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Stability and compatibility studies of zorubicin in intravenous fluids and PVC infusion bags

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Abstract

The stability of zorubicin (ZOR) in admixtures for continuous intravenous infusion was studied. ZOR was reconstituted and diluted to 600 μg ml^{-1} for simulated infusion and to 250 and 1000 μg ml^{-1} for storage in poly(vinyl chloride) (PVC) bags containing 5% dextrose injection or 0.9% sodium chloride injection (0.9% NaCl). Bags were then stored at refrigerated temperature (4°C) and in the dark for 24 h. ZOR concentrations in each admixture were tested by stability-indicating high-performance liquid chromatographic (HPLC) assay with ultraviolet detection. No substantial loss of ZOR was observed during simulated infusions ($n = 4$) using PVC infusion bags and administration sets over a 1 h infusion. The drug stored at 4°C in the dark in PVC bags showed that it is highly unstable at 250 μg ml^{-1} in 0.9% NaCl injection and in 5% dextrose injection. On the other hand, under the same storage conditions, at 1000 μg ml^{-1}, ZOR is more stable in 0.9% NaCl injection (6 h) than in 5% dextrose (4 h). The reported superior stability of the 1000 μg ml^{-1} in 0.9% NaCl can be explained, at least in part, by the difference in pH. Changes in pH, particularly a decrease, seem to affect adversely the stability of ZOR. In fact, ZOR is rapidly converted into daunorubicin, the dominant degradation product, which is more cardiotoxic than the parent drug. Therefore, several precautions must be observed when the commercial product (Rubidazone) is prepared and reconstituted in i.v. fluids and containers.

Keywords: Administration sets; Compatibility; Dextrose; PVC infusion bags; Sodium chloride; Storage; Zorubicin

1. Introduction

Zorubicin (ZOR; Fig. 1) belongs to the anthracycline compounds, a group of tetracyclic amino sugar-linked antibiotics, used in the treatment of various acute leukaemia (lymphoblastic and non-lymphoblastic) and solid tumours. It is generally administered when the patients show resistance to the most commonly used anthracyclines: doxorubicin (DOX) and daunorubicin (DNR). ZOR is

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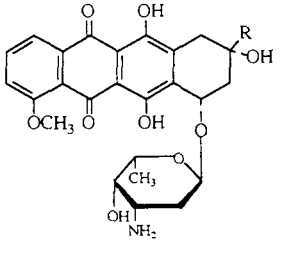
the benzoylhydrazone derivative of daunorubicin, but it introduces lower cardiomyopathy and bone-marrow toxicity than DNR or DOX [1,2]. The cytotoxic effect results from intercalation between DNA pairs. To minimize toxicity, individualized dose regimens are given preferentially over prolonged periods of time by carefully inspecting i.v. administration to prevent extravasion. Many studies on anthracyclines stability (DOX, DNR, epiribucin) [3–5] have been reported but, to the authors' knowledge, little information [6] is available on ZOR. Although the drug is stable in the lyophilized form, its long-term stability in infusion solutions and its compatibility with poly(vinyl chloride) (PVC) containers in which it is used clinically have not been determined. Information about ZOR stability is important because infusions of ZOR degradation products may be cardiotoxic or less effective than infusions of parent drug [7–9].

There is evidence from recent studies that the therapeutic index of anticancer agents may be improved if traditional intravenous bolus injection schedules are replaced with continuous regimens [10].

The continuous infusion therapy has been studied. Hence, the therapeutic advantages of continuous infusion vs. intermittent small-volume infusion or intravenous (i.v.) push have been suggested [11]. Now, for practical reasons (unbreakable, easily stockable, etc.), PVC containers are gradually replacing the conventional glass bottles used in continuous i.v. infusions. Therefore, it is imperative that the stability and compatibility of antitumour agents in administration vehicles and PVC containers be investigated. Consequently, when drugs are administered by continuous i.v. infusion with PVC material, the knowledge of the rate of drug delivery to the patient is essential [12,13].

Previous studies [14,15] have reported the loss of certain drugs (diazepam, nitroglycerine) from aqueous solutions stored in PVC infusion bags for various periods of time. Generally, these losses have been attributed to interaction (adsorption or absorption) between the drug and the plastic infusion bag and, in some cases, may diminish the therapeutic response owing to a reduced drug delivery to the patient. Considering binding, the loss of drug from aqueous solutions stored in plastic infusion bags is well documented [13–16]. A container–formulation combination is compatible if the magnitude of ingredient loss is within acceptable limits over the entire shelf-life of the product. Other studies have showed a loss of anthracyclines with glass bottles due to adsorption [14,17]. A minimal amount of information is available concerning the stability of ZOR in injection solutions. Only one study [18] at room temperature has been performed at a low concentration of ZOR (50 μg ml^{-1}). No stability study at refrigerated temperature and no information on the compatibility of ZOR with PVC infusion bags and intravenous administration sets are available in the literature. However, such information is important when drug solutions are to be infused over long periods of time.

The present study was undertaken with the following objectives: (i) to study the behaviour of ZOR in simulated infusion using PVC containers and administration sets, in conformity with conditions of infusion used in clinical practice (infusion flow rate, dose, volume, temperature and light); (ii) to determine the differences in possible interactions between PVC containers and PVC giving



Compound	—R	Molecular weight
Zorubicin	—C(CH$_3$)=N—NH—COC$_6$H$_5$	645.67
Daunorubicin	—COCH$_3$	527.53
Epirubicin	—COCH$_2$OH	543.53

Fig. 1. Structures of the anthracyclines.

sets, and the differences in stability of the drug in 0.9% sodium chloride injection and 5% dextrose injection; and (iii) to study the stability of ZOR in i.v. fluids and its compatibility with PVC bags over a wide concentration range (250–1000 μg ml^{-1}) at refrigerated temperature.

A stability-indicating high-performance liquid chromatographic (HPLC) method was developed and used in the present study. Trissel [19], then Hagen [20] reported that it is imperative to demonstrate that the method used to investigate the stability of drugs to be stability-indicating. This method allowed the rapid determination of intact drug and its dominant decomposition product in injection solutions (5% dextrose and 0.9% NaCl) using a suitable chromatographic column and mobile phase. This analytical technique was used to investigate the compatibility of ZOR with PVC containers and PVC infusion sets both during simulated infusions and storage at 4°C in PVC bags used in a hospital pharmacy department where the reconstitution of cytostatics is centralized.

2. Experimental

2.1. Chemicals and materials

The drug substance studies is a commercial product suitable for clinical use. ZOR was used as the current clinical formulation Rubidazone (RBZ) intravenous injection and was obtained from Bellon Laboratories (Neuilly sur Seine, France) in vials of 50 mg of sterile powder for injection. RBZ was reconstituted for assay with 4 ml of sodium glycinate buffer as indicated by the manufacturer. Epiribucin (EPI), used as an internal standard (IS), was obtained from Farmitalia Carlo Erba Laboratories (Milan, Italy). Daunorubicin (DNR), used as a standard, was obtained from Aldrich Chemical (Saint-Quentin-Fallavier, France). Sodium dihydrogenphosphate and tetraethylammonium (TEA) were of analytical grade and obtained from Prolabo (Paris, France). All organic solvents were of HPLC grade and obtained from Alchym (Marchiennes, France). The water used for buffers and dilutions was deionized and purified by distillation.

For simulated infusions, we used a volumetric infusion pump (ref. P3000) and PVC infusion sets (ref. S05, 72201) obtained from Becton Dickinson Laboratories, Division Vial Medical (Saint-Etienne de Saint-Geoirs, France). Infusion bags of PVC (Macoflex) containing either 0.9% sodium chloride or 5% dextrose were kindly provided by Macopharma Laboratories (Tourcoing, France). Glass bottles used as reference were obtained from Biosedra Laboratories (Louviers, France). The pH meter, used to measure the pH of injection solutions during storage in PVC bags, was a Model HI 8417 microprocessor (Hanna Instruments, Lingolsheim, France). Mass spectra were measured with a API-I mass spectrometer (Perkin-Elmer SCIEX) working in the anion spray mode.

2.2. Chromatographic conditions and instrumentation

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

Analyses were performed on a 5 μm C18 Ultremex ODS column (150 × 4.6 mm i.d.) (Phenomenex, Torrance, CA, USA) operating at room temperature. ZOR separation was based on the method using a mobile phase consisting of sodium dihydrogenphosphate buffer (0.03 M) with 0.1% TEA–acetonitrile–THF (65:33:1, v/v/v). The phosphate buffer was prepared in water adjusted to pH 4.5, giving both optimal resolution and separation of peaks. After degassing with a helium stream for 15 min, the mobile phase was pumped through the column at a flow rate of 2 ml min^{-1}. The chromatographic separation was carried out with detection at 254 nm and the injection volume was 10 μl.

2.3. Calibration curve

To obtain a stock standard solution, RBZ (50 mg) was reconstituted with 4 ml of glycinate buffer containing 148.8 mg of glycine and 1.448 mg of sodium glycinate, then with distilled water to give a drug concentration of 1 mg ml^{-1}.

Working standard solutions were prepared daily from the stock standard solution by suitable dilutions with phosphate buffer adjusted to pH 6.8 in polypropylene tubes. Calibration curves were constructed between 5 and 25 μg ml^{-1}. Before HPLC analysis, each dilution of the calibration curve was prepared in phosphate buffer (pH 6.8). The internal standard stock solution (EPI) (1 mg ml^{-1}) was diluted with mobile phase to give a final concentration of 40 μg ml^{-1}. Calibration curves were constructed as a linear plot of peak-area ratio (ZOR/EPI) versus ZOR concentration. The assay was validated by establishing the within-day and between-day variations. Five sets of samples were prepared on the same day to establish the within-day variation. The assay was repeated weekly for 5 weeks to establish the between-day variation. All dilutions were carried out in the shortest possible time with solutions previously cooled to 4°C to avoid potential degradation of ZOR. Each sample analysed was always quantified at least twice by HPLC and the average value recorded.

Preliminary studies were performed to access the stability-indicating capacity of our HPLC method. Samples of the drug solution were studied intentionally at extremes of pH to lead to degradation. This was done with dilute solutions of hydrochloric acid and sodium hydroxide (0.1 *N*). An oxidant, such as hydrogen peroxide, was used to speed up the degradation of the drug. The chromatographic conditions were then adjusted until a satisfactory separation was achieved and validation proceeded.

2.4. Simulated i.v. infusions of zorubicin formulation diluted in injection solutions

Infusions of ZOR were carried out under laboratory conditions simulating those routinely used in clinical practice in the hospital. ZOR injections were prepared according to standard hospital procedures by using a polyethylene syringe. Immediately before infusions, the ZOR formulation (RBZ) was added to 250 ml PVC infusion bags and glass bottles, used as reference, containing 5% dextrose injection or 0.9% sodium chloride injection to yield an initial nominal concentration of 600 μg ml^{-1} the mean concentration mostly used in clinical practice. The ZOR formulation was added to the diluents as follows. Three vials containing 50 mg of powder were reconstituted with commercial solvent and were added to 250 ml containers of i.v. fluid. The containers containing the drug were agitated by turning and shaking for about 1 min after preparation to simulate the agitation a bag may undergo during preparation, transportation and administration. The container was then attached to an administration set connected to the infusion pump that allowed the solution to flow through at a constant rate. The flow rate was adjusted to 4.16 ml min^{-1} and the infusion was started. The infusion time was set at 1 h.

At specified times of infusion, 1 ml of solution was withdrawn at times 0, 5, 15, 30, 45 and 60 min from the container and at the same time, an aliquot of effluent (1 ml) was collected from the administration set, in order to evaluate bags and giving sets separately for the stability and compatibility of zorubicin.

The samples were then kept frozen in polypropylene tubes at −20°C until analysis by HPLC to assay for zorubicin concentrations. Preliminary stability trials after freezing were made in 1 month during reproducibility assays. After thawing at room temperature, one portion of sample was immediately diluted in phosphate buffer and analysed for zorubicin concentration.

Simulated infusions were prepared in quadruplicate (two infusions in 0.9% sodium chloride injection and two infusions in 5% dextrose injection) at ambient temperature (22 ± 0.5°C) under normal room fluorescent light.

2.5. Storage in infusion bags

To infusion bags containing 50 and 250 ml of 0.9% NaCl injection or 5% dextrose injection, a

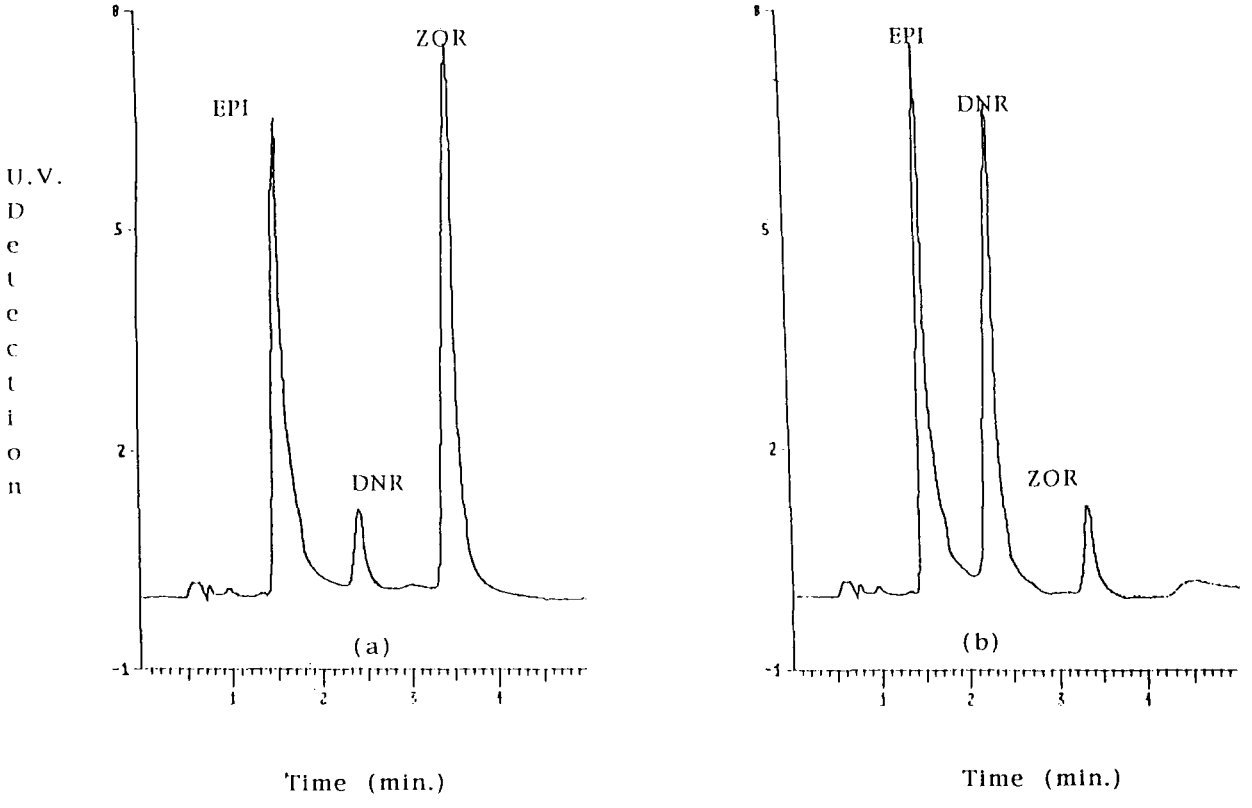


Fig. 2. Chromatograms of (a) standard ZOR with internal standard epirubicin (EPI) and contamination product daunorubicin (DNR); (b) sample obtained after 24 h of storage in a PVC bag at 4°C.

known amount of ZOR was added to achieve the following two extreme concentrations: 12.5 mg in 50 ml of solution (250 μg ml^{-1}) and 250 mg in 250 ml of solution (1000 μg ml^{-1}).

After mixing the solution in the bag by rapid shaking, samples (1 ml) were withdrawn at time zero and at regular intervals with a polyethylene syringe. At the same time, the pH values of the solutions were measured immediately after mixing and during the course of the experiment using a properly standardized pH meter. After agitation at each time point, the samples were placed in clear glass test-tubes and were visually inspected for colour and clarity by following European Pharmacopoeia protocols V.6.1 (1983) and V.6.2 (1980). Then the samples were stored in polypropylene tubes at −20°C until HPLC analysis. Infusion bags containing ZOR were stored at +4°C over a period of 24 h in the dark.

3. Results and discussion

3.1. HPLC

A representative sample of the chromatograms produced with the HPLC conditions used in this work is given in Fig. 2. A decomposed solution of ZOR showed a dominant chromatographic peak for a degradation product, identified as daunorubicin (DNR).

The injection of an authentic sample of DNR produced a peak at 2.28 min, which is identical with the retention time of the peak seen in the decomposition mixture. Hence these results suggest that this is a stability-indicating assay for ZOR because DNR was well separated from the parent drug (ZOR).

The preliminary study showed that the acidic conditions of the chromatographic procedure (pH 2.3–4.3) revealed that ZOR is highly unstable

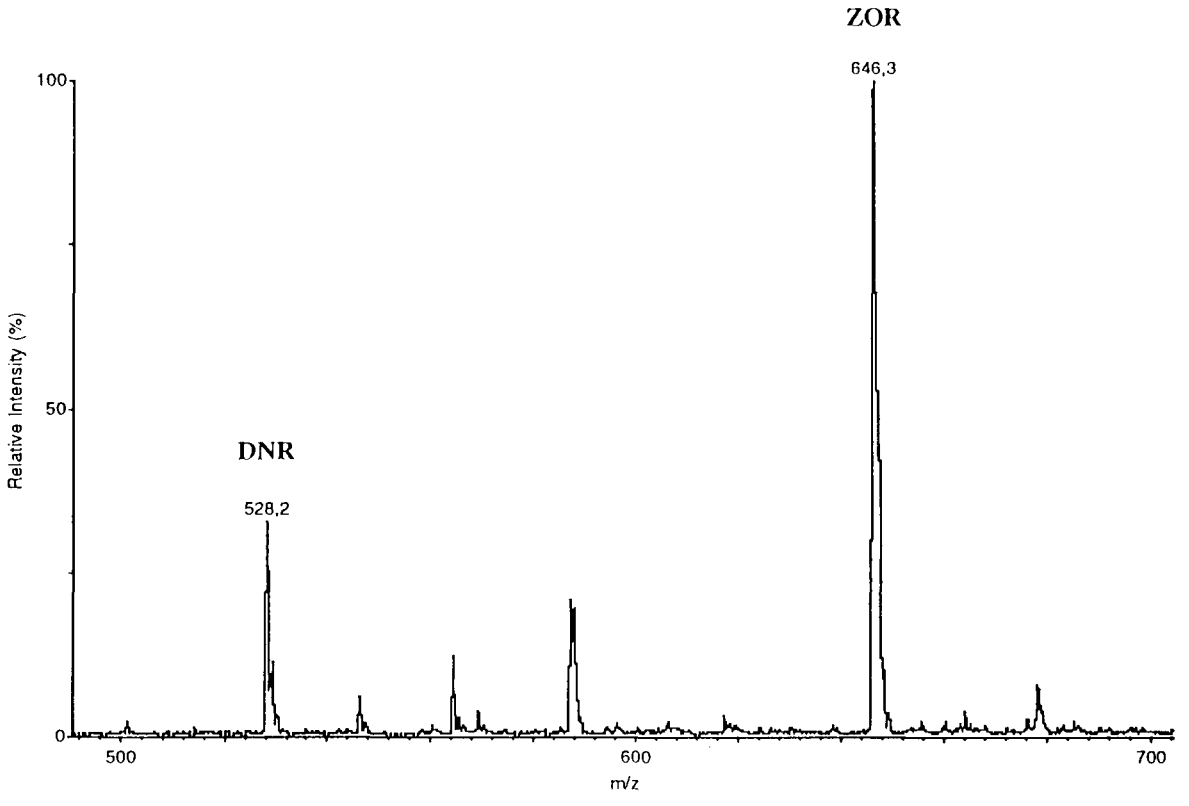


Fig. 3. Mass spectrum of formulated product (RBZ), including the peak mass corresponding to the main degradation product DNR (M_r: 527.53 + 1H$^+$ = 528.53) and ZOR (M_r: 645.67 + 1H$^+$ = 646.67).

under mildly acidic conditions. Under these analytical conditions, the presence of 0.9% NaCl or 5% dextrose, rapid degradation of ZOR is observed. When using a mobile phase with a phosphate buffer (pH 4.5) and when the commercial form RBZ was initially dissolved in commercial diluent, sodium glycinate buffer (pH 7.4), before reconstitution in 0.9% NaCl or 5% dextrose, two cases were obtained: (i) when dilutions were made with distilled water or with phosphate buffer 5 < pH < 6, degradation of ZOR was observed which increased with time; and (ii) when dilutions were

Table 1
Validation data for HPLC assay procedure (sample substance = ZOR; $n = 5$)

Concentration (μg ml^{-1})	Average concentration found ± SD (μg ml^{-1})	Between-day RSD (%)	Within-day RSD (%)	Accuracy (%)	Linear regression equation ($y = ax + b$)[a]	Correlation coefficient (r)
25	25.04 ± 0.33	0.74	0.53	100.16		
20	20.14 ± 0.17	2.63	0.64	100.70		
15	14.55 ± 0.11	2.71	1.82	97.00	$y = 0.0794x + 0.0754$	0.9998
10	9.82 ± 0.18	1.40	1.84	98.20		
5	5.31 ± 0.23	1.40	2.99	106.20		

SD = standard deviation; RSD = relative standard deviation.
[a] $a \pm$ SD = 0.0794 ± 0.001; $b \pm$ SD = 0.0754 ± 0.006.

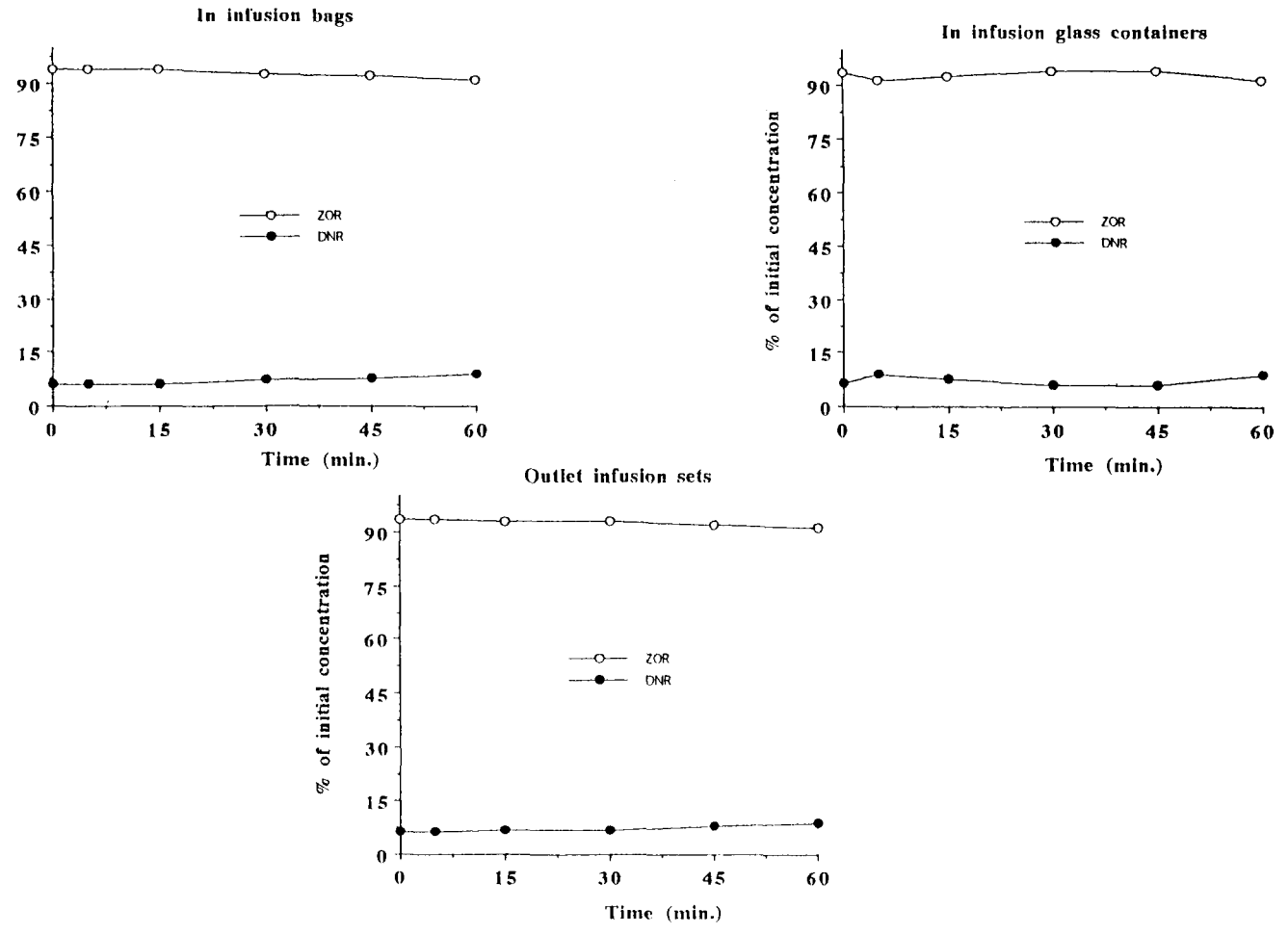


Fig. 4. Concentration kinetics of ZOR during simulated infusion (1 h) using plastic infusion bags ($n = 4$), glass containers ($n = 2$) and infusion sets ($n = 6$).

made with phosphate buffer (pH 6.8), perfect stablility of ZOR with time was observed. However, in this study, contamination by 6–10% DNR was observed, already described by Bosanquet [4]. Hence subsequent studies were carried out according to the latter conditions.

Under the latter conditions, the mobile solvent used, allowing chromatograms with satisfactory resolution to be obtained, contained phosphate buffer adjusted to pH 4.5, and nevertheless maintained the stability of ZOR on the column. These results disagreed with those described by Kovach et al. [6]. They used Tris–HCl (pH 7.6) in the mobile phase to maintain the stability of ZOR and observed that the use of buffers below pH 7.0 produced sharper peaks but causes breakdown of ZOR to DNR. This last problem was not observed in the present study, as shown in Fig. 2, all the more because a mobile phase more basic than pH 7.5 caused progressive and irreversible damage of the reversed-phase material (C18-bonded phase).

Chromatograms of ZOR and IS in solution obtained immediately after mixing and after 24 h of storage at 4°C are illustrated in Fig. 2. Drugs (ZOR and IS) were rapidly well separated, identified and quantified. The components were satisfactorily resolved by this HPLC method. Hence

Table 2
Degradation of ZOR with time: solution at 250 μg ml^{-1} in PVC bags stored at 4°C

Infusion solution	0.9% NaCl		5% Dextrose	
	ZOR (%)	DNR (%)	ZOR (%)	DNR (%)
Initial	89.36	10.64	78.35	21.68
15 min	83.59	16.41	70.00	30.00
30 min	80.59	19.41	65.34	34.66
1 h	65.63	34.37	54.69	45.31
2 h	69.72	30.28	40.38	59.62
4 h	59.80	40.28	27.84	72.16
6 h	53.10	46.90	16.67	83.33
8 h	37.32	62.67	9.90	90.10
22 h	21.16	78.94	6.41	93.59
24 h	20.35	79.65	2.38	97.62

Drug concentration: 12.5 mg of zorubicin per 250 ml (250 μg ml^{-1}).

the HPLC assay allowed the rapid measurement of ZOR and DNR in standard preparations of the drug. Pharmaceutical preparations (RBZ) of ZOR used in this study contained 6–10% of DNR. Indeed, as indicated by the manufacturer, the formulated product (RBZ) contained ca. 8% (range 6–10%) DNR and 92% ZOR by HPLC assay. These percentages were the same whether the formulated product was dissolved in the sodium glycinate buffer (pH 7) supplied by the manufacturer or in phosphate buffer (pH 6.8).

Table 3
Time course of ZOR concentration: solution at 1000 μg ml^{-1} in PVC bags stored at 4°C

Infusion solution	0.9% NaCl		5% Dextrose	
	ZOR (%)	DNR (%)	ZOR (%)	DNR (%)
Initial	93.74	6.26	95.39	4.61
15 min	92.66	7.44	94.80	5.20
30 min	93.24	6.76	94.40	5.60
1 h	93.61	6.39	93.05	6.95
2 h	90.61	9.39	93.62	6.38
4 h	92.07	7.93	91.98	8.12
6 h	92.47	7.53	87.89	12.11
8 h	88.82	11.18	88.25	11.75
22 h	87.86	12.14	86.09	13.91
24 h	87.49	12.51	85.32	14.68

Drug concentration: 250 mg of zorubicin per 250 ml (1000 μg ml^{-1}).

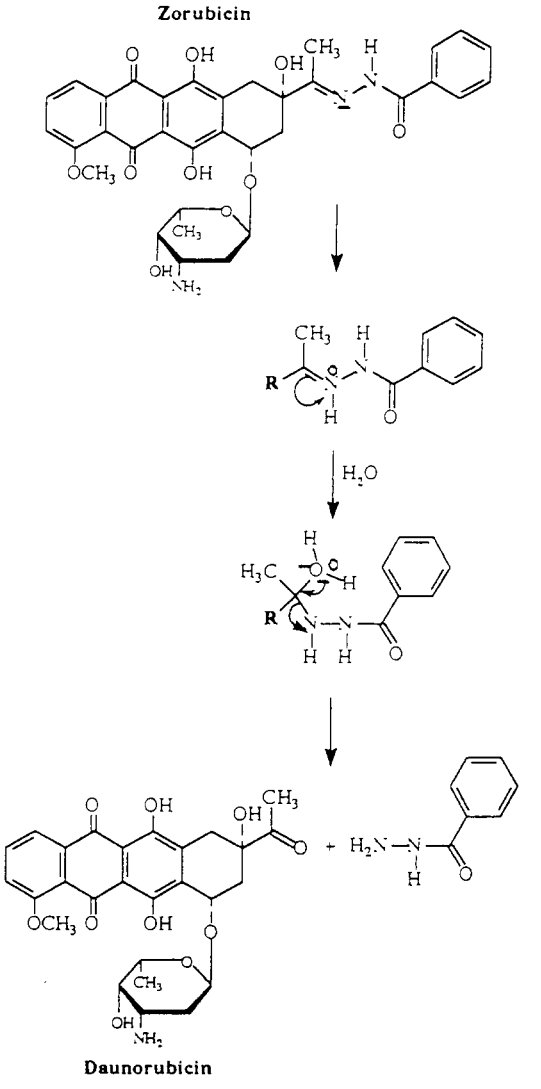


Fig. 5. Reaction mechanism of degradation of zorubicin to daunorubicin in unbuffered infusion solution.

This observation was strengthened by mass spectral studies of samples, showing the peak molecular weight corresponding to DNR and ZOR (Fig. 3). The other peak molecular weights were not detected under the HPLC conditions used; these peaks certainly corresponded to synthetic intermediate products or minor decomposition products or other components of the solution such as excipients, but did not interfere with the response of the drug. It was found that the ratio of ZOR to

DNR in the formulated product was maintained for several hours during the assay procedure. Retention times for ZOR, DNR and IS,were 3.09, 2.28 and 1.52 min, respectively.

Table 1 summarizes the validation data of the assay procedure for ZOR. The precision of the ZOR assay was determined by using five series of five measurements at five theoretical concentrations. As shown in Table 1, the within-day and between-day relative standard derivations (RSD) were lower than 2.99% and 2.71%, respectively, indicating good reproducibility for ZOR. Calibration curves were constructed from a linear plot of peak-area ratio (ZOR/IS) versus concentration (5–25 $\mu g\ ml^{-1}$). The ZOR calibration curve did not differ significantly from the ZOR injection curve (Student's t-test, $p > 0.05$).

No significant differences were observed between equation parameters. The correlation coefficient of the calibration graph was greater than 0.999, indicating good linearity. The calibration graph did not pass through the origin.

During preliminary assay, the stability of drug was investigated on one cycle of freezing at −20°C during 1 month. Then, thawing was undertaken at room temperature. Freezing and thawing under these conditions had no effect on ZOR concentrations (5–25 $\mu g\ ml^{-1}$) in phosphate buffer (pH 6.8). The results suggest that the samples could be frozen and thawed without significant deterioration or reduction in drug concentration, and are in agreement with similar observations by others [4].

3.2. Stability of zorubicin in i.v. fluids during simulated infusions using PVC infusion bags and giving sets

The analysis of each sample was performed by HPLC after suitable dilution in phosphate buffer (pH 6.8) in order to fit the calibration curve. For all the RBZ infusion solutions, the initial concentration of drug (150 mg per 250 ml, corresponding to 600 $\mu g\ ml^{-1}$) was designated as 100% RBZ (ZOR + DNR). All subsequently measured concentrations were expressed as a percentage of the initial concentration. Stability was defined as a concentration of 90–105% of the initial concentration, in accordance with the Health Registration of France, the French regulatory agency for drug and drug-related products.

Fig. 4 depicts the concentration kinetics of RBZ, ZOR and DNR during simulated infusions at room temperature under conditions of normal room lighting using PVC infusion bags and sets ($n = 4$), and glass bottles and sets ($n = 2$) used as reference. When RBZ solutions were infused through PVC infusion sets from PVC infusion bags for 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 ZOR concentrations at time zero and at any subsequent time points. No significant difference was observed between RBZ concentrations of solution collected in PVC bags and outlet giving sets. This demonstrates that the drug was not sorbed by the plastic infusion bags and sets during infusion at ambient temperature. Except for the peak of the dominant degradation product corresponding to DNR, no additional peak corresponding to other degradation products was observed on the chromatograms.

No significant difference was observed between 5% dextrose injection and 0.9% sodium chloride injection with respect to drug stability during 1 h simulated infusions.

Hence, no degradation and no loss of drug were observed during simulated infusion using PVC bags containing 5% dextrose injection or 0.9% sodium chloride injection and administration sets, suggesting that RBZ formulation was chemically stable for up to 1 h. On the other hand, there was no substantial difference between PVC bags and glass containers with regard to stability and compatibility of ZOR. Based on HPLC analysis, it can be concluded that there was an initial contamination of the commercial product by 6–10% DNR, and no degradation of the drug occurred during simulated infusion over a period of 1 h.

3.3. Stability of ZOR in i.v. fluid stored in PVC bags

The analysis of each sample was performed by HPLC after suitable dilution in phosphate buffer

(pH 6.8) in order to fit the calibration curve. Each sample analysed was always quantified at least twice by HPLC and the average value recorded.

Over 24 h of storage at 4°C with protection from light (almost all anthracycline antitumour agents are sensitive to light during prolonged exposure [4]), there was a substantial difference between ZOR concentrations at time zero and at any subsequent time points during the course of experiment. The ZOR concentrations present in solution after various times of storage in PVC infusion bags are presented in Tables 2 and 3, expressed as a percentage of the initial concentration. Because of the use of a large therapeutic range of the ZOR, two concentrations (250 and 1000 μg ml^{-1}) were studied. When stored over 24 h at 4°C, RBZ (formulated product) reconstituted in 0.9% NaCl or 5% dextrose injection was unstable at 250 μg ml^{-1}, and almost all of drug was converted into its dominant degradation product. For instance, after 4 h, nearly 75% of ZOR in 5% dextrose and more than 40% of ZOR in 0.9% NaCl was converted into DNR. On the other hand, under the same conditions of storage but at 1000 μg ml^{-1}, ZOR was found to have greater stability. Indeed, after 24 h of storage, ZOR at 1000 μg ml^{-1} had only lost 10–15% of its activity in the two solutions. The reaction mechanism of degradation ZOR to DNR is explained in Fig. 5 and involves protonation of a nitrogen atom. This reaction is increased by acidic conditions of the infusion solution. Hence, the rapid conversion of ZOR into DNR is a possible limitation to the usefulness of ZOR, because DNR is more cardiotoxic than ZOR [6].

The better stability of ZOR observed at higher concentrations was certainly due to the pH of the admixture and solution composition rather than the storage temperature. The stability of ZOR has never been investigated at refrigerated temperatures but Poochikian et al. [18] and Kovach et al. [6] reported similar observations when solutions were stored at ambient temperature. Therefore, reported superior stability in this study of the ZOR solution at 1000 μg ml^{-1} can be explained, at least in part, by the large difference in pH. The pH infusion solutions was stable during the storage period, but the initial pH of 0.9% NaCl (5.5–6) was slightly superior to the pH of 5% dextrose (4.5–5). Therefore, the pH of reconstituted solutions remained constant ($\pm$0.30) over the 24 h period, ranging from 4.5 in 5% dextrose injection to 6 in 0.9% NaCl. That is why, when low concentrations of ZOR are prepared by diluting the formulated product, the sodium glycinate diluent used has insufficient buffering capacity to maintain the pH at a value allowing the stability of ZOR.

Therefore, to avoid a rapid conversion of ZOR to DNR, the formulated product (RBZ) must be reconstituted with the commercial solvent, glycinate diluent. For storage, dilutions in i.v. fluids will be made preferably in 0.9% NaCl at high concentration (1000 μg ml^{-1}) in order to maintain adequately the pH of the reconstituted solution. The more dilute solution used in this study (250 μg ml^{-1}) was not adequately buffered by the original diluent to maintain the initial pH of the reconstituted solution.

Finally, dilution of the drug in each of the two infusion fluids resulted in no visual evidence of incompatiblity upon periodic macroscopic examination during the course of the experiment. However, visual inspection of RBZ solution indicated a red colour of the RBZ admixtures. No colour variation and no precipitation were observed during the 24 h study. All the diluted RBZ formulation solutions initially appeared non-hazy over 24 h. No additional peak corresponding to degradation products was observed on the chromatograms.

4. Conclusions

With the increasing use of continuous i.v. infusion of cytostatic agents, the present study examined the kinetics of ZOR concentration during simulated infusion using PVC infusion bags and administration sets. The results demonstrate a satisfactory compability of this anticancer drug with PVC infusion material during an infusion period commonly used in hospitals (1 h). Except for initial contamination of RBZ by 6–10% of DNR, no degradation of ZOR to DNR was observed over 1 h infusion. After storage in

PVC bags at 4°C, RBZ at 250 μg ml^{-1} was unstable in 9% NaCl solutions and in 5% dextrose. For such low concentrations, the degradation product DNR is an active agent and, consequently, it is preferable not to use ZOR at low dilutions. At 1000 μg ml^{-1}, ZOR solutions are stable at 4°C for 6 h in 9% NaCl and 4 h in 5% dextrose. Consequently, ZOR solutions may be infused using PVC bags during 1 h at 600 μg ml^{-1} immediately after their preparation or stored at 4°C in PVC containers at concentrations $\geqslant$1000 μg ml^{-1}. In fact, the degradation of ZOR in solution is pH and concentration dependent.

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