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SHORT COMMUNICATION

Stability of Etoposide Solutions in Disposable Infusion Devices for Day Hospital Cancer Practices

Alison Klasen · Romain Kessari · Lionel Mercier · Cyril Valade · Jacques Grill · Romain Desmaris · Angelo Paci

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Abstract In a context of day hospital care of cancer patients, a protocol combining etoposide and carboplatin is used in paediatrics. Disposable infusion devices can be used to improve patient quality of life and to optimize nursing time. Stability data are available for carboplatin in these devices but not for etoposide. The aim of this study was to determine the stability of etoposide solutions in these devices by monitoring the changing etoposide concentration. To study the changing etoposide concentration, we investigated three different concentrations, each in two different solvents: sodium chloride (NaCl) 0.9 % and dextrose 5 %, in Intermate® disposable infusion devices. Quantitative analyses were performed by high-performance liquid chromatography coupled with ultraviolet (UV) detection on samples collected over a 24-h study period. The results showed that 100 mg/L etoposide solutions were stable for 24 h in NaCl 0.9 % and for 12 h in dextrose 5 %, whatever the temperature. The 400-mg/L solutions were stable for 24 h in both diluents, whatever the temperature, whereas the 600-mg/L solutions when diluted in NaCl 0.9 % and dextrose 5 % in water were stable for 8 and 6 h, respectively. We found that precipitation was the main phenomenon responsible for decreased etoposide concentrations. This study allowed us to conclude that etoposide solutions prepared in Intermate® infusion devices are stable for day hospital administration in paediatrics. It will also allow us to conduct a future clinical study that will focus on the medico-economic feasibility of this protocol and on the evaluation of patient and nurse satisfaction.

A. Klasen · R. Kessari · L. Mercier · A. Paci (✉)
Service Interdépartemental de Pharmacologie et d'Analyse du Médicament (SIPAM), Institut Gustave-Roussy, 114, rue Edouard Vaillant, 94805 Villejuif Cedex, France
e-mail: angelo.paci@gustaveroussy.fr; angelo.paci@igr.fr

A. Klasen · L. Mercier · A. Paci
Département de Biologie et Pathologie médicales, Institut Gustave-Roussy, 114, rue Edouard Vaillant, 94805 Villejuif Cedex, France

R. Kessari · L. Mercier · C. Valade · R. Desmaris · A. Paci
Département de Pharmacie Clinique, Institut Gustave-Roussy, 114, rue Edouard Vaillant, 94805 Villejuif Cedex, France

J. Grill
Département de Cancérologie de l'Enfant et de l'Adolescent, Institut Gustave-Roussy, 114, rue Edouard Vaillant, 94805 Villejuif Cedex, France

1 Introduction

In the context of day hospital care of cancer patients, some chemotherapy preparations can be administered using disposable infusion devices in order to improve the patient's quality of life. These devices are particularly useful for this purpose in paediatrics as they provide young patients with more mobility during drug administration, enabling them to continue their social and educational programmes instead of being bedridden. Disposable infusion devices consist in a latex- and polyvinyl chloride (PVC)-free polyisoprene elastomer reservoir along with an anti-ultraviolet (UV) protective shell that can be worn around the waist.

In the Department of Paediatrics at Gustave Roussy, a regimen combining etoposide and carboplatin (VP-Carbo) was designed for day hospital paediatrics (DHP) treatment of neuroblastomas or medulloblastomas in children. Currently, etoposide is administered via a 1-h infusion of a diluted solution, while carboplatin is administered using a disposable infusion device because stability data concerning the latter drug are already available in the literature [1, 2].

Etoposide (Fig. 1) is an antineoplastic agent, semi-synthetically derived from podophyllotoxin (epipodophyllotoxin), which acts through the inhibition of DNA topoisomerase II. It can be used as a single agent but is more usually used in combined multi-agent regimens to treat several malignancies: embryonic carcinoma of the testis, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), non-Hodgkin malignant lymphoma, Hodgkin's disease (intensified therapy) and acute leukaemia. In paediatrics, etoposide is mainly used to treat central nervous system tumours such as neuroblastoma and medulloblastoma.

Etoposide can be administered orally using 25- or 50-mg capsules or via a slow intravenous perfusion (a 1- to 2-h infusion) using a 20-mg/mL solution diluted in sodium chloride or dextrose. The infusion should start within the hour following its preparation. Dosages may range from 50 to 400 mg/m^2/day over 1–8 days, but typical dosages are from 50 to 150 mg/m^2/day over 1–3 consecutive days of treatment every 3 or 4 weeks. The oral dose is twice its intravenous counterpart.

Regarding stability data, the summary of product characteristics (SPC) for etoposide describes a solution prepared in PVC infusion bags or polyethylene syringes. The manufacturers recommend that the diluted solution be stored up to 48 h at room temperature. Nevertheless, the French Society of Oncology Pharmacy reported that sodium chloride 0.9 % (NaCl 0.9 %) diluted solutions stored at a temperature below 25 °C and under ambient light remain stable up to 96 h for a 200-mg/L concentration and up to 24 h for a 400-mg/L concentration. Beijnen et al. [3] reported that etoposide is supposed to be stable up to 96 h at 400 mg/L in a NaCl 0.9 % solution and in dextrose 5 % in water (D5W).

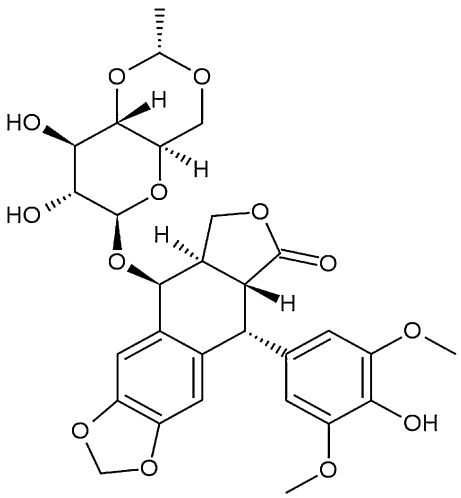

Fig. 1 Chemical structure of etoposide

The stability studies previously carried out using infusion bags filled with solutions reported that etoposide stability is a function of the pH (optimum pH between 4 and 5) [3]. Neither light nor the container had an impact on solution stability [3, 4]. However, the temperature did have an impact on the stability of the solution, since a room temperature of 20–24 °C was reportedly more suitable than a refrigerated one (4–12 °C) [5, 6]. Etoposide stability is also concentration dependent without drug degradation. Changes in content were reportedly due to the formation of a fine white precipitate, which corresponds to pure *trans*-etoposide [6]. Precipitation does not always appear but is more frequent when concentrations exceed 400 mg/L [3].

The aim of this study was to determine the stability of etoposide solutions in disposable infusion devices in order to allow the use of DHP protocols. Such devices could improve the quality of life of young patients and could permit better management of day hospital room availability, thereby reducing treatment costs through a decrease in nursing time.

As the only available stability data on etoposide solutions found in the literature concerned solutions in soft infusion bags and since there are no data on etoposide stability at 33 °C, which is the temperature attained by the solutions prepared in the devices worn around the patient's waist, we decided to investigate the stability of several etoposide solutions in these devices.

The study was to be conducted over a period of 24 h, at three different concentrations; 100, 400 and 600 mg/L, to fulfil the clinical protocol for the paediatric day hospital. The methodology consisted in monitoring changes in concentration by high-performance liquid chromatography coupled with ultraviolet spectrophotometric detection (HPLC-UV) of a given number of samples per testing condition. This technique makes it possible to detect degradation products in order to explain any possible degradation of etoposide over time. The objective was to obtain an adequate stability period in order to be able to administer the preparation during the period, taking into account the time required to prepare the solution for injection (i.e. the time between the preparation and the end of administration, being about 6 h), for the three concentration solutions. A further aim of the study was to investigate the physico-chemical phenomena involved in the stability of etoposide solutions.

2 Materials and Methods

2.1 Materials

Etoposide solutions are prepared from an initial solution at 20 mg/mL of etoposide Teva injectable solution. To study

changes in the active ingredient, the dilution solvents used were NaCl 0.9 % and D5W from Fresenius Kabi (Louviers, France). Thirty-six Intermate® disposable infusion devices from Baxter SAS (Maurepas, France) were used. Twelve had a nominal volume of 100 mL (SV100) and 24 had a nominal volume of 250 mL (LV100). For the degradation study, 0.1 M hydrochloric acid (0.1 M HCl) and 0.1 M sodium hydroxide (0.1 M NaOH) were provided by Prolabo-VWR International SA (Fontenay-sous-bois, France) and 10 % hydrogen peroxide (10 % H_2O_2) by Cooper (Melun, France). Eighteen borosilicate tubes with a capacity of 10 mL were used. The mobile phase was composed of ultrapure water; of HPLC grade acetonitrile (ACN) and RP grade acetic acid at 99 % from Prolabo-VWR International SA (Fontenay-sous-bois, France). Water was produced by a USF Elga dialyser.

2.2 Methods

2.2.1 Chromatographic Conditions

The chromatographic system comprises a Merck-Hitachi L2200® injector, a Merck-Hitachi pump L-7100®, equipped with an HPLC Nucleodur® column (C18*endcapped*/100 Å/5 µm/250 × 4 mm), a Jasco 2075 PLUS® UV detector along with Merck-Hitachi HSM D-7000® software for computerized processing. The mobile phase consisted of a water/ACN/99 % acetic acid mixture (64/35/1, v/v/v). Chromatographic separation was done at a 0.7 mL/min flow rate, with detection conducted at a 288 nm wavelength. The injected volume was 20 µL. The analysis took 10 min and etoposide retention time was 6.4 min (Fig. 2). Chromatographic analysis makes it possible to identify one or more compounds characterized by a chromatographic peak and its retention time. The area under the peak represents the concentration of each compound. Thus, component concentration in solution was monitored by comparing peak areas against a calibration plot.

2.2.2 Validation of the Analytical Method

Validation is essential to demonstrate that the method is adapted to its use. Validation was conducted by evaluating common parameters defined by the International Conference on Harmonization (ICH) [7] such as specificity, response function, linearity, accuracy, precision (repeatability and intermediate precision) and limits of detection (LOD) and quantification (LOQ). The parameters were determined by the statistical analysis of six calibration plots.

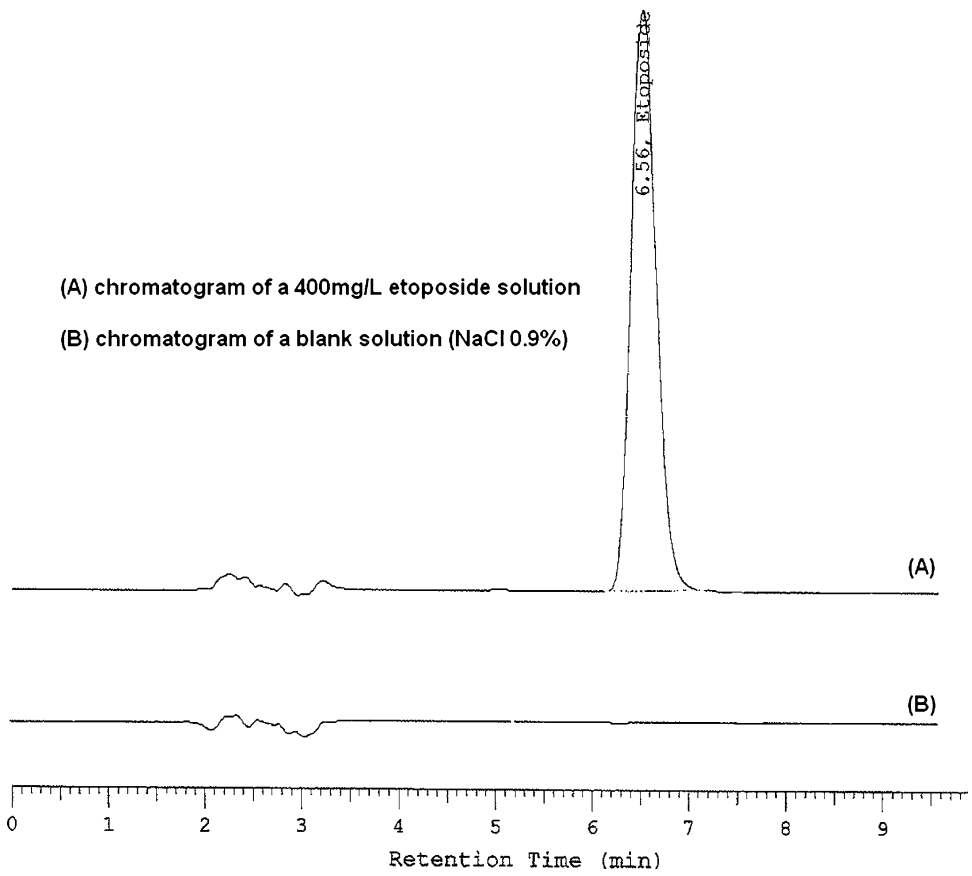

Fig. 2 Chromatograms of a 400-mg/L etoposide solution and a NaCl 0.9 % blank

2.2.2.1 Specificity Specificity was investigated by comparing the chromatogram of a blank sample with the chromatogram of the solution under study. HPLC is a selective method that separates different components on a column. The specificity of the method was assessed by analysing an etoposide solution. Figure 2 shows the chromatogram resulting from the injection of a 400 mg/L etoposide solution and of a blank sample of NaCl 0.9 %.

2.2.2.2 Linearity The calibration range was constructed based on 11 calibration standards (25, 50, 100, 150, 200, 250, 500, 750, 1,000, 1,250 and 1,500 mg/L). Linearity was investigated for six calibration plots recorded on six different days (one plot a day). The average equation parameters for the six linear regressions were:

$y = 3{,}787{,}945x + 29{,}207\ (r^2 = 0.999)$.

A statistic comparison of the calibration curves was conducted through normalised analysis of variance. Variances were found homogenous by a Bartlett–Levine test ($p < 0.00001$).

2.2.2.3 Accuracy Accuracy expresses the closeness of agreement between the values accepted as conventionally true (referred to as the standard) and an estimated value (called the medium) obtained by applying the analysis technique a number of times. As shown in Table 1, accuracy values expressed by the theoretical value were below 5 % except for the lowest quality control established at 6.7 %.

Table 1 Fidelity and accuracy data of the analytical method

Quality controls (mg/L)	35	180	220	900	1,100	1,350
Repeatability						
CVr (%)	2.8	0.5	4.8	4.9	1.2	0.9
Bias (%)	6.7	4.5	6	4.4	0.5	0.2
Intermediate fidelity						
CVi (%)	2.2	0.3	1.6	2.6	1.7	1.6
Bias (%)	2.3	2.1	0.1	0.6	−2	−2.1

2.2.2.4 Precision Precision allows one to assess the repeatability and intermediate precision. The latter are expressed by the coefficients of variation of repeatability and intermediate precision that do not exceed 4.9 and 2.6 %, respectively (Table 1).

2.2.2.5 Limits of Detection (LOD) and of Quantification (LOQ) LODs and LOQs are determined by the slope (a) and the standard deviation of the y intercept of the linear regressions (SDb) of calibration plots. The computed value of the LOD is equal to 14.1 mg/L (LOD = 3.3 × SD$b/a$) and that of the LOQ is equal to 42.8 mg/L (LOQ = 10 × SD$b/a$) (Table 2).

2.3 Changing Concentration of the Active Ingredient

2.3.1 Preparation of Devices

Manufacturing was carried out under conditions consistent with Good Manufacturing Practices [8] in sterile isolation boxes. Etoposide solutions were prepared at three different concentration levels: 100 mg/L, 400 and 600 mg/L. Twelve Intermate® SV100 disposable perfusion devices were filled with 100-mg/L solutions, twelve Intermate® LV100 disposable perfusion devices with 400-mg/L solutions and twelve Intermate® LV100 disposable perfusion devices with 600-mg/L solutions.

2.3.2 Sampling and Pre-analytical Treatment

The stability study was conducted over three consecutive days (one per concentration). The first sample was collected at H0, immediately after filling the device. The following samples were collected at H2, H4, H6, H8, H12 and H24. The samples ($n = 12$) were analysed in duplicate via the HPLC-UV chromatographic system. The samples for the analysis were collected from each mobile perfusion device using a luer syringe after bleeding air from the pipe, under a laminar air flow hood. The samples (approximately 0.5 mL) were then placed directly in vials that were positioned in the chromatographic system (without prior dilution). Etoposide concentrations were determined against the linear regression plot.

Table 2 Calibration data and Detection and quantification Limits

	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Mean	SD
Slope (a)	3,750,686	3,713,734	3,695,046	3,875,442	3,879,179	3,813,583	3,787,945.0	80,202.7
Intercept (b)	17,962.0	30,672.0	39,300.0	20,002.0	11,776.0	55,528.0	29,206.7	16,197.7
Determination coefficient (R^2)	0.9999	0.9998	0.9998	0.9995	0.9995	0.9995	0.9997	0.0002
Limit of detection (mg/L)							14.1	
Limit of quantification (mg/L)							42.8	

2.3.3 *Etoposide and Determination of Related Degradation Products*

For the forced degradation study in 0.1 M HCl, 0.1 M NaOH and 10 % H_2O_2 as recommended by ICH, 100-, 400- and 600-mg/L solutions were prepared in three borosilicate tubes. The study was conducted over three days. Ten microlitres of a 100-µL volume of the sample were injected into the chromatographic system. The first sample was analysed at H0 immediately after preparation of solutions. The following samples were tested at H24 and at H48.

2.3.4 *Analysis of the Precipitation Phenomenon*

The stability data available in the literature for etoposide in solution in these diluents prepared in PVC soft bags suggest that the main cause of decreased etoposide concentration over time is a precipitation phenomenon. The kinetics of the degradation process is reported to be dependent largely on the concentration [6]. That is why we conducted a further experiment to quantify this phenomenon. The stability of the etoposide solution in the disposable perfusion devices was studied in NaCl 0.9 % and in D5W at 600 mg/L.

2.3.4.1 Sampling and Analytical Pre-treatment After preparing the devices, a sample (S1) was tested at H0 in order to determine the initial concentration of the solution. A second sample (S2) was tested at H24 to quantify the concentration in the device after 24 h. The samples were placed in a vial and then directly into the chromatographic system. A volume of 10 µL was injected.

At H24, we drilled through the balloon drug reservoir via the shell of the device and recovered 100 mL of the solution that were then placed in two 50 mL-Falcon® tubes (F1 and F2). The contents of each tube were centrifuged for 5 min at 3,000 rpm; the supernatant was then eliminated to obtain the precipitate. To obtain the whole precipitate in the device, the inside of the shell and of the balloon was rinsed twice with 10 mL of water using a syringe with a needle (L1 and L2). L1, L2 and the precipitate were mixed and centrifuged for 5 min at 3,000 rpm. After elimination of the supernatant, the precipitate was dissolved in 25 mL of methanol. Concentrations of etoposide methanolic solutions were determined by HPLC-UV in the conditions described above. Finally, the L1 and L2 samples were analysed by injecting 10 µL into the chromatographic system. Etoposide concentrations were determined to evaluate the efficiency of the washing and thus the reliability of the precipitate recovery method.

3 Results

3.1 Forced Degradation Study

Exposition of etoposide solutions to studied conditions led to precipitation after 48 h for ambient and 33 °C storage conditions except for alkaline conditions, where coloration of solution was observed instead of a precipitation.

Figure 3 shows results of the forced degradation study for 600-mg/L etoposide solutions in various dissolution media. Curve A shows the results of an injection of etoposide solution diluted in NaCl 0.9 %; curve B shows the chromatogram resulting from the injection of a solution of etoposide diluted in NaOH 0.1 M injected right after dilution; curve C shows the chromatogram resulting from the injection of a solution of etoposide diluted in H_2O_2 10 % after 48 h of exposition; curve D shows the chromatogram resulting from the injection of a solution of etoposide diluted in HCL 0.1 M after 48 h of exposition; curve E shows the chromatogram resulting from the injection of a solution of etoposide diluted in NaOH 0.1 M after 48 h of exposition. Exposition to alkaline conditions yields a main degradation product eluted around 6.0 min, its content is increased after 48 h of exposition. Exposition to acidic conditions yields another degradation product eluted around 4.0 min. Exposition to strong oxidative conditions yields a degradation product eluted around 8.4 min. Therefore, the chromatographic method is able to separate etoposide from its main degradation products.

Evolution of etoposide content in supernatant in different stress testing conditions is shown in Fig. 4. Those results show that etoposide content is greatly decreased in the supernatant in acidic and alkaline conditions while it remains stable in oxidative conditions. For alkaline conditions, decrease in etoposide concentration is probably caused by chemical degradation, as suggested by the chromatographic elution of by-products of etoposide and coloration of solution. For acidic conditions, it is unclear whether the decrease is due to the precipitation phenomenon or to a chemical degradation caused by stress factor, or a combination of both. Those results are consistent with previous observation of pH-related degradation of etoposide in solution [3].

3.2 Changing Concentration of the Active Ingredient

We decided to work with a confidence interval of ±5 % (i.e. [95, 105 %] of the nominal value) for concentrations in this study, although a confidence interval of ±10 % is stipulated for hospital preparations (i.e. [90, 110 %]) in the literature [9, 10]. For the sake of simplicity, by definition, the value of 100 % represented the concentration values

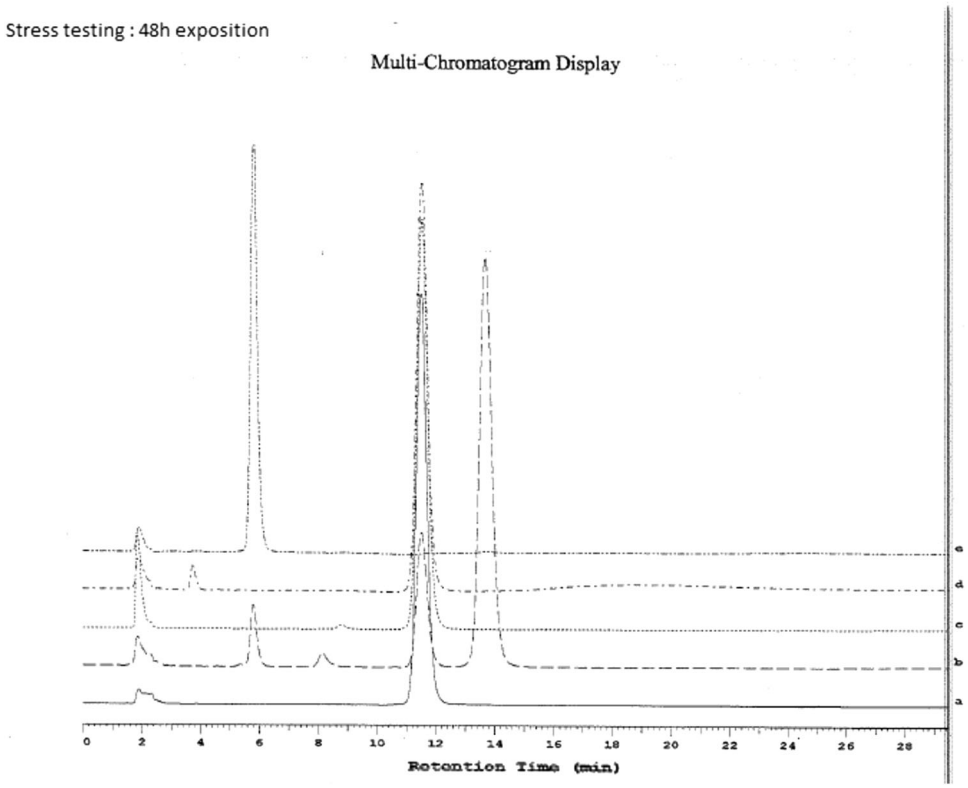

Fig. 3 Chromatograms of 600-mg/L etoposide solution submitted to various stress testing of forced degradation study

observed at H0. For the 100-mg/L concentration (Table 3), we observed that the solution was stable for 24 h in the NaCl 0.9 % and 12 h in the D5W, both at room temperature and at 33 °C. Regarding the 400-mg/L solution, etoposide was stable for 24 h in both diluents, both at room temperature and at 33 °C (Table 4), which is consistent with reported data [3, 5]. We retained a 24-h stability period for NaCl 0.9 % and D5W solutions at 400 mg/L.

We observed dissimilar stability data for the 600-mg/L solutions. Stability periods of 8 and 6 h were found for NaCl 0.9 % and D5W solutions stored at room temperature and at 33 °C, respectively (Table 5). According to the literature [3–5], above 400 mg/L, etoposide concentrations decrease dramatically, with precipitate formation resulting in a 24-h stability period. In our study, the 600-mg/L solutions were found to be stable up to 8 h at room temperature and up to 6 h at 33 °C (Fig. 5). It is noteworthy that the temperature has an impact on etoposide stability, especially in the case of high-concentration solutions.

Concentrations at 100 and 400 mg/L are suitable for paediatric regimens, whereas the concentration of 600 mg/L is intended for adults. The minimum stability period required is approximately 5–6 h for the use of these disposable perfusion devices in this context. If stability above 24 h is required, etoposide phosphate (Etopophos®), between 0.1 and 20 mg/mL in PVC bags [11], known to be stable up to 48 h at 37 °C, up to 96 h at 25 °C and up to 7 days between 2 and 8 °C in solution could be used. However, no stability data are available concerning this active substance in these devices.

As changes in the concentration did not reveal any degradation products similar to those observed during stress testing, whereas precipitation was observed, we investigated the precipitation phenomenon.

3.3 Precipitation Phenomenon

3.3.1 Reliability of the Precipitate Recovery Method

Normalised etoposide data after quantification of the wash solution (L1 and L2) yielded the following results. For solutions in NaCl 0.9 % (samples 1–3), the average etoposide concentration found in L1 was 7.3 % of the initial concentration and 3.3 % for L2. For solutions in D5W (samples 4–6), the average etoposide concentration found was 19.5 % of the initial concentration for L1 and 3.2 % for L2. Using this method, overall recovery was 102.1 and 97.9 % of initial content of etoposide in D5W and NaCl 0.9 %, respectively. Moreover, less than 4.0 % of the initial content of etoposide was found in the second wash elution, indicating a 96.0 % extraction yield for our method. Thus, the recovery method was considered reliable for our purpose.

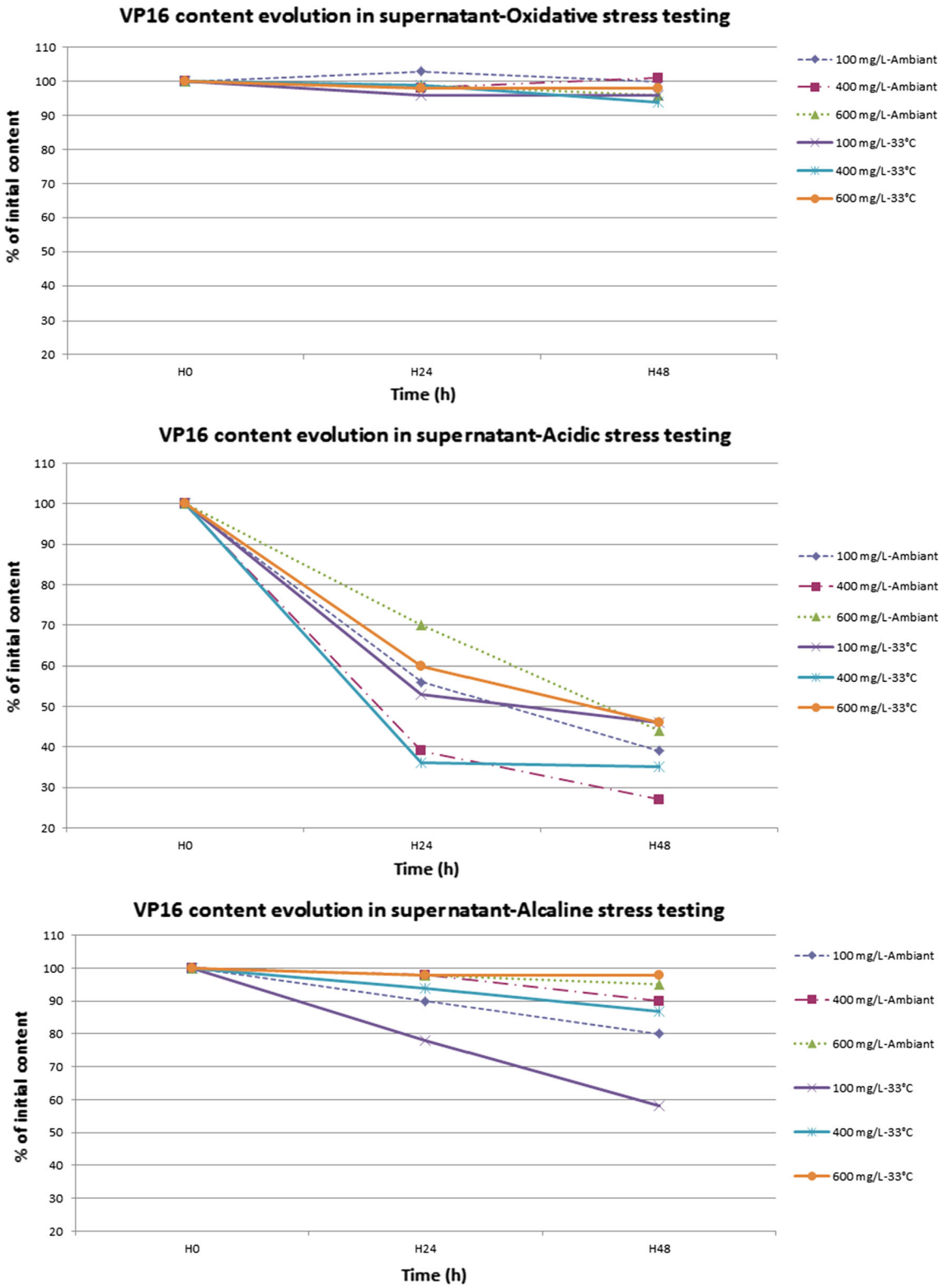

Fig. 4 Changing concentration as a function of time 100-, 400- and 600-mg/L etoposide solutions exposed to various stress factors

Table 3 Variation of the concentration values for the 100-mg/L etoposide solution

h	0	2	4	6	8	12	24
NaCl 0.9 %							
RT							
Mean	100.0 %	102.8 %	99.9 %	104.1 %	98.6 %	99.5 %	99.4 %
RSD	0.000	0.072	0.042	0.023	0.038	0.038	0.026
δ (%)	0.0	2.8	−0.1	4.1	−1.4	−0.5	−0.6
33 °C							
Mean	100.0 %	100.6 %	101.1 %	98.9 %	98.4 %	99.3 %	99.6 %
RSD	0.000	0.003	0.013	0.001	0.001	0.001	0.003
δ (%)	0.0	0.6	1.1	−1.1	−1.6	−0.7	−0.4
D5W							
RT							
Mean	100.0 %	99.9 %	98.5 %	99.1 %	99.5 %	101.1 %	93.7 %
RSD	0.000	0.013	0.012	0.019	0.001	0.011	0.012
δ (%)	0.0	−0.1	−1.5	−0.9	−0.5	1.1	*−6.3*
33 °C							
Mean	100.0 %	100.2 %	100.9 %	99.7 %	100.7 %	98.3 %	93.8 %
RSD	0.000	0.007	0.016	0.003	0.009	0.012	0.019
δ (%)	0.0	0.2	0.9	−0.3	0.7	−1.7	*−6.2*

The mean and RSD values were calculated on six different measurements. δ is the variation between each time and H0

Italic values correspond to content shift >5 % between stability samples and native samples

D5W dextrose 5 % in water

Table 4 Variation of the concentration values for the 400-mg/L etoposide solution

h	0	2	4	6	8	12	24
NaCl 0.9 %							
RT							
Average	100.0 %	100.8 %	101.9 %	101.0 %	100.9 %	101.5 %	102.5 %
RSD	0.000	0.003	0.004	0.001	0.001	0.004	0.016
δ (%)	0.0	0.8	1.9	1.0	0.9	1.5	2.5
33 °C							
Average	100.0 %	100.4 %	102.6 %	99.7 %	100.1 %	99.5 %	99.9 %
RSD	0.000	0.010	0.009	0.007	0.008	0.025	0.019
δ (%)	0.0	0.4	2.6	−0.3	0.1	−0.5	−0.1
D5W							
RT							
Average	100.0 %	100.1 %	100.4 %	100.7 %	100.1 %	100.0 %	103.9 %
RSD	0.000	0.008	0.003	0.014	0.007	0.009	0.004
δ (%)	0.0	0.1	0.4	0.7	0.1	0.0	3.9
33 °C							
Average	100.0 %	100.2 %	98.8 %	99.3 %	99.9 %	99.6 %	98.7 %
RSD	0.000	0.009	0.009	0.011	0.018	0.008	0.001
δ (%)	0.0	0.2	−1.2	−0.7	−0.1	−0.4	−1.3

The mean and RSD values were calculated on six different measurements. δ is the variation between each time and H0

D5W dextrose 5 % in water

Table 5 Variation of the concentration values for the 600-mg/L etoposide solution

h	0	2	4	6	8	12	24
NaCl 0.9 %							
RT							
Average	100.00 %	99.40 %	101.90 %	101.70 %	103.10 %	74.40 %	51.70 %
RSD	0.000	0.014	0.021	0.018	0.031	0.028	0.03
δ (%)	0.0	−0.6	1.9	1.7	3.1	*−25.6*	*−48.3*
33 °C							
Average	100.00 %	100.30 %	100.80 %	100.50 %	91.20 %	68.90 %	50.40 %
RSD	0.000	0.005	0.016	0.005	0.004	0.012	0.021
δ (%)	0.0	0.3	0.8	0.5	*−8.8*	*−31.1*	*−49.6*
D5W							
RT							
Average	100.00 %	100.00 %	99.80 %	100.00 %	99.20 %	89.20 %	71.30 %
RSD	0.000	0.001	0.003	0.005	0.001	0.05	0.038
δ (%)	0.0	0.0	−0.2	0.0	−0.8	*−10.8*	*−28.7*
33 °C							
Average	100.00 %	100.00 %	101.70 %	102.40 %	106.80 %	99.80 %	65.60 %
RSD	0.000	0.008	0.012	0.019	0.076	0.048	0.019
δ (%)	0.0	0.0	1.7	2.4	*6.8*	*−0.2*	*−34.4*

The mean and RSD values were calculated on six different measurements. δ is the variation between each time and H0

Italic values correspond to content shift >5 % between stability samples and native samples

D5W dextrose 5 % in water

3.3.2 Results of the Precipitation Phenomenon

The quantitative results of the study are presented in Table 6, taking into account a confidence interval of ±5 % (i.e. [95, 105 %] of the nominal value) for the concentrations. For the sake of simplicity, by definition, the value of 100 % represented the concentration values observed at H0. The same retention time (6.97 min) found for each assayed solution indicated that the substance forming the precipitate and that in the solution were the same compound (i.e. etoposide). This showed that the precipitate found in the devices was etoposide, as previous studies suggested. We observed a precipitate at H24 for the six devices prepared.

For solutions in NaCl 0.9 % after 24 h, the amount of etoposide (L1 + L2) in solution (S_{NaCl}) represented an average of 37.0 % of the initial etoposide concentration, while the concentration from the precipitate (P_{NaCl}) represented an average of 65.2 % of the initial etoposide concentration. For solutions in D5W after 24 h, the amount of etoposide (L1 + L2) in the solution (S_{D5}) represented an average of 42.4 % of the initial etoposide concentration, while the concentration from the precipitate (P_{D5}) represented an average of 55.5 % of the initial etoposide concentration. The decreased etoposide concentration in disposable infusion devices was therefore only due to the formation of an etoposide precipitate. This decrease in concentration may be considered as entirely due to the phenomenon of precipitation, and not to the formation of degradation products.

4 Conclusion

Regarding changes in the concentration of the active substance, we can conclude that (i) in low-dose solutions (100 mg/L), etoposide was stable up to 12 h in D5W and up to 24 h in NaCl 0.9 %, both at room temperature and at 33 °C; (ii) etoposide was stable up to 24 h in 400-mg/L solutions, in NaCl 0.9 % and D5W, both at room temperature and at 33 °C; and (iii) etoposide was stable in 600-mg/L solutions for 8 h at room temperature and for 6 h at 33 °C, in NaCl 0.9 % and D5W.

After 24 h, quantification of the precipitate and of etoposide in solution showed that 100 % of the initial etoposide concentration is recovered, with a 5 % confidence interval. No known etoposide degradation products were found while monitoring changes in the content of the active ingredient. Moreover, the amount of etoposide found in the form of a precipitate corresponded to the missing amount. This allowed us to conclude that precipitation was the only cause of instability in the etoposide solution in these devices.

This study allowed us therefore to conclude that etoposide was stable enough, especially at low and medium

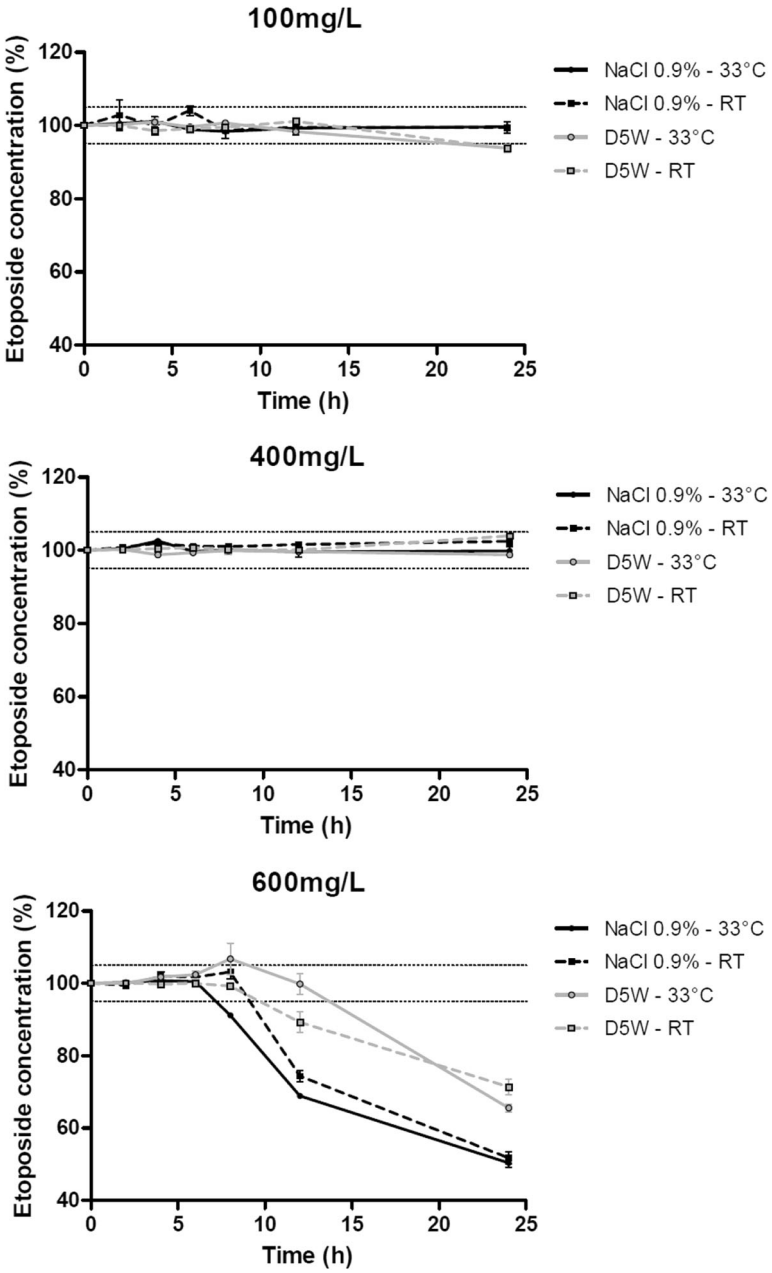

Fig. 5 Changing concentration as a function of time in 100-, 400- and 600-mg/L etoposide solutions in infusion devices

Table 6 Distribution of etoposide in solution and in its precipitate form (600 mg/L)

Time	Etoposide amount in %				Time	Etoposide amount in %			
	NaCl 0.9 % H0	Solution H24	Precipitate H24	Sum of etoposide amounts H24		D5W H0	Solution H24	Precipitate H24	Sum of etoposide amounts H24
Sample 1	100	36.6	63.7	100.3	Sample 4	100	43.8	54.5	98.2
Sample 2	100	37.4	64.9	102.3	Sample 5	100	41.1	56.5	97.6
Sample 3	100	36.9	67.0	103.9	Sample 6	100	42.3	55.6	97.8

concentrations, for use in disposable infusion devices such as Intermate® prepared in the Central Chemotherapy Production Facility for day hospital administration in a Paediatrics Unit. It will also allow our clinical team to conduct a future clinical study that will focus on the medico-economic feasibility of using these infusion devices and on the evaluation of patient and nurse satisfaction.

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References

1. Baxter report 93/REP/NIV/PD/4249/0155. Review of data generated on the stability of ifosfamide, carboplatin, mitomycin and mitoxantrone in infusor and shelf lives allocation. I. Wilmet.
2. Rochard E, Barthes D, Courtois P. Stability and compatibility study of carboplatin with three portable infusion pump reservoirs. Int J Pharm. 1994;101(3):257–62.
3. Beijnen JH, Beijnen-Bandhoe AU, Dubbleman AC, et al. Chemical and physical stability of etoposide and teniposide in commonly used infusion fluids. J Parenter Sci Technol. 1991;45(2):108–12.
4. Mueller HJ, Gensmer-Traexler J, Haker I. Stability of cytostatic drugs stored in a new type of infusion container. Hospital Pharmacist. 2004;11:429–34.
5. Barthes DM, Rochard EB, Pouliquen IJ, et al. Stability and compatibility of etoposide in 0,9 % sodium chloride injection in three containers. Am J Hosp Pharm. 1994;51(21):2706–9.
6. Joel SP, Clark PI, Slevin ML. Stability of the i.v. and oral formulations of etoposide in solution. Cancer Chemother Pharmacol. 1995;37(1–2):117–24.
7. Validation of analytical procedures: text and methodology. ICH Q2 (R1) (November 2005) CPMP/ICH/381/95.
8. Bonnes Pratiques de Préparation publiées au JO du 21/11/2007.
9. Trissel LA. Avoiding common flaws in stability and compatibility studies of injectable drugs. Am J Hosp Pharm. 1983;40:1159–60.
10. Trissel LA, Flora KP. Stability studies: five years later. Am J Hosp Pharm. 1988;45(7):1569–71.
11. Zhang Y, Trissel LA. Physical and chemical stability of etoposide phosphate solutions. J Am Pharm Assoc. 1999;39(2):146–50.