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Stability, compatibility and plasticizer extraction of quinine injection added to infusion solutions and stored in polyvinyl chloride (PVC) containers

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Abstract

The stability of quinine was determined in various diluents and in polyvinyl chloride (PVC) containers. The release of diethyhexyl phthalate (DEHP) from PVC bags into intravenous infusions of quinine was also measured. We used an injection of two doses of quinine; quiniforme at 500 mg and quinimax at 400 mg in either 250- or 500-ml PVC infusion bags containing 5% dextrose, to give initial nominal concentrations of 2 or 1 mg ml^{-1} quiniforme and 1.6 or 0.8 mg ml^{-1} quinimax, the mean concentrations commonly used in clinical practice. Samples were assayed by stability-indicating high-performance liquid chromatography (HPLC) and the clarity was determined visually. Experiments were conducted to determine whether the stability and compatibility of quinine would be compromised, and whether DEHP would be leached from PVC bags and PVC administration sets during storage and simulated infusion. There was no substantial loss of quiniforme and quinimax over 1- or 2-h simulated infusion irrespective of the diluent, and storage during 8 h at 22°C, 48 or 72 h at 4°C and 96 h at 45°C. Leaching of DEHP was also detected during simulated infusion delivery using PVC bags and PVC administration sets. The quantity was less than 2 μg ml^{-1}. During storage at 4°C and room temperature the leaching of DEHP was low, but when the temperature was 45°C the quantity was high, 21 μg ml^{-1}. To minimise patient exposure to DEHP, quinine solutions with all drugs should be infused immediately or stored for a maximum of 48 h at 4°C.

Keywords: Quinine; Diethyhexyl phthalate (DEHP); Stability; Compatibility; Polyvinyl chloride (PVC) infusion bags; Administration sets; Infusion solutions

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1. Introduction

Quinine was the first antimalarial drug to be used and also the first to which resistance was reported. After 1960, quinine was reintroduced in routine therapy alone or in combination [1,2]. A review of the principal antimalarial drug is presented as the basis for specific recommendations for the treatment of malaria. Considering that the majority of imported plasmodium falciparum infections are acquired in areas with a high prevalence of chloroquine resistance, mefloquine in generally considered the first-line drug for the treatment of uncomplicated falciparum malaria. For severe tropical malaria, or if parasitemia exceeds 2%, quinine remains the drug of choice [3,4]. Quinine is given by infusion, and manufacturers recommend that the injection be diluted with at least 250 or 500 ml of 5% dextrose before administration.

For intravenous infusions of drugs, the containers may be glass or plastic. Polyvinyl chloride (PVC) bags for infusion solutions offer several advantages over conventional glass containers, such as easier storage and shipping because of their relative resistance to breakage. However, several problems are reported with their use such as the loss of substantial amounts of drugs from the solution by adsorption onto the plastic bags [5–7] and the leaching of potentially harmful substances into the solution [8–10] particularly a plasticizer, diethylhexyl phthalate (DEHP), that is incorporated into PVC to make the bags soft and pliable.

Like Venkataramanan et al. [9] who examined cyclosporin injection, Pearson and Trissel [11] reported that the agent responsible for the leaching of DEHP into solutions was probably the non-ionic surfactant Cremophor EL or the lipophil of the solution. DEHP appears to have a low order of acute toxicity when given either by injection or orally [12]. However, toxicity studies in animals have demonstrated an association between prolonged exposure to DEHP and changes in hepatocellular structure and liver function [13,14]. In addition, DEHP can induce in animals the development of hepatocellular carcinoma [15], and has been found to be teratogenic in rats [16,17].

Therefore, with the increasing use of continuous i.v. infusion and intermittent small-volume i.v. infusion modes of administration, it is imperative that the stability and the compatibility of quinine in administration vehicles and PVC containers be investigated. Consequently, when drugs are administered by continuous i.v. infusion with PVC material, knowledge of the rate of
drug delivery to the patient is essential [5], like the innocuousness of the infusion solution.

The objectives of this study were to observe the physical appearance of the quinine formulation diluted to concentrations that may be used clinically in 5% dextrose for injection for 1 and 2 h, respectively, and stored in PVC bags, and to determine if the stability of quinine is compromised during storage. In tropical and subtropical countries, quinine is usually perfused with many drugs such as: calcium in hypocalcemia cases, Aspegic® for fever states, Hydrosol Polyvitaminé® for deficiency of vitamins and Heptamyl® in low blood pressure.

Consequently, in each perfusion bag, there were also:

- one ampoule of calcium (glubionate of calcium, 10 ml)
- one ampoule of Aspegic® (0.5 g)
- one ampoule of Hydrosol B.N.O. Polyvitaminé®
- one ampoule of Heptamyl® (5 ml) (chlorhydrate of heptaminol)

Finally, the extent of DEHP leaching was also determined during simulated infusion and storage because leaching of the plasticizer from PVC bags was known for quinine mixed with other drugs. The objective was to evaluate the real and potential risks of the exposure of patients to DEHP during an infusion using PVC bags and administration sets.

For these studies, a stability-indicating high performance liquid chromatographic (HPLC) method of determining quinine was developed; an

HPLC method was also developed to determine DEHP.

2. Experimental

2.1. Chemical and materials

The drug substance studied was a commercial product suitable for clinical use. Quinine exists in two dosages quiniforme (500 mg) and quinimax (400 mg), and they were kindly supplied by Synthelabo and Labaz Laboratories, respectively. Phosphoric acid and triethylamine were analytical grade and obtained from Prolabo (Paris, France). All organic solvents were HPLC grade and were obtained from Alchym (Marchiennes, France). The water used for buffers and dilutions was de-ionised and purified by distillation.

For simulated infusion, a volumetric infusion pump (P3000) and PVC infusion sets (S05, 72201) were obtained from Becton Dickinson Laboratories, Division Vial Medical (Saint-Etienne de Saint-Geoirs, France). Infusion bags of polyvinyl chloride (PVC, Macoflex) containing 5% dextrose were kindly provided by Macopharma Laboratories (Tourcoing, France). To measure the pH of infusion solutions during storage in PVC bags, a model HI 8417 pH meter (Hanna Instruments, Lingolsheim, France) was used.

2.2. Chromatography

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 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 recorded onto a Hewlett-Packard Thinkjet terminal printer.

Quinine concentrations were determined by a stability-indicating HPLC assay. Chromatography was performed on 5-μm C18 Hypersil ODS column (150×4.6 mm i.d.) (Interchim, Montluçon, France) operating at room temperature. Quinine (quiniforme or quinimax) was eluted isocratically with a mobile phase of acetonitrile-methanol and buffer (triethylamine 0.2% adjusted to pH 3.5 with phosphoric acid) mixture (40:20:40, v/v/v). The flow rate was 0.4 ml min^{-1}, and detection was set at 330 nm. A total of 20 μl of each sample was injected on to the analytical column. No internal standard was used because we obtained a near 100% average recovery of drug.

DEHP concentrations were measured with the same liquid chromatograph. The mobile phase was acetonitrile-triethylamine (0.2% adjusted to pH 2.5 with phosphoric acid) (90:10, v/v), eluted isocratically at a flow-rate of 1.5 ml min^{-1} on C18 Hypersil ODS column (250×4.6 mm i.d.) (Interchim, Montluçon, France) operating at room temperature. Detection was set at 222 nm. A total of 20 μl of each sample was injected on to the analytical column and di(*n*-nonyl) phthalate (DNNP) was used as the internal standard.

2.3. Calibrations curves

Quinine (quiniforme and quinimax) and DEHP calibrations curves were constructed at concentration ranges of (2.5–20 and 5–20 μg ml^{-1}) and 2.5–20 μg ml^{-1}. Standard stock solutions (1 mg ml^{-1}) of quinine in water and DEHP, DNNP were prepared daily in methanol. A series of dilutions was made to prepare the standard solutions of the desired concentrations. Samples were diluted with the mobile phase before injection into the column. Calibration curves were constructed from a linear plot of peak area ratio (quinine, DEHP/DNNP) versus concentration.

The assay was validated by establishing the within-day and between-day variations. On the same day five sets of samples were prepared to establish the within-day variation. The assay was repeated weekly for 5 weeks to establish the between-day variation.

Since DEHP is a persistent environmental pollutant [18], 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 previously with a methanol/acetonitrile mixture, and were analysed in duplicate.

2.4. Simulated i.v. infusion of quiniforme and quinimax diluted with injection solutions

Insofar as it was possible, we employed conditions in conformity with the drug concentrations normally used in hospital pharmacy departments for the storage of drugs in infusion bags. To infusion bags containing quiniforme (500 mg) and quinimax (400 mg) in either 250 or 500 ml of 5% dextrose solution, a known amount of quiniforme and quinimax was added to achieve the following concentrations which are most often used in hospitals: 2 or 1 mg ml^{-1} and 1.6 or 0.8 mg ml^{-1} in the bags.

Infusion solutions of drug were prepared in PVC bags immediately before infusion. The bags containing the drug were agitated by bending, flexing, massaging and shaking for ~ 1 min after preparation to simulate the agitation a bag may undergo during preparation, transportation and administration. The bags were then attached to an administration set connected to the infusion pump that allowed the solution to flow through at a constant rate. The flow was adjusted and the infusion was started.

At specified times of infusion, 1 ml of solution was withdrawn at zero time and at regular intervals from the PVC bags; at the same time, an aliquot of effluent (1 ml) was collected from the administration set in order to evaluate the bags and administration sets separately for stability, compatibility of quinine and leaching of DEHP.

Samples were kept frozen in polypropylene tubes at -20°C until analysis by HPLC for quinine and DEHP. Preliminary stability trials after freezing were conducted for 1 month during reproducibility assays. After thawing, one portion of the sample was immediately diluted with the mobile phase and analysed for quinine. A second portion was diluted with acetonitrile and water and analysed for DEHP.

Simulated infusions were prepared in duplicate (two infusions in 5% dextrose injection) at ambient temperature (22–26°C) without protection from light.

2.5. Stability of quiniforme and quinimax injection in i.v. fluids in PVC bags

One ampoule of quiniforme 500 mg was diluted in 500 ml 5% of dextrose injection in PVC bags to produce a nominal quiniforme concentration of 2 mg ml^{-1} and one ampoule of quinimax 400 mg was diluted in either 250 or 500 ml of 5% dextrose to produce a nominal quinimax concentration of 1.6 or 0.8 mg ml^{-1} by the same procedure. All bags were prepared in duplicate. After preparation, the bags were agitated by flexing and shaking for ~ 1 min; two of each type of container were then stored at:

- room temperature without protection from light (22°C),
- 4°C during 48 or 72 h, or
- 45°C during 96 h, to mimic storage conditions found in warm climates where ambient temperature often exceeds 40°C.

A total of 3 ml was removed from each bag at time zero and regular intervals, and the pH was immediately measured. 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 Pharmacopoeia protocols V.6.1. (1983) and V.6.2. (1980). The samples were then kept frozen in polypropylene tubes at -20°C until analysis by HPLC for quinine and DEHP.

3. Results and discussion

3.1. Chromatography

Quiniforme and quinimax were rapidly identified and quantified by comparison with standard products. The components were satisfactorily resolved by the HPLC method and had retention times of 4.7 and 4.2 min respectively. DEHP was eluted at 10.11 min.

During the specific stability-indicating assay, quinine was chemically stable under both acidic and alkaline conditions. The homogeneity of the quinine peak was confirmed by determining the drug at the three wavelengths of 300, 330 and 360 nm, using the respective calibration curves. The

resulting chromatograms were compared with chromatograms of intact quiniforme, quinimax, DEHP and 5% dextrose. No degradation products interfered and the drug was eluted with the same retention time as that of the parent quiniforme and quinimax peak. Preliminary studies showed that quiniforme and quinimax were stable for 1 month at −20°C.

The validation data of the assay procedure for quiniforme, quinimax and DEHP was studied. The precision of the quiniforme and quinimax was determined by using five series of five measurement at three theoretical concentrations. The between-day RSD values were lower than 2.35 and 2.96% for quiniforme and quinimax, respectively, indicating good reproducibility for the two drugs. Calibration curves were constructed from a linear plot of peak-area ratio (quinforme or quinimax) versus concentration (2.5–20 and 5–20 μg ml^{-1}). The correlation coefficient of the standard curve was greater than 0.999 for the two drugs indicating good linearity.

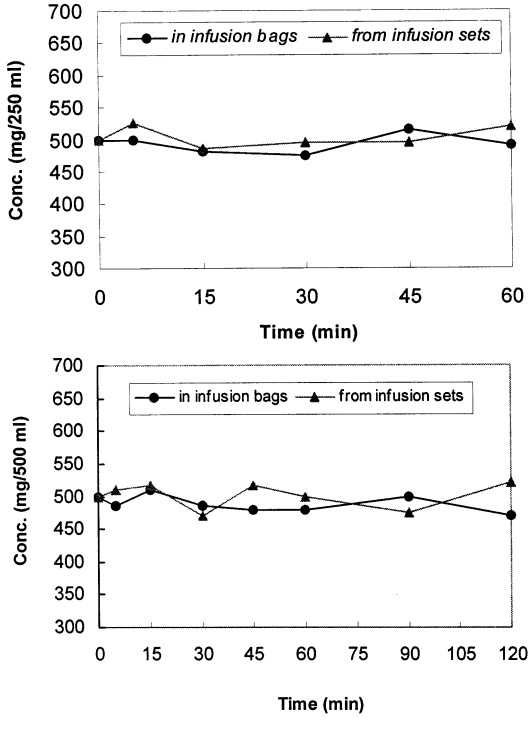

Fig. 1. Concentration kinetics of quiniforme during simulated infusion (1 and 2 h) using plastic infusion bags and sets.

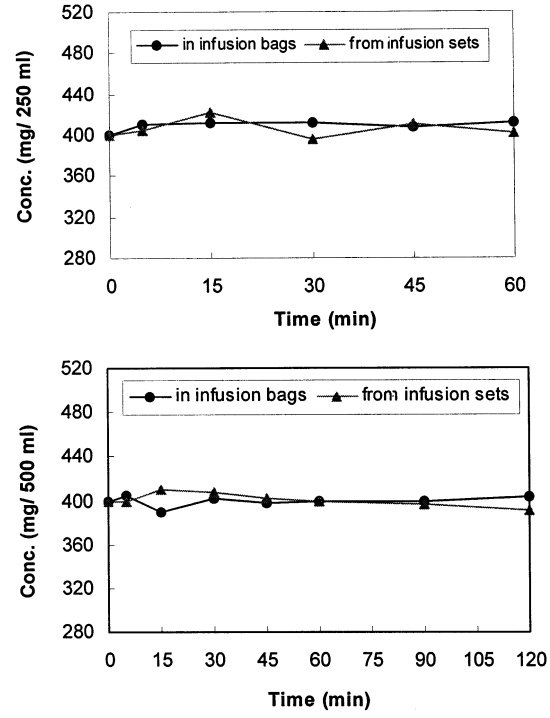

Fig. 2. Concentration kinetics of quinimax during simulated infusion (1 and 2 h) using plastic infusion bags and sets.

For the assay of DEHP, the between-day RSD values were lower than 3.17%. The lower limit of detection was 160 ng ml^{-1}. The calibration curves covered the range of 2.5–20 μg ml^{-1} with a correlation coefficient better than 0.998.

3.2. Stability of quiniforme and quinimax in i.v. fluids during simulated infusion using PVC infusion bags and administration sets

The analysis of each point was performed by HPLC after suitable dilution in the mobile phase in order to fit the calibration curve. For all the quiniforme and quinimax infusion solutions, the initial concentrations were 500 mg/500 ml and 400 mg/250 or 500 ml. All concentrations were measured thereafter at a particular time. Stability was defined as a concentration within 90–105% of the initial concentration, in accordance with the Health Registration of France, the French regulatory agency for drug and drug-related products.

As shown in Figs. 1 and 2, when quiniforme and quinimax were infused through PVC infusion sets from PVC infusion bags for 1 and 2 h, the variation in drug concentration in both the PVC bags and effluent never exceeded 10%. There was no substantial difference between quiniforme or quinimax concentrations at zero time and at any subsequent time intervals. This demonstrates that the drug was not sorbed by the plastic infusion bags and sets during infusion at ambient temperature. No additional peak corresponding to degradation products was observed on chromatograms. No significant difference was observed between quiniforme or quinimax concentrations of solutions collected in PVC bags and from administration sets. In conclusion the quiniforme or quinimax infusions solution are chemically stable for up to 1 and 2 h.

Table 1
Total concentrations (mg/250 ml) of quiniforme and quinimax after storage in plastic bags at room temperature

Storage time (h)	Drugs (infusion solution: dextrose 5%)	
	Quiniforme	Quinimax
0	500	400
1	509	397
2	482	404
3	507	407
4	496	423
5	500	401
6	507	404
7	511	394
8	504	397

Table 2
Total concentrations (mg/250 ml) of quiniforme and quinimax after storage in plastic bags at 4°C[a]

Storage time (h)	Drugs (infusion solution: dextrose 5%)	
	Quiniforme	Quinimax
0	500	400
1	510	401
2	513	409
3	517	376
4	513	374
5	466	384
24	502	403
48	486	ND
72	ND	414

[a] ND, not determined.

Table 3
Total concentrations (mg/250 ml) of quiniforme and quinimax after storage in plastic bags at 45°C

Storage time (h)	Drugs (infusion solution: dextrose 5%)	
	Quiniforme	Quinimax
0	500	400
1	491	413
2	508	404
3	477	390
4	516	394
5	498	380
6	501	396
24	514	397
48	481	417
72	502	401
96	505	407

3.3. Compatibility of quiniforme and quinimax and extent of DEHP leaching from PVC bags during simulated infusion

The amounts of DEHP released after 1- or 2-h perfusion were sampled at the exit from the sets and were less than 2 μg ml^{-1} for quiniforme and quinimax.

3.4. Stability of quiniforme and quinimax in i.v. fluids stored in PVC bags

After storage for 8 h at room temperature, 48 or 72 h at 4°C and 96 h at 45°C without protection from light, there was no substantial difference between quiniforme or quinimax concentrations at zero time and at any subsequent time intervals. The concentrations of quiniforme and quinimax present in solution after various periods of storage in PVC infusion bags at different temperatures are listed in Tables 1–3.

No precipitation of drugs, no alteration and no change of color of the solution were observed

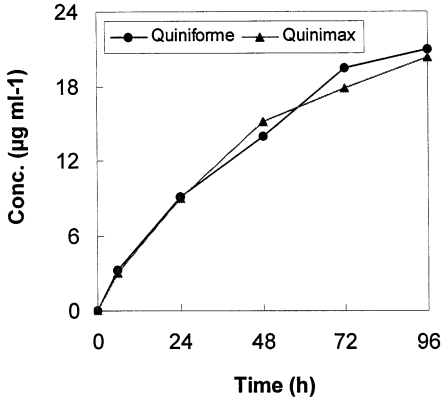

Fig. 3. Graph of concentration against time of DEHP leached from 500-ml polyvinyl chloride (PVC) infusion bags containing 500 mg of quiniforme and 400 mg of quinimax ($n = 2$) at 45°C.

during storage at room temperature, at 4 or at 45°C for quiniforme and quinimax after reconstitution in PVC bags.

3.5. Compatibility of quiniforme, quinimax and extent of DEHP leaching from PVC bags during storage

When quiniforme was stored at 4°C for 48 h, DEHP release was below 4.5 μg ml^{-1}, whereas storage at room temperature for 8 h yielded DEHP concentrations less than 5.5 μg ml^{-1}.

In the case of a 72-h storage period of quinimax at 4°C, the amount of DEHP recovered reached an average value of 13.3 μg ml^{-1} whereas only 4 μg ml^{-1} were released. Quinimax was kept in the bag for 8 h at room temperature.

We have also studied the release of DEHP during storage of quiniforme alone at 45°C in a PVC bag (average of two determinations with 5% dextrose solution) and were unable to detect the presence of DEHP.

Fig. 3 illustrates the release of DEHP observed during a 4-day storage period of quiniforme or quinimax at 45°C in association with other drug. The quantity of DEHP leached was 21 μg ml^{-1} for quiniforme, and DEHP release reached a maximum of 20.35 μg ml^{-1} for quinimax. However, when quiniforme or quinimax were stored alone in a 5% dextrose solution for 4 days at 45°C no DEHP was released. Since the drug mixture added to the bags was strongly lipophilic, the release of DEHP must be due to the presence of the multivitamin cocktail.

4. Conclusion

The results of the present study clearly demonstrate that quinine (quiniforme at 500 mg in either 250 or 500 ml; quinimax at 400 mg in either 250 or 500 ml) remains stable in 5% dextrose solution when delivered from soft Macoflex bags over a 1–2-h perfusion period at room temperature and light conditions. Moreover, quiniforme (500 mg/500 ml) and quinimax (400 mg in either 250 or 500 ml) in a 5% dextrose solution are compatible with the Macoflex bags when stored for 8 h at room temperature and ambient light for 48 or 72 h at 4°C, or for a 4-day period at 45°C without protection from light.

However, a significant release of DEHP reaching up to 21 μg ml^{-1} was observed when quiniforme and quinimax were associated with other drugs during a 4-day storage period at 45°C.

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