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New liquid oral formulations of hydroxychloroquine: a physicochemical stability study

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Abstract

Objectives: Hydroxychloroquine (HCQ) presents many drug properties that increase its therapeutic use. There are, indeed, different research pathways in numerous autoimmune, inflammatory, and infectious diseases, as well as in cancerology. HCQ is only marketed as HCQ sulfate in film-coated or coated tablets for oral use. No pediatric liquid form is currently available on the market. The purpose of the present study is to develop oral liquid formulations for HCQ at 50 mg/mL with two different oral vehicle suspensions, namely ORA-Plus®/ORA-Sweet® (ORA) and Syrspend® SF PH 4 (SYR).

Methods: The suspension stability was assessed in different storage conditions (4 and 25 °C). A high-pressure liquid chromatography (HPLC) stability-indicating method with UV detection was developed to determine HCQ concentrations in the different formulations, and detect potential degradation products. Physical parameters, e.g. pH and osmolality were also monitored during the period of the stability study.

Results: HCQ concentration, osmolality, and pH remained stable for 90 days at 4 and 30 °C for HCQ in 50% ORA-Plus®/50% ORA-Sweet®. For HCQ suspension in

SYR, the suspension remained stable 90 days at 4 °C and 60 days at 30 °C.

Conclusions: For all preparations, no significant physical or chemical modification was noticed during the period of the study.

Keywords: an antimalarial agent; HPLC; hydroxychloroquine sulfate; oral suspension; stability study.

Introduction

Hydroxychloroquine (HCQ) is an old molecule from the class of 4-aminoquinolines. It was originally developed as an antimalarial drug [1]. Many additional drug properties of HCQ increase its therapeutic use. There are, indeed, different research pathways in numerous autoimmune, inflammatory, and infectious diseases, as well as in cancerology, with variable outcomes depending on the disease [1–3]. It is a weak base that interacts with phagocytic functions by raising the intracellular pH, leading to an alteration in the selective autoantigens presentation. HCQ inhibits the production and the release of cytokines by T cells: IL-1, IL-2, IL-6, IL-18, TNF- α , and IFN- γ . These effects are achieved by inhibiting the activation of the *toll-like receptor* involved in innate immunity and autoimmune diseases [1–3]. HCQ is associated with multiple metabolic benefits. It improves lipid profiles and lowers blood glucose levels [1–3]. It also decreases platelet aggregation. Furthermore, HCQ is a chloroquine derivate that accumulates in lysosomes, which leads to neutralization of the lysosome and inhibition of autophagy [2, 4, 5]. In light of these data, in clinical practice HCQ is recommended for the management of systemic lupus erythematosus [5–9] and rheumatoid arthritis [10, 11]. Numerous studies report its efficacy more or less established in other diseases such as scleroderma [12], sarcoidosis [13], Gougerot–Sjorgren’s syndromes [14], antiphospholipid syndrome [15], disseminated interstitial lung disease [16], idiopathic thrombocytopenic purpura [17], preeclampsia, repeated spontaneous abortions [18] and diabetes [19]. HCQ is also evaluated in infectious diseases such as Q fever and Whipple’s disease [20, 21]. The combination of HCQ with chemotherapies is being tested in

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oncology, particularly as a strategy for modulating autophagy in order to reduce chemoresistance [22, 23].

HCQ pharmacokinetics properties are characterized by a good oral bioavailability of about 75%, a good diffusion into tissues with very important volumes of distribution (up to 44,000 L) with long mean residence time (1,300 h), and a long plasma elimination half-life of approximately 40 days with a high interindividual variation [1, 3]. HCQ is mainly eliminated by the kidneys (40–60%), with a very long tissue half-life. There is no evident therapeutic concentration threshold. For example, on the suppressive treatment of malaria, suggested plasma or serum concentrations should be up to 10 ng/mL, while on systemic lupus erythematosus, target concentrations should be above or equal to 500 ng/mL [24].

Because of this variability, pharmacological monitoring is recommended in order to obtain effective plasma concentrations and to prevent possible overdosage which may contribute to the occurrence of adverse events, in particular ocular events [1–3]. HCQ reaches its steady-state concentration by about 6 months of therapy [1].

Recommended adult doses range from 100 to 600 mg per day. In pediatrics, the doses for the different diseases vary from 2 to 13 mg/kg/day divided into 1 or 2 doses according to the indications [25, 26].

In all countries where HCQ is currently marketed, the drug is available as HCQ sulfate in film-coated or coated tablets for oral use. No other pharmaceutical form is currently available on the market. For pediatric therapies, HCQ tablets can be crushed but still have an off-flavor bitter taste [27]. In fact, tablets are not indicated for children younger than 6 years, and old persons are sometimes not able to swallow solid pharmaceutical forms. HCQ is a compound belonging to class 1 of the Biopharmaceutics Classification System (BCS) proposed by L.G Amidon in 1995 to predict the bioavailability of oral drugs [28]. According to BCS, class 1, is the most favorable situation corresponding to high permeability and solubility drugs. Thus, modification of HCQ oral formulation is unlikely to change its bioavailability [29]. In light of the various points discussed, the formulation of an oral liquid pharmaceutical form of HCQ and its stability evaluation is essential. This must ensure the safety of HCQ administration in adult or pediatric patients who cannot swallow the tablets. An oral liquid solution with a concentration of 50 mg/mL allows covering both adult and pediatric dosages with adapted volumes. The availability of an oral liquid form would also allow adjustment of doses in light of drug monitoring, which is necessary considering the interindividual variation of plasmatic dosage of HCQ and to prevent some adverse events.

As explained above oral liquid forms are not commercially available, but HCQ stability in Oral Mix[®] and Oral

Mix SF[®] bases, at a concentration of 25 mg/mL is already reported [29]. Oral Mix[®] and Oral Mix SF[®] are suspending bases products of Medisca Pharmaceutique Inc, mainly available in North America, while in Europe, ORA[®] and Syrspend[®] products, for instance, are available. All of these products' composition and physicochemical properties are summarised in Table 1.

As compared to Syrspend[®] SF PH4, ORA[®] and Oral[®] ranges are more similar in term of qualitative composition, but they differ from their antifoaming agent: dimethicone for ORA[®] and simethicone for Oral[®]. Also, ORA-Plus[®] contains calcium sulfate and trisodium phosphate as buffering agents, while Oral Mix[®] contains sodium citrate. They also differ from their osmolality, 3,200 mOsm/kg for ORA-Sweet[®] while Oral Mix[®] osmolality is 1,231 mOsm/kg. Furthermore, no data concerning the quantitative composition of these bases are available.

For all these reasons, extrapolating HCQ stability from Oral[®] Mix [31] to ORA[®] is, according to us, not possible. Thus, there was a need to study the stability of HCQ in alternative ready-to-use bases. We here propose stability studies in two different bases at a concentration of 50 mg/mL of HCQ: 50/50 mix of ORA-Plus[®] – ORA-Sweet[®], and in liquid Syrspend[®] SF PH4, with the idea to offer maximum solutions to pharmacists for compounding preparations.

Material and methods

Materials and reagents

As HCQ is not available as a raw pharmaceuticals grade powder, the oral suspensions were performed from commercially available HCQ PLAQUENIL[®] 200 mg tablets (ingredients: Corn starch, Hypromellose, Lactose monohydrate, Macrogol 4000, Magnesium stearate, Povidone, Titanium dioxide) batch number: ORO27 exp. 12/2022 (Sanofi, Paris, France). Oral suspension vehicles used were ORA-Plus[®] and ORA-Sweet[®] (INRESA, Bartenheim, France) or Syrspend[®] SF PH4 (Fagron, Thiais, France). For the analytical validation method, HCQ used was Pharmacopoeia analytical grade, provided by ACROS organics (Geel, Belgique). All ingredients were of Pharmacopoeia grade.

For HPLC, methanol (Hipersolv Chromanorm, VWR, Fontenay sous Bois France), potassium dihydrogen phosphate (ACROS Organics, Geel, Belgique), trimethylamine, sodium heptanesulfonate (Sigma-Aldrich, Saint-Louis, USA) were used. Water was obtained from a Prima reverse osmosis system (Elga Labwater, Antony, France). Sodium hydroxide, chlorhydric acid (VWR, Fontenay sous Bois France), H₂O₂ 30% (Carlo Erba Reagent, Val de Reuil France) were used for the forced degradation study. All reagents and solvents were of analytical grade.

For concentration measurements with the HPLC method, the system used a Perkin Elmer Series 200 pump, an injector, and an oven. The detector was a diode array detector (Flexar PDA detector, Perkin Elmer, Waltham, USA) operating between 200 and 700 nm. Chromera software (v4.1.0) (Perkin Elmer, Waltham, USA) was used

Table 1: Comparison of ready to use liquid bases for oral suspension compounding.

Composition	ORA-Plus®	Ora-Sweet®	Syrspend® SF pH 4	Oral Mix®	Oral Mix® SF
Purified water	X	X	X	X	X
Modified food starch			X		
Microcrystalline cellulose	X			X	X
Carboxymethylcellulose sodium	X			X	X
Xanthan gum	X			X	X
Carrageenan	X			X	X
Calcium sulfate	X				
Trisodium phosphate	X				
Citric acid	X	X	X	X	X
Malic acid			X		
Sodium phosphate	X	X			
Dimethicone	X				
Sucrose		X		X	
Sucralose			X		
Glycerin		X		X	X
Sorbitol		X		X	X
Sodium saccharine					X
Sodium citrate			X	X	X
Cherry flavor			X	X	X
Sodium benzoate			X		
Potassium sorbate	X	X		X	X
Methylparaben	X	X		X	X
Propylparaben					X
Simethicone			X	X	X
Citrus-berry flavor		X			
Physico-chemical properties					
Appearance	Translucent, milky white, thixotropic liquid,	Clear liquid with slight tint	Hazy with translucent syrup	Off-white aqueous	Off-white aqueous
pH	4–4.5	4.2	4.2	4–5	4–5
Osmolality, mOsm/kg	157	3,240	<50	1,231	795
Viscosity, cps	Thixotropic range 1,300–6,700 at 25 °C via Brookfield viscosimeter	Not available	3,290	100–350	100–350
Taste	No taste	Sweet citrus-berry flavor	Cherry	Cherry	Cherry
Flask volume, mL	473	473	473	473	473

to quantify the peaks of the chromatograms. Development of stability indicating method was performed with several types of equipment to obtain forced degradation of HCQ. Indeed, light degradation was accomplished with a UV-A exposition of 366 nm under a 300 $\mu\text{W}/\text{cm}^2$ intensity (Chromato-Vue system model CC-20, Ultra Violet Product, Upland, California). Heat degradation was performed with a lab oven at 80 °C (Mettler, Schwabach, Germany). The two different formulations were stored in different conditions: either in a refrigerator meaning temperature between 2 and 8 °C or in a climatic chamber fully qualified and validated according to ICH with a temperature of 30 °C $\pm$ 2 °C under 65% $\pm$ 5% relative humidity (Mettler, Schwabach, Germany).

Preparation of oral HCQ suspensions

HCQ tablets were crushed in a mortar to obtain a fine powder. As PLAQUENIL® powder represents a negligible amount of the

preparation (<1% w/w), it was not necessary to adjust the final volume of the suspension vehicle. Indeed, progressive incorporation of the total volume of 50% ORA-Plus® then 50% of ORA-Sweet® was simply performed. A similar compounding process was performed for the preparation with Syrspend® SF PH 4.

Study design and sample storage

All formulations of HCQ suspensions were performed at a target concentration of 50 mg/mL, and were packaged in 30 mL amber type I glass bottle (COOPER, Melun, France) with bakelite cap (COOPER, Melun, France). Amber glass bottle is necessary to avoid HCQ photodegradation [32]. At predetermined time points (0, 1, 3, 7, 14, 30, 60, and 90 days), bottles were shaken, before each sample preparation for the study. One sample per storage condition was tested. Storage was performed in two different conditions: refrigerator or climatic chamber.

Analyses performed

Samples preparation for analytic assays: Previous to starting assays, the absence of interaction between ORA-Sweet®/ORA-Plus® or Syrspond® SF PH4, and HCQ was tested. Indeed chromatographic assay was performed just with HCQ without matrix such as oral suspension vehicle, which could interfere with the assay. To check this, chromatograms of pure HCQ powder and HCQ suspension in the different formulations were compared. Moreover, two standard curves, one realized with HCQ and one with HCQ in oral suspension vehicle were compared.

For each checkpoint of stability study, the concentration of HCQ suspension at 25 or 50 mg/mL was determined by diluting respectively 1 mL or 0.5 mL sample with 25 mL of aqueous mobile phase (Phase A) and put in an ultrasound bath for 15 min. In order to remove vehicles (ORA-Sweet®/ORA-Plus® or Syrspond® SF®), 4 mL of this diluted mix were centrifuged for 10 min at 2,860 g. Then, 0.5 mL of the supernatant was diluted with 0.5 mL of the aqueous mobile phase (Phase A). We added an internal quality control (IQC) at 0.5 mg/mL for each run day assay.

Chromatographic conditions: An HPLC method was developed after the adaptation of a previous work [33] and European Pharmacopeia 10th Ed (monography n°2849) [34]. The mobile phases consisted of an aqueous phase (Phase A) composed of a mixture of sodium dihydrogen phosphate buffer, sodium 1-pentanesulfonate (11.3 mM) which pH was adjusted with trimethylamine solution to pH 7.0, and an organic phase (Phase B), composed of methanol. This mobile phase was a binary mixture obtained after using a gradient elution mode at a flow-rate of 1.2 mL/min. A linear gradient elution was programmed as 90% A-10% B (v/v, 0 min), 90% A-10% B (v/v, 0.2 min), 45 % A-55% B (v/v, 10 min), 90% A-10% B (v/v, 15 min). A C18 ODS HypersilPhenyl (100 mm × 4.6 mm, 3 µm) (Thermo-Scientific, Villebon-sur-Yvette, France) was used and maintained at 40 °C. The sample injection volume was 10 µL and the analysis time was 17 min. HCQ detection and quantification were processed at 254 nm and 3D chromatograms were acquired to allow post-processing at a wavelength between 210 and 400 nm in order to detect potential degradation products.

Forced degradation conditions (of HCQ and oral vehicles): An analytical method is stability-indicating if it is able to distinguish the drug from its degradation products. To check this property, HCQ was submitted to different degradative conditions (alkaline, acidic, light, oxidation, temperature), and the degradation of HCQ was quantified, then the degradation products were sought [35]. For the following steps, a 2.5 mg/mL HCQ solution was performed by dissolving analytical grade HCQ in demineralized water. The alkaline degradation was performed by adding a NaOH solution (1 M) in the 2.5 mg/mL HCQ solution (1:1 v:v) and neutralizing it after 3 h with a hydrochloric acid (1 M) solution. The acidic degradation was carried out by adding a hydrochloric acid solution (1 M) in the 2.5 mg/mL HCQ solution (1:1 v:v) and neutralizing it after 3 h with NaOH solution (1 M). The light degradation was accomplished using UV-A exposition of 366 nm under a 300 µW/cm² intensity for four days. Oxidation degradation was performed by adding the H₂O₂ 30 vol solution (equivalent to 9% w/w) in the 2.5 mg/mL HCQ solution (1:10 v:v) for one day. The temperature degradation study was carried out with an HCQ solution (2.5 mg/mL) placed in a lab oven at 80 °C for five days.

Finally, to complete this forced degradation study, it was decided to add a specific degradation study of HCQ suspension in its oral suspension vehicles. HCQ suspension was incubated with sodium hydroxide 1 M in a lab oven at 80 °C for 60 and 240 min (n=3) for each vehicle's suspension, ORA-Plus®/ORA-Sweet®, and Syrspond® SF PH4.

Others analyses performed

Organoleptic appreciation: The odor, appearance, and color of the preparation were assessed. Odor determination was performed by smelling the suspensions. Visual examination was performed to detect a potential crystallization or color change.

pH study: Measurements were performed using a pH meter Mettler Toledo FP20 (Mettler-Toledo, Columbus, USA). It was carried out for each checkpoint of stability study, after coming back to ambient temperature, on 500 µL samples of suspension.

Figure 1: Chromatogram of HCQ reference solution in water at 0.5 mg/mL.

The pH of each suspension within every storage condition was monitored from the initial day of experience to the end of the study.

Osmolality: Measurements of osmolality were performed using an osmometer Vapro® (EliTech group, Puteaux, France). It was carried out for each checkpoint of stability study, on samples of 10 µL of suspension.

The osmolality of each suspension within every storage condition was monitored from the initial day of experience to the end of the study.

Data analysis and acceptability criteria

Method validation for high liquid chromatographic study: Method validation was performed according to the International Conference on Harmonization [36] (ICH Q2 R1). The linearity of the method was evaluated by three standard curves performed on three different days from 0.1 to 1 mg/mL (0.1, 0.25, 0.5, 0.75, and 1 mg/mL). The method was considered as linear if the correlation coefficient was over 0.99 for the mean standard curve. The accuracy of the method was assessed using nine determinations of three different concentrations (0.3, 0.45, and 0.625 mg/mL) measured three times a day, for three days. The accuracy was measured as the difference between the mean and the accepted true value. Then the coefficient of variation (CV) of accuracy was calculated from the ratio of the difference between true and observed value over the true value expressed in percentage. For each concentration, CV had to be less than 10% to be accepted. The repeatability was assessed by measuring a 0.45 mg/mL solution six times. For intermediate precision, this concentration was determined six times a day for three days. Repeatability and intermediate precision were determined using the standard deviation of the repeated assays; the threshold value for acceptability was 5%.

Working standard solutions for the calibration curves were prepared by dissolution of HCQ in the aqueous mobile phase, to reach a final HCQ concentration of 0.1–0.25–0.5–0.75 and 1 mg/mL.

Stability study criteria: HCQ formulations are considered stable if physical and chemical characteristics have not significantly changed: pH was considered stable if its measure was found between plus or minus 0.2 units of initial pH measurement (pH meter precision: ± 0.1 pH unit). For the osmolality study, a change of less than 10% around the initial osmolality measurement was considered acceptable.

HCQ formulation was considered stable if HCQ concentration did not fall under 90% of initial concentration, without any specific degradation products found according to forced degradation.

Results

Assay chromatographic characteristics

The retention time of HCQ was found to be 6.20 min (Figure 1).

The three standard curves were very similar and the mean equation of the relationship between HCQ concentration (y) and peak area (x) was: $y = 0.00006x + 5.4875$. The correlation coefficient (r^2) was found greater than 0.999. The accuracy of the analytical method was 4.87, 3.41 and 3.59% respectively for 0.3, 0.45 and 0.625 mg/mL concentrations. The repeatability of the method was systematically inferior

to 5% (2.25, 0.9 and 0.49% for the day 1, 2 and 3 respectively) and the intermediate precision was evaluated to be 1.21%. Limit of quantification determination is not necessary in the case of a stability study whose objective is to quantify near 100% of HCQ concentration.

Degradations were observed just for three conditions (alkaline, oxidation, and light) with approximatively 10–30% of HCQ degradation, and the apparition of degradation products on chromatogram (Figure 2). Conversely, we observed no modification of chromatogram for acid or heat stress degradation conditions.

Figure 2: Examples of the chromatogram of HCQ standard solution after degradation in different conditions: oxidation condition (H_2O_2 30 V, 24 h) (A) and alkaline stress condition (NaOH 1 M, 3 h) (B).

Figure 3: Chromatograms of fresh HCQ suspensions on ORA-Plus/ORA-Sweet® (A), and Syrspend® SF PH4 (B). Chromatograms of HCQ suspension on ORA-Plus/ORA-Sweet® (C, E) and Syrspend® SF PH4 (D, F), after stress degradation alkaline (NaOH, 1 M) associate with heat (80 °C) respectively after 1 h (C, D) and 4 h (E, F).

For forced degradation of oral suspension vehicles alone, we can see for ORA-Plus®/ORA-Sweet® and Syrspend® SF PH4 the chromatogram in Figure 3. These results demonstrated the degradation product peaks are separated from the HCQ peak.

Table 2: Relative concentrations of HCQ (%) in each oral vehicle suspension after NaOH (1 M) and Heat (80 °C) stress condition during 1 or 4 h(%).

	HCQ suspension ORA-Plus®/ ORA-Sweet® NaOH (1 M)/80 °C		HCQ suspension Syrspend® SF PH4 NaOH (1 M)/80 °C	
	1 h	4 h	1 h	4 h
Sample 1	88.3	44.68	89.6	53.6
Sample 2	92.78	47.06	87.6	66.9
Sample 3	95.04	47.70	81.4	74.2
Mean	92.0	46.5	86.2	64.9
Standard deviation	3.4	1.6	4.3	10.5

Table 3: Area under the curve (AUC) of different samples after preparation before analytical assays for each hydroxychloroquine suspension in comparison with the reference solution.

	HCQ 0.5 mg/mL ORA-Plus/ ORA-Sweet®	HCQ 0.5 mg/mL Syrspend® SF PH4	Reference solu- tion HCQ 0.5 mg/mL
Sample 1	$8.09 \cdot 10^6$	$8.16 \cdot 10^6$	$8.38 \cdot 10^6$
Sample 2	$8.17 \cdot 10^6$	$8.20 \cdot 10^6$	$8.15 \cdot 10^6$
Sample 3	$8.25 \cdot 10^6$	$8.30 \cdot 10^6$	$8.28 \cdot 10^6$
Mean	$8.17 \cdot 10^6$	$8.22 \cdot 10^6$	$8.27 \cdot 10^6$
Standard deviation	$8.23 \cdot 10^5$	$4.75 \cdot 10^5$	$1.16 \cdot 10^5$
CV, %	1.2	0.6	0.1

Relative HCQ concentrations were measured and presented in Table 2. The diminution of the area under the curve of HCQ peak with the progressive appearance of degradation products was observed.

The results of sampling preparation to check the matrix effect after the different steps (US, centrifugation, dilution) are presented in Table 3. The difference between the reference solution and each HCQ suspension is less than 2%. Thus, there is no influence of oral vehicle suspension.

The comparison between all standard curves, either prepared with HCQ solution or HCQ suspension (Ora-Plus/Ora-sweet and Syrspend SF pH 4), shows that equation curves are very similar. Respectively, $y = 6.20E-08x + 0.01$ or $y = 5.86E-08x + 0.01$ and $y = 5.91E-08x + 0.01$. These results are confirmed with the graphical representation because standard curves are totally overlaid (Figure 4). Thus, in this case, it was considered not necessary to perform specific statistical analysis. These results add supplementary proof for the absence of the matrix effect of oral suspension vehicles. For the realization of working standard solutions for method validation, it is thus possible to avoid using an oral suspension vehicle.

Suspensions stability

The pH remained stable at about 4.2 for all formulations during 90 days (Table 4). A slight alkalinization (<to 0.1 pH unit) of suspensions was observed for each tested condition. This result is in the range of the pH meter precision.

Osmolality remained stable during 90 days, at about 2,800 mOsm/kg for 50 mg/mL ORA suspensions and 190 mOsm/kg for 50 mg/mL SYR suspensions (Table 4).

Figure 4: Comparison of HCQ standard curve with and without oral vehicle suspension. (square: HCQ solution, without oral vehicle suspension; round: HCQ suspension, with oral vehicle suspension)

Table 4: Mean of pH and osmolality of eight measures during 90 days of each HCQ suspension in different storage conditions.

		pH		Osmolality	
		Mean $\pm$ SD	CV, %	Mean $\pm$ SD, mOsm/kg	CV, %
HCQ 50 mg/mL ORA-Plus/ORa-Sweet®	5 $\pm$ 3 °C	4.24 $\pm$ 0,03	0.76	2,796 $\pm$ 110.6	3.96
	30 $\pm$ 2 °C	4.29 $\pm$ 0,04	0.88	2,826 $\pm$ 150.7	5.33
HCQ 50 mg/mL Syrspend® SF PH4	5 $\pm$ 3 °C	4.23 $\pm$ 0,04	0.89	184 $\pm$ 10.7	5.84
	30 $\pm$ 2 °C	4.27 $\pm$ 0,06	1.34	195 $\pm$ 13.5	6.92

Figure 5: Representation of relative HCQ concentration during 90 days of stability study of HCQ suspension (50 mg/mL), in two different oral vehicles suspension ORA (OraSweet®/OraPlus®) and SYR (Syrspend®SF PH4), at both ICH conditions 5 and 30 °C. (Triangle: ORA HCQ at 30 °C; cross: ORA HCQ at 5 °C; square: HCQ SYR at 5 °C; diamond: HCQ SYR at 30 °C).

No modification of the visual aspect of all HCQ suspensions was observed during the stability study: the cloudy suspensions remained white without crystallization after 90 days whatever the storage conditions. Slight reversible sedimentation occurred, but suspensions easily resuspended when shaking the flask. SYR suspension kept its cherry odor while ORA suspension gave a faint sweet smell.

As shown in Figure 5, after 90 days, the 50 mg/mL HCQ suspension in OraSweet®/Ora Plus® remained stable for both storage conditions (5 and 30 °C).

For HCQ suspension in Syrspend® SF PH 4, stability is only approved at 5 °C during 90 days, indeed at 30 °C at 90 days concentration measured reaches 112% of initial relative concentration.

Discussion

The HPLC stability-indicating method with forced degradation has demonstrated its suitability for testing the stability of HCQ suspensions in OraSweet®/Ora Plus® and Syrspend® SF PH 4. Indeed, to be able to distinguish degradation products from HCQ and oral suspension vehicles, we have added to the forced degradation of HCQ a forced degradation of HCQ in oral suspension vehicles. Because oral vehicles are certified stable for a long time (until one year) we choose

just one extreme condition, and we applied simultaneously alkaline (NaOH 1 M) and heat (80 °C) stress conditions during two exposition times i.e. one or 4 h. In these conditions, no degradation was observed for both oral suspensions.

All chromatographic parameters meet the acceptance criteria for the HCQ assay. In contrast to the study of Singh et al. [33], it was decided to performed reversed-phase HPLC with gradient method instead of an isocratic method to allow elution in short runtime of impurities or probable degradations products like HCQ-O-Acetate (HCA), HCQ-O-Sulphate (HCS), or dichloroquinoline (DCQ) as cited by Dongala et al. [37]. Indeed, gradually increasing organic mobile phase proportion permits to elute these impurities, which are more apolar. Concerning the degradation products, Saini et al. performed a specific study on HCQ degradation product in ICH conditions. We obtained similar results. HCQ is susceptible only to alkaline, photolysis, and oxidative conditions. To avoid this reaction of photo-degradation, the use of amber bottles is justified.

Among the parameters monitored in this study, osmolality remained stable for 90 days whatever the formulations and storage conditions. A strong difference exists between ORA and SYR osmolality, ORA being much more hyperosmolar than SYR. To avoid side effects like bloating, diarrhea, a hypo osmolar oral vehicle suspension will often be preferred.

Whatever the chosen oral base, the presence of carboxymethyl cellulose sodium or sodium citrate, coupled with a sweetener and flavor in ORA-Plus® or Syrspend® SF PH4 is attempted to reduce the bitter taste of HCQ [30].

Finally, the obtained results allow an optimal organization of the compounding process due to the extended stability of the formulation. Indeed, except for the final point of HCQ suspension in Syrspend SF PH4 at 30 °C, all other assay values were within 10% of the initial concentration without detectable signs of decomposition after 90 days.

Concerning the last point at 90 days for HCQ suspension in Syrspend SF PH4® at 30 °C, we suspect that this result is due to slight evaporation. Indeed, for Syrspend SF PH4®, better preservation was observed at the refrigerated condition. We hypothesize that this result is due to solvent loss because of evaporation which was more important at ambient temperature. We do not think HCQ degrades in this condition, especially since (1) HCQ is really hard to degrade (as seen in Table 2) and (2) no degradation products appeared at this time of analysis.

The formulations of HCQ suspensions at 50 mg/mL are stable for at least 90 days at 4 °C. At 30 °C, these HCQ suspensions are stable, for 90 and 60 days respectively, in ORA and SYR. These stability data are comparable to the results obtained by Henry et al. [31], although the study was performed in different oral suspension vehicles.

This study constitutes the first step of the development of oral suspensions of HCQ. It could be improved by a microbiological stability study, as recommended by the GERPAC guidelines [38].

Conclusion

We propose two novel easy-to-handle formulations of HCQ in oral suspensions. The suspension in the ORA vehicle remained stable for 90 days at 4 and 30 °C. For HCQ suspension in SYR, it remained stable for 90 days at 4 °C and 60 days at 30 °C. For all preparations, no significant physical or chemical modification was noticed during the period of the study.

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References

1. Shippey EA, Wagler VD, Collamer AN. Hydroxychloroquine: an old drug with new relevance. *Cleve Clin J Med* 2018;85:459–67.
2. Shukla AM, Wagle Shukla A. Expanding horizons for clinical applications of chloroquine, hydroxychloroquine, and related structural analogues. *Drugs Context* 2019;8:9–1.
3. É Liozon. Hydroxychloroquine (Plaquenil): un vieux médicament au profil toujours très intéressant [Hydroxychloroquine: an old medicine with a profile that is always very interesting]. *Rev Prat* 2019;69:587–90.
4. Liu T, Zhang J, Li K, Deng L, Wang H. Combination of an autophagy inducer and an autophagy inhibitor: a smarter strategy emerging in cancer therapy. *Front Pharmacol* 2020;11:408.
5. Fanouriakis A, Kostopoulou M, Alunno A, Aringer M, Bajema I, Boletis JN, et al. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis* 2019;78:736–45.
6. Basta F, Fasola F, Triantafyllis K, Schwarting A. Systemic lupus erythematosus (SLE) therapy: the old and the new. *Rheumatol Ther* 2020;7:443–6.
7. Lever E, Alves MR, Isenberg DA. Towards precision medicine in systemic lupus erythematosus. *Pharmgenomics Pers Med* 2020;13:39–49.
8. Rodriguez-Smith J, Brunner HI. Update on the treatment and outcome of systemic lupus erythematosus in children. *Curr Opin Rheumatol* 2019;31:464–70.
9. Bertsias GK, Tektonidou M, Amoura Z, Aringer M, Bajema I, Berden JHM, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012;71:1771–82.
10. Hazlewood GS, Barnabe C, Tomlinson G, Marshall D, Devoe D, Bombardier C. Methotrexate monotherapy and methotrexate combination therapy with traditional and biologic disease modifying antirheumatic drugs for rheumatoid arthritis: abridged Cochrane systematic review and network meta-analysis. *BMJ* 2016;353:i1777.
11. Rempenault C, Combe B, Barnette T, Gaujoux-Viala C, Lukas C, Morel J, et al. Clinical and structural efficacy of hydroxychloroquine in rheumatoid arthritis: a systematic review. *Arthritis Care Res* 2020;72:36–40.
12. Kumar AB, Blixt EK, Drage LA, el-Azhary AR, Wetter DA. Treatment of morphea with hydroxychloroquine: a retrospective review of 84 patients at Mayo Clinic, 1996–2013. *J Am Acad Dermatol* 2019;80:1658–63.
13. El Jammal T, Jamilloux Y, Gerfaud-Valentin M, Valeyre D, Sève P. Refractory sarcoidosis: a review. *Ther Clin Risk Manag* 2020;16:323–45.
14. Ramos-Casals M, Brito-Zerón P, Bombardieri S, Bootsma H, De Vita S, Dörner T, et al. EULAR recommendations for the

- management of Sjögren's syndrome with topical and systemic therapies. *Ann Rheum Dis* 2020;79:3–18.
15. Petri M. Improvements in diagnosis and risk assessment of primary and secondary antiphospholipid syndrome. *Hematology Am Soc Hematol Educ Program* 2019;2019:415–20.
 16. Centre de Référence des Maladies Respiratoires Rares (RespiRare). Protocole National de Diagnostic et de Soins (PNDs). Pneumopathies interstitielles diffuses de l'enfant; 2017. Available from: https://epar.iplesp.upmc.fr/site_respirare/index.php/fr/professional-access/pnds/175-pnds-pid [Accessed 7 Aug 2020].
 17. Weber E, Reynaud Q, Fort R, Durupt S, Cathébras P, Durieu I, et al. Immunomodulatory treatments for persistent and chronic immune thrombocytopenic purpura: a PRISMA-compliant systematic review and meta-analysis of 28 studies. *Medicine* 2017;96:e7534.
 18. de Moreuil C, Alavi Z, Pasquier E. Hydroxychloroquine may be beneficial in preeclampsia and recurrent miscarriage. *Br J Clin Pharmacol* 2020;86:39–49.
 19. Wondafrash DZ, Desalegn TZ, Yimer EM, Tsige AG, Adamu BA, Zewdie KA. Potential effect of hydroxychloroquine in diabetes mellitus: a systematic review on preclinical and clinical trial studies. *J Diabetes Res* 2020;2020:5214751.
 20. Delsing CE, Warris A, Bleeker-Rovers CP. Q fever: still more queries than answers. *Adv Exp Med Biol* 2011;719:133–43.
 21. Lagier JC, Raoult D. Whipple's disease and *Tropheryma whipplei* infections: when to suspect them and how to diagnose and treat them. *Curr Opin Infect Dis* 2018;31:463–70.
 22. Xu R, Ji Z, Xu C, Zhu J. The clinical value of using chloroquine or hydroxychloroquine as autophagy inhibitors in the treatment of cancers: a systematic review and meta-analysis. *Medicine* 2018;97:e12912.
 23. Jones TM, Carew JS, Nawrocki ST. Therapeutic targeting of autophagy for renal cell carcinoma therapy. *Cancers* 2020;12:1185.
 24. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis, and related diseases. *Inflammopharmacology* 2015;23:231–69.
 25. Hydroxychloroquine: drug information. In: Uptodate, [Internet]. Waltham, MA: UpToDate. Available from: <https://www.uptodate.com/contents/hydroxychloroquine-drug-information#F180880> [cited 2020 November, 23].
 26. Plaquenil 200 mg. Base de données publique des médicaments de l'Agence Nationale de Sécurité du Médicament [Internet]. ANSM; 2020. [modified 2020 October 26, cited 2020 November, 23] Available from: <http://base-donnees-publique.medicaments.gouv.fr/affichageDoc.php?specid=67767535&typedoc=R>.
 27. Société Française de Pharmacie Clinique. Ecrasement des comprimés et/ou l'ouverture des gélules. Available from: <http://geriatrie.sfpcc.eu/application/choose> [Accessed 4 Aug 2020].
 28. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res (N Y)* 1995;12:413–20.
 29. Plöger GF, Hofsäss MA, Dressman JB. Solubility determination of active pharmaceutical ingredients which have been recently added to the list of essential medicines in the context of the biopharmaceutics classification system-biowaiver. *J Pharm Sci* 2018;107:1478–88.
 30. Pauli E, Joshi H, Vasavada A, Brackett J, Towa L. Evaluation of an immediate-release formulation of hydroxychloroquine sulfate with an interwoven pediatric taste-masking system. *J Pharm Sci* 2020;109:1493–7.
 31. McHenry AR, Wempe MF, Rice PJ. Stability of extemporaneously prepared hydroxychloroquine sulfate 25-mg/ml suspensions in plastic bottles and syringes. *Int J Pharm Compd* 2017;21:251–4.
 32. Saini B, Bansal G. Characterization of four new photodegradation products of hydroxychloroquine through LC-PDA, ESI-MSn, and LC-MS-TOF studies. *J Pharm Biomed Anal* 2013;84:224–31.
 33. Singh A, Roopkishora, Singh CL, Gupta S, Kumar S, Kumar M. Development and validation of reversed-phase high-performance liquid chromatographic method for hydroxychloroquine sulphate. *Indian J Pharm Sci* 2015;77:586–91.
 34. European pharmacopoeia, 10th ed. monography 2849, Hydroxychloroquine sulphate; 2020:2896–7 pp.
 35. Rivas-Granizo P, Jorge Santos SRC, Ferraz HG. Development of a stability-indicating LC assay method for determination of chloroquine. *Chromatographia* 2019;69:137–41.
 36. International Conference on Harmonization. Validation of analytical procedures: text and methodology Q2(R1); 1994.
 37. Dongala T, Katari NK, Palakurthi AK, Narasimha Rao Katakam L, Mariseti V. Stability indicating LC method development for hydroxychloroquine sulfate impurities as available for treatment of COVID-19 and evaluation of risk assessment prior to method validation by quality by design approach. *Chromatographia* 2020;83:1269–81.
 38. Sautou CV. Guide méthodologique des études de stabilité des préparations, 1ère éd. SFPC/GERPAC; 2013. ISBN : 978-2-9526010-6-1.