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In vitro release and stability of an artesunate rectal gel suitable for pediatric use

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Abstract

The rectal route is indicated to treat patients with rapidly evolving malaria who cannot take oral medication to prevent progression to severe forms of the disease. Improvement can be made in terms of rectal bioavailability and stability of current formulations. We studied a new two-compartment, muco-adhesive gel formulation of artesunate which is adapted for use in children and storage in tropical climates. The formulation contains 50 mg of artesunate per gram of gel. Because of its instability in aqueous solutions, artesunate is in the dry component of the gel with Carbopol® and separate from the liquid phase until reconstitution. Artesunate is stable in the dry blend for 6 months at 45 °C and 60% RH. The gel should be used between 1 and 72 h after being reconstituted.

Artesunate release was measured by with a rapid, simple and reliable HPLC-UV which allowed the analysis of artesunate and dihydroartemisinin with an analysis time at 3 min. The amount of artesunate released over 6 h was 56 ± 0.97%. Compared to the reference suspension, total release and dissolution efficiency were lower and rate of release was slower (time to 50% dissolution 271 ± 21 min), probably because of the higher viscosity of the gel, but the drug release profiles were similar. The calculated *in vitro* release exponent (*n*) value suggested that artesunate is released from the gel by non-Fickian transport.

Keywords: Artesunate; Rectal gel; Malaria; Carbopol® 974P NF; HPLC method; Dihydroartemisinin; Validation

1. Introduction

The artemisinin-type compounds are the most potent anti-malarial drugs available today. They are very safe and well tolerated. Artesunate (ART, Fig. 1), a relatively water-soluble, semi-synthetic derivative of extractive artemisinin, is the most researched of this class (Myint et al., 2004) and is available for oral, injectable and rectal treatment.

If not promptly treated, malaria caused by *Plasmodium falciparum* can evolve rapidly in subjects with inadequate immune response, such as children in endemic areas and travellers, to more severe and lethal complications, including severe anaemia, organ failure lactic acidosis and cerebral malaria. Severe malaria can be fatal even when adequately treated, with mortality rates even in the best facilities across the tropics of 17% with intravenous quinine, and 14% with intramuscular artemether (Stepniewska et al., 2001), although intravenous ART reduced the mortality of paediatric and adult severe malaria in South-East Asia by 35% compared with quinine (Checkley and Whitty, 2007). Furthermore, the majority of malaria deaths occurs outside health facilities and patients in rural areas do not have access to care. Mortality could be significantly reduced if malaria was diagnosed and treated early, when patients can take oral medications (http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf). Unfortunately, this is often not the case, and a child would soon unable to take medicines orally because the disease causes the patient to vomit and become unconscious. At this stage treatment is urgently needed

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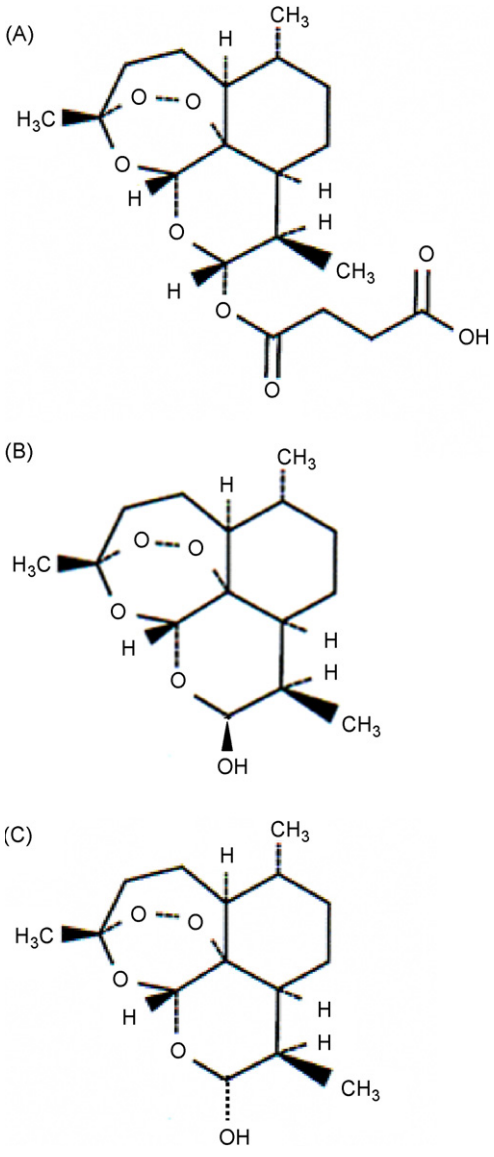

Fig. 1. Chemical structures: (A) ART; (B) β-DHA and (C) α-DHA.

to prevent progression to severe malaria and coma, but an oral medication is no longer of use and injectable treatments are not available outside the health system.

The rectal route would obviate these problems. Rectal formulations of artemisinin compounds have been in use for quite some time, including ART suppositories and soft-gelatine capsules. The latter are as effective as intravenous quinine as one-off treatment for "non-per-os" patients to prevent disease progression (Barnes et al., 2004; Pengsaa et al., 2005) and are recommended by the WHO (http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf). Rectal administration of ART seems to be an easy and earlier treatment specially suitable for children who are unable to take any oral medications. ART by rectal route was well tolerated and there was no evidence of major or minor toxicity. Furthermore this experience and accumulating evidence elsewhere indicate that rectal administration is acceptable in diverse cultures, even in adults. The soft-gelatine capsules used in these experiments at the currently recommended 10 mg/kg dose are 50% bioavailable versus intravenous in moderately severe malaria (Krishna et al., 2001), albeit with high inter-individual variability. On the other hand, this formulation is not without its limitations and can be perfected especially to prevent rejection, prolong shelf-life and reduce variability of absorption.

The purpose of this work was to develop a rectal formulation of ART which is easy to administer to children and is stable in the hot and humid tropical climates. We opted for a mucoadhesive gel as this formulation would be less prone to rejection and proved bioavailable and locally well tolerated with other antimalarials (Fawaz et al., 2004). As ART is readily transformed to dihydroartemisinin (DHA), its bioactive metabolite (Brockman et al., 2000) (Fig. 1) in aqueous solutions (Batty et al., 1996; Gabriëls and Plaizier-Vercammen, 2004), it must be separated from the other aqueous components in a two-compartment formulation. Having in one compartment ART in organic solvent and in the other the neutral gel ensured ART solubility, but not its stability beyond 3 months (Gaudin et al., 2007). We thus opted for a formulation whereby ART was in one compartment with the dry constituents of the gel and ART, separated from a second compartment filled with the liquid components. A dry ART formulation would ensure chemical stability of the active compound until reconstitution when needed for treatment.

Drug release was measured *in vitro*. ART stability was determined both in the dry blend and in the gel after reconstitution.

2. Materials and methods

2.1. Materials

ART was purchased from Knoll BASF Pharma (Liestal, Switzerland) by DNDi (Drug for Neglected Disease initiative) (Geneva, Switzerland). The gel-forming polymer Carbopol® 974P NF was from Noveon (USA). Tween® 80 was from Sigma (St. Quentin Fallavier, France). Acetonitrile isocratic HPLC grade was from Prolabo VWR (Leuden, Belgium). Absolute ethanol was purex analytical grade 99% from SDS (Peypin, France). H_3PO_4 was from JT Baker chemicals (Deventer, Holland). KH_2PO_4, HCOOH and Na_2HPO_4, 12 H_2O were from Merck (Darmstadt, Germany). Dichloromethane stabilized with 0.2% ethanol was from Merck (Munich, Germany). Infrared quality potassium bromide (Merck, Darmstadt, Germany) was used for the KBr pellet technique.

2.2. Preparation of ART formulations

2.2.1. Preparation of the extemporaneous ART gel

ART gel preparation included two steps: (i) separate preparation of the dry blend and the liquid phase and (ii) mixing the two components, extemporaneously.

2.2.1.1. Preparation of the dry and liquid blends. The dry blend was 75 g of ART (mean particle size <150 μm) mixed with 12 g of Carbopol® 974P NF using Turbula blender (10 min).

The liquid phase was a mixture of absolute ethanol (10 g), NaOH 5M (1.64 g) and deionised water (86.36 g).

2.2.1.2. Extemporaneous preparation of the ART gel. 9.42 g of the liquid phase were introduced in a glass vial containing 0.58 g of the dry blend, then vigorously stirred with vortex during 90 s and left at room temperature. The ART gel became homogeneous and ready to use after 1 h. Thus, 100 g of gel contained ART (5 g), Carbopol® 974P NF (0.8 g), ethanol (10 g), water (86.36 g) and NaOH 5M (1.64 g) to neutralize Carbopol® 974P NF.

All determinations on the gel were carried out 1 h after its preparation when a gelled suspension of ART crystals was homogenously white.

2.2.2. Preparation of ART suspension

Under permanent magnetic stirring, 5 g of ART were added to a mixture of 10 g of absolute ethanol, 0.1 g of Tween® 80 and 84.99 g of deionised water.

2.3. Measurement of pH and dynamic viscosity of the ART gel

A Knick pH-Meter was used to measure the pH of the ART gel at 22 °C. The electrode was inserted into the sample 5 min prior to taking the reading.

Viscosity of the ART gel was measured in a cone viscometer (Model DV-3, Brookfield, USA) using a T-D spindle rotated at a speed of 50 rpm. A 50 g sample of ART gel was placed in a 100 ml beaker. Viscosity was recorded at 20 °C and then temperature was raised to 37 °C by a water jacket through which water was circulated at 37 °C from a thermostat bath. Each measurement was repeated three times and the mean value calculated.

2.4. In vitro drug release kinetics from ART formulations

In vitro release of ART from formulations (gel and suspension) was monitored by the USP basket method (Sotax AT 7, Switzerland) at a rotating speed of 100 rpm in 1000 ml phosphate buffer medium (pH 7.4) at 37 ± 0.5 °C. Phosphate buffer was obtained by dissolving of Na_2HPO_4, $12H_2O$ (19.167 g) and KH_2PO_4 (1.787 g) in 1000 ml of deionised water and adjusted to pH 7.4 with either 80% *o*-phosphoric acid or NaOH 1 M. A 2 g sample of each ART formulation was introduced in a dialysis tubing (Spectra/Pore®, MC CO 12,000–14,000, 16 mm diameter, from Spectrum Laboratories, Rancho Dominguez, CA, USA) after one end was tightly tied. Then, the other end was sealed to obtain a bag which was immediately put into a basket. Six bags were prepared for each formulation. At defined times, samples (0.25 ml) were withdrawn and ART concentrations were determined using ART quantification method by HPLC (Gaudin et al., 2007). The dissolution time of 50% (DT50) of the initial drug content of each tested formulation was calculated from the dissolution profiles. Dissolution efficiency (ED%) was calculated over 6 h (Khan, 1975).

2.5. Stability study of ART in the dry blend and in the gel

2.5.1. ART quantification by HPLC

ART was quantified using a HPLC method previously developed (Gaudin et al., 2007). The HPLC method consists of an X-Terra RP C18 column (50 mm × 3 mm i.d., 3.5 μm particle size) with an isocratic mobile phase composed by acetonitrile: potassium phosphate buffer at 10 mM (40:60, v:v; pH 2.9) at 0.7 ml min^{-1}, with UV detection at 220 nm. The sensibility of this method is 0.012 and 0.060 mg/ml for ART and DHA, respectively.

2.5.2. Stability study of ART in the dry blend

2.5.2.1. Sampling. Immediately after preparation, the dry blend was distributed under vacuum in 5 ml glass vials (0.29 g/vial) with rubber stopper. Vials containing dry powder were stored in a temperature and humidity controlled chamber (45 °C and 60% RH). Every two weeks, 5 samples were checked of ART (Gaudin et al., 2007). IR measurements were performed on dry blend samples at the beginning and at the end of the stability study. In addition, and ART gel was prepared from this dry blend; the dynamic viscosity and the pH of the gel were measured.

2.5.2.2. Validation of the ART quantification HPLC method for the dry blend. The range of solutions containing the dry blend prepared for the validation of the HPLC method is detailed in Table 1. All samples were prepared by weighing. The stock solutions were sonicated 10 s before centrifugation at 5000 rpm during 5 min. Then the solutions were diluted in acetonitrile:water (40:60, v:v) and analysed by HPLC (Gaudin et al., 2007). The linearity obtained with these data was assessed and compared to that obtained from solutions containing ART only (Table 2).

2.5.2.3. IR experiment. Spectra were obtained with a Mattson model Genesis II FTIR™ spectrometer controlled by Winfirst software from Mattson instruments Inc. (Win. Lab. Instruments, Bagnolet, France). Calibrated pellets for each sample in proportion of 1% in KBr were performed and recorded in the transmittance mode with 20 scans acquired at 2 cm^{-1} resolution, between 4000 and 400 cm^{-1}.

2.5.3. Stability study of ART in the ART gel

2.5.3.1. Gel samples. As the ART gel is not ready for use until after 1 h of reconstitution, and ART is very unstable in aqueous media, ART stability in the gel was tested at room temperature in order to establish the maximum useful life-span of this rectal gel after reconstitution. Vials with rubber stoppers containing ART gel were kept at 22 °C. For the procedure of ART extraction from the gel, a reduced amount of ART gel was prepared using 0.116 g of dry blend mixed with 1.884 g of liquid phase.

2.5.3.2. Liquid/liquid extraction procedure. The entire amount of ART gel prepared was diluted with twice 15 ml of aqueous formic acid (pH 2.5) and transferred into decantation flask. Then ART was extracted with 3 ml × 30 ml of dichloromethane. The

Table 1
Preparation of the ART solutions for the validation study

Solution	ART%				
	50	70	90	100	110
Stock solution Weight (g) of the dry blend in 9 g of absolute ethanol (Corresponding ART mass (g))	0.174 (0.15)	0.244 (0.21)	0.313 (0.27)	0.348 (0.30)	0.383 (0.33)
Analytical solution Final concentration (mg ml^{-1}) (0.5 g of stock solution in acetonitrile:water (40:60), Qsp 25 ml)	0.33	0.47	0.6	0.67	0.73

Table 2
Comparison of validation results

	ART[a]	ART + Carbopol
Linearity		
Correlation coefficient r	0.9998	0.9919
Slope	866.41	833.05
Intercept	9.58	20.14
Accuracy		
% Recovery	99.99	99.99
% R.S.D.	0.20	0.88
Precision		
Intra-day precision (% R.S.D.)	0.70	1.15
Day-to-day precision (% R.S.D.)	0.74	1.56

[a] Results from reference (Krishna et al., 2001).

phase separation was accelerated by centrifugation for 5 min at 5000 rpm. Then organic layer was evaporated to dryness with a rotary evaporator. The extraction yield was 95.4% (0.8% R.S.D.).

2.5.3.3. Quantification of ART in gel extract. The dry residue obtained from the extraction procedure was dissolved in 3 g of ethanol. Then 0.5 g of this solution was weighed, placed into a 25 ml flask and completed with acetonitrile:water (40:60, v:v) before injection into the LC system (Gaudin et al., 2007). The quantification method was not validated for ART analysis in gel samples as the time required for each sample extraction was ≥1 h, and this measurement would not be performed in routine analysis.

2.6. Statistics

Data from the *in vitro* drug release study were expressed as mean value ± S.D. Statistical analysis was performed using ANOVA test (Scheffe test). Mean differences were considered statistically significant at a level of $P < 0.05$.

3. Results and discussion

3.1. In vitro kinetic drug release of ART

The *in vitro* kinetic drug release study was performed on an ART gel with a pH of 5.7 and a viscosity of 5490 mPas (20 °C) (viscosity was 4445 mPas at 37 °C). An ART suspension was used as reference. Profiles of drug release from the ART gel and the suspension are shown in Fig. 2. Drug release was generally delayed by the addition of the polymer and there was no burst effect. The DT50 of the gel (271 ± 21 min) was longer than with the suspension (230 ± 37 min) ($P = 0.0377$) and the cumulative amount of drug released over the 6 h test lower (56 ± 0.97% versus 66.67 ± 4.61%, respectively ($P = 0.0002$), while efficiency of dissolution (ED%) was the same (37.04 ± 1.38 and 38.4 ± 4.55, respectively $P = 0.4972$).

ART dissolution profiles obtained from the ART gel and the suspension were compared using the model independent mathematical approach proposed by Moore et al. (Moore and Flanner, 1996) and recommended by the FDA and the European Guidance for quality control. It consisted in comparison of the difference factor (f_1) and the similarity factor (f_2) (Shah et al., 1998) calculated using the following equations (Moore and Flanner, 1996):

$$f_1 = \frac{\sum_t^n (R_t - T_t)}{\sum_t^n R_t} \times 100 \quad (1)$$

$$f_2 = 50 \times \log\left(\left[1 + \frac{1}{n}\sum_{t=1}^{t=n}(R_t - T_t)^2\right]^{-0.5} \times 100\right) \quad (2)$$

where R_t and T_t are the cumulative percentages dissolved at each of the selected n time points of the reference and test product, respectively. The calculated values for f_1 (3.15%) and for f_2 (62.2%) showed that dissolution profiles of ART were similar from the ART gel and suspension.

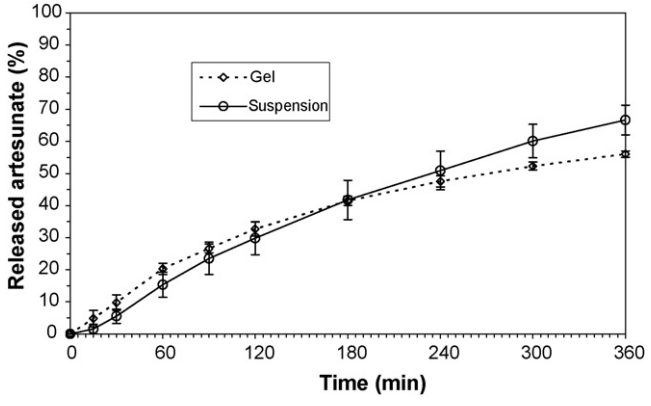

Fig. 2. *In vitro* dissolution profiles of ART from gel and suspension (mean ± S.D.).

Table 3
Calculated correlation coefficients

	Zero order	First order (Wagner)	Higuchi model	Peppas model
Gel	0.9634	0.8172	0.9531	**0.9825**
Suspension	0.9880	0.8103	**0.9989**	0.9866

The description of dissolution profiles by a model function was attempted using four mathematical models namely zero and first order kinetics (Wagner, 1969), the Higuchi square-root model (Higuchi, 1961) and the Krosmeyer–Peppas model (Korsmeyer et al., 1983; Peppas, 1985). The correlation coefficients calculated for each of these (Table 3) indicated that ART release was better described by the Peppas model for the ART gel and the Higuchi model for the suspension.

To understand the release mechanism of ART from the ART gel, we attempted to describe the rate of release using the semi-empirical model of Korsmeyer et al. and more precisely the equation proposed by Peppas:

$$\frac{M_t}{M_\infty} = at^n \tag{3}$$

or the logarithmic form of this equation:

$$\log\left(\frac{M_t}{M_\infty}\right) = \log(a) + n\log(t) \tag{4}$$

where (M_t/M_∞) is the fraction of released drug at time (t), (a) is a characteristic constant of the dosage form and (n) is the release exponent indicative of the drug release mechanism. The kinetic parameters (n and a) were calculated from the plot of $\log(M_t/M_\infty)$ versus $\log(t)$ where $(M_t/M_\infty) < 0.6$.

The mean (n) value obtained from the ART gel was of 0.6873 suggesting that ART was released from the gel formulation following a non-Fickian transport. Moreover, the (n) value obtained from the suspension (1.079) suggested a super case-II transport. The a values reveal that drug was released slower from the ART gel (18.34%/h^n) than from the suspension (13.023%/h^n). This difference could be attributed to the higher ART gel viscosity. A similar result has already been reported for acetaminophen in liquid suppositories (Choi et al., 1998; Kim et al., 1998), for insulin in liquid suppository (Yun et al., 1999) and for quinine in rectal gel (Fawaz et al., 2004) and was attributed to the high gel strength of the liquid suppository.

3.2. ART stability study

3.2.1. ART stability study in the dry powder blend

Since the ART gel was extemporaneously prepared by mixing together the dry blend containing ART and the liquid phase, the stability of ART was established in the dry blend. To determine ART stability in the dry gel, we used the same chromatographic method for ART quantification developed previously (Gaudin et al., 2007), validated for samples with Carbopol® 974P NF. To avoid carbopol injection in the chromatographic system, ethanol was added to the dry blend samples thus solubilizing ART but not carbopol which was removed by centrifugation. In order to establish if excipient (i.e. carbopol) and/or sample manipulation interfered with ART analysis, a blank sample was prepared with carbopol and analyzed following the same procedure. No peak was observed at ART retention time (2.76 ± 0.02 min) (Fig. 3A). The specificity of the chromatographic method was then tested for samples containing carbopol.

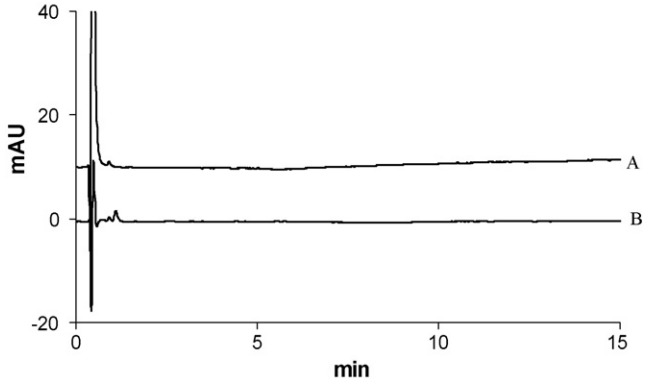

Fig. 3. Blank extract of carbopol (A) and neutral gel (B).

Linearity, accuracy and precision of the HPLC method were assessed with five ART solutions containing carbopol and compared to the values obtained with ART solutions without carbopol (Table 2). The slopes and the intercepts were not significantly different ($P = 0.05$), while both intercepts were comparable to zero ($P < 0.05$). Therefore ART can be quantified in a sample of dry blend using a solution of ART at 100% as the reference.

ART remained stable in the dry blend after 3 months at room temperature (100.8% recovery and 0.5% R.S.D.). After storage at 45 °C and 60% RH for 175 days, the percentage of recovery was 99.6 and 0.5% R.S.D. Over this period, the minimum and the maximum percentage of recovery were 98.8 and 100.8%, respectively. And the maximum R.S.D. was 1.2%. The pH and viscosity of gel using aged-dry blend were found unchanged. The IR spectra of fresh-prepared dry blend and blend kept one leaved at 45 °C and 60% RH for 175 days were similar (see Fig. 4). We concluded that ART is stable in the dry blend in tropical conditions.

3.2.2. ART stability in the ART gel

Since ART is unstable in aqueous media and gel administration occurs 1 h after extemporaneous preparation with the aqueous liquid phase, ART stability in the ART gel after reconstitution was studied following liquid/liquid extraction from the ART gel using the HPLC-analytical developed in reference (Gaudin et al., 2007).

In order to verify the specificity of the chromatographic method, the same extraction procedure was carried out with a neutral gel prepared without ART (Fig. 3B). No peak was observed on the chromatogram at the ART retention time (2.76 ± 0.02 min).

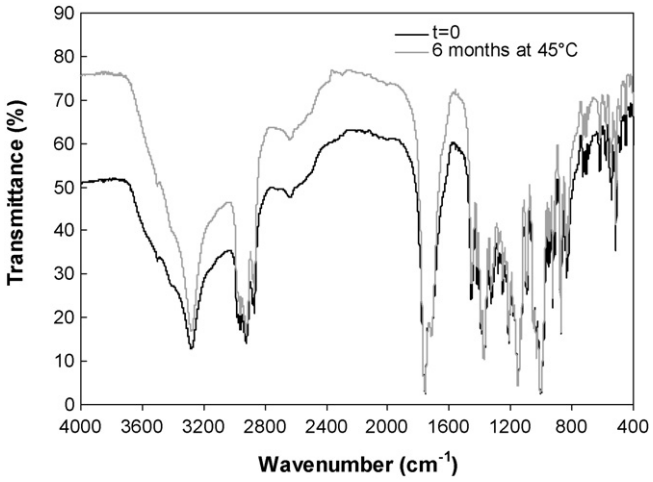

Fig. 4. IR spectra of ART:carbopol blend at 5:0.8 (w:w).

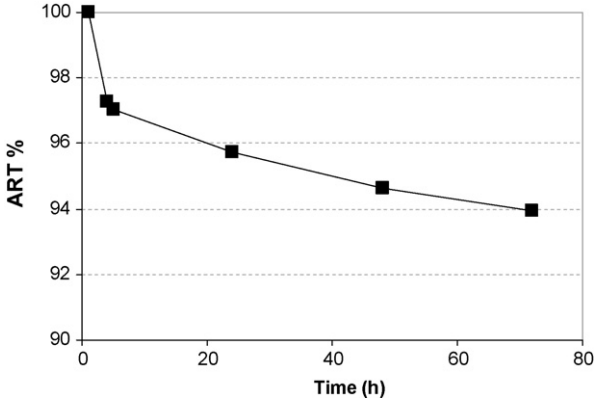

Fig. 5. ART content in gel depending on its lifetime.

The yield of ART extraction was first established after 1 h following the extemporaneous preparation. Fig. 5 shows that after 72 h the remaining amount of ART was 94%. DHA was the only product detected in 7-day old gels. Fig. 6 shows the peaks of ART, α-DHA and β-DHA at 2.76, 1.48 and 2.25 min, respectively. DHA peaks were identified by the standard injection of these products (Gaudin et al., 2007). In conclusion ART remains stable in the gel for 3 days. This would allow some flexibility with the use of the product after reconstitution.

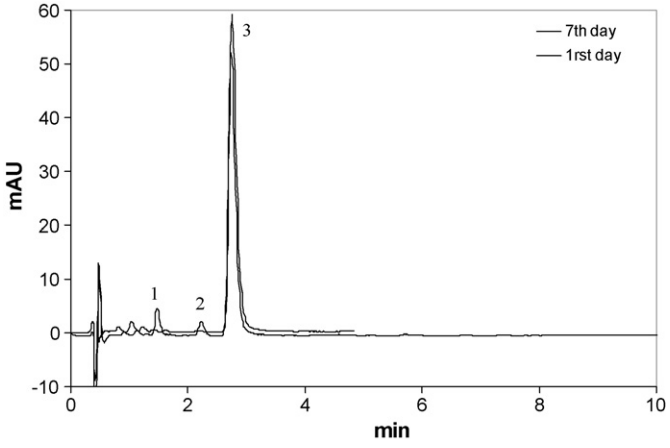

Fig. 6. Results of the stability study of ART in gel. (1) α-DHA; (2) β-DHA and (3) ART.

4. Conclusion

This work proposes a new, two-compartment formulation of ART for rectal administration to treat malaria which is adapted for use in children and stable in tropical climates. The gel should be administered between done 1 and 72 h after reconstitution which ensures a flexible use of this new formulation, especially for clinical evaluation.

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