AAPS PharmsciTech 2001; 2 (3) article 14
(http://www.pharmscitech.com)

Pharmaceutical Development and Manufacturing of a Parenteral Formulation of a Novel Antitumor Agent, VNP40101M

Submitted: March 16, 2001; Accepted: August 14, 2001; Published: August 26, 2001.

Gopal Krishna,* William F. Hodnick, Wengsheng Lang, Xu Lin, Srinivasa Karra, John Mao, Bijan Almassian
Vion Pharmaceuticals, Inc., 4 Science Park, New Haven, CT 06511

ABSTRACT

The objective of this study was to develop and manufacture a stable parenteral formulation for Phase I clinical trials of VNP40101M (1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(2-methylamino)carbonyl] hydrazine), a novel antitumor agent. The solubility and stability of the drug was determined. Solubility studies suggested that VNP40101M exhibited poor aqueous solubility but showed appreciable solubility in nonaqueous solvents. The aqueous solubility of the drug could not be increased by adjusting the pH. At a pH above 7, base-catalyzed decomposition of VNP40101M occurred. The low octanol-water partition coefficient of 0.75 suggested poor solubility in lipophilic solvents. Based on these preformulation observations, a parenteral formulation containing 10 mg/mL of VNP40101M was prepared in a solvent system consisting of 30% ethyl alcohol and 70% polyethylene glycol-300 (PEG-300). To minimize base-catalyzed hydrolytic degradation, citric acid at 0.6% concentration was included to acidify the formulation. Rubber closures, filter membranes, and liquid transfer tubing were selected on the basis of compatibility studies and absence of loss of drug due to adsorption of these components. The formulation was subjected to accelerated stability studies and dilution studies with large volume parenteral (LVP) solutions, normal saline, and 5% dextrose injection (D5W). The results of the dilution study indicated that the formulation could be diluted in these solutions up to 2 mg/mL for 8 hours without drug precipitation and degradation. Accelerated stability studies suggested that the product should be kept at 2°C to 8°C for long-term storage. The developed formulation was successfully scaled up and manufactured for use in clinical trials.

***Corresponding author:** Gopal Krishna, Vion Pharmaceuticals, Inc., 4 Science Park, New Haven, CT 06511; Telephone: 203-498-4210; Facsimile: 203-498-4211; E-mail: Gkrishna@vionpharm.com

INTRODUCTION

Chemotherapeutic drugs are a major tool in anticancer therapy. However, most chemotherapeutic drugs are nonspecific or become less effective as tumor cells acquire multidrug resistance [1-3]. Therefore, development of novel anticancer agents that are more effective against tumor cells resistant to currently available agents is expected to complement existing treatments in the fight against this deadly disease. Several derivatives of the sulfonylhydrazine class of compounds have been found to be potent alkylating agents and have been shown to have broad antitumor activity in animal models [4-8]. VNP40101M (1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(2 methylamino)carbonyl] hydrazine) belongs to this class of compounds and has been shown to be active against a variety of transplanted murine and human solid tumors, including tumors that are resistant to other alkylating agents such as cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea, and melphalan [8,9]. Shyam et al [8] found complete cures (survival < 60 days) in L1210 carcinoma bearing mice administered with VNP40101M at doses of 10 and 15 mg/kg given daily for 6 days following tumor implantation. VNP40101M was also found to be active against B16F10 melanoma in vivo [] and against mer+ (high alkyl guanine transferase activity) cell lines [10] and cell lines with high glutathione and glutathione tranferase activity, which are known mechanisms of resistance to alkylating agents [11]. VNP40101M was also shown to cross the blood-brain barrier and eradicate leukemia cells with a log kill of greater than 6 [12]. On the basis of these observations, the clinical development of VNP40101M was undertaken.

The objective of this study was to design, formulate, and manufacture a sterile parenteral formulation of VNP40101M suitable for clinical Phase I testing. For this purpose, analytical methods were developed and validated. The solubility in different solvents, pH conditions, and the octanol-water partition coefficient

was assessed. Forced degradation studies were conducted, and the degradation pathway was elucidated. A liquid formulation was developed and subjected to accelerated stability studies and to compatibility studies with large volume parenteral (LVP) solutions. Manufacturing issues, such as cleaning and vial filling parameters, were also addressed.

MATERIALS AND METHODS

Materials

VNP40101M and degradation product 90CE were synthesized in-house at Vion Pharmaceuticals, Inc (New Haven, CT). Polyethylene glycol 300 (PEG-300) *(National Formulary*) and anhydrous glycerol were obtained from Spectrum Chemicals Manufacturing Corp (Gardena, CA); dehydrated ethyl alcohol 200 proof (*US Pharmacopeia* [USP]) was from Pharmaco Products (Brookfield, CT); anhydrous citric acid and octanol were obtained from J.T. Baker (Phillipsburg, NJ). Propylene glycol was from Fisher Scientific (Fair Lawn, NJ); acetonitrile (high-performance liquid chromatography [HPLC] grade), Tween 80, and phosphoric acid were from Aldrich Chemical Co (Milwaukee, WI). Five percent dextrose injection (USP) and 0.9% sodium chloride injection (USP) were obtained from Baxter Healthcare (Deerfield, IL). The USP Type I glass vials and the rubber stoppers were obtained from Kimble Glass Company (Vineland, NJ) and The West Company (Lionville, PA), respectively.

PREFORMULATION STUDIES

Analytical Techniques: HPLC analysis of VNP40101M

A stability-indicating HPLC (model 1090, Hewlett Packard, Avondale, PA) assay was developed and validated to analyze VNP40101M in formulation samples. The separation was conducted on a reverse phase Prodigy ODS-3 (250 x 4.6 mm, 5 μm) column (Phenomenex, Torrance, CA) at 40°C. The mobile phase consisted of 35% acetonitrile and 65% water with 0.1% phosphoric acid. The flow rate was 1 mL/min, and the injection volume was 10 mL. VNP40101M (retention time, approximately 6.7 minutes) was monitored at 200 nm. Prior to HPLC analysis, the formulation samples were diluted 10-fold in acetonitrile. The quantitation of VNP40101M was conducted using an external calibration method based on integrated peak area. Calibration standards were prepared in acetonitrile containing 10% of formulation vehicle and were analyzed with the samples. All analysis was done in duplicate, and the mean peak concentration was used to determine the concentration of VNP40101M in the formulation.

Liquid Chromatography/Mass Spectrometry Analysis of 90CE

90CE (1,2 Bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine) is a known degradation product of VNP40101M. To control the quality of VNP4010M drug product, a method for the determination of 90CE in formulation samples was developed and validated using LC/MS with electrospray ionization. An HPLC device (model 1100, Hewlett Packard) and a Navigator mass spectrometer (Finnigan, San Jose, CA) were used. Using a Prodigy ODS 3 (Phenomenex, Torrance, CA) column (250 ´ 2 mm, 5 μm) at 40°C, 90CE was separated from the parent compound VNP40101M. An isocratic elution with 30% acetonitrile in 10 mM acetic acid solution was performed at a flow rate of 0.25 mL/min. The injection volume was 5 μL. Under the negative ion mode, 90CE was monitored at m/z 248.9. The MS operation parameters were capillary voltage, -2.2 kV; ion source temperature, 180°C; cone voltage, -40 V; and drying gas flow rate, 350 L/h.

Solubility studies in different solvents

The solubility of VNP40101M was determined by mixing an excess quantity of drug with approximately 2 mL of the solvent taken in a screw-capped bottle. The bottles were rotated on a Glas-Col (Terre Haute, IN) laboratory rotator at 30 rpm for 24 hours at room temperature. Preliminary studies indicated that this time period was adequate to obtain equilibrium solubility. After the particles had settled, the supernatant was carefully withdrawn and filtered through a 0.22-µm filter and analyzed by HPLC.

Partition coefficient

The octanol-water partition coefficient of VNP40101M was determined by the shake flask method. The drug was added to 2 mL of water and 2 mL of octanol in a glass bottle. The mixture was shaken for 24 hours at 22°C to 23°C. The two phases were carefully separated and analyzed for VNP40101M by HPLC.

pH solubility profile

The pH solubility of VNP40101M was determined at a pH ranging from 3 to 7.7 in phosphate-citric acid buffer adjusted to constant ionic strength of 0.5 M with potassium chloride. An excess quantity of drug was mixed with 4 mL of the buffer solutions and stirred for 24 hours at room temperature (22°C-23°C). The suspension was filtered with a 0.22 µm filter and analyzed by HPLC.

Forced degradation study

The stability of VNP40101M was assessed by subjecting it to thermal, acidic, basic, and oxidative stress. A 1 mg/mL solution was prepared in a 10:90 acetonitrile:water mixture and heated to 100°C for 30 minutes and then assayed. Acidic stress was performed by mixing 0.1 mL of a 10 mg/mL VNP40101M in acetonitrile solution with 0.1 mL of either 1 N or 12.1 N hydrochloric acid and allowed to react for 24 hours. For the basic stress, VNP40101M solution in acetonitrile was treated with TRIS-buffered saline with a pH of 8. Samples were removed at 0, 25, 60, and 90 minutes for assay. Oxidative stress was induced by mixing an equal volume of drug solution in acetonitrile with 30% hydrogen peroxide solution and allowed to react for 24 hours. The final concentration of all the samples prepared for this study was 1 mg/mL. The samples were analyzed for VNP40101M by HPLC.

FORMULATION DEVELOPMENT

Preparation of VNP40101M solution

Solutions of VNP40101M were prepared by dissolving the drug in a mixture of 30% ethyl alcohol and 70% PEG-300. In the case of solutions containing a stabilizer, the stabilizer was dissolved in ethyl alcohol prior to adding PEG-300.

Compatibility of rubber closures with VNP40101M injection

The formulation was filled in 10-mL glass vials and stoppered with West gray butyl 4432/50 S127 serum stoppers. Vials were also stoppered with West 4432/50 gray Teflon-coated stoppers and served as controls. The vials were placed upright and inverted in the stability chambers maintained at 60°C, 40°C with 75% RH, 25°C with 60% RH, and 5°C (refrigeration). The vials were inverted to obtain maximum exposure of the rubber closure to the formulation. The contents of the vials were periodically analyzed for VNP40101M.

Effect of silastic tubing on VNP40101M injection

In pharmaceutical manufacturing, silicone tubing is typically classified as a process aid. It is used in transfer processes and as such should not interact with the drug product. The effect of silastic tubing on VNP40101M formulation was tested by immersing the tubing in the drug solution for 24 hours at room temperature. The tubing was also immersed in the formulation vehicle (PEG-300/ethyl alcohol) for the same amount of time and served as a control. The solution was analyzed by HPLC to determine any loss of drug via adsorption. Because the VNP40101M injection was formulated in a nonaqueous solvent vehicle, the tubing used for filling operations may lose its elasticity and thus cause filling problems. Silastic tubing was tested for its ability to dispense the solution into vials consistently without variation in fill volumes. An Adtech 4 head filling machine (Hulmeville, PA) was used to dispense 5 mL or 10 mL into vials. Vials were removed periodically and their fill weights were determined.

Effect of sterilizing filters

To evaluate the effect of a sterilizing filter on VNP40101M solution, the solution was filtered twice through a Millipore (Bedford, MA) 0.2-μm Durapore® polyvinylidene fluoride (PVDF) membrane filter. The solution was analyzed before and after filtration by HPLC.

Cleaning method development

A cleaning method was established using isopropanol (IPA) as the solvent. The formulation was drained off from a stainless steel beaker and then rinsed 3 times with IPA, washed and scrubbed with Alconox detergent, and triple rinsed with water for injection (WFI). After each IPA rinse and the final WFI rinse, a rinse sample was collected. An area of the beaker was also swabbed. Both the rinse samples and swab samples were analyzed by HPLC for VNP40101M, and the final WFI rinse sample was also analyzed for total organic carbon (TOC).

Compatibility with infusion solution

Admixtures of VNP40101M were prepared in 0.9% sodium chloride injection (normal saline) and 5% dextrose injection (D5W) in glass containers. The nominal concentration of VNP40101M in the admixtures was 0.1, 0.5, 1, and 2 mg/mL. At periodic intervals, the diluted solution was examined for visual

precipitation and pH and was assayed for VNP40101M and 90CE using HPLC and LC/MS, respectively.

Stability of VNP40101M injection

The final formulation of VNP40101M was put into USP Type I clear glass vials and subjected to stability testing following International Conference on Harmonization (ICH) guidelines [13]. The vials were stored at 40°C with 75% RH, 25°C with 60% RH, and 5°C (refrigeration). The solution was periodically analyzed for VNP40101M and 90CE by HPLC and LC/MS, respectively.

RESULTS AND DISCUSSION

Preformulation studies: Analytical method development

The development of a suitable stability-indicating assay of a new drug is very critical for the successful development of a dosage form. Stability-indicating methods for VNP40101M and its degradation product, 90CE, were developed and validated according to the ICH guidelines. The HPLC assay of VNP40101M was found to be robust and linear over the concentration range of 0.6 to 1.4 mg/mL with an r^2 of 0.9966. The method quantitation limit was 5 μg/mL for VNP40101M. Precision of the assay determined from 6 repetitive injections of a solution containing 1 mg/mL VNP40101M was found to be 0.5% expressed as percent relative standard deviation (RSD).

Because of its poor ultraviolet (UV) absorption, the degradation product 90CE could not be analyzed using HPLC-UV. An LC/MS method was therefore developed to quantitate 90CE. Regression analysis of 90CE calibration curves suggested a quadriatic (second order) relationship between the MS response (total ion current) at m/z 248.9 and 90CE concentration (Figure 1). An r^2 value of 0.997 was obtained over the concentration range of 0.1 to 5 μg/mL. The average accuracy in terms of recovery was 109% to 110%, and the precision in terms of RSD was 5%.

Solubility of VNP40101M

The solubility of VNP40101M in various solvents was estimated and is given in Table 1. The solubility of VNP40101M in water was only 0.66 mg/mL. VNP40101M was fairly soluble in parenterally acceptable cosolvents. In ethyl alcohol, propylene glycol, and 30% ethyl alcohol, VNP40101M was soluble to the extent of 1.4 mg/mL. In PEG-300, it was soluble to the extent of 16.8 mg/mL.

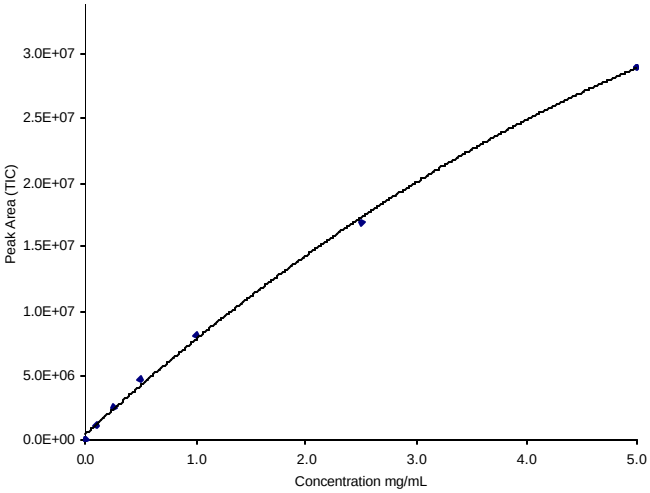

Figure 1. Calibration curve: total ion current versus 90CE concentration

Table 1. Solubility of VNP40101M at room temperature

Solvent	Solubility (mg/mL)
Water	0.66
Propylene glycol	1.4
Polyethylene glycol 300	16.8
Ethyl alcohol	1.4
30% ethanol	1.4
30% glycerol	0.47
50% glycerol	0.41
0.1% Tween 80	0.64

The octanol-water partition coefficient of VNP40101M was estimated to be 0.75, suggesting poor solubility in lipophilic solvents.

pH solubility profile of VNP40101M

To determine if an aqueous formulation could be prepared by adjusting the pH of the solution, the solubility profile of VNP40101M was investigated at different pHs. The solubility of VNP40101M as a function of pH is given in Figure 2. The solubility of VNP40101M showed a gradual but slow decrease from pH 3 to pH 6. At pH 3.43, the solubility was 0.63 mg/mL, but solubility decreased to 0.58 at pH 6.16. The solubility dropped to 0.36 and 0.1 mg/mL at pH 7.18 and pH 7.77, respectively. At these high pH values, rapid degradation of VNP40101M occurred, which was evident from several degradation peaks in the HPLC chromatograms. This indicated that VNP40101M is unstable at alkaline pH.

Forced degradation study

Thermal stress caused about 40% degradation of VNP40101M (Table 2). Acidic stress with strong acid, such as hydrochloric acid, did not degrade VNP40101M. However, basic stress caused a time-dependent loss of VNP40101M. No degradation was seen under oxidative stress. The above observations are consistent with the degradation pathway reported in the literature [8,9]. VNP40101M undergoes a pH-specific base-catalyzed hydrolytic breakdown into 90CE and methyl isocyanate (Figure 3). Both 90CE and methyl isocyanate react with water and break down further to form gaseous nitrogen, sulfinic acid and chloroethanol, methylamine, and carbon dioxide.

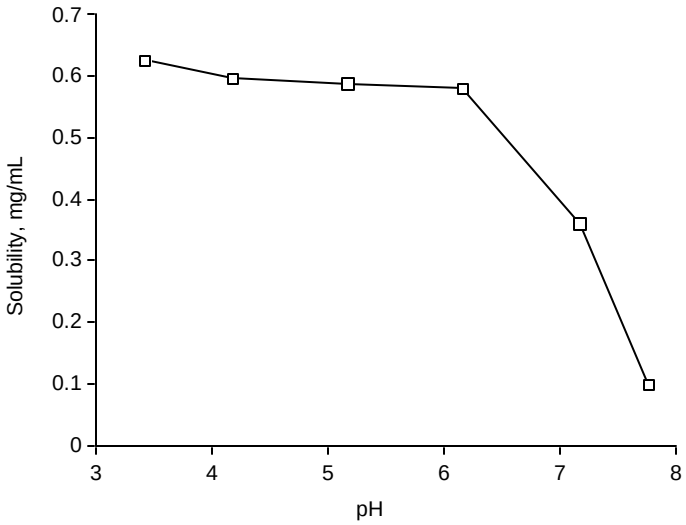

Figure 2. pH solubility profile of VNP40101M

Table 2. Effect of degradation stress on the stability of VNP40101M

Stress condition	Duration of stress	VNP40101M concentration (mg/mL) post stress
Heat at 100°C	30 minutes	0.58
Oxidation	24 hours	1.06
1 N HCl	24 hours	1.11
12 N HCl	24 hours	1.17
pH 8 buffer	0 hours	0.91
	25 minutes	0.79
	60 minutes	0.50
	90 minutes	0.45

Note: Initial concentration before stress for all samples was 1 mg/mL

FORMULATION DEVELOPMENT

Formulation of VNP40101M

The low aqueous solubility precluded development of an aqueous formulation. Many poorly water-soluble drugs are maintained in solution by the use of water-miscible solvents. Based on solubility characteristics, PEG-300 and ethyl alcohol were selected as vehicles for VNP40101M. Combinations of PEG-300 and ethyl alcohol are common cosolvent vehicles that are considered safe for use in the preparation of parenteral solutions [14,15]. At a ratio of 70% PEG-300 and 30% ethyl alcohol, VNP40101M could be dissolved to a high concentration of 10 mg/mL.

This formulation presented a processing problem. Because VNP40101M undergoes hydrolytic breakdown, it was essential to avoid contact with moisture. However, both the vehicles are relatively hygroscopic, and the prevention of moisture absorption during the normal course of manufacturing was difficult. Dissolution of VNP40101M in the vehicle was slow and required overnight mixing. Mixing in an open vessel resulted in approximately 2.5% moisture ingress. Covering the manufacturing vessel containing an overhead mixer was at best incomplete and provided only partial protection. On assay, the formulation was found to contain 3% to 4% of 90CE as a degradation compound.

Because the hydrolytic breakdown of VNP40101M is base catalyzed, hydrolysis can be minimized with the inclusion of a small amount of a weak acid. Formulations of VNP40101M (10 mg/mL) were therefore prepared in PEG-300 and ethyl alcohol with 0%, 0.3%, and 0.6% citric acid in a closed system. Figure 4 shows the markedly decreased level of 90CE in formulation containing 0.6% citric acid as compared to the control. A similar result was obtained with formulation containing 0.3% citric acid. The concentration of 90CE as a percentage of 101 M label was found to be 2.2%, 0.02%, and 0.015% for formulations containing 0% (control), 0.3%, and 0.6% citric acid, respectively. It is clear from this result that acidifying the formulation minimized 90CE formation. Based on these results, 0.6% citric acid was included in the formulation as a stabilizer.

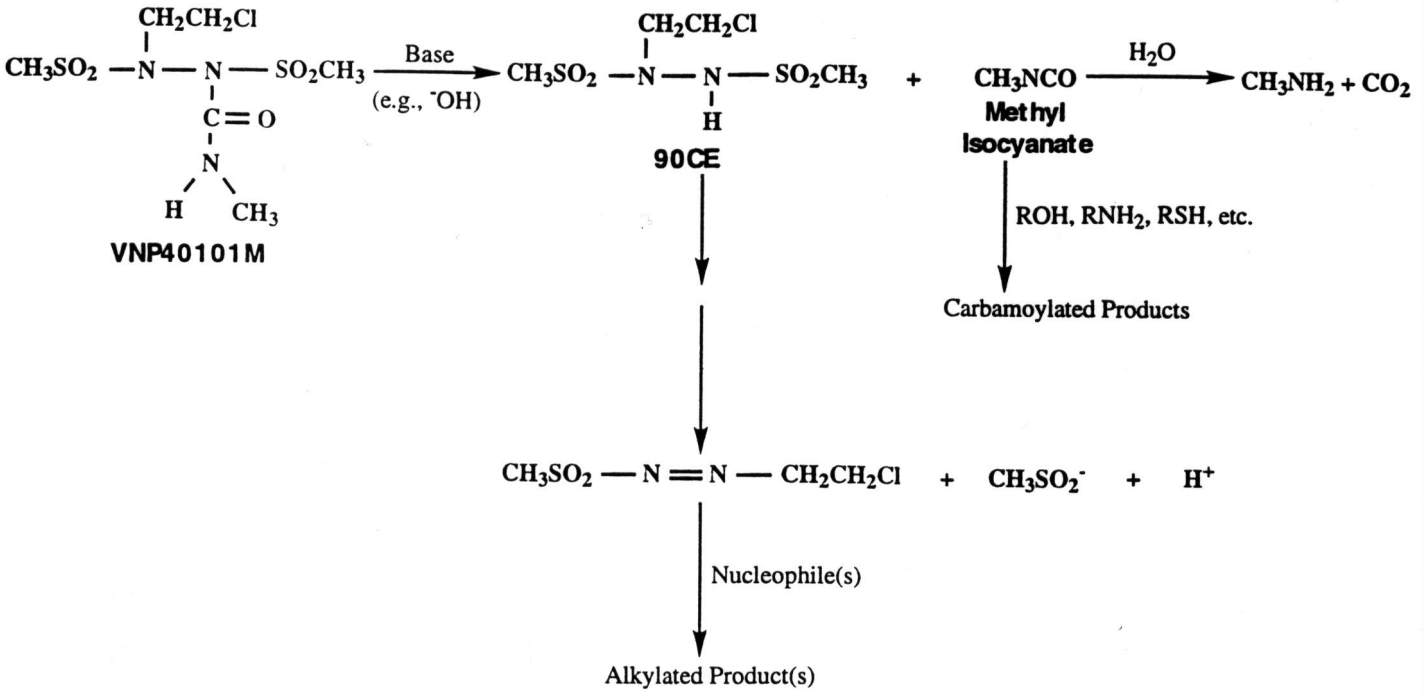

Figure 3. Scheme for breakdown of VNP40101M

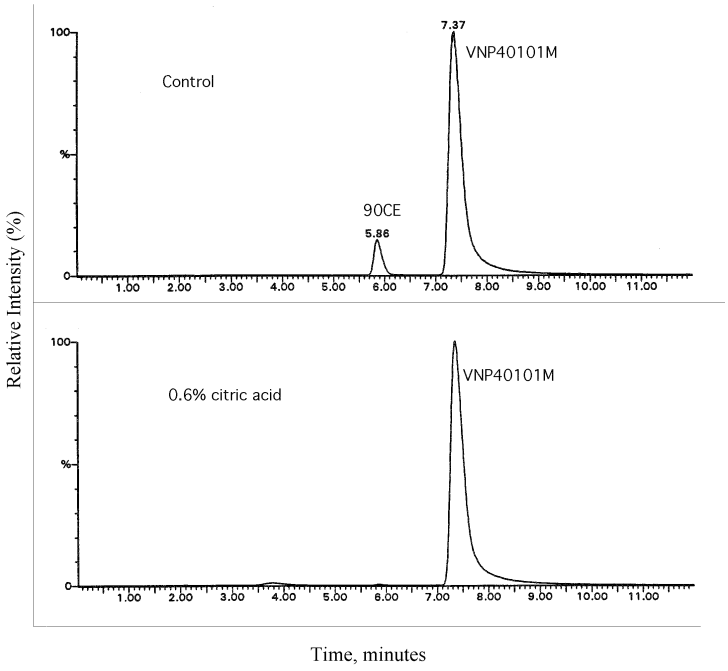

Figure 4. Effect of citric acid on stability of VNP40101M

Compatibility with rubber closures

Selection of rubber closures should be based on universal acceptability in all markets (European Union/Japan/United States) as well as on compatibility with the drug solution. West gray butyl stopper 4432/50 was selected for evaluation because of its global acceptability, as it meets *European Pharmacopoeia* (EP), *Japanese Pharmacopeia* (JP), and USP requirements. Adsorption of drug to rubber closures is a common problem associated with injectable solutions. Further, incompatibility could also arise between the vehicle components and the rubber formulation that could result in deformation of the rubber closure with subsequent loss of container-closure integrity. Adsorption is generally prevented by using a Teflon-coated stopper that forms a barrier between the solution and the rubber components. The results of this study indicated that the formulation was compatible with the rubber stoppers studied. The recovery of VNP40101M was nearly 100% from solutions stoppered with either Teflon-coated or uncoated stoppers and stored for 2 months at accelerated temperatures. Further, the sealability of the rubber closures was unaltered as determined by absence of leakage from vials punctured and stored

inverted. Also, a low extractable profile was reported for this stopper with polyethylene glycol and 20% ethanol [16].

Effect of silastic tubing

Silastic tubing is generally used to transfer the product from one container to another and for vial filling operations. Drug adsorption into these tubing materials may result in substantial loss of potency. The recovery of VNP40101M from a solution that had been in contact with silastic tubing for 24 hours was 100%, indicating no loss of drug via absorption into the tubing.

The nature of solvents may affect the ability of the tubing to dispense the solution uniformly during the filling operation. When silastic tubing alone was used, significant drifts in fill volume were observed over 20 minutes. This was probably caused by the softening of the tubing due to interaction with the nonaqueous vehicle. Replacing the silastic tube with a more rigid polypropylene tube presented a different problem. The pinching clamps at the dispensing nozzle could not be completely closed due to the rigidity of the tubing. As a result, the solution dripped extensively from the filling nozzle. When a combination of the rigid polypropylene tubing extending from the filling tank and joined to the filling nozzle via silastic tubing was used, fill volume drifts were minimized. The average fill weights after filling 1000 vials with 5 mL each using the above combination of tubings was 5.12 mL with a standard deviation of 0.03 and a CV% of 0.58.

Filter sterilization

The forced degradation study indicated that the drug is heat sensitive (Table 2). Further, the formulation contains 30% ethyl alcohol, which does not allow for terminal sterilization by heat. Therefore, sterilization by filtration was opted for VNP40101M injection using the sterilization decision tree for nonaqueous liquid or dry powder products [17]. However, loss of drug to filter surfaces could be a problem leading to out-of-specification results. In this study, filtration through a Durapore® PVDF membrane filter did not alter the drug concentration. The recovery of VNP40101M after filtering twice through the filter was 100%, indicating absence of adsorption by the filter. Filtration through a Millipore Opticap® hydrophilic PVDF membrane filter was recommended on the basis of this study.

Table 3. Accelerated stability of VNP40101M

Storage conditions	Storage interval (months)	VNP40101M Recovery (n = 4) (% ± SD)	90CE Formation (n = 2) (% ± SD)
5°C	Initial	100.5 ± 0.4	0.04 ± 0.00
	1	105.3 ± 0.6	0.10 ± 0.14
	3	101.7 ± 1.1	0.12 ± 0.01
	6	104.5 ± 1.0	0.16 ± 0.00
25°C with 60% RH	Initial	100.5 ± 0.4	0.04 ± 0.00
	1	101.0 ± 2.5	0.17 ± 0.03
	3	101.7 ± 1.8	0.37 ± 0.02
	6	101.8 ± 0.3	0.75 ± 0.01
40°C with 70% RH	Initial	100.5 ± 0.4	0.04 ± 0.00
	1	104.9 ± 0.4	0.64 ± 0.03
	3	101.1 ± 0.4	1.24 ± 0.02
	6	100.9 ± 0.5	2.06 ± 0.03

Cleaning studies

The ability to adequately clean equipment and vessels used in the clinical manufacture of drugs is an essential step in the drug development program. Product-specific assay and TOC analysis were used to evaluate the adequacy of cleaning. Rinsing with isopropanol 3 times followed by a detergent wash with water adequately removed all traces of VNP40101M. No VNP40101M was detected in the HPLC assay, which has a limit of detection of 0.05 mg/mL. TOC results of rinse and swab samples were below the USP purified water specification of 0.5 ppm. A cleaning method was established on the basis of these results.

Stability of VNP40101M injection

The final formulation was subjected to accelerated stability testing. VNP40101M was chemically and physically stable at 5°C for 6 months, and no measurable degradation product was observed as tabulated in Table 3. On the other hand, a slight degradation of VNP40101M to 90CE was observed at 25°C and 40°C. A long-term stability study is in progress.

Compatibility with LVP solutions

VNP40101M injection is a nonaqueous solution that should be diluted prior to intravenous infusion. The compatibility of VNP40101M with LVP solutions was assessed. Dilution of VNP40101M formulation up to a concentration of 1 mg/mL in normal saline or D5W and

stored at room temperature was found to be stable without drug loss for up to 24 hours (Tables 4 and 5). When diluted to 2 mg/mL or above, precipitation was seen at 8 hours (admixture in D5W) and 24 hours (normal saline) with a consequent drop in VNP40101M concentration. In 4 mg/mL dilutions, precipitation was seen sooner (2 hours after dilution) for both admixtures. The pH of all the dilutions in either admixture ranged between 3 and 4 and remained unchanged during the 24-hour period.

Dilutions greater than 2 mg/mL showed a decrease in VNP40101M concentration over time as a result of drug precipitation. The 90CE concentrations did not significantly increase in any of the dilutions. Further, the 90CE values represent less than 0.1% of the total VNP40101M concentration in the total admixture.

The admixture study indicates that the drug could be diluted in normal saline or D5W up to 2 mg/mL and should be administered preferably within 2 to 4 hours of preparation. Solutions containing nonaqueous solvents are reported to extract plasticizers from polyvinyl chloride (PVC) containers and infusion sets [18]. Therefore, it is recommended that VNP40101M injection formulation should be diluted and administered using polypropylene rather than PVC containers and administration sets.

CONCLUSION

In summary, the solubility and stability of VNP40101M was evaluated to design a suitable parenteral formulation. Because of its poor aqueous solubility, the formulation was prepared in a totally nonaqueous system consisting of 70% PEG-300 and 30% ethyl alcohol. Inclusion of citric acid helped in minimizing hydrolytic degradation of VNP40101M to 90CE. Manufacturing issues such as cleaning, filling studies, and compatibility with silastic tubing were also addressed. Compatibility studies with LVP solutions indicated that the formulation could be readily diluted in D5W or normal saline up to a concentration of 2 mg/mL for at least 8 hours. Although a slight degradation was seen at higher temperatures (25°C and 40°C), greater than 95% of the drug was intact at 4°C for 6 months. The formulation was successfully manufactured for Phase I clinical trials.

ACKNOWLEDGEMENTS

The authors wish to thank Joe Tunner and Susan Hochschwender of Matrix Pharmaceuticals, Inc, San Diego, CA, for their support in the development of this project.

Table 4. Effect of dilution with D5W on the stability of VNP40101M injection

Theoretical admixture concentration (mg/mL)	Concentration of VNP40101M (mg/mL) after storage for					Concentration of 90CE (µg/mL) after storage for				
	0 h	2 h	4 h	8 h	24 h	0 h	2 h	4 h	8 h	24 h
0.1	0.10	0.10	0.10	0.10	0.10	0.04	0.03	0.03	0.04	0.02
1.0	0.99	0.99	0.99	0.98	0.99	0.49	0.52	0.48	0.35	0.41
2.0	2.02	2.01	1.99	1.87	1.19	1.04	1.44	.03	0.74	0.87
4.0	4.06	3.92	3.63	3.09	1.71	2.23	2.68	2.08	1.33	2.06

Table 5. Effect of dilution with normal saline on the stability of VNP40101M injection

Theoretical admixture concentration (mg/mL)	Concentration of VNP40101M (mg/mL) after storage for					Concentration of 90CE (µg/mL) after storage for				
	0 h	2 h	4 h	8 h	24 h	0 h	2 h	4 h	8 h	24 h
0.1	0.09	0.09	0.09	-	0.09	ND	ND	ND	ND	ND
1.0	1.00	1.01	1.02	1.01	1.01	0.23	0.48	0.67	0.54	0.28
2.0	2.01	2.02	2.00	2.01	1.80	0.51	1.86	1.18	0.91	0.61
4.0	4.09	-	3.08	2.33	1.40	2.22	2.33	2.32	2.33	1.87

ND = not determined

REFERENCES

1. Takemura H, Urasaki Y, Yoshida A, Fukushima T, Ueda T. Simultaneous treatment with 1-ß-D Arabinofuranosylcytosine and daunorubicin induces cross resistance to both drugs due to a combination specific mechanism in HL60 cells. Cancer Res. 2001;61:172-177.

2. Brisco MJ, Sykes PJ, Dolman G, et al. Early resistance to therapy during induction in childhood acute lymphoblastic leukemia. Cancer Res. 2000;60:5092-5096.

3. Yu DS, Sun GH, Ma CP, Chang SY. Verapamil modulation of multidrug resistance in renal cell carcinoma. J Formos Med Assoc. 2000;99:311-316.

4. Shyam K, Cosby LA, Sartorelli AC. Synthesis and evaluation of N,N'-bis(arylsulfonyl)hydrazines as antineoplastic agents. J Med Chem. 1985;28:525-527.

5. Shyam K, Furubayashi R, Hrubiec RT, Sartorelli AC. 1,2-bis(arylsulfonyl)hydrazines. 2. The influence of arylsulfonyl and aralkylsulfonyl substituents on antitumor and alkylating activity. J Med Chem. 1986;29:1323-1325.

6. Shyam K, Hrubiec RT, Furubayashi R, Cosby LA, Sartorelli AC. 1,2-Bis(sulfonyl)hydrazines. 3. Effects of structural modification on antineoplastic activity. J Med Chem. 1987;30:2157-2161.

7. Shyam K, Penketh PG, Divo AA, Loomis RH, Rose WC, Sartorelli AC. Synthesis and evaluation of 1-acyl-1,2-bis(methylsulfonyl)-2-(2- chloroethyl)hydrazines as antineoplastic agents. J Med Chem. 1993;36:3496-3502.

8. Shyam K, Penketh PG, Loomis RH, Rose WC, Srtorelli AC. Antitumor 2-(aminocarbonyl)-1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-hydrazines. J Med Chem. 1996;39:796-801.

9. Penketh PG, Shyam K, Sartorelli AC. Comparison of DNA lesions produced by tumor-inhibitory 1,2-bis(sulfonyl)hydrazines and chloroethylnitrosoureas. Biochem Pharmacol. 2000;59:283-291.

10. Pratviel G, Shyam K, Sartorelli AC. Cytotoxic and DNA-damaging effects of 1,2-bis(sulfonyl)hydrazines on human cells of the Mer+ and Mer- phenotype. Cancer Biochem Biophys. 1989;10:365-375.

11. Penketh PG, Shyam K, Sartorelli AC. Mechanisms of resistance to alkylating agents. Cancer Treat Res. 1996;87:65-81.

12. Finch RA, Shyam K, Penketh PG, Sartorelli AC. 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-2-(methylamino)carbonylhydrazine (101M): a novel sulfonylhydrazine prodrug with broad spectrum antineoplastic activity. Cancer Res. 2001;61:3033-3038.

13. ICH Guideline on the validation of analytical procedures: Methodology, Food and Drug Administration. Federal Register. 1997;62:27463-27467.

14. Powell M, Nguyen T, Baloian L. Compendium of excipients for parenteral formulations. PDA J Pharm Sci Technol. 1998;52:238-311.

15. Mottu F, Laurent A, Rufenacht D, Doelker E. Organic solvents for pharmaceutical parenterals and embolic liquids: a review of toxicity data. PDA J Pharm Sci Technol. 2000;54:456-469.

16. The West Company Technical Report, 1999/002 and 1999/006.

17. Morris JM. Sterilization decision trees development and implementation. PDA J Pharm Sci Technol. 2000;54:64-68.

18. Krishna G, Mao J, Almassian B. Development of a parenteral formulation of an investigational anticancer drug, 3-aminopyridine-2-carboxaldehyde thiosemicarbzone. Pharm Dev Technol. 1999;4:71-80.