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Pharmaceutical development of a parenteral lyophilized formulation of the novel indoloquinone antitumor agent EO9

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Abstract. The aim of this study was to design a stable parenteral dosing form of the investigational cytotoxic drug, encoded EO9. EO9 exhibits poor aqueous solubility and stability characteristics. Freeze-drying was selected as the manufacturing process. Differential scanning calorimetry studies were conducted to determine the freeze-drying cycle parameters. A stable lyophilized formulation of EO9 was developed. The prototype, containing 8.0 mg EO9 and 200 mg lactose/vial, was found to be the optimal formulation in terms of solubility, length of the freeze-drying cycle, stability, and dosing requirements for phase I clinical trials. Quality control of the freeze-dried formulation showed that the manufacturing process does not change the integrity of EO9. Shelf-life studies demonstrated that the formulation remains stable for at least 1 year when stored at +4° C in a dark environment.

Key words: EO9 – Parenteral formulation – Lyophilization

Introduction

EO9 (3-hydroxymethyl-5-aziridinyl-1-methyl-2-(1*H*-indole-4,7-dione)-propenol; NSC 382,456; Fig. 1) is the lead compound in a series of novel and fully synthetic bioreductive alkylating indoloquinones with potent antitumor properties [6, 15]. EO9 requires metabolic activation to give the cytotoxic species. Some of the pathways by which this can occur have been identified and include one-electron reduction by reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome P450 reductase and two-electron reduction by DT-diaphorase [1, 2, 8, 13]. Studies have been conducted dealing with the bioanalysis [3, 9]; the pharmacokinetics, distribution, and metabolism [16]; and the chemical stability of EO9 in aqueous solution [12]. However, data have thus far not been presented on the pharmaceutical formulation of EO9.

The New Drug Development and Coordinating Committee (NDDCC) of the European Organization for Research and Treatment of Cancer (EORTC), in collaboration with the Cancer Research Campaign (CRC) and the United States National Cancer Institute (NCI), has recently formalized a procedure for the preclinical development of investigational drugs in Europe. The aspects of formulation development and pharmaceutical production are coordinated by the EORTC/CRC/NCI Joint Formulation Working Party (JFWP). The JFWP has drawn up guidelines, which are intended to organize the pharmaceutical and preclinical development processes and to reduce the lag time between the discovery of promising new drugs and the initiation of phase I clinical trials [5]. The pharmaceutical development

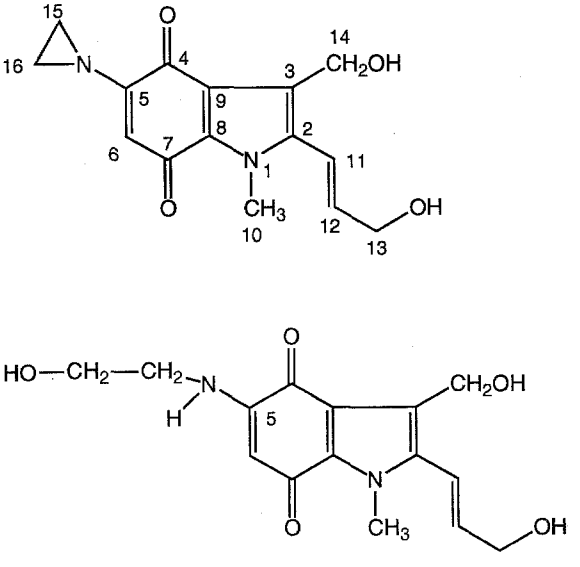

Fig. 1. Chemical structures of EO9 (*top*) and EO5A (*bottom*), the major degradation product of EO9

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of the investigational cytotoxic drug EO9 is based on the EORTC/CRC/NCI guidelines as described by Davignon et al. [5].

Pharmaceutical development of EO9 for clinical use has been hampered because of its chemical instability and low aqueous solubility. In the present report, the development of a stable lyophilized, parenteral formulation of EO9 is presented.

Materials and methods

Chemicals

EO9 was synthesized by Drs. E.A. Oostveen and W.N. Speckamp (Laboratory of Organic Chemistry, University of Amsterdam, The Netherlands) and was provided by the EORTC NDDO (Amsterdam, The Netherlands). Quality control of the raw material indicated that EO9 could be used without any further purification. All other chemicals were of analytical grade and deionized water was used throughout.

Characterization of EO9 bulk drug

The interim reference standard of EO9, i.e., the batch of highest purity, was structurally characterized by means of nuclear magnetic resonance (NMR) spectrometry, mass spectrometry (MS), and infrared (IR) spectrometry and was analytically characterized with high-performance liquid chromatography (HPLC), ultraviolet/visible (UV/VIS) spectrophotometry, and high-performance thin-layer chromatography (HPTLC). Furthermore, the melting point and the moisture content of the interim reference standard were determined. On the basis of these results, specifications were drawn up for EO9 bulk drug.

Stability and solubility of EO9

Stability studies. The chemical stability and degradation kinetics of EO9 have been reported in an earlier study [12].

Solubility studies. A solubility-time profile was determined by UV/VIS spectrophotometry. A 0.001-*M* borax buffer solution (pH 9.0) was used instead of water because at this pH, EO9 shows the best stability [12]. EO9 (50 mg; two different batches, batches I and II) was added to 50.0 ml 0.001 *M* borax buffer (pH 9.0) and the solution was continuously stirred during the course of the experiment. At 5, 15, 35, 75, 155, and 315 min and at 24 h, a sample was taken and centrifuged for 5 min at 3000 rpm, and an aliquot of the supernatant was diluted 100 times with the borax buffer. The absorbance at 269 nm was used for quantification purposes. Calibration curves generated for EO9 in borax buffer were linear ($r > 0.999$) in the concentration range of interest (0–20 μg ml^{-1}). For batch II, the solubility-time profile was also determined by HPLC analysis.

Formulation of EO9

Pre-formulation. Transition temperatures and freezing characteristics of EO9 solutions were determined by differential scanning calorimetry (DSC). The DSC experiments were performed with a Netzsch low-temperature DSC system equipped with a Model 200 controller and a Model 43 measuring cell (Netzsch-Gerätebau, Selb, Germany). The heating rate was 1.0° C/min^{-1}. Analyses were performed under a nitrogen purge. The temperature scale and heat flux were calibrated with gallium and mercury. Aluminum sample pans were used. An empty sample pan was used as the reference.

Formulation process. The particle size of EO9 bulk drug was decreased by grinding with a mortar and pestle. EO9 (1.6 g) and lactose (40 g) were then added to 8 l Water for Injection, which had been adjusted to pH 9–9.5 with 1 *N* sodium hydroxide solution, and dissolution was achieved by magnetically stirring the solution for 15 min. Sterilization was achieved by filtration of the admixture through a 0.22-μm filter (Milli-fil GS; Millipore, Bedford, Mass., USA). Aliquots (40 ml), theoretically containing 8.0 mg EO9 and 200 mg lactose, were filled into 50-ml glass vials (Aluglas B.V., Uithoorn, The Netherlands). The recovery of the formulation process was approximately 200 vials/run. Freeze-drying closures (grey butyl V9032; Helvoet Pharma N.V., Alken, Belgium) were partially inserted into each vial and the vials were loaded into the freeze-dryer (Lyovac GT 4, Finn Aqua Santasalo-Sohlberg, Hürth, Germany) at room temperature. The product temperature was monitored continuously with Model Pt-100 resistance thermometers (Finn Aqua Santasalo-Sohlberg). The freezing stage was carried out at –40° C for 10.5 h. A vacuum (10^{-1} mbar) was then established in 1 min. Primary drying of the product was subsequently accomplished by increasing the temperature of the shelves from –40° to +10° C in 2 h and by maintaining the temperature at +10° C for 82.5 h. The secondary drying phase was carried out at +20° C for approximately 12 h. After completion of the lyophilization cycle the vials were stoppered pneumatically in a vacuum and capped with aluminum caps (Helvoet Pharma N.V.).

All manipulations took place, as far as possible, under laminar down-flow conditions in a Model MKV 183 II biological safety cabinet (Class 100; Interflow B.V., Wieringerwerf, The Netherlands) in a clean room (Class 10,000; Interflow B.V.). Before use, the glass vials, rubber stoppers, mortar and pestle, and all glassware were washed thoroughly and then sterilized by autoclaving for 20 min at 120° C.

Quality control of the lyophilized final product

Quality control was performed by visual inspection of the appearance and the color of the pharmaceutical product. HPLC analysis and UV/VIS spectrophotometry were used to determine the content and purity of the product. The residual moisture content in the lyophilized product was determined by the Karl-Fischer titration method. Furthermore, a limulus amoebocyte lysate (LAL) test for the presence of endotoxins and a sterility test were performed.

HPLC analysis. Analysis of EO9 was performed using a Model 510 solvent-delivery system (Waters Associates Inc., Milford, Mass., USA) and a Model ISS-100 autosampler (Perkin-Elmer, Uberlingen, Germany). An LC-UV detector (Pye Unicam, Cambridge, England) with a fixed-wavelength filter for detection at 254 nm was used. The sensitivity was 0.05 AUFS. The stainless-steel analytical column (12.5 cm × 4 mm internal diameter) was packed with Lichrosorb 5 μm RP-8 material (Merck, Darmstadt, Germany). The mobile phase consisted of methanol/water (30:70, w/w) to which 1% (v/w) 0.5 *M* sodium phosphate buffer (pH 7.0) was added. The flow rate was 1.0 ml min^{-1} and the column was kept at ambient temperature. Quantitation of EO9 was based on peak area measurements using a Model DataJet CH1 integrator (Spectra Physics Analytical Inc., San Jose, Calif., USA).

The accuracy and precision of the analytical method were determined by replicate injections of known concentrations of the highest and lowest standard solutions on three different occasions. Estimates of the between-day and within-day precision were obtained by means of one-way analysis of variance (ANOVA). The linearity of the analytical method was investigated by least-squares regression analysis. The limit of detection of the HPLC assay was determined as the drug quantity at which the UV 254-nm response was equal to 3 times the average signal-to-noise ratio.

The purity of EO9 final product was determined by HPLC analysis (UV detection, 254 nm) and was calculated by dividing the peak area of the EO9 peak by the total peak area (except the solvent front) in the chromatogram times 100%.

UV/VIS spectrophotometry. UV/VIS spectra were recorded on a Model UV/VIS 918 spectrophotometer (GBC Scientific Equipment Ltd, Victoria, Australia) equipped with a LEO personal computer and an Epson LX-400 printer/plotter. Lyophilized EO9 (8 mg) was dissolved in 1 l water to a final concentration of 8 μg ml^{-1}. The absorbance of EO9 was determined at the absorption maxima, and using the absorbance at 269 nm, the concentration and, subsequently, the content per vial were calculated.

Residual moisture content. The residual moisture content in EO9 final product was determined by the Karl-Fischer titration method. The analyte was dissolved in a solvent (ReAquant Solvent Sprint; J.T. Baker B.V., Deventer, Holland) and was then titrated with a titrant (ReAquant Titrant, 3.5 mg H_2O ml^{-1}). The end point of the titration was determined biamperometrically. Before titration, a 2.00-ml volume of acetic acid (100%) was added to the product to enhance the water withdrawal.

LAL test. The LAL test was performed with the modified Pyrogent Gel Clot test (Mallinckrodt, St. Louis, Mo., USA; U.S. License 709). Instead of test tubes, microscope glass plates were used, which had been thoroughly cleaned with 70% alcohol. The limulus amoebocyte lysate was reconstituted in LAL reagent water (final concentration, 69.4 EU ml^{-1}). The *Escherichia coli* endotoxin standard was prepared by reconstitution with sterile distilled water to a final concentration of 4.55 EU ml^{-1}. The test solution was prepared by reconstitution of the lyophilized EO9 with sterile 0.9% sodium chloride (final concentration, 0.5 mg ml^{-1}). The positive controls were prepared by adding 100 μl endotoxin standard (4.55 EU ml^{-1}) to 1 ml test solution or to 1 ml 0.9% sodium chloride, respectively. Subsequently, 10 μl lysate reagent was added to 10 μl test solution, to 10 μl 0.9% sodium chloride, and to 10 μl positive control. The samples were incubated for 30 min at 37° C. A positive reaction occurred when the endotoxin concentration was * 3.28 EU/vial and was indicated by a firm gel that remained intact when the glass plate was inverted 180° .

Sterility test. The freeze-dried EO9 was reconstituted in sterile 0.9% sodium chloride solution to a final concentration of 0.5 mg ml^{-1}. This solution was filtered through a 0.45-μm cellulose nitrate filter. The filter was then placed onto a Caso Nutrient Pad (Sartorius Instr., Abcoude, The Netherlands) and was incubated for at least 1 week at 37° C and checked every day for microbial growth.

Table 1. Identification and characterization of the interim reference standard of EO9 bulk drug

Analytical method	Results
Appearance	Dark red crystalline powder, free of visible signs of contamination
FD-MS	Molecular weight 288.33 Molecular formula $C_{15}H_{16}N_2O_4$
[^{1}H]-NMR δ ($CDCl_3$)	Proposed assignments [11]: 6.4–6.55: m, 1H, C*H* = $CHCH_2OH$ 6.12: dt, 1H, J = 16.0 and 4.6 Hz, $CH = CHCH_2OH$ 5.79: s, 1H, H-6 4.68: d, 2H, J = 7.1 Hz, $ArCH_2OH$ 4.38: m, 2H, $CH = CHCH_2OH$ 4.13: t, 1H, J = 7.1 Hz, $ArCH_2OH$ 3.90: s, 3H, $N\text{-}CH_3$ 2.20: s, 4H, CH_2N 1.76: t, 1H, J = 5.6 Hz, $CH = CHCH_2OH$
Chromatography	HPLC: t_r is 6.2 min HPTLC: R_f is 0.52
UV/VIS	Absorption maxima at 270, 312, and 508 nm
IR	Characteristic absorption bands (approximately): 3500–3000 cm^{-1}: (broad) O-H stretching of the two hydroxyl groups 1660 and 1630 cm^{-1}: C = O stretching of the quinone carbonyls 1585 cm^{-1}: C = C stretching of the quinone ring 990 cm^{-1}: C-O stretching of the α,β-unsaturated primary alcohol(s) 960 cm^{-1}: C-H deformation of the *trans* double bond C_{11}-C_{12}
Melting point (° C)	182–184 (uncorrected)
Purity (%)[a]	
EO9	99.4
EO5A	0.3
unknown	0.3
Moisture content (%)	0.23

[a] The purity of EO9 bulk drug was calculated by dividing the peak area of the EO9 peak by the total peak area (except the solvent front) in the chromatogram times 100%

Shelf life studies

The content and purity of the lyophilized EO9 was determined by means of HPLC analysis after storage for 6 and 12 months respectively, at 4° C in a dark environment. Furthermore, the appearance and the color of the lyophilized EO9 were registered, and after its reconstitution in 0.9% sodium chloride, the pH and the clarity of solution were established.

Stability of EO9 after reconstitution and dilution

The stability of EO9 after reconstitution and dilution of the pharmaceutical product in 0.9% sodium chloride solution was investigated by means of HPLC analysis. The stability of a 0.5-mg ml^{-1} and a 0.05-mg ml^{-1} solution was studied in glass vials at room temperature (22° C) and under normal light conditions. EO9 concentrations were determined at 0, 2, 4, 6, and 24 h after reconstitution and dilution.

Results and discussion

Characterization of EO9

In the first stage of the pharmaceutical development of a new drug substance, an interim reference standard should be established. This is the batch of the highest purity available. This batch of EO9 bulk drug has been fully characterized. At first, a structural characterization of EO9 was performed with [^{1}H]-NMR, MS, and IR spectrometry. Subsequently, EO9 bulk drug was analytically characterized by chromatographic and spectrometric techniques. Table 1 shows the results of the characterization of the interim reference standard.

HPLC analysis. A stability-indicating HPLC analysis was developed and was validated in terms of accuracy, precision, and linearity. The accuracy and precision characteristics are shown in Table 2. Calibration curves generated for EO9 in water were linear ($r > 0.999$) in the concentration range of interest (0–100 μg ml^{-1}). The limit of detection was 0.12 ng/injection, and standard solutions remained

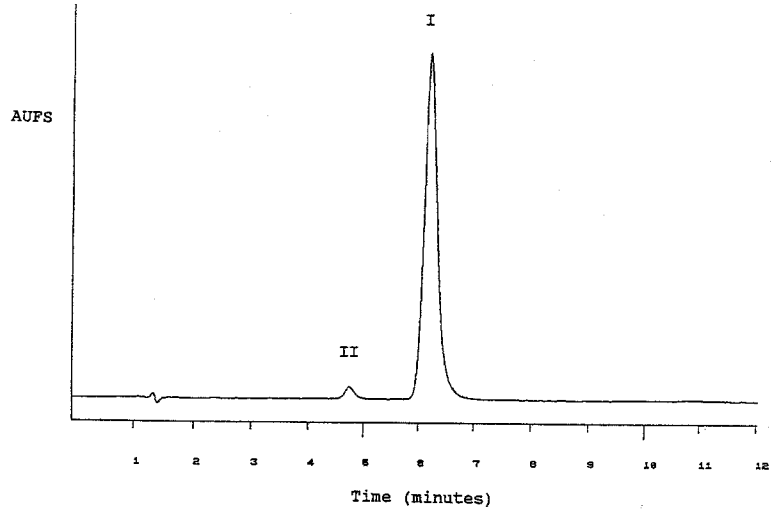

Fig. 2. HPLC chromatogram of EO9 (40 μg ml^{-1}). The retention times of EO9 (*I*) and EO5A (*II*) are 6.2 and 4.7 min, respectively

Table 2. Accuracy and precision of the HPLC method

Theoretical conc. (μg ml^{-1})	Measured conc. (μg ml^{-1})	Accuracy (%)	Precision within-day (%)[a]	Precision between-day (%)[b]	*P*
99.2	99.5	100.3	0.70	0.49	0.0665
19.8	20.2	100.8	1.76	0.97	0.1231

conc., concentration
[a] Within-day precision (n = 5)
[b] Between-day precision (n = 3)

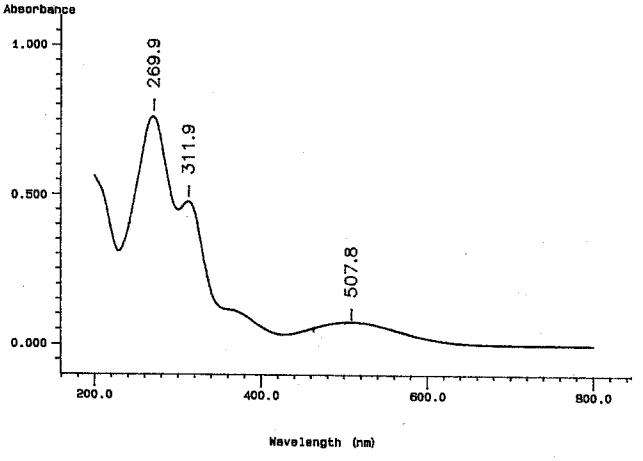

Fig. 3. UV spectrum of EO9 (10 μg ml^{-1}) in methanol/water (1:49)

stable for at least 12 h at room temperature as verified by HPLC analysis. An example of an HPLC chromatogram of EO9 (concentration, 40 μg ml^{-1}) is shown in Fig. 2. The peak at 6.2 min corresponds to EO9 and that at 4.7 min corresponds to EO5A, the major degradation product of EO9.

UV/VIS spectrophotometry. Figure 3 shows the UV spectrum of EO9 bulk drug in methanol/water (1:49). EO9 possesses three absorption maxima at 270, 312, and 508 nm, respectively.

Solubility of EO9

The solubility-time profile of EO9 in a 0.001-*M* borax buffer solution (pH 9.0) was determined for two different batches of EO9. The effect of the particle size on the solubility rate could be investigated, since the physical appearance of the two batches was completely different. The appearance of the particles of batch I was regular and small, whereas the particles of batch II were irregular and contained large fragments (estimated size range, 2–4 μm). A 0.001-*M* borax buffer solution (pH 9.0) was used instead of water because at this pH, EO9 shows the best stability [12]. The solubility of EO9 increased from 0.44 mg ml^{-1} (after 5 min) to 0.57 mg ml^{-1} (after 24 h) for batch I and from 0.42 mg ml^{-1} to 0.52 mg ml^{-1} for batch II as determined by UV/VIS spectrophotometry. The results obtained with HPLC analysis are similar and demonstrate that the drug does not degrade during the solubility experiments. Furthermore, these results indicate that the particle size of EO9 does influence the solubility rate of EO9 in borax buffer. The solubility of batch I was significantly higher than that of batch II (P = 0.0354 at 5 min; P = 0.0102 at 24 h). Thus, reducing the particle size of EO9 bulk drug is an important step during scaling-up of the manufacturing process to reach a solubility of at least 0.2 mg ml^{-1} (e.g., 1.6 g in 8 l).

Differential scanning calorimetry

DSC was used to determine the freezing and melting characteristics of EO9 in water and in 0.5% (w/v) lactose solution. The EO9 solution [0.2 mg ml^{-1} in 0.5% (w/v) lactose] was cooled to –60° C. Thereafter, the frozen EO9 solution was heated at a rate of 1° C min^{-1} to +20° C (Fig. 4). No eutectic behavior was observed. The extrapolated onset temperature (starting temperature of melting) of the solution was +0.1° C, which indicates that the behavior is almost that of pure water, as would be expected

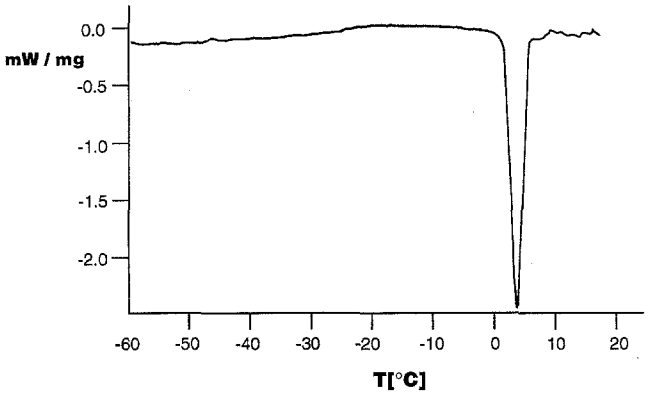

Fig. 4. DSC melting curve generated for EO9 (0.2 mg ml^{-1}; heating rate, 1° C min^{-1}) in a 0.5% (w/v) lactose aqueous solution

for these low amounts of additives. The use of lactose makes freeze-drying below the collapse temperature of lactose (–31° C) necessary. Basically, freeze-drying can be selected as the manufacturing process for any substance when this substance does not show eutectic behavior and when the lyophilization process takes place below the collapse temperature. On the basis of these data, the following freeze-drying cycle was selected. The solution was frozen at –40° C for approximately 10 h, primary freeze-drying was performed at 10^{-1} mbar, and the plate temperature was kept at +10° C. Secondary drying was performed at +20° C and 10^{-1} mbar.

Formulation of EO9

In an earlier study, the chemical stability of EO9 was investigated as a function of pH, buffer composition, ionic strength, and temperature. EO9 is highly unstable in aqueous solution. The degradation rate is strongly pH-dependent and is affected by phosphate-buffer components [12]. Because of the chemical instability of EO9 in aqueous solution, freeze-drying was selected for the pharmaceutical formulation. One of the more important advantages of freeze-drying is that the water content of the product can be reduced to very low levels, which is important for a compound such as EO9, which degrades in aqueous media. Furthermore, since the product is sealed in vacuo, oxidative denaturation is greatly reduced [4]. Another important advantage is that the solubility rate of lyophilized products during reconstitution is generally increased. The selection of the content of the EO9 vials (8 mg) was based on the expected doses for animal toxicology and clinical phase I trials.

During the development stage, EO9 was freeze-dried without the use of a bulking agent. However, a bulking agent appeared to be necessary to obtain an acceptable freeze-dried cake. Lactose enhanced the freeze-drying properties of EO9 sufficiently and was therefore selected as the bulking agent.

Quality control of the lyophilized final product

The results of the quality control of several batches of lyophilized EO9 are listed in Table 3. All batches appeared as a pink/red-colored freeze-dried cake. EO9 was identified by HPLC analysis; the retention time (t_r) of the main peak obtained in the chromatogram of the reconstituted lyophilized EO9 corresponded to the t_r of the main peak in the chromatogram of the interim reference standard. The average content of the vials was 7.9 ± 0.2 mg/vial. EO9 was also identified by UV/VIS spectrophotometry. Recon-

Table 3. Quality control of several batches of lyophilized EO9

Test method/item	Specification	Batch 1	Batch 2	Batch 3
Visual inspection/appearance	Pink/red lyophilized cake	Conforms	Conforms	Conforms
HPLC/				
1. t_r	t_r is approx. 6.2 min	Conforms	Conforms	Conforms
2. Content (mg)	8.0 ± 0.4 mg	7.7	7.9	8.0
3. Purity[a]:				
EO9	>98%	99.3	99.6	99.8
EO5A	total impurities (EO5A + unknown)	0.4	0.3	0.2
Unknown	<2%	0.3	0.1	0.0
UV/VIS/				
1. λ_{max} (nm)	λ_{max}: 269–273, 311–315, and 505–512 nm	Conforms	Conforms	Conforms
2. Content (mg)	8.0 ± 0.4 mg	8.0	8.1	7.9
Koal-Fischer titration method/ residual moisture content (%)	<1%	0.6	0.3	0.7
LAL test/pyrogens	<3.28 EU/vial	Conforms	Conforms	Conforms
Sterility test	Sterile	Conforms	Conforms	Conforms

[a] The purity of EO9 final product was calculated by dividing the peak area of the EO9 peak by the total peak area (except the solvent front) in the chromatogram times 100%

Table 4. Shelf-life studies with lyophilized EO9

Stability after	Content[a] ± SD (%)	n[b]	pH[c]	n[b]
6 months	101.0±3.4	10	8.6	3
12 months	98.1±2.2	4	8.1	4

[a] The content (%) is calculated relative to the content of the lyophilized formulation at t = 0
[b] Number of determinations
[c] pH after reconstitution

Table 5. Stability of lyophilized EO9 at room temperature after reconstitution and dilution

Time (h)	Average amount of EO9 ± SD (%)[a] (0.5 mg ml^{-1}; n = 3)	Average amount of EO9 ± SD (%)[a] (0.05 mg ml^{-1}; n = 3)
0	100.0	100.0
2	100.9±0.7	98.4±2.0
4	99.7±0.7	95.2±0.2
6	102.3±1.1	94.5±2.7
24	99.6±2.9	94.5±1.1

[a] Percentage of the initial concentration

stituted lyophilized EO9 exhibited absorption maxima at 269, 311, and 506 nm. The average content of the vials as determined by UV/VIS spectrophotometry was 8.0± 0.1 mg/vial (mean, 3 batches). The average purity was 99.6%±0.3% and the average residual moisture content was 0.5%±0.2% (mean, 3 batches). Moisture contents of <1% are usually desired for pharmaceuticals [14]. All batches passed the LAL test; the absolute amount of endotoxins in the reconstituted lyophilized EO9 was <3.28 EU/vial. The endotoxin limit for a given product as defined by the Food and Drug Administration (FDA), expressed as the maximal allowable endotoxin concentration given in 1 h, is equal to 5 EU/kg body weight [7]. Thus, the final product of EO9 is far within the FDA norm for sterile fluids. All batches passed the sterility test; no microbial growth occurred.

Manufacturing documentation is delivered with each lot of finished product, appropriately detailing the preparation and formulation procedures. All freeze-dried batches of EO9 were specified by a lot number and a certificate of analysis. The certificate of analysis provides information about the appearance, content, identity, residual moisture content, sterility, pyrogens and excipient of the formulated product. Furthermore, details on the storage, reconstitution instructions, and stability data of the reconstituted product as well as an example of the label are included with the certificate of analysis. The label contains the drug name, content, and storage conditions, the name of the manufacturer, the lot number, the expiration date, and the "investigational use" statement.

Shelf-life studies

Shelf-life studies were performed to assure the integrity of the final product during storage. The stability of lyophilized EO9 was investigated by HPLC analysis after 6 and 12 months of storage at +4° C, the recommended storage temperature. Table 4 shows the results of these studies. After 6 and 12 months of storage at +4° C, the lyophilized product maintained its appearance as an acceptable pink/red cake, with no change being visible. The solution was clear after reconstitution. The content of the lyophilized EO9 was 101.0%±3.4% (n = 10) and 98.1%±2.2% (n = 4) after 6 and 12 months of storage at +4° C, respectively. Thus, EO9 remains stable in a freeze-dried solid form for at least 1 year when stored at +4° C in a dark environment.

Extensive-shelf life studies with the formulation, evaluating three storage conditions, i.e., the recommended storage temperature (+4° C) as well as a temperature one level above (+22° C) and a temperature one level below the recommended storage temperature (–30° C), are ongoing. The color and appearance and, after reconstitution, the content, clarity, and pH of the solution will be determined at regular intervals.

Stability of EO9 after reconstitution and dilution

The stability of EO9 after reconstitution and dilution was investigated. The experimental conditions mimicked the situation of preparation and dilution of EO9 for intravenous injection as carried out in a hospital pharmacy. The results of the stability studies are shown in Table 5. For the 0.5-mg ml^{-1} solution, the amount of EO9 remaining after reconstitution was 99.6%±2.9% (n = 3) after 24 h. For the 0.05 mg ml^{-1} solution, the amount of EO9 remaining after reconstitution was 94.5%±2.7% (n = 3) after 6 h, exceeding the 5% threshold of degradation. It is therefore advisable to use the 0.05-mg ml^{-1} solution within 4 h of its preparation.

Conclusions

EO9, an investigational cytotoxic drug, shows poor aqueous solubility and stability characteristics. A stable lyophilized formulation of EO9 has been designed according to the guidelines drawn up by the EORTC/CRC/NCI Joint Formulation Working Party. Quality control of the lyophilized formulation demonstrated that the manufacturing process does not change the integrity of EO9. Shelf-life studies showed that the lyophilized formulation remains stable for at least 1 year when stored at +4° C in a dark environment. A phase I study with EO9 has recently been completed. In several patients a partial response was observed [10]. EO9 will soon enter phase II trials within the framework of the EORTC.

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