

Stability and Compatibility of Etoposide in Normal Saline

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ABSTRACT

Objective: At concentrations greater than 0.4 mg/mL, solutions of etoposide in normal saline are unstable, and the drug precipitates quickly. However, the stability of this drug at much higher concentrations has not been examined. This study was undertaken to evaluate the stability and compatibility of solutions of etoposide in 0.9% sodium chloride (normal saline [NS]), ranging in concentration from 0.20 to 12.00 mg/mL and stored in glass test tubes for 22 days at room temperature (24°C) and in the refrigerator (4°C).

Method: Solutions of etoposide in NS (0.20 to 12.00 mg/mL) were prepared and stored at room temperature (24°C) and in the refrigerator (4°C). A validated, stability-indicating high-pressure liquid chromatographic method was used to determine the concentration of the drug on days 0, 1, 2, 5, 7, 14, and 22. Samples were inspected visually to check for the formation of a precipitate.

Results: Etoposide solutions from 0.20 to 0.50 mg/mL and those of 9.50 mg/mL or more were stable for at least 24 h at both temperatures, retaining more than 90% of their initial concentration. The 8.00 mg/mL solution stored at 4°C was also stable for this period. Solutions of 2.00, 4.00, and 6.00 mg/mL precipitated within 2 h. Solutions of 1.00 to 8.00 mg/mL lost more than 10% of their initial concentration within 24 h. Solutions of 0.20, 11.00, and 12.00 mg/mL retained more than 90% of the initial concentration over the full 22-day study period, and no precipitate was observed.

Conclusions: The compatibility of etoposide in 0.9% NS is concentration-dependent. Etoposide solutions at lower concentrations (up to 0.50 mg/mL) retained most of their initial concentration for at least 24 h, and stronger solutions (10.00 mg/mL or more) retained most of their initial concentration for at least 7 days. It is recommended that storage of etoposide solutions in NS (concentration 10 mg/mL or more) at 4°C or 24°C not exceed 5 days, depending on solution sterility and bacterial contamination rate in the particular IV admixture program. Under these conditions, more than 94% of the initial concentration of the drug should be retained.

Key words: etoposide, stability, precipitate, concentration dependent, compatibility, normal saline, stability-indicating, liquid chromatography

RÉSUMÉ

Objectif : Les solutions d'étoposide, à des concentrations supérieures à 0,4 mg/mL de solution physiologique salée, sont instables et forment rapidement un précipité. Toutefois, la stabilité de cet agent à des concentrations supérieures n'a pas été évaluée. Cette étude a été menée afin d'évaluer la stabilité et la compatibilité de l'étoposide préparée dans solutions de chlorure de sodium à 0,9 % (solution physiologique salée; NS), à des concentrations allant de 0,20 à 12,00 mg/mL, puis entreposées dans des éprouvettes en verre durant 22 jours, à la température ambiante de 24 °C et au réfrigérateur à 4 °C.

Méthode : Les solutions d'étoposide dans du NS (0,20 à 12,00 mg/mL) ont été préparées puis entreposées à la température ambiante (24 °C) et au réfrigérateur (4°C). La concentration des solutions en médicament a été déterminée au moyen d'une épreuve de stabilité par chromatographie liquide à haute pression validée, aux jours 0, 1, 2, 5, 7, 14, et 22. Les échantillons ont été inspectés visuellement à la recherche d'un précipité.

Résultats : Les solutions d'étoposide aux concentrations allant de 0,20 à 0,50 mg/mL et celles de 9,50 mg/mL ou plus sont restées stables durant au moins 24 heures aux deux températures et ont conservé plus de 90 % de leur concentration initiale en médicament. Les solutions de 8,00 mg/mL conservées à 4 °C sont également restées stables durant cette période. Les solutions de 2,00, 4,00, et 6,00 mg/mL ont formé un précipité après deux heures. Les solutions de 1,00 à 8,00 mg/mL ont perdu plus de 10 % de leur concentration initiale en 24 heures. Les solutions de 0,20, 11,00 et 12,00 mg/mL ont conservé plus de 90 % de leur concentration initiale pendant les 22 jours qu'a duré l'étude et n'ont formé aucun précipité.

Conclusion : La compatibilité de l'étoposide préparée dans un NS à 0,9 % est fonction de la concentration. Les solutions d'étoposide de plus faible concentration (jusqu'à 0,50 mg/mL) ont conservé la plus grande partie de leur concentration initiale en médicament pendant au moins 24 heures, et les solutions à plus forte concentration (10,00 mg/mL ou plus) ont conservé leur concentration initiale en médicament pendant au moins sept jours. Il est recommandé de ne pas conserver les solutions d'étoposide dans du NS (à des concentrations de 10 mg/mL ou plus) à 4 °C ou à 24 °C pendant plus de cinq jours, en tenant compte de la stérilité de la solution et du taux de contamination bactérienne relatif au programme d'additifs aux solutés intraveineux de chaque établissement. Dans ces conditions, les solutions devraient retenir plus de 94 % des concentrations initiales en médicament.

Mots clés : étoposide, stabilité, précipité, fonction de la concentration, compatibilité, solution physiologique salée, épreuve de stabilité, chromatographie liquide

Can J Hosp Pharm 2000;53:338-45



INTRODUCTION

Etoposide (VP 16-213) is a semisynthetic epipodophyllotoxin. It is an antineoplastic agent used in a variety of malignant hematopoietic and tissue lesions, including small-cell lung cancer, leukemia, lymphoma, ovarian carcinoma, and breast cancer. The drug must be infused in large volumes because of its concentration-dependent stability.¹ Clinical resistance may be overcome with prolonged low-dose therapy.¹ The agent is typically given in a dose of 50 to 100 mg/m² per day IV over at least 60 min.² Too rapid an infusion of etoposide can cause hypotension.³ It has been recommended that etoposide solutions be administered immediately after dilution and that they not exceed a concentration of 0.4 mg/mL.⁴

A number of previous studies have reported wide variation in etoposide stability. Joel and colleagues⁴ reported that 0.4 mg/mL etoposide in normal saline (NS; 0.9% sodium chloride) was stable for 4 to 5 days at room temperature. However, others have recommended shorter expiry dates.⁵⁻⁷ Canetta and colleagues⁵ reported that a 0.25 mg/mL solution of etoposide was stable for 72 h in NS, whereas at 0.4 mg/mL the solution was stable for only 4 h. Barthes and colleagues⁸ recommended that 0.4 mg/mL solutions of etoposide should be stored for no longer than 24 h at room temperature because of the possibility of precipitation. Barthes and colleagues⁸ observed a precipitate in 0.4 mg/mL solutions stored for 72 h in glass bottles, polyvinylchloride bags, and polyethylene bags. Arnold⁷ reported that solutions of 0.2 mg/mL were stable for 6 h and those of 0.4 mg/mL were stable for 3 h. Phillips and Lauper⁹ and McCollam and Garrison¹⁰ both reported that, at higher concentrations, 1 mg/mL of etoposide crystallized in 30 min in a standing solution and in only 5 min if stirred. However, McLeod and Relling⁶ found that etoposide for injection, diluted to 10 mg/mL in NS, was stable for 22 days. Although these results present a confusing picture, they might be explained by concentration-dependent compatibility of etoposide in NS.

This study evaluated the stability and compatibility of etoposide in NS over a range of concentrations (0.20 to 12.00 mg/mL) at room temperature (24°C) and under refrigeration (4°C) over a 22-day period.

METHODS

Apparatus and Material

Drug concentrations were determined according to a modified version of a previously published, validated

liquid chromatographic method.¹¹ Modifications were made to the mobile phase, injection volume, and flow rate to enhance chromatographic separation of etoposide and degradation products. Assay development was performed with a 600E System Controller solvent delivery system (Waters Corporation, Mississauga, Ontario), a WISP 712 autoinjector (Waters Corporation, Mississauga, Ontario), a Spectra System UV6000LP ultraviolet detector (Thermo Separation Products, Fremont, California) at a wavelength of 230 nm, and an Ultrasphere ODS (octadecylsilane) reverse-phase C₁₈, 5- μ m column (4.6 mm x 25 cm) (distributed in Canada by Beckman, Mississauga, Ontario). The injection volume was 20 μ L. The mobile phase was 40% acetonitrile and 60% 0.01 mol/L potassium dihydrogen phosphate (KH₂PO₄) (high-pressure liquid chromatography grade, Fisher Scientific Company, Toronto, Ontario) at pH 5.60. The flow rate was 1 mL/min. An acidic mobile phase was selected to prevent epimerization of etoposide during the assay.¹¹ Chromatograms were evaluated from peak areas using chromatographic acquisition and integration software (Chromquest, ThermoQuest Inc., San Jose, California). Least-squares regression was then used to interpolate the etoposide concentration from a standard curve.

Assay Validation — Accelerated Study

Following the set-up of the chromatographic separation, etoposide was intentionally degraded at neutral, acidic, and basic pH to ensure that the separation method could indicate stability. A sample of 1.25 mL of etoposide (etoposide, 20 mg/mL; Novopharm, lot 71766, expiry June 2000) was diluted in 25 mL of methanol to produce a 1.00 mg/mL solution, which had a pH of 4.88. To this, 0.6 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide were added to make acidic (pH 1.19) and basic (pH 8.93) solutions of etoposide (1.00 mg/mL). These solutions were placed in sterile glass vials and incubated in a water bath at 96°C \pm 2°C. Samples were subjected to chromatography just before incubation (time zero), 10 min after incubation started, and every 30 min for 240 min. Chromatograms were inspected for the appearance of additional peaks, and the etoposide peak was examined with respect to area, retention time, and peak shape.

Accuracy and Reproducibility

The method was validated with respect to accuracy and reproducibility before the study period. Five standard curves for etoposide were constructed on

5 separate days, each consisting of 5 standards and 3 quality control samples. Standards with concentrations of 0.20, 0.50, 0.80, 0.90 and 1.00 mg/mL and quality control samples of 0.35, 0.65, and 0.95 mg/mL were prepared fresh with etoposide and methanol each day and subjected to chromatography in duplicate. Standard curves were assessed for linearity, accuracy, and reproducibility. Within-day reproducibility was assessed by means of the coefficient of variation of the peak area between replicate samples. Accuracy was determined by calculating the percent deviation of the calculated concentration from the known concentration of the standards and quality control samples.

Sample Preparation

On study day zero, 17 samples were prepared in glass test tubes from a 20 mg/mL stock solution of etoposide (Novopharm, lot 71766, expiry June 2000) with NS (Baxter Inc., lot W9C041C1, expiry December 1999).

One sample of each of the following etoposide solutions was prepared: 0.20, 0.30, 0.35, 0.40, 0.45, 0.50, 1.00, 1.50, 2.00, 4.00, 6.00, 8.00, 9.50, 10.00, 10.50, 11.00, and 12.00 mg/mL. Immediately after preparation, the pH of several of the samples spanning the concentration range was determined. Each sample was subjected to chromatography and then split into 2 aliquots; one was stored at room temperature (24°C) and the other at 4°C. All samples were covered with Parafilm barrier film to prevent evaporation and contamination. All samples were exposed to conventional laboratory cool-white fluorescent light. The samples were analyzed for etoposide content immediately after preparation (day 0) and on days 1, 2, 5, 7, 14, and 22.

A standard curve, consisting of samples with concentrations of 0.05, 0.20, 0.50, 1.00, and 2.00 mg/mL, was prepared before each analysis. Quality control samples of 0.10, 0.40, and 1.00 mg/mL were used to validate the standard curve. All samples were run in duplicate. Standards and quality control samples were prepared with methanol to avoid precipitation during analysis. A blank (methanol) was used to establish baseline. The standard curve and the quality control samples were analyzed first, followed by the samples of etoposide in NS. All samples in the concentration range above 1.50 mg/mL were diluted, so that they would be within the range of the standard curve with methanol, to avoid further unwanted precipitation during analysis. Concentrations with a noticeable precipitate were centrifuged for 10 min at 2000 g to separate the precipitate from the solution. Samples were drawn from the super-

nate. The precipitate was later analyzed by high-pressure liquid chromatography and was confirmed to be etoposide.

Physical Inspection

Before the preparation of samples for chromatographic analysis, each sample was inspected visually, under conventional laboratory cool-white fluorescent light, for the formation of particulate matter. Samples were also inspected for clarity and colour.

Data Reduction and Statistical Analysis

Means were calculated for replicate analysis, and these means are reported in the tables. Sample reproducibility was assessed by means of the coefficient of variation (standard deviation divided by the mean), and samples were considered reproducible if the coefficient of variation was less than 15%.¹² Average error was determined by calculating the mean of the individual concentration errors. Individual concentration errors were determined from the equation (observed concentration – expected concentration)/expected concentration.

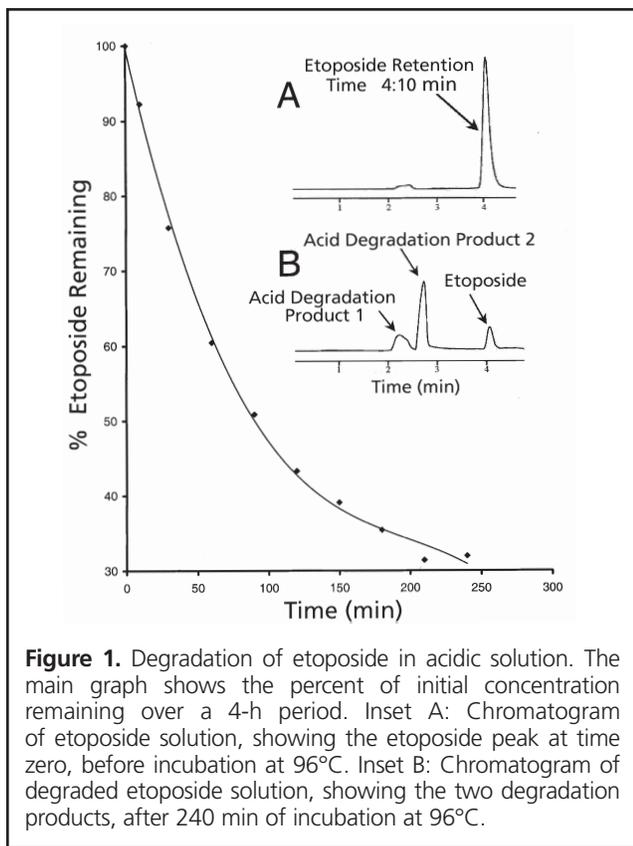
The concentration of etoposide samples stored at both room and refrigerated temperatures was determined by interpolation from the standard curve. The concentrations are presented as the percent remaining, based on the observed concentration on study day zero. Etoposide concentrations were considered “acceptable” or “within acceptable limits” if the concentration on any day of analysis was not less than 90% of the concentration on day zero. A solution was judged to be physically stable if no change in colour or clarity of the mixture was visible, if there was no evidence of a precipitate or other particulate formation, and if the concentration remained at greater than or equal to 90% of the day zero concentration.

RESULTS

Assay Validation — Accelerated Study

At 96°C ± 2°C, etoposide was degraded in acidic pH to yield 2 degradation products (Figure 1). Etoposide degraded by more than 10% within 30 min, and only 32% of the initial etoposide concentration remained after 4 h. Figure 1 shows the percent concentration remaining as a function of time at pH 1.19. Alkaline degradation was extremely rapid, and etoposide disappeared almost completely after 10 min, although degradation products were not observed.





Accuracy and Reproducibility

Five standard curves with quality controls were constructed and validated. Etoposide was measured accurately, with average deviation less than 4% (range 0.02% to 10.68%). Average error for the 9.50 mg/mL etoposide solution was 1.22% and never exceeded 4.03%. Correlation coefficients for the standard curves all exceeded 0.997. The average coefficient of variation for the quality control samples was 3.50%, and the average deviation of the quality control samples from the known concentration was less than 3.00% (for 0.35 mg/mL, 2.48%; for 0.65 mg/mL, 4.22%; and for 0.95 mg/mL, 1.22%). The average error (as coefficient of variation, in percent) on duplicate analysis of standards and the average percent deviation of the standards were both less than 2.00% (1.97% and 1.18%, respectively). These data indicate that the etoposide was measured accurately and reproducibly and that differences of up to 10% could be confidently detected with duplicate injection.¹³

Storage at Room Temperature (24°C)

Analyzed concentrations of samples stored at 24°C are presented in Table 1, which also lists the percent of

etoposide remaining relative to the initial concentration on day zero. Figure 2 depicts the expected concentration compared to the actual concentration of etoposide over the 22-day study period at room temperature. It is clear from Figure 2 and Table 1 that concentrations in the middle range (0.40 to 9.50 mg/mL) lost more than 10% of their initial concentration, generally within 24 h but certainly within 48 h. In samples with an initial etoposide concentration of 2.00, 4.00, or 6.00 mg/mL, more than 40% of the initial concentration was lost within 24 h. In the sample with an initial concentration of 8.00 mg/mL, the amount of precipitate increased over the study period, and the percent remaining gradually decreased from 85% at 24 h to 58% at 48 h and finally to 30% after 22 days. Conversely, samples with a concentration of 11.00 mg/mL or greater did not drop below 90% of their initial concentration after at least 7 days of storage. Although chromatographic analysis was not completed for samples from these initial concentrations collected on days 14 and 22, it was assumed that the solutions maintained adequate concentration, on the basis of the trend observed up to 7 days. In addition, no precipitate was visible in either of these solutions after 22 days at room temperature.

Storage at Refrigerated Temperature (4°C)

Analyzed concentrations of samples stored at 4°C are presented in Table 2, which also lists the percent of etoposide remaining relative to the initial concentration on day zero. Figure 3 depicts the expected concentration compared with the actual concentration of etoposide over the 22-day study period at 4°C. Similar to the samples stored at room temperature, only samples in the midrange of concentration (1.00 to 6.00 mg/mL) lost more than 10% of their initial concentration. The solution with concentration 10.00 mg/mL maintained more than 90% of its initial concentration for 7 days (chromatography not completed at 7 days for 10.50, 11.00, and 12.00 mg/mL). Furthermore, samples of 10.50 mg/mL and greater did not drop below 90% of their initial concentration during the 22-day study period, and no precipitate was visible.

Visual Inspection

All samples were clear and free of particulate matter when initially prepared. At low concentrations, etoposide solutions were colourless. As the concentration of etoposide in NS increased, the colour gradually increased to a transparent, yellowish colour. This colour remained the same throughout the entire study period.

Table 1. Concentration of Etoposide (in Normal Saline, 0.9% Sodium Chloride) Remaining after Storage at Room Temperature (24°C)*

Nominal	Day 0		Day 1		Day 2		Day 5		Day 7		Day 14		Day 22		
	Conc'n (mg/mL)	Conc'n (mg/mL)	% Remaining												
0.20	0.19	0.21	111	0.19	100	0.19	100	0.19	100	0.19	100	0.19	100	0.19	100
0.30	0.29	0.34	117	0.29	100	0.25	86	0.18	62	0.17	59	0.14	48	0.14	48
0.35	0.33	0.36	109	0.32	97	0.25	76	0.21	64	0.18	55	0.17	52	0.17	52
0.40	0.37	0.41	111	0.32	86	0.21	57	0.18	49	0.15	41	0.15	41	0.15	41
0.45	0.41	0.43	105	0.27	66	0.17	41	0.16	39	0.16	39	0.16	39	0.19	46
0.50	0.48	0.58	121	0.27	56	0.19	40	0.15	31	0.16	33	0.16	33	0.16	33
1.00	0.89	0.69	78	0.70	79	0.36	40	0.25	28	0.24	27	0.54	61	0.54	61
1.50	1.23	0.65	53	0.54	44	0.52	42	0.40	33	0.32	26	0.48	39	0.48	39
2.00	1.58	0.79	50	0.62	39	0.50	32	0.48	30	0.25	16	0.22	14	0.22	14
4.00	3.93	1.34	34	1.03	26	0.91	23	0.67	17	0.73	19	1.09	28	1.09	28
6.00	5.80	2.54	44	2.09	36	1.87	32	—	—	1.49	26	0.98	17	0.98	17
8.00	8.30	7.04	85	4.79	58	3.57	43	2.75	33	2.72	33	2.51	30	2.51	30
9.50	9.45	9.46	100	7.30	77	5.10	54	5.37	57	4.16	44	3.87	41	3.87	41
10.00	10.02	9.91	99	10.16	101	9.46	94	6.09	61	5.90	59	4.99	50	4.99	50
10.50	10.74	10.69	100	10.52	98	10.97	103	10.42	97	10.25	95	9.06	84	9.06	84
11.00	11.08	10.95	99	10.97	99	11.08	100	10.10	91	—	—	—	—	—	—
12.00	11.73	11.96	102	11.99	102	11.52	98	12.04	103	—	—	—	—	—	—

Dash = data not available.

*Shading indicates samples that retained at least 90% of the initial analyzed concentration.

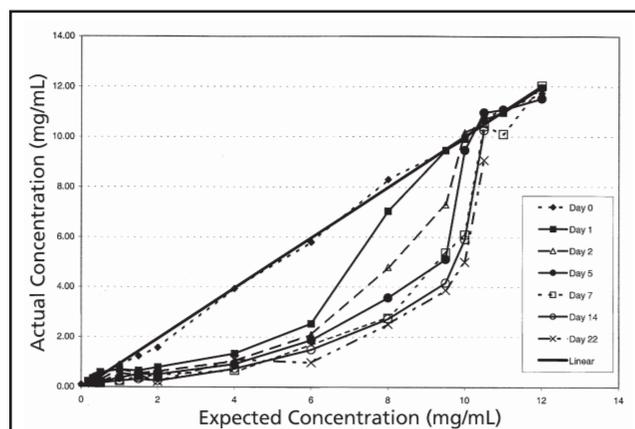


Figure 2. Actual concentration of etoposide as a function of expected concentration over a 22-day period at room temperature (24°C). Etoposide concentrations remained within 10% of the initial observed concentration (day zero) at low (0.20 to 0.50 mg/mL) and high (10.50 to 12.00 mg/mL) concentrations for several days. At midrange concentrations (1.00 to 8.00 mg/mL), the solutions lost more than 10% of the concentration within 24 h. Concentrations in the lower midrange (1.00 to 4.00 mg/mL) quickly precipitated and lost more than 10% concentration and then remained at that concentration for 3 weeks. Higher midrange concentrations (6.00 to 10.00 mg/mL) precipitated and lost concentration more gradually over the 22-day study period.

Samples at concentrations between 1.00 and 8.00 mg/mL precipitated within hours of being prepared. These concentrations also produced the most precipitate by the end of the study. The amount of precipitate was greatest for the 4.00 mg/mL sample, and there was progressively less precipitate for samples on either side of this concentration. Solutions of high (9.50 and 10.00 mg/mL) and low (0.35 and 0.30 mg/mL) concentrations also precipitated later than solutions of concentrations in the middle range. Samples stored at room temperature (24°C) formed a white fluffy precipitate, whereas samples stored in the refrigerator formed a more solid, dense, crystalline precipitate. The crystalline precipitate formed on the bottom of the test tube, whereas the fluffy precipitate was often found floating in the sample.

Sample pH

Immediately after preparation, samples had the pH values presented in Table 3.

DISCUSSION

This study demonstrated concentration-dependent compatibility and stability of etoposide in NS. Previous studies have demonstrated incompatibility and instability at concentrations greater than 0.40 mg/mL.^{2,4-10} Once a solution has been found to precipitate at low concentrations, stability studies at



Table 2. Concentration of Etoposide (in Normal Saline, 0.9% Sodium Chloride) Remaining after Refrigerated Storage (4°C)*

Nominal	Day 0		Day 1		Day 2		Day 5		Day 7		Day 14		Day 22		
	Conc'n (mg/mL)	Conc'n (mg/mL)	% Remaining												
0.20	0.19	0.21	111	0.18	95	0.18	95	0.20	105	0.18	95	—	—	—	—
0.30	0.29	0.33	114	0.29	100	0.27	93	0.30	103	0.23	79	0.23	79	0.23	79
0.35	0.33	0.37	112	0.33	100	0.29	88	0.30	91	0.22	67	0.22	67	0.22	67
0.40	0.37	0.40	108	0.34	92	0.20	54	0.21	57	0.23	62	0.16	43	0.16	43
0.45	0.41	0.44	107	0.33	80	0.21	51	0.18	44	0.20	49	0.21	51	0.21	51
0.50	0.48	0.46	96	0.24	50	0.21	44	0.21	44	0.19	40	0.18	38	0.18	38
1.00	0.89	0.56	63	0.56	63	0.45	51	0.39	44	0.16	18	0.33	37	0.33	37
1.50	1.23	0.77	63	0.51	41	0.43	35	0.42	34	0.49	40	0.95	77	0.95	77
2.00	1.58	0.86	54	0.71	45	0.76	48	0.58	37	0.69	44	1.10	70	1.10	70
4.00	3.93	1.61	41	1.29	33	1.18	30	1.16	30	0.93	24	1.56	40	1.56	40
6.00	5.80	3.40	59	2.44	42	2.56	44	2.65	46	1.88	32	2.11	36	2.11	36
8.00	8.30	8.01	97	—	—	4.52	54	—	—	2.90	35	2.86	34	2.86	34
9.50	9.45	8.80	93	9.27	98	6.80	72	6.68	71	5.77	61	5.81	61	5.81	61
10.00	10.02	10.10	101	9.27	93	9.51	95	9.16	91	7.64	76	7.87	79	7.87	79
10.50	10.74	10.53	98	10.31	96	10.79	100	—	—	10.36	96	9.82	91	9.82	91
11.00	11.08	11.20	101	10.31	93	11.13	100	—	—	11.43	103	11.75	106	11.75	106
12.00	11.73	11.86	101	11.97	102	11.63	99	—	—	11.74	100	—	—	—	—

Dash = data not available.

*Shading indicates samples that retained at least 90% of the initial analyzed concentration.

higher concentrations are rarely conducted. Etoposide demonstrated an interesting phenomenon in this study, in that it was stable at low concentrations, precipitated quickly at midrange concentrations, and was also stable at high concentrations. The formation of a precipitate was observed within hours for etoposide solutions between 2.00 and 8.00 mg/mL. This precipitation is directly related to the poor aqueous solubility of etoposide.¹⁴ Etoposide 20 mg/mL formulations on the Canadian market contain polyethylene glycol and polysorbate 80.¹⁵ As these solvents are diluted in water or saline, they become less able to support the solubility of etoposide. As a result, etoposide will remain dissolved only in very dilute solutions or in more concentrated solutions. This property is very similar to the concentration-dependent compatibility observed with pentobarbital in saline and dextrose solutions, as a result of the dilution of its propylene glycol and alcohol solvents.¹⁶

It is currently recommended that etoposide be diluted in NS to a concentration of 0.40 mg/mL and that it then be administered immediately.² Because etoposide must be infused over 1 h or more, a concentration that precipitates within hours is unacceptable for clinical use. A very dilute solution necessitates an extended infusion time, and too high a concentration or too rapid IV administration may result in hypotension. These constraints can be taxing not

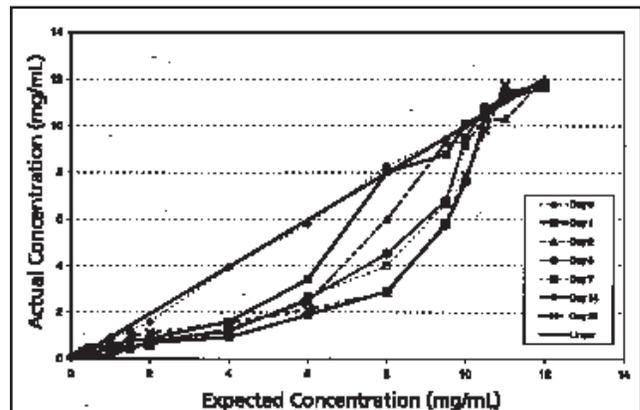


Figure 3. Actual concentration of etoposide as a function of expected concentration over a 22-day period under refrigeration (4°C). Etoposide concentrations remained within 10% of the initial observed concentration (on day zero) at low (0.20 to 0.50 mg/mL) and high (10.50 to 12.00 mg/mL) concentrations for several days. At midrange concentrations (1.00 to 6.00 mg/mL), the solutions lost more than 10% of the concentration within 24 h. Concentrations in the lower midrange (1.00 to 4.00 mg/mL) quickly precipitated and lost more than 10% concentration and then remained at that concentration for 3 weeks. Higher midrange concentrations (6.00 to 10.00 mg/mL) precipitated and lost concentration more gradually over the 22-day study period.

only on nursing staff but also on pharmacy staff, especially where high doses of etoposide are required, as for patients undergoing bone marrow transplant. A concentration that can be easily prepared in advance, stored for long periods (e.g., 7 days), and administered with minimal fluid volumes can make the work of the health-care professional easier and will result in less wastage.^{17,18}

Sample pH

All samples had a pH in the range of 3.6 to 4.2 upon preparation. This pH provides optimal stability and avoids epimerization to the less active *cis*-etoposide.¹⁴

Comparison of Storage Temperatures

Although drug solubility is often dependent on temperature, in general there was no difference in etoposide stability in NS at 4°C or 24°C. At both temperatures, the etoposide concentration gradually decreased, in some cases continually over the 22-day study period. The most rapid loss in concentration was observed in the 4.00 mg/mL sample, which precipitated within hours of preparation. Approximately 60% of the initial concentration of this sample was lost within 24 h at both 4°C and 24°C. At room temperature, the 0.40 mg/mL etoposide solution precipitated within 2 days and lost 14% of its initial concentration within 48 h, whereas the 0.40 mg/mL sample stored at 4°C did not have a visible precipitate and lost only 8% of its initial concentration at 48 h. A precipitate was evident in this sample after 5 days.

At concentrations of 10.50, 11.00, and 12.00 mg/mL, etoposide in NS was stable at room temperature for up to 2 weeks, although the 10.50 mg/mL sample did have a noticeable precipitate on day 22 and dropped below 90% of its initial concentration. The 11.00 and 12.00 mg/mL samples did not lose more than 9% of their initial concentration over 22 days and did not precipitate. The 8.00 mg/mL sample remained stable for 24 h when refrigerated.

Concentrations of etoposide below 0.30 mg/mL did not precipitate and retained adequate concentration for 22 days. When stored in the refrigerator, some samples (8.00 and 10.50 mg/mL) maintained stability slightly longer than at room temperature. Yet a general trend of decreasing concentration over time was observed at both temperatures.

Although infusion of highly concentrated etoposide solutions has been reported to cause hypotension, in the authors' experience, which has involved more than 50

Table 3. pH of Etoposide Samples Immediately after Preparation

Sample Concentration (mg/mL)	pH
0.20	4.2
0.40	3.9
2.00	3.7
4.00	3.7
6.00	3.6
10.00	3.8

bone marrow transplant patients given 60 mg/kg of etoposide, 10 mg/mL solutions are extremely well tolerated (unpublished observation). By increasing the concentration of the solution, very concentrated solutions of etoposide can be mixed, in glass bottles, in small volumes (typically under 500 mL) and infused into a running IV that the nurse has already established. One-to-one nursing has been maintained to monitor vital signs over the 5-h infusion. In this series of 50 patients, there was only one easily managed case of hypotension. Etoposide at these doses, prepared at the conventional 0.4 mg/mL concentration, would require administration of 10 to 12 L of fluid. Concentrated solutions (10 to 11 mg/mL) require less pharmacy preparation and nursing time.

The authors recommend that, after consideration of solution sterility and the bacterial contamination rate of each IV admixture program, solutions of etoposide and NS of at least 10 mg/mL be assigned an expiry date not exceeding 5 days when stored either at 4°C or 24°C. With such an expiry date, it is estimated that more than 94% of the initial etoposide concentration will remain at the end of the expiry period.

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