ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 373–381

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Stability study of fotemustine in PVC infusion bags and sets under various conditions using a stability-indicating high-performance liquid chromatographic assay

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Received 20 November 1997; received in revised form 5 February 1998; accepted 16 February 1998

Abstract

The stability and compatibility of fotemustine, a nitrosourea anticancer agent, in 5% dextrose solution with polyvinyl chloride (PVC) containers and administration sets were studied under different conditions of temperature and light. The drug was diluted to 0.8 and 2 mg ml^{-1} in 100 or 250 ml 5% dextrose injection solutions for 1-h simulated infusions using PVC bags and administration sets with protection from light. After preparation in the PVC bags containing 5% dextrose, fotemustine was also prepared at the same concentrations and stored at 4°C for 48 h and at room temperature (22°C) or at sunray exposure (> 30°C) over 8 h with or without protection from light. The solution samples were removed immediately at various time points of simulated infusions and storage, and stored at − 20°C until analysis. The physical compatibility with PVC and chemical stability in solution of fotemustine were assessed by visual examination and by measuring the concentration of the drug in duplicate using a stability-indicating high-performance chromatographic assay. When admixed with a 5% dextrose solution, fotemustine 2 and 0.8 mg ml^{-1} was compatible and stable over 1-h of simulated infusion using PVC bags through PVC administration sets with protection from light. On the other hand, in the same diluent, fotemustine was compatible and stable with PVC bags for at least 8 h at 22°C with protection from light and for at least 48 h at 4°C with protection from light. There were no pH variation, no visual change, no color change, no visible precipitation and no loss of the drug. Conversely, when the solutions were exposed to light (ambient or solar), the drug concentration decreased rapidly, leading to the production of a degradation product as shown by mass spectral analysis and a discoloration of the solutions. Finally, in all cases, no DEHP (di-2-ethylhexyl phthalate) was detected in the injection solution.

Keywords: Fotemustine; Stability; Compatibility; PVC bags; Dextrose (5%); HPLC

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0731-7085/98/$ - see front matter
PII S0731-7085(98)00096-X

1. Introduction

Fotemustine (FM) is a chloroethylnitrosourea, chemically characterized by the graft of an aminophosphonic acid on the chloronitrosourea radical which makes it highly lipophilic. So, FM readily penetrates the blood brain barrier. Several clinical studies using FM have been conducted in malignant primary brain tumours, brain metastasis of non-small cell lung cancer and disseminated malignant melanoma with encouraging results [1,2].

To obtain a clinically high response-rate and a decreased toxicity, FM (100 mg m^{-2}) must be delivered by a 1-h infusion after dilution of the commercial product in injection solution [3]. For infusion, the clinical formulation is diluted in 5% dextrose solution present in containers of glass or plastic. Polyvinyl chloride (PVC) bags of the infusion solutions offer several advantages over conventional glass containers, such as easier storage and shipping because of their relative resistance to breakage [4]. However, several problems are reported with their use such as the loss of substantial amounts of drug from the solution by adsorption onto the plastic bags [5–7], and the leaching of potentially harmful substances into the solution, particularly a plasticizer, di-2-ethylhexyl phthalate (DEHP), that is incorporated into PVC to make the bags soft and pliable [8–10].

The purpose of this study was to determine under various conditions (temperature, concentrations, light and infusion duration) the chemical stability and the physical compatibility of FM with PVC materials when admixed in 5% dextrose solution. As the FM manufacturers recommended, FM must not be diluted in 0.9% sodium chloride injection because the drug is unstable in saline solution. At the same time, the extent of DEHP leaching was also determined during simulated infusion and storage. To the authors knowledge, no compatibility study of FM with PVC has been reported in the literature. However, the stability and compatibility of drugs in clinical preparations is of great interest to many pharmaceutical professionals.

Indeed, when drugs are delivered by continuous i.v. infusion with PVC material or prepared and stored over an indeterminate time, knowledge of the rate of drug delivery to the patient is essential.

To conduct the stability and compatibility studies, a prerequisite is to develop a stability-indicating high-performance liquid chromatographic (HPLC) method for FM at various pH, light and temperature conditions [11,12]. In the literature, many HPLC procedures are described to analyse FM [13]. This paper describes a simple assay using a reverse-phase octadecyl column for stability-indicating analysis.

2. Experimental

2.1. Chemicals and materials

The drug substance studied is a commercial product suitable for clinical use. The FM used was the current clinical formulation Muphoran* i.v. injection and was generously donated by Servier Laboratories, (Courbevoie, France) in vials of 200 mg sterile powder for injection and ampules of 4 ml commercial solvent consisting of 95% ethyl alcohol (3.35 ml)–water for injection (0.65 ml). Sodium phenobarbital (PB), used as the internal standard, was obtained from Sigma (Saint-Quentin-Fallavier, France). DEHP and di-*n*-nonyl phthalate (DNNP, internal standard) were obtained from Aldrich (Saint-Quentin-Fallavier, France). Acetic acid and ammonium acetate were analytical grade and purchased from Prolabo (Paris, France). Acetonitrile and all the other organic solvents used were high-performance liquid chromatography (HPLC) grade and obtained from Alchym (Marchiennes, France). The water used for the buffers and dilution was de-ionized and purified by distillation, and obtained from Macopharma Laboratories (Tourcoing, France).

For simulated infusions, a volumetric pump (ref. no. P3000) and PVC administration sets (ref. no. S05, 72201) were used and obtained from Becton Dickinson Laboratories, Division Vial Medical (Saint-Etienne de Saint-Geoirs, France). PVC infusion bags (PVC, Macoflex®) containing 100 or 250 ml 5% dextrose injection were kindly provided by Macopharma Laboratories (Tourcoing, France).

The pH meter, used to measure the pH of the injection solutions during storage in the PVC bags, was a model HI 8417 microprocessor (Hanna Instruments, Lingolsheim, France). Electrospray ionization (ESI) mass spectra were recorded on a single quadrupole mass spectrometer API-I (Perkin-Elmer-Sciex, Toronto, Canada) equipped with an ion-spray (nebulizer-assisted electrospray source) (Sciex, Toronto, Canada). The FM samples were dissolved in 20% acetonitrile and 80% water (v/v) and the solutions were continuously infused with a medical infusion pump at a flow-rate of 5 μl min^{-1}. Electrospray was performed by applying a potential of 5 kV to the syringe needle. The quadrupole was calibrated using polypropyleneglycol (Aldrich). Ionspray mass spectra were acquired in the multichannel analyser (MCA) mode from 150 to 1500 m/z with a scan time of 27 s. Data from five spectra was pooled. The computer program used was Mac Spec for spectra processing to calculate the molecular masses of the samples.

2.2. Chromatographic conditions and instrumentation

The HPLC analyses were performed using a Hewlett-Packard 1090M HPLC system equipped with a variable-volume injector, an automatic sampling system and a Hewlett-Packard 79994 linear photodiode array UV detector operating at suitable wavelenghts. The output from the detector was connected to a Hewlett-Packard 9000 model 300 integrator to control data acquisition and integration. The retention times and peaks areas were determined by computer recorded to a Hewlett-Packard Thinkjet terminal printer.

The analyses of FM were performed on a 5 μm C18 Kromasil BDS column (150 mm × 4.6 mm i.d.) (Life Sciences International, Eragny, France) operating at room temperature. The drug separation was based on an isocratic method using a mobile phase consisting of acetonitrile–aqueous buffer mixture (30:70 v/v). The buffer was prepared in water with ammonium acetate (0.05 M) adjusted to pH 4.5 with acetic acid giving both optimal resolution and separation of the peaks. After degassing with a helium stream for 15 min, the mobile phase was pumped through the column at a flow-rate of 1 ml min^{-1}. The samples (10 μl) were injected into the analytical column and the chromatographic separation was carried out with final detection at 230 nm. PB was used as the internal standard.

Analyses of DEHP were performed as previously described by Faouzi et al. [14], using a C18 Hypersil ODS column and UV detector operating at 222 nm. DNNP was used as the internal standard.

2.3. Calibration curves and stability-indicating capacity

The calibration curves were constructed at a concentration range of 5–20 μg ml^{-1}. Standard stock aqueous solutions of FM and PB at 1 mg ml^{-1} were prepared in polypropylene tubes. The standards were immediately aliquoted (1 ml) and stored at −20°C. After thawing of an aliquot, suitable dilutions were made to prepare the standard solutions of the desired concentrations. The samples were diluted with the mobile phase before injection into the column. The calibration curves were constructed from a linear plot of the peak area ratio (FM:PB) versus concentration. The peak ratio was calculated and the amount of drug determined by reference to the calibration curve.

The accuracy and precision of the assay were validated by establishing the intra-assay and the inter-assay variations evaluated by R.S.D. At four different levels (5, 10, 15 and 20 μg ml^{-1}), five sets of samples were prepared on the same day to establish the intra-assay variation. The assay was repeated weekly for five weeks to establish the inter-assay variation. The HPLC method was validated as stability-indicating by accelerating the decomposition of FM. The stability-indicating assay for FM was established by boiling and by adding concentrated hydrochloric acid for acidic conditions, 1 N sodium hydroxide solution for alkaline conditions and 1% hydrogen peroxide to samples of FM in 5% dextrose. In addition, the purity and homogeneity of the FM peak in the samples was confirmed by quantitating the drug at three wavelengths (230, 250 and 280 nm) using the corresponding calibration curves.

At the same time, standard stock solutions of DEHP and DNNP were prepared daily in methanol at 1 mg ml^{-1}. Since DEHP is a persistent environmental pollutant, rigorous precautions were taken to avoid contamination during both sample handling and sample analysis. All the samples were prepared and diluted in glass or polypropylene tubes washed beforehand with a methanol–acetonitrile mixture, rinsed with an acidic solution, and analyzed in duplicate.

2.4. Simulated infusions of FM formulation diluted in injection solutions

Infusions of FM were carried out under laboratory conditions simulating those routinely used in hospital clinical practice. For this purpose, an infusion pump and PVC administration sets were used. FM (200 mg) was reconstituted with commercial solvent for injection according to standard hospital procedures and the recommandations of the manufacturer by using a 10-ml glass syringe with a 22-gauge needle. The FM solution was then added to 100 or 250 ml PVC infusion bags containing 5% dextrose to yield the initial nominal concentrations of 2 and 0.8 mg ml^{-1}, averaging the concentrations mostly used in clinical practice. The infusion solutions of the drug were prepared in PVC bags immediately before the infusion. The bags containing the drug were agitated by bending, flexing, massaging and shaking for about 1 min after preparation to simulate the agitation that a bag may undergo during preparation, transportation and administration. The bags were then attached to an administration set connected to the infusion pump which allowed the solution to flow through at a constant rate. The flow rate was adjusted to either 1.66 or at 4.16 ml min^{-1}, and the simulated infusion was started for 1-h with protection from light.

A solution (1 ml) was withdrawn at time zero and at regular intervals (5, 15, 30, 45 and 60 min) from the PVC bags, and at the same time, an aliquot of effluent (1 ml) was collected from the administration set, in order to evaluate the bags and sets separately for stability, compatibility of FM and leaching of DEHP. Then, the samples were kept frozen in polypropylene tubes at -20°C until analysis by HPLC to assay for FM and DEHP concentrations. Preliminary stability trials after freezing were made over one month during reproducibility assays. After defrosting, one portion of the sample was immediately diluted in the mobile phase and analyzed for FM concentration. A second portion was diluted in acetonitrile and water and analyzed for DEHP. Simulated infusions were prepared in duplicate in 5% dextrose solution at ambient temperature (22°C) with protection from light.

2.5. Stability of FM injection in i.v. fluids stored in PVC bags

FM (200 mg) was reconstituted with a commercial solvent and were diluted either in 100 or 250 ml 5% dextrose injection in PVC bags to produce a nominal FM concentration of 2 or 0.8 mg ml^{-1} respectively, according to the same procedure.

All the bags were prepared in duplicate. After preparation, the bags were agitated by flexing and shaking for about 1 min. Then, two of each type of container were stored at room temperature (22°C) for 8 h with protection from light, two at room temperature (22°C) for 8 h at normal light exposure (8 h/24 h) without protection from light, two at sunlight exposure (>30°C, 8 h/24 h) without protection from light and two at 4°C for 48 h with protection from light. A sample (1 ml) was removed from each bag at time zero and at regular intervals (1, 2, 4, 6, 8, 24, 30 and 48 h). After agitation at each time point, the samples were placed in clear glass test tubes and were visually inspected for color and clarity by following European Pharmacopeia protocols V.6.1. (1983) and V.6.2. (1980). At the same time, the pH values of solutions were measured immediately after mixing and during the course of the experiment using a properly standardized pH meter. Then, samples were kept frozen in polypropylene tubes at -20°C until analysis by HPLC to assay for FM and DEHP concentrations.

3. Results and discussion

Optimally, drug compatibility and stability trials should include both visual and chemical tests. On the other hand, the target concentrations of FM tested in our study were selected on the basis of either standardized concentrations of solutions recommended by the manufacturer or the usual doses administered to cancer patients. Since the doses of FM investigated are usually titrated to achieve a clinical effect, an infinite number of possible concentration combinations could have been tested. Finally, further investigations are needed to evaluate compatibility and stability when other drugs are administered concomitantly by the same infusion, as might be required in cancer patients.

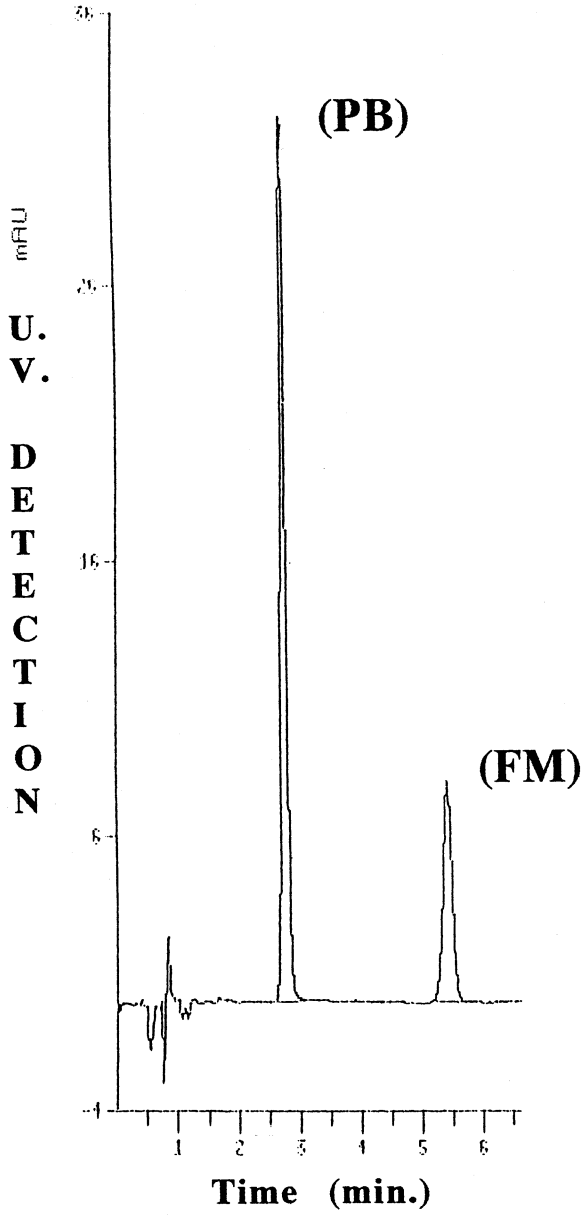

Fig. 1. Typical chromatogram of FM with its internal standard PB.

3.1. Chromatography

FM was resolved with a baseline separation from the internal standard under the conditions developed. A typical chromatogram of FM (5 μg ml^{-1}) with the internal standard PB (10 μg ml^{-1}) in solution obtained immediately after mixing is illustrated in Fig. 1. The retention times of FM and PB were 5.43 and 2.74 min, respectively.

During the stability-indicating assay and the specificity assay, FM was chemically stable in acidic conditions, but highly unstable in alkaline conditions leading to a reduction in the peak for the intact drug. Neither PB nor the decomposition products interfered with the intact FM peak. To prove the stability-indicating capability, the FM peak was analyzed at the end of an incubation at high and low pH by mass spectrometral analysis which showed that the analyzed chromatographic peak is indeed FM. The homogeneity of the FM peak was confirmed by quantitating the drug at the three wavelengths (230, 250 and 280 nm) using the respective calibration curves. Monitoring at 230 nm yielded the best R.S.D. and was thus selected for further studies. The four-points calibration curve of FM at 5–20 μg ml^{-1} was constructed with absorbance at 230 nm.

The resulting chromatograms were compared with chromatograms of the intact FM solution and 5% dextrose solution. No degradation product interfered or was eluted with the same retention time of the parent FM peak. 5-Hydroxymethylfurfural (HMF), a dextrose degradation product which emerges after sterilization, was not detected under these HPLC conditions.

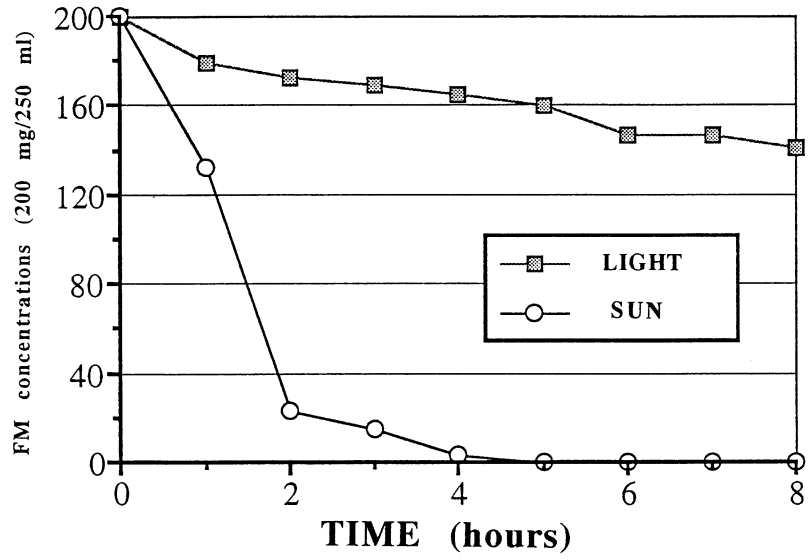

Fig. 2. FM concentration kinetics of the solutions stored in PVC bags without protection from light (ambient and solar).

The precision of the FM assay was determined by using five series of five measurements at four theoretical concentrations. The intra-assay and inter-assay coefficient of variation expressed as percent R.S.D. were lower than 1.60 and 2.36%, respectively, indicating good reproducibility for FM. The calibration curves were constructed from a linear plot of peak area ratio (FM:PB) versus FM concentration (5–20 μg ml^{-1}). The correlation coefficient of the standard curve was greater than 0.999.

For the assay of DEHP, the intra-assay and inter-assay coefficients of variation (R.S.D. values) were lower than 0.75 and 4.36%, respectively. The limit of detection was 100 ng ml^{-1} and showed that the assay was sensitive enough. The calibration curve covered the range 3.12–50 μg ml^{-1} with a correlation coefficient better than 0.999.

3.2. Stability of FM in i.v. fluids during simulated infusions

The analysis of each sample was performed by HPLC after a suitable dilution in the mobile phase in order to fit the calibration curve. At time zero, the initial concentration of FM was designated as 2 or 0.8 mg ml^{-1} for 1-h infusion with protection from light. All subsequently measured concentrations were expressed with respect to the initial one. Stability was defined as a concentration representing 90–105% of the initial one, in accordance with the Health Registration of France, the French regulatory agency for drug and drug-related products. Instability of the drug and incompatibility with PVC were defined as a $>10\%$ decrease from the initial drug concentration.

When the FM solutions were infused through PVC infusion sets from PVC infusion bags over 1 h, the variation in drug concentration in both the PVC bags and effluent in no case exceeded 10%. There was no substantial difference between the FM concentrations at time zero and at any subsequent time point. This demonstrates that the drug was not absorbed by the plastic infusion bags and sets during infusion at ambient temperature and with protection from light. No additional peak corresponding to degradation products was observed on the chromatograms. No significant difference was observed between the FM concentrations of solutions collected in the PVC bags and from the infusion sets.

No visible precipitation, no discoloration of solution, no degradation and no loss of drug was observed during the simulated infusions using PVC bags containing 100 or 250 ml 5% dextrose solution and administration sets, suggesting that the FM formulation was chemically stable and compatible with PVC for up to 1-h infusion.

A drug is considered incompatible with the PVC containers if DEHP was detected in the drug solutions. In the case of FM, no DEHP (< 100 ng ml^{-1}) was detected in the FM injections used as a 1-h simulated infusion.

3.3. Stability of FM in 5% dextrose stored in PVC bags

For at least 8 h of storage at 22°C with light protection and 48 h at 4°C, there was no substantial difference between the FM concentrations at time zero and at any subsequent time points.

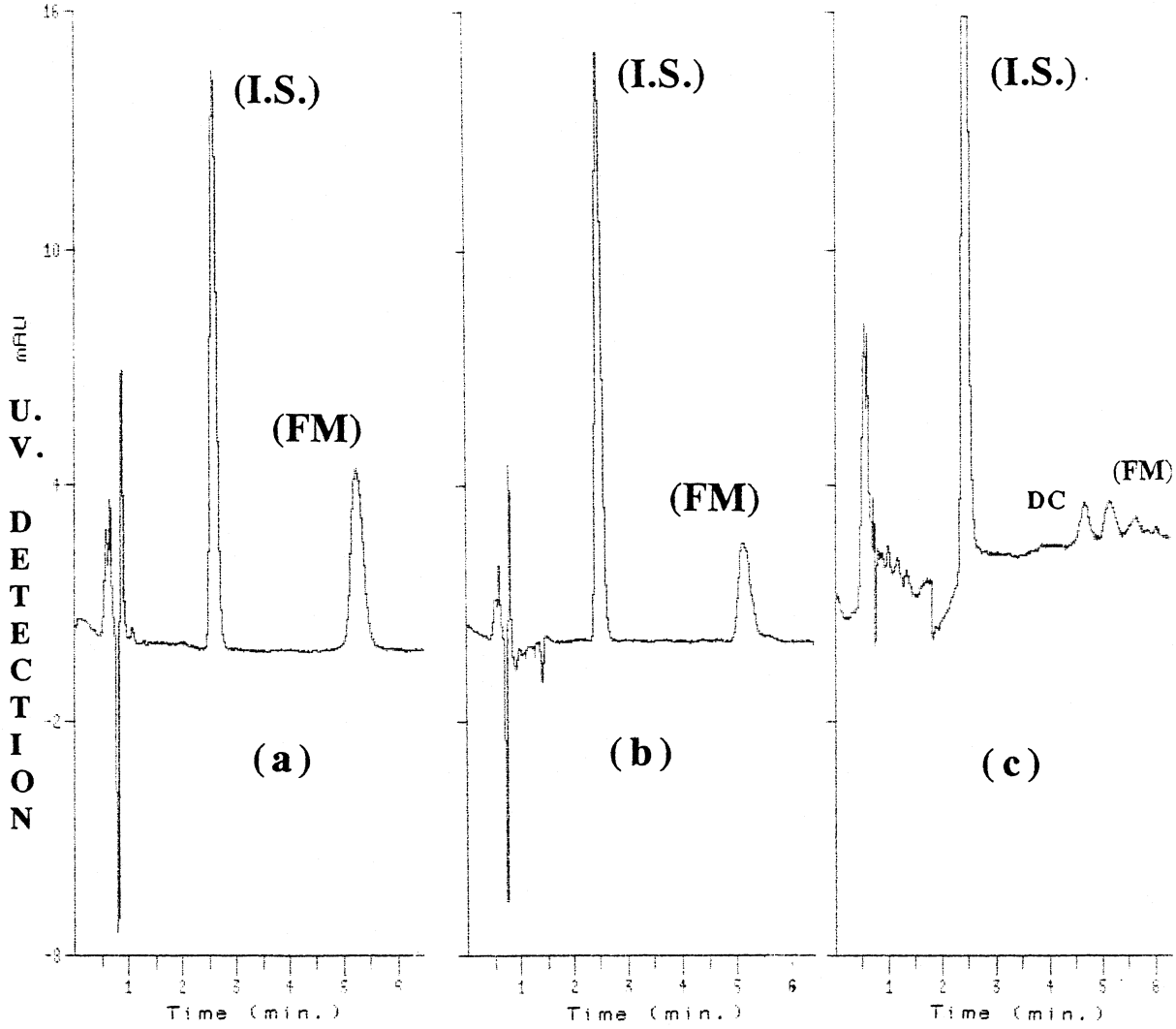

Fig. 3. HPLC chromatograms of light-induced degradation of FM stored in 5% dextrose after light exposure: (a) an FM solution in 5% dextrose at time T_0, (b) an FM solution in 5% dextrose at $T_0 + 8$ h after ambient light exposure, (c) an FM solution in 5% dextrose at $T_0 + 8$ h after sunlight exposure. (DC, degradation compound).

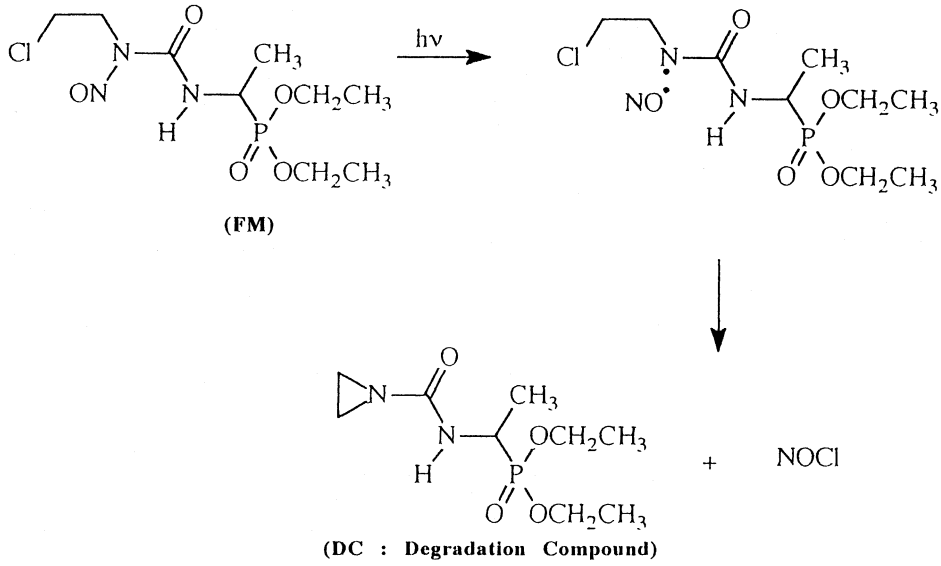

Fig. 4. The reaction mechanism of light on an FM solution producing a degradation compound.

No color variation was observed during storage, as well as no precipitation. No additional peaks corresponding to degradation products was observed on the chromatograms. So, the stability and compatibility of FM were satisfactory (loss $< 10\%$) when the solutions were protected from light during 48 h at 4°C and 8 h at room temperature for 0.8 and 2 mg ml^{-1} concentrations.

However, when the FM solutions were not protected from ambient light during storage at 22°C or exposed to sunlight, the drug concentration decreased rapidly, as shown by Fig. 2, leading to the production of a degradation compound. For example, at room temperature, the FM concentration decreased by 10% within 1 h and 30% over 8 h, and at sunray exposure, they decreased by 75% after 2 h of storage in the PVC containers. So, FM is highly unstable under light exposure (ambient or solar). An HPLC chromatogram of the light-induced degradation is presented in Fig. 3.

On the other hand, visual inspection of the FM injection indicated a discoloration of the solution, but no precipitation. The degradation of FM under light exposure led to a degradation product detected by our HPLC method and identified by mass spectra. The mass spectral study of the FM solution samples showed a molecular weight corresponding to FM and its degradation product. At time T_0, the molecular ion peak of FM was identified at M^+ 316, but after three days storage under light, the molecular ion peak was not detected. On the contrary, we observed a new mass peak corresponding to the main degradation product at M^+ 250. The reaction mechanism of light on the FM solution leading its degradation is described in Fig. 4.

On the other hand, in all cases, the pH of the infusion solutions was stable during the storage period and did not vary from the initial pH of 4.2–4.6 for 5% dextrose. Finally, in all cases, no DEHP was detected in the FM solutions.

In conclusion, the HPLC procedure described in this paper is stability-indicating for the determination of the FM concentrations in parenteral solutions. This stability study shows that the FM solutions must be protected from light during infusion or storage at 22 or 4°C to avoid drug degradation.

Acknowledgements

The authors wish to thank Macopharma (Tourcoing) and Servier (I.R.I.S., Courbevoie) Labora-

tories for their cooperation. The technical assistance of X. Meersseman is also acknowledged.

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