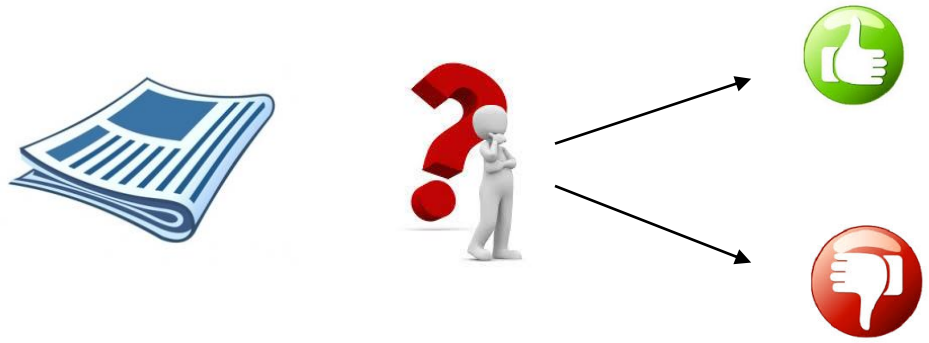
Lecture détaillée d'une publication de stabilité



COURS DES - Nancy

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Original article

Physicochemical stability of etoposide diluted at range concentrations between 0.38 and 1.75 mg/mL in polyolefin bags

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1. Introduction : but de l'étude ?

The manufacturers determined a 96-hour stability for the etoposide diluted at 0.2 mg/mL up to 48 hours for 0.4 mg/mL etoposide solutions at 25°C. Summaries of product characteristics specify that etoposide needs to be diluted with 5% glucose or 0.9% sodium chloride (0.9% NaCl) with volumes ranging from 250 to 1000 mL. The final concentration should not exceed 0.4 mg/mL and diluted solutions should not be stored in the refrigerator because precipitation may occur.[1,2]

The recommended dose of etoposide is 60 to 120 mg/m² administered by intravenous infusion (IV) daily for 3 to 5 days over 30 to 60 min.[1] Sometimes, the dose may increase up to 300 mg/m² for the treatment of non-Hodgkin's lymphoma. For patients with a body surface area of 2 m² (slightly above the mean value for women and men), the dose of etoposide will be 600 mg. This solution should be diluted with 1.5 litres of solvent so as not to exceed a concentration of 0.4 mg/mL. This fluid intake is not always feasible for patients. For these high doses, the etoposide phosphate salt, Etopophos, can be used without concentration limits. In fact, for a dose of 300 mg/m² and a patient with a body surface area equal to 2 m², the volume of dilution can be 250 mL.[7]

The first objective of this work was to study the stability of etoposide in 0.9% NaCl and 5% glucose over a range of concentrations equal or higher than the recommended limit concentration at 0.4 mg/mL, (0.38 mg/mL to 1.75 mg/mL) at 25°C and between 2°C to 8°C after 9, 16, 21, 28 and 61 days in polyolefin bags. Stability data at higher concentrations of etoposide than 0.4 mg/mL would overcome Etopophos shortage. That would

- Etudier la stabilité de l'étoposide à une concentration > 0,4 mg/mL
- Surmonter les problème de rupture d'Etopophos®
- Gain économique

2. Matériels et méthodes : conditions d'étude

Chemicals and reagents

All solvents were of high-performance liquid chromatography (HPLC) grade from VWR Chemicals (VWR Prolabo, Fontenay sous-Bois, France) or Merck Sigma. Formic acid (batch: 15I140502), triethylamine (batch: 14J280506) and acetonitrile (batch: 17F211398) were used for the mobile phase. Hydrochloric acid 1M (batch: 16090003) and 0.1M (batch: 171101), sodium hydroxide 1M (Merck; batch HC61977) and 0.1M (batch 170808) and hydrogen peroxide 30% (Merck; lot no. K487438107B) were used for the forced degradation. Water for chromatography was obtained from a reverse osmosis system (Millipore Iberica, Madrid, Spain). Etoposide 20 mg/mL, Concentrate for Solution for Infusion (Etoposide Mylan, batch 2049), Easyflex 0.9% NaCl (MacoPharma, batch 17I01B) or 5% glucose 250 mL (MacoPharma, batch 17E30C) were used for test solutions.

- Produits utilisés
  - Etoposide Mylan
  - Poches Easyflex®
- Extrapolation des données ?
  - Même produits que dans notre pratique quotidienne ?
  - Composition ?

2. Matériels et méthodes :

Etude de la stabilité chimique : choix de la méthode analytique

HPLC assay

Etoposide concentrations were analysed by a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method with photodiode array detection adapted from European pharmacopoeia.[11]

The HPLC system consisted of an ELITE LaChrom VWR/Hitachi plus autosampler, a VWR photodiode array (PDA) detector L-2455 and a VWR L-2130 HPLC-pump. Data was acquired and integrated by using EZChrom Elite (VWR, Agilent). The column used was LiChrospher 100 RP-18, LiChroCART 125–4, length 12.5 cm and particle size 5 µm (Analytical Chromatography, Merck) with a gradient from formic acid (VWR, France), triethylamine (VWR, France) and ultrapure water (solution A) to formic acid, triethylamine and acetonitrile (acetonitrile HPLC gradient grade (VWR, France)) (solution B). The gradient was set up as follows: start (0 min), 75% A; 25% B; 12 min: 27% A; 73% B.

The flow rate was set at 1 mL/minute, with an injection volume of 50 µL. The detection wavelength was set at 285 nm. The temperature of the injector was set at 23°C and the temperature of the column at 40°C. Under these conditions, the retention time of etoposide was around 9.5 min. The calibration curve was

- Choix de la méthode analytique ?
  - SEPARATIVE !!!
- Descriptions des conditions d'étude
- Réalisation de la validation de la méthode analytique ?

2. Matériels et méthodes

Etude de la stabilité chimique : validation de la méthode analytique

- Validation de la méthode analytique ?
  - Linéarité : gamme étalon en 5 points
  - Répétabilité et précision intermédiaire
  - *Stability indicating capability*
    - Dégradation forcée de solution d'étoposide

time of etoposide was around 9.5 min. The calibration curve was constructed from plots of peak area versus concentration. The linearity of the method was evaluated for five concentrations (10, 30, 50, 70, 90 µg/mL).

One millilitre of etoposide 20 mg/mL was diluted in 0.9% NaCl 200 mL. This solution was used to prepare standard curves by diluting with 0.9% NaCl. The intraday and interday precisions were evaluated at 50 µg/mL. The intra-day reproducibility was evaluated as recommended by ICH Q2 (R1),[12] using six determinations at 100% of the test concentration: 50 µg/mL. For interday precision, six injections of etoposide at 50 µg/mL were assayed daily on three consecutive days. To demonstrate the specificity of the method and the absence of interaction between etoposide and its excipients, a solution for each excipient of etoposide (polysorbate 80, polyethylene glycol 300, benzyl alcohol, citric acid) was realised and analysed by HPLC.

The stability-indicating capability was evaluated by analysing forced degraded etoposide solutions.

Acidic conditions: a solution of 200 µg/mL etoposide 1 mL was diluted with 1 mL HCl 0.5M, stored at 25°C for 15 min, neutralised by 1 mL of NaOH 0.5M and diluted with 1 mL of 0.9% NaCl to obtain a theoretical concentration of 50 µg/mL.

Alkali degradation: a solution of 200 µg/mL etoposide 1 mL was diluted with 1 mL NaOH 0.01M, stored at 25°C for 5 min, neutralised by 1 mL of HCl 0.01M and diluted with 1 mL of 0.9% NaCl to obtain a theoretical concentration of 50 µg/mL.

Oxidative degradation: a solution of 200 µg/mL etoposide 1 mL was diluted with H_2O_2 3% 1 mL stored at 25°C and diluted with 2 mL of 0.9% NaCl to obtain a theoretical concentration of 50 µg/mL.

UV degradation: a solution of 50 µg/mL etoposide was exposed for 15 min, 30 min, 1 hour and 12 hours under a sun-like spectrum lamp at 254 nm (Vilbert Lourmat).

Heat degradation: a solution of 50 µg/mL etoposide was exposed to a temperature of 60°C for 15 min, 30 min, 1 hour and 12 hours.

3. Matériels et méthodes
Autres méthodes ?

pH measurement

pH measurement was performed using a Crison pH25 pH-meter. Analysis was carried out for each concentration, each solvent and each temperature after preparation and on days 9, 16, 21, 28 and 61 days. pH values were considered to be acceptable if they did not vary by more than 1 pH unit from the initial measurement. We measured pH only on one bag for each condition.

4. Matériels et méthodes
Etude de la stabilité physique ?

Determination of physical stability

Physical stability was realised with a visual examination: colour changes and particulate matter every day of the assay. The subvisual evaluation was assessed by using a Safas Monaco UV mc2 spectrophotometer. After using an infusion pump, we measured the effect on turbidity by UV spectrophotometry. During the study, we measured turbidity only on one bag for each condition. The absorbance light was scanned at 550 nm. The absorbance of more than 0.010 AU was considered as evidence of turbidity, providing a quantitative determination of incompatibility.[14] An absorbance reading less than 0.010 AU was considered to be a noise level.

- Examen visuel et examen subvisuel

5. Résultats
Validation de la méthode

Reversed phase HPLC

The calibration curve was linear, the correlation coefficient was 0.99998. The equation of the calibration curve was y=81158.17833x −12234.183. The intra-day precision expressed as relative standard deviation (RSD) was 1.01%. The inter-day precision expressed as relative standard deviation (RSD) was 2.25%. The absence of interference by excipients was validated.

Stability indicating capacity was proved by using various stressed conditions. The retention time of etoposide was 9.7 min. Chromatogram obtained after alkaline stressed conditions is presented in figure 1 for example.

The mass balance was evaluated and is presented in table 1. Area for exclusion limit was established at 2020.

- Linéarité ✔
- Précision ✔

5. Résultats
Validation de la méthode

- Stability indicating ✔

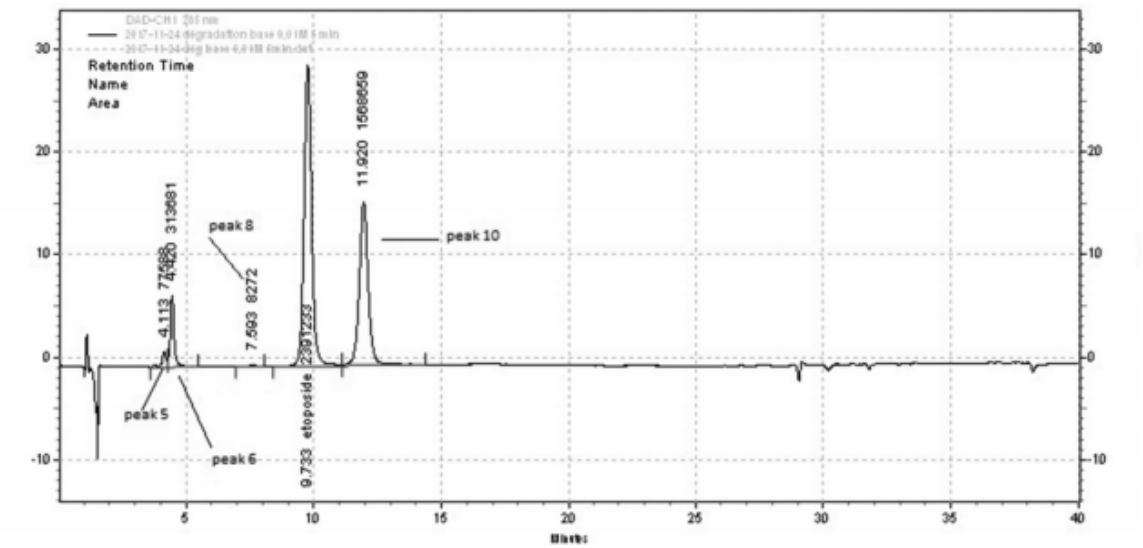


Table 1 Mass balance of etoposide solutions after various stressed degradations

			Area		
Peaks	Retention time (min)	Relative retention	Without stressed degradation	HCl 0.5M 15 min	NaOH 0.01M 5 min
1	2.2	0.23		2748	
3	2.6	0.27		22 167	
4	3	0.31		10 313	
5	4.1	0.42		22 391	77 588
6	4.4	0.45		282 109	313 681
7	6.5	0.67		8075	
8	7.6	0.78		10 537	8272
Etoposide	9.7	1	4 151 813	3 237 978	2 391 233
10	11.9	1.23		491 595	1 568 659
11	38.1	3.93	64 887	84 555	
Total mass balance			4 216 700	4 172 468	4 359 433
% degradation				22%	42%

6. Résultats

Stabilité CHIMIQUE

- Glucose 5%

Table 3 Stability of etoposide diluted with 5% glucose at 25°C and 2°C to 8°C

Theoretical concentration (mg/mL)	% initial concentration remaining			
	9 days	21 days	28 days	61 days
		25°C		
0.38	97.56%	95.84%	96.97%	98.72%
0.38	100.58%	99.65%	101.23%	101.89%
0.74	99.73%	97.18%	98.53%	95.15%
0.74	96.04%	96.93%	95.07%	94.79%
1.26	98.37%	98.79%	96.80%	101.71%
1.26	98.18%	97.30%	99.42%	97.89%
1.75	99.92%	98.82%	98.89%	P
1.75	98.67%	98.34%	95.09%	94.21%
		2–8°C		
0.38	93.78%	99.46%	99.88%	P
0.38	99.93%	102.58%	100.41%	104.07%
0.74	98.17%	99.35%	99.58%	P
0.74	97.69%	100.99%	98.86%	103.78%
1.26	91.87%	99.31%	98.08%	105.31%
1.26	94.83%	99.85%	99.01%	105.34%
1.75	96.67%	99.53%	99.33%	P
1.75	97.87%	P	–	–

Note, Drug concentrations in samples taken at time zero were designated as 100%. P=precipitations.

- NaCl 0,9%

Table 2 Stability of etoposide diluted with 0.9% sodium chloride at 25°C and 2°C to 8°C

Theoretical concentration (mg/mL)	% initial concentration remaining				
	9 days	16 days	21 days	28 days	61 days
			25°C		
0.38	93.10%	97.54%	98.77%	97.08%	91.77%
0.38	95.12%	96.80%	96.91%	97.46%	97.14%
0.74	95.84%	96.27%	95.84%	93.70%	P
0.74	94.64%	97.99%	93.80%	95.76%	P
1.26	95.11%	97.00%	98.24%	98.08%	93.06%
1.26	89.39%	94.65%	94.36%	95.26%	91.62%
1.75	97.80%	96.23%	95.23%	97.90%	93.00%
1.75	93.68%	95.59%	92.59%	93.19%	P
			2–8°C		
0.38	99.83%	98.78%	98.65%	99.86%	102.74%
0.38	98.53%	97.27%	98.40%	99.40%	98.46%
0.74	95.17%	100.87%	98.99%	101.80%	P
0.74	103.76%	100.75%	103.51%	105.44%	107.71%
1.26	95.11%	97.09%	98.27%	P	–
1.26	95.11%	98.38%	100.25%	99.97%	P
1.75	102.75%	P	–	–	–
1.75	P	–	–	–	–

Note, Drug concentrations in samples taken at time zero were designated as 100%. P=precipitations.

7. Résultats

Stabilité PHYSIQUE

Physical stability of solutions

Visual aspect

An extensive white precipitate was observed as shown in figure 3 in different solutions (see tables 2 and 3).

- Etoposide – 2-8°C – NaCl 0,9% - 1,75 mg/mL

Figure 3 The precipitated etoposide solution, stored at 2°C to 8°C.

Autres méthodes : pH

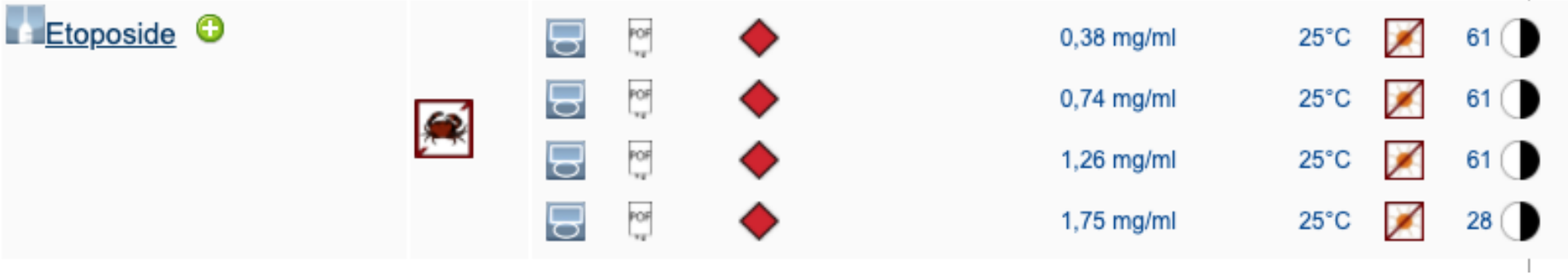
pH measurements

All samples diluted in 0.9% NaCl had a pH in the range of 3.32 to 3.97 and in the range of 3.36 to 3.87 in 5% glucose during the study. No significant modification of pH was observed during the whole stability study. For all solutions, the maximum variation obtained between the day of assay and day 0 was 0.15 pH unit.

8. Conclusion

CONCLUSION

Etoposide diluted in 5% glucose at 0.38, 0.74 and 1.26 were stable over 61 days at 25°C. Etoposide solutions diluted in 5% glucose 1.75 mg/mL were stable 28 days at 25°C.



9. Retour d'expérience

- Données complémentaires sur la stabilité physique