

Stability and compatibility of topotecan hydrochloride for injection with common infusion solutions and containers

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Received 13 September 1996; received in revised form 17 December 1996; accepted 17 December 1996

Abstract

The stability and compatibility of topotecan hydrochloride with common infusion solutions and containers were studied. During this study, the leaching of diethylhexyl phthalate (DEHP), a major plasticizer of some polyvinyl chloride (PVC) materials was also investigated. A formulation of topotecan hydrochloride was added to 50 ml PVC infusion bags, polyolefin infusion bags and 150 ml glass bottles containing either 5% dextrose injection or 0.9% sodium chloride injection at an initial nominal topotecan concentration of 0.05 mg ml^{-1} . Additionally, the topotecan hydrochloride formulation was added to 50 ml PVC infusion bags containing either 5% dextrose injection or 0.9% sodium chloride injection at an initial nominal topotecan concentration of 0.025 mg ml^{-1} . Containers were maintained at 5°C for 7 days or $23\text{--}24^\circ\text{C}$ for 24 h. Samples were analyzed using a stability-indicating HPLC method to determine the concentration of topotecan and the presence of any degradates. The samples were also analyzed by separate HPLC methods to detect the presence of DEHP and the hydrolyzed lactone ring form (SKF 105992) of topotecan hydrochloride. In addition, the pH of each sample was measured initially and at the end of the storage time. There was no significant loss of topotecan observed for any of the conditions studied and no significant increase in degradates was observed. The pH remained unchanged for all samples between the start and end of the study. At the concentrations studied, topotecan hydrochloride was stable for up to 24 h at room temperature and for up to 7 days at 5°C , in PVC and polyolefin infusion bags and glass bottles containing either 5% dextrose injection or 0.9% sodium chloride injection. The presence of topotecan hydrochloride did not contribute to leaching of DEHP in the PVC infusion bags. © 1997 Elsevier Science B.V.

Keywords: Dextrose injection; Diethylhexyl phthalate; Leaching; Polyvinyl chloride; Sodium chloride injection; Topoisomerase I; Topotecan hydrochloride

1. Introduction

Topotecan hydrochloride is a semisynthetic, water soluble analog of camptothecin with signifi-

cant in vitro and in vivo antitumor activity. Topotecan hydrochloride is an inhibitor of topoisomerase I, which like other camptothecin analogs, stabilizes the covalent complex between topoisomerase I and DNA, resulting in enzyme linked DNA cleavage and single strand breaks [1]. Topotecan hydrochloride contains a lactone ring

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which must be intact in order to have biological activity. Hydrolysis of the lactone ring, which forms SKF 105992 (Fig. 1), is known to occur if the pH of the solution rises above pH 4 [2,3]. Thus, the formulation developed, and currently marketed for the treatment of ovarian cancer, contains tartaric acid as a buffering agent to prevent hydrolysis of the lactone ring upon reconstitution and dilution. The physical and chemical stability and compatibility, as well as any hydrolysis of the lactone ring, of topotecan hydrochloride in common infusion solutions has not been previously reported.

Studies have demonstrated that the plasticizer diethylhexyl phthalate (DEHP) can be leached from polyvinyl chloride (PVC) in the presence of various solvents or drug products [4–7]. Since hepatotoxicity due to DEHP has been reported [8] and topotecan hydrochloride will be given to patients whose health is impaired, the possible effect on leaching was investigated.

The study objectives were to determine the stability and compatibility, under varying storage conditions, of topotecan hydrochloride, at concentrations expected to be used clinically, with commonly used infusion solutions and containers. Additionally, the effects of topotecan hydrochloride on the leaching of DEHP was determined. Also, since the biological activity of topotecan hydrochloride requires that the lactone ring is intact, analysis to detect the presence of SKF 105992 was performed.

2. Experimental

2.1. Materials

A commercial formulation of topotecan hydrochloride for injection (4 mg per vial) was supplied by SmithKline Beecham Pharmaceuticals (King of Prussia, PA). The 5% dextrose injection and 0.9% sodium chloride injection were in 50 ml fill PVC bags from Baxter Healthcare (Deerfield, IL) and 50 ml fill polyolefin infusion bags and 150 ml fill glass bottles from McGaw (Irvine, CA). DEHP obtained from Aldrich (Milwaukee, WI) was used as a standard for the DEHP analysis.

All solvents were of HPLC grade, and deionized water filtered through a Millipore Milli-Q Water Purification System (Bedford, MA) was used. Reference standard topotecan hydrochloride supplied by SmithKline Beecham Pharmaceuticals (King of Prussia, PA) was used for the topotecan assay and for preparing standards for the SKF 105992 analysis.

2.2. Topotecan method

The concentrations of topotecan (free base) were determined using a stability indicating gradient HPLC procedure. The liquid chromatograph consisted of a Shimadzu 6A HPLC system equipped with a Hitachi L-4000 UV detector and a Cosmosil 5C₁₈-AR column (4.6 × 250 mm, 5 μm) (Nacalai Tesque, Kyoto, Japan) at room temperature. Data was acquired and integrated using a Nelson System 6000 and Nelson Access*Chrom (version 1.8) software. Mobile phase A was water–acetonitrile–trifluoroacetic acid (85:15:0.1, v/v/w) and mobile phase B was water–acetonitrile–trifluoroacetic acid (60:40:0.1, v/v/w). The flow rate was 1.0 ml min⁻¹ with detection at 228 nm and an injection volume of 40 μl. The analysis time was 30 min with the gradient going from 0% of mobile phase B at the start of the analysis to 100% at 20 min and 0% again at 21 min.

2.3. SKF 105992 method

In order to more easily detect the presence of SKF 105992 from hydrolysis of the lactone ring a separate HPLC procedure was used. This proce-

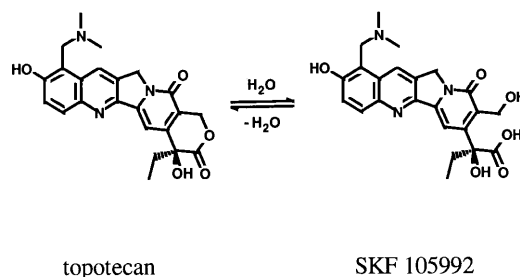


Fig. 1. Hydrolysis of topotecan to SKF 105992.

cedure was an isocratic HPLC procedure using a Shimadzu 6A HPLC system equipped with a Shimadzu SPD-6AV UV detector with a Hypersil ODS column (4.6×150 mm, $5 \mu\text{m}$) (Shandon, Pittsburgh, PA) at room temperature. Data was acquired and integrated using a Nelson System 6000 and Nelson Access*Chrom (version 1.8) software. The mobile phase consisted of buffer (sodium phosphate dibasic-tetrabutyl ammonium phosphate (1 M)–triethylamine (0.4:4:5, w,v,v) (pH* 6.0))–methanol–tetrahydrofuran (90:6:4, v/v/v). The flow rate was 1.0 ml min^{-1} with detection at 382 nm and an injection volume of $20 \mu\text{l}$.

2.4. DEHP method

Several methods for the detection of DEHP have been reported [9–13]. For this study, detection of DEHP was determined using an isocratic HPLC procedure using a Shimadzu 6A HPLC system equipped with a Shimadzu SPD-6AV UV detector with a Cosmosil 5C₁₈-AR column (4.6×250 mm, $5 \mu\text{m}$) (Nacalai Tesque) at room temperature. Data was acquired and integrated using a Nelson System 6000 and Nelson Access*Chrom (version 1.8) software. The mobile phase consisted of acetic acid (1%)–methanol (13:87, v/v). The flow rate was 2.0 ml min^{-1} with detection at 235 nm and an injection volume of $100 \mu\text{l}$.

2.5. Chromatography

The HPLC analysis method for topotecan was a modification of a previously validated stability-indicating method for topotecan [14]. Modification of this method was to change from isocratic to gradient in order to elute all impurity and degradation products more quickly, resulting in shortening the analysis time from 60 to 30 min. The stability-indicating nature of the modified method was determined by analyzing solutions of 5% dextrose injection, 0.9% sodium chloride injection, topotecan hydrochloride formulation prepared in 5% dextrose injection and 0.9% sodium chloride injection and all known impurity and degradation products of topotecan prepared in 5% dextrose injection and 0.9% sodium chloride injection.

For the topotecan analysis, working standards were prepared daily, at the level of concentration of the control sample (0.025 or 0.05 mg ml^{-1}), in either 5% dextrose injection or 0.9% sodium chloride injection. Variations of within-day and between day analysis for topotecan were determined using the R.S.D. of standard response factors (ratio of weight versus peak area) from each individual HPLC run.

For the DEHP analysis, standard stock solutions (5 mg ml^{-1}) and first dilutions ($50 \mu\text{g ml}^{-1}$) were prepared daily in methanol–water (87:13, v/v). Subsequent dilutions (0.5 , 0.2 and $0.1 \mu\text{g ml}^{-1}$) were prepared daily in either 5% dextrose injection or 0.9% sodium chloride injection. The selectivity of the DEHP analysis was determined by analyzing solutions of methanol–water (87:13 v/v), topotecan hydrochloride formulation in 5% dextrose injection and 0.9% sodium chloride injection to yield a nominal topotecan concentration of 0.08 mg ml^{-1} , 5% dextrose injection and 0.9% sodium chloride injection.

2.6. Sample preparation

Solutions were prepared at a nominal topotecan concentration of either 0.025 or 0.05 mg ml^{-1} . Separate solutions at 0.05 mg ml^{-1} were prepared with 5% dextrose injection and 0.9% sodium chloride injection from Baxter PVC bags, McGaw polyolefin bags and McGaw glass bottles. The 5% dextrose injection and 0.9% sodium chloride injection solutions were prepared at different times using the same procedure. For each, twenty topotecan vials were reconstituted with 4 ml Sterile Water for Injection, USP, from Abbott Laboratories (Chicago, IL). The contents of the vials were combined and mixed in a flask before transferring separate 12.5 ml aliquots into 250 ml volumetric flasks and a 35 ml aliquot into a 1 l volumetric flask. The 250 ml flasks were diluted to volume with 5% dextrose injection from Baxter PVC bags, 5% dextrose injection from McGaw polyolefin bags, 0.9% sodium chloride injection from Baxter PVC bags or 0.9% sodium chloride injection from McGaw polyolefin bags. Into the 1 l flask was added 665 ml of 5% dextrose injection or 0.9% sodium chloride from McGaw glass bottles.

Table 1
Validation data for the topotecan assay

IV Diluent	Concentration (mg ml ⁻¹)	Mean concentration found \pm S.D. (mg ml ⁻¹)	Accuracy (%)
5% dextrose	0.0206	0.0209 \pm 0.0005	101.5
	0.02575	0.0263 \pm 0.0004	102.1
	0.0309	0.0312 \pm 0.0006	101.1
	0.0412	0.0425 \pm 0.001	103.1
	0.0515	0.0520 \pm 0.0006	100.9
	0.0618	0.0606 \pm 0.001	98.0
0.9% sodium chloride	0.0206	0.0210 \pm 0.0001	101.7
	0.02575	0.0260 \pm 0.0003	101.0
	0.0309	0.0311 \pm 0.0003	100.6
	0.0412	0.0423 \pm 0.0005	102.6
	0.0515	0.0519 \pm 0.0006	100.8
	0.0618	0.0607 \pm 0.0007	98.2

$n = 4$.

The solutions at 0.025 mg ml⁻¹ were prepared using 5% dextrose injection, or 0.9% sodium chloride injection from Baxter PVC bags. These solutions were prepared at the same time by reconstituting five topotecan vials with 4 ml of Sterile Water for Injection, USP, and then combining the contents in a flask. Aliquots of 6.25 ml were placed in separate 250 ml volumetric flasks, and diluted to volume using 5% dextrose injection or 0.9% sodium chloride injection from Baxter PVC bags.

An aliquot was removed, from each flask prepared, as a control and analyzed, initially and after 24 h, for topotecan, DEHP and SKF 105992 by the HPLC methods described. The appropriate volume (50 or 150 ml) of the solution was then transferred to empty infusion containers (bags or bottles), and the containers agitated by shaking and/or squeezing. Duplicate infusion containers were prepared for all variations of infusion solution (5% dextrose injection or 0.9% sodium chloride injection), topotecan concentration (0.025 or 0.05 mg ml⁻¹) and storage temperature (room temperature or 5°C).

From the room temperature infusion containers, approximately 10 ml were withdrawn immediately after transfer to the containers (initial) and at 6 and 24 h and analyzed for topotecan, DEHP, and SKF 105992. From the 5°C infusion containers, approximately 10 ml were withdrawn immedi-

ately after transfer to the containers (initial) and at 7 days, and analyzed for topotecan, DEHP and SKF 105992. The infusion containers were stored at room temperature, under normal laboratory lighting (\sim 30–35 ft. candles) and at 5°C (without light). In addition, the pH of each sample, at the beginning and end of the storage period, was measured using an Orion SA 520 pH meter.

3. Results and discussion

3.1. Chromatography

Topotecan hydrochloride formulation prepared in 5% dextrose injection and 0.9% sodium chloride injection were assayed using the modified topotecan method, and the results were not different than samples assayed by the unmodified method. Also, there was no change in the impurity/degradation profile. Chromatograms of 5% dextrose injection, 0.9% sodium chloride injection, and all known impurity and degradation products of topotecan in 5% dextrose injection and 0.9% sodium chloride injection showed none of these solutions eluted any peaks which interfered with the topotecan peak.

For each infusion solution (5% dextrose injection or 0.9% sodium chloride injection) and concentration (0.05 or 0.025 mg ml⁻¹), the

Table 2
Topotecan analysis as percent of initial concentration

IV Diluent	Storage condition	Storage time	Topotecan % initial concentration ^a		
			PVC bags	Polyolefin bags	Glass bottles
5% dextrose ^b	23–24°C	6 h	100.4 ± 0.41	100.5 ± 0.41	100.0 ± 0.00
5% dextrose ^b	23–24°C	24 h	100.2 ± 0.14	100.6 ± 0.28	100.1 ± 0.14
5% dextrose ^b	5°C	7 days	99.8 ± 0.00	99.6 ± 0.00	99.8 ± 0.27
5% dextrose ^c	23–24°C	6 h	100.0 ± 0.00	NA	NA
5% dextrose ^c	23–24°C	24 h	99.2 ± 0.56	NA	NA
5% dextrose ^c	5°C	7 days	100.0 ± 0.00	NA	NA
0.9% sodium chloride ^b	23–24°C	6 h	100.1 ± 0.14	100.1 ± 0.14	99.6 ± 0.28
0.9% sodium chloride ^b	23–24°C	24 h	100.4 ± 0.00	100.1 ± 0.14	100.0 ± 0.28
0.9% sodium chloride ^b	5°C	7 days	100.0 ± 0.28	100.1 ± 0.14	100.4 ± 0.85
0.9% sodium chloride ^c	23–24°C	6 h	99.6 ± 0.00	NA	NA
0.9% sodium chloride ^c	23–24°C	24 h	99.0 ± 0.27	NA	NA
0.9% sodium chloride ^c	5°C	7 days	100.0 ± 0.56	NA	NA

$n = 2$. NA, not analyzed.

^a Mean ± SD.

^b Nominal concentration of topotecan = 0.05 mg ml⁻¹.

^c Nominal concentration of topotecan = 0.025 mg ml⁻¹.

within-day and between-day variations were determined using three sets of replicate measurements. As shown in Table 1, the topotecan method was accurate in both 5% dextrose injection and 0.9% sodium chloride injection at each of six concentrations. The linearity of topotecan in 5% dextrose injection and 0.9% sodium chloride injection was determined by plotting peak area versus concentration for six concentrations ($n = 4$ for each) covering the range of 0.02–0.06 mg ml⁻¹. The correlation coefficients of the 5% dextrose injection and 0.9% sodium chloride injection calibration curves were 0.998 and 0.999, respectively, indicating good linearity. The within-day variations for topotecan at 0.05 mg ml⁻¹ in 5% dextrose injection and 0.9% sodium chloride injection ranged from 0.20–0.57% and 0.25–0.55%, respectively ($n = 9, 11$ or 13). The within-day variations for topotecan at 0.025 mg ml⁻¹ in 5% dextrose injection and 0.9% sodium chloride injection ranged from 0.14–1.07% and 0.27–1.09%, respectively ($n = 7, 9$ or 11). The between day variations for topotecan at 0.05 mg ml⁻¹ in 5% dextrose injection and 0.9% sodium chloride injection ($n = 33$), and topotecan at 0.025 mg ml⁻¹ in 5% dextrose injection and 0.9% sodium chloride injection ($n = 27$) were 1.21, 1.00, 0.63 and 0.32%, respectively.

Chromatograms of the solutions of methanol–water (87:13, v/v), topotecan vials (0.08 mg ml⁻¹) in 5% dextrose injection and 0.9% sodium chloride injection, 5% dextrose injection and 0.9% sodium chloride injection showed no peaks eluted which interfered with the DEHP peak.

3.2. Stability of topotecan

Once the samples were withdrawn from the infusion containers, the HPLC analysis was performed without any dilution. The concentration of each sample was determined in mg ml⁻¹ and the concentration at each sampling time was compared to each condition's initial concentration. Stability was defined as 95–105% of the initial concentration, and no greater than 0.5% increase in total degradation products or 0.1% increase in an individual product, from initial to final sample time. Table 2 summarizes topotecan analysis results, as percent of initial concentration, for Baxter PVC bag, McGaw polyolefin bag and McGaw glass bottle solutions.

All samples appeared clear, upon visual observation, throughout the storage times. There was no significant difference (range of 98.8–101.0%)

in toptecan concentration from the initial sample to the final storage sample for all three types of infusion containers with either 5% dextrose injection or 0.9% sodium chloride injection. Comparison of the control, initial, and final storage samples for each storage condition showed no significant difference (total products < 0.2% and any individual product < 0.1% for all samples) in any of the impurity/degradation peaks detected, and no peaks were detected in the final storage samples which were not detected in the control and initial storage samples (Figs. 2 and 3 show chromatograms of samples in PVC bags for 24 h at room temperature and 7 days at 5°C, respectively). There was no SKF 105992 detected in any of the samples. This, coupled with the pH results for all samples ranging from 3.31 to 3.58, is consistent with the referenced studies which showed hydrolysis of the lactone ring at pH > 4.

3.3. DEHP leaching during storage

No DEHP was detected in any of the initial or stored polyolefin infusion bag or glass bottle sam-

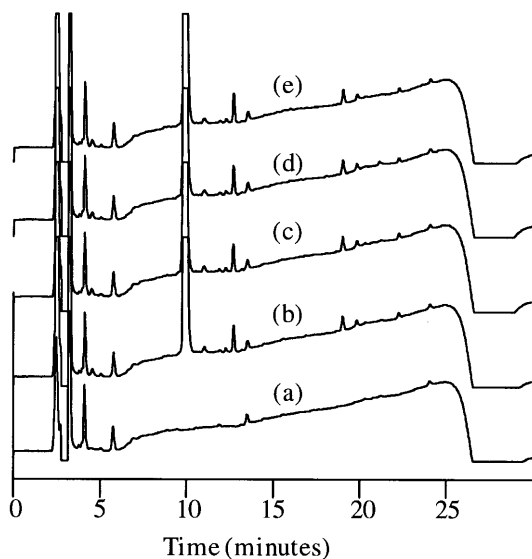


Fig. 2. Chromatogram of (a) 5% dextrose injection, (b) control sample, (c) initial sample, (d) 6 h sample and (e) 24 h sample for toptecan determination at 0.05 mg ml^{-1} in Baxter PVC Bags containing 5% dextrose injection stored at 23–24°C over 24 h.

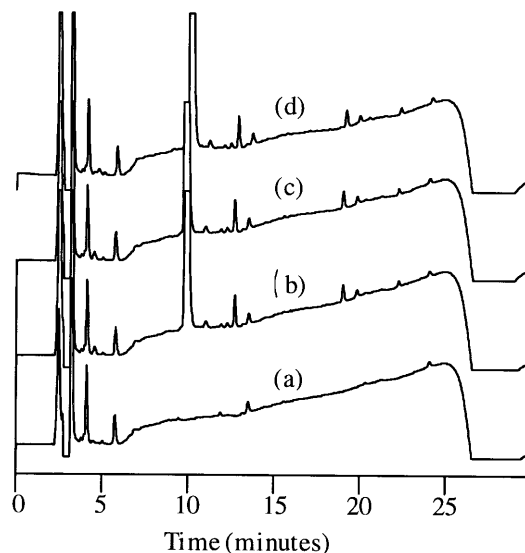


Fig. 3. Chromatogram of (a) 5% dextrose injection, (b) control sample, (c) initial sample and (d) sample for toptecan determination at 0.05 mg ml^{-1} in Baxter PVC Bags containing 5% dextrose injection stored at 5°C over 7 days.

ples. Less than $0.2 \text{ } \mu\text{g ml}^{-1}$ DEHP was detected in the initial samples from PVC infusion bags, and the difference in DEHP detected between the initial and stored samples was $< 0.1 \text{ } \mu\text{g ml}^{-1}$ for all samples.

4. Conclusions

At concentrations of 0.05 mg ml^{-1} , toptecan was stable for up to 24 h at room temperature and for up to 7 days at 5°C, in PVC and polyolefin infusion bags and glass bottles containing either 5% dextrose injection or 0.9% sodium chloride injection. At concentrations of 0.025 mg ml^{-1} , toptecan was stable for up to 24 h at room temperature, and for up to 7 days at 5°C, in PVC bags containing either 5% dextrose injection, or 0.9% sodium chloride injection. There were no significant differences observed in toptecan stability between 5% dextrose injection and 0.9% sodium chloride injection. The presence of toptecan hydrochloride did not contribute to significant leaching of DEHP in the PVC infusion bags. There were no significant differences observed in

leaching of DEHP between 5% dextrose injection and 0.9% sodium chloride injection.

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