31 days percentage of initial concentration at room temperature was 105.23% and 103.94% for the volumes of 1 and 4 mL (Tables 1 and 3). On the other hand, after 31 days the mean change in the concentrate concentration at refrigerator temperature was 105.34% and 103.08% for the volumes of 1 and 4 mL (Tables 1 and 3). The analysis also comprised a topotecan solution at a concentration of 0.03 mg/mL, which had been prepared by adding the concentrate of 1 mg/mL and the volumes of 1 or 4 mL to 0.9% NaCl solution and storing it at room temperature (22.6 ± 1.1°C) and at refrigerator temperature (3.6 ± 0.5°C) without access to light. In order to label the concentration and specify the percentage change the samples were collected at the same time intervals as the concentrate. After 31 days percentage of initial concentration of the solution stored at room temperature was 101.71% and 100.32% when the solution had been prepared from the concentrate of the volumes of 1 and 4 mL (Tables 2 and 4). On the other hand, after 31 days percentage of initial concentration of the solution stored at refrigerator temperature was 100.66% and 106.29% when the solution had been prepared from the concentrate of the volumes of 1 and 4 mL (Tables 2 and 4).

Conclusion

Topotecan appears to be physically and chemically stable for atleast 31 days in concentrate in glass containers or diluted with 0.9% sodium chloride in PE bags at a concentration of 0.03 mg/mL, at refrigerator temperature (2-8°C) and room temperature (15-25°C) when protected from light.

The study was carried out in the Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, Karol Marcinkowski University of Medical Sciences, Poznań, Poland

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Konflikt interesów / Conflict of interest
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Piśmiennictwo

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