Fate of hydroxychloroquine in the aquatic environment

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Abstract. Hydroxychloroquine (HCQ) is a drug used to prevent or treat malaria infections and to treat certain auto-immune diseases such as lupus and rheumatoid arthritis. It is a high production volume pharmaceutical and has a potential for being persistent and bioaccumulative. In this work hydrolytic and photolytic stability of hydroxychloroquine was investigated. Hydrolytic stability was examined according to the OECD guideline 111 and preliminary results show that hydroxychloroquine is resistant to hydrolytic degradation. The photolytic degradation of HCQ was investigated under simulated solar radiation in MilliQ water and spring water. The results showed that investigated pharmaceutical degrade very slowly with half-laves of 5.5 h and 11.6 h in spring and MilliQ water, respectively. Effect of pH value on photodegradation rate was examined and results show significantly influence, faster degradation was observed at higher pH value. Obtained half-lives ranged from 23.1 h for pH 4 to 5.5 min for pH 9. Photodegradation followed first order kinetics with coefficients of determination (r^2) higher than 0.99. Samples from hydrolytic and photolytic experiments were analyzed using high performance liquid chromatography.

Keywords: pharmaceuticals, hydroxychloquine, hydrolysis, photolysis

1. Introduction

Hydroxychloroquine (2-({4-[(7-chloroquinolin-4-yl) amino]pentyl}(ethyl)amino)ethan-1-ol, CAS number: 000118-42-3) is 4-aminoquinolone derivative. Together with chloroquine, hydroxychloroquine has been used for years in antimalarial prevention. Since 1950s, they gained popularity in the treatment of diseases in both rheumatology and dermatology. They were used for diseases such as rheumatoid arthritis and systemic lupus erythematosus in rheumatology, and polymorphic light eruptions and porphyria cutanea tarda in dermatology. It has been reported that hydroxychloroquine can cause ocular toxicity, with the most serious being an irreversible retinopathy (Browing, 2014; Tehrani R *et al*., 2008). HCQ is excreted as unchanged or metabolized drug mainly through kidney (40–60%) and feces (8–25%). Substantial portion of hydroxychloroquine excreted unchanged (Daughton, 2014); published data cover range from 6% to 60%, with median value of 23% (Browing, 2014). The metabolites of HCQ are pharmacologically active (Browing, 2014).

After administration, excreted HCQ and its metabolites enter sewage system and potentially can reach the environment. In the environment, its fate and behavior are determined by their physicochemical properties. HCQ is a weak base highly soluble in water with log K_{ow} value of 3.03[1] and BIOWIN values less than 0.5 (BIOWIN1=0.1249, BIOWIN5=-0.0746)[2]. Due to the fact that HCQ is potentially persistent and bioaccumulative and produced in large quantities, it has the high potential for being the next emerging pharmaceutical contaminant (Daughton, 2014; EMEA/ CHMP/SWP/4447/00 corr2, 2006; Howard and Muir, 2011). Nevertheless, the data on environmental fate and behavior of HCQ are very scarce. Recently, a monitoring study reports on the detection of HCQ in surface sediment from tidal sections of the river in southeast China (Chen *et al*., 2013).

In order to better understand fate and behavior of HCQ in water matrices, the aim of presented study was to investigate its hydrolytic and photolytic stability. Photostability during exposure to simulated solar radiation was investigated in MilliQ and spring water. The influence of water pH value on photolytic behavior of HCQ was also investigated.

2. Experimental

2.1. Materials and chemicals

High purity (≥98%) analytical standard of hydroxy-chloroquine sulfate was supplied from Sigma-Aldrich (St. Lous, MO, USA). All chemicals used in experiments (citric acid, formic acid, NaH_2PO_4, Na_2HPO_4, NaOH, H_2SO_4, H_3BO_3) were analytical grade reagents and supplied by Kemika (Zagreb, Croatia). Ultrapure water was prepared by the Millipore Simplicity UV system (Millipore Corporation, Billerica, MA, USA). Acetonitrile was HPLC grade (J.T. Baker, Deventer, The Netherlands). Aqueous solutions of

[1] Value estimated by the KOWWIN v1.68 program, EPI Suite.

[2] Values estimated by the BioWin v4.10 program, EPI Suit.

sodium hydroxide (c=0.1 mol L^{-1}) and sulfuric acid (c=0.01 mol L^{-1}) were used for the adjustment of the water pH value.

2.2. Hydrolytic experiments

The hydrolytic stability of HCQ was examined according to the procedure described in OECD 111 (2004). In order to quantify the hydrolysis rate of HCQ all the tests were done in three replicates and the conditions of chromatographic determination were the same as for the photolysis experiments.

2.3. Photodegradation experiments

The photolysis experiments were conducted in a Suntest CPS+ simulator (Atlas, Germany) equipped with a Xenon lamp and a temperature sensor. The device emitted radiation in the wavelength range of 300−800 nm to simulate natural sunlight. During the experiments the radiation intensity was maintained at 500 W m^{-1} and the reaction temperature was kept at 25 °C. The initial mass concentration of HCQ was 10 mg L^{-1}. The test solutions of HCQ were prepared in MilliQ and filtered spring water (pH 5.07, electrical conductivity 35.9 µS cm^{-1}, Cl^- 1.7 mg L^{-1}, NO_3^- 7.8 mg L^{-1}, SO_4^{2-} 1.9 mg L^{-1}, NPOC 0.2622 mg L^{-1}). Sodium phosphate (monobasic and dibasic), boric acid, sulfuric acid and sodium hydroxide were used for the preparation of 10 mM buffer solutions (pH 4.0, 7.0 and 9.0). Solutions of HCQ were irradiated in 50 mL quartz vessels. The depth of the solution was 3 cm and the distance between the lamp and the solution surface was 14 cm. Aliquots of 300 µL were withdrawn at regular time intervals and directly analyzed by HPLC-DAD. Control samples had the same composition as test solutions and were performed under the same conditions as test solutions but they were protected from the irradiation of the light.

2.4. Analytical determination

To determine photodegradation rate of HCQ and its photodegradation products, samples were analyzed by HPLC-DAD (Waters 2795 Alliance HPLC System with 2996 DAD Detector). The separation was carried out on Kinetex C18 column (150 mm x 4.6 mm, particle size 5 µm, pore size 100 Å, Phenomenex). The analysis were performed using 0.1% formic acid in MilliQ water as eluent A and 0.1% formic acid in acetonitrile as eluent B in gradient elution mode at the flow rate 0.5 mL min^{-1} with the column temperature of 40 °C. The time of each analysis was 20 min. The elution gradient started with 94% of eluent A and held for 8 min, and then eluent A started linearly decreasing to 50% over 15 min. During the last five minutes eluent A was linearly increased to 94%. Equilibration time was 5 min. The injection volume was 20 µL. All compounds were detected at a wavelength of 343.4 nm. Instrument control, data acquisition and evaluation were done using MassLynx V4.1 SCN 714 Waters Inc. software.

3. Results and discussion

3.1. Hydrolytic degradation

The experiment investigating hydrolysis of HCQ was performed according to the OECD 111 (2004) procedure in capped glass vials, under dark conditions. Hydrolysis experiments were performed at three different pH values, 4, 7, and 9. The experiments were performed at 50 °C in five days. Obtained results showed very small degree of hydrolytic degradation (less than 1%) of HCQ under the studied conditions. Hydrolytic degradation of 10% at 50 °C corresponds to a half-life of approximately of 30 days, which is equivalent to the half-life of 1 year at 25 °C (OECD 111, 2004). Based on obtained result, HCQ was considered stable and further testing was not required.

3.2. Photolytic degradation

Photodegradation kinetics of HCQ was investigated in MilliQ water and spring water. Initial concentration of HCQ in the solutions was 10 mg L^{-1}. During all photodegradation experiments, in the dark controls no obvious losses of HCQ were detected.

When exposed to simulated solar light, HCQ degrade slowly with half-lives of 11.6 days and 5.5 days in MilliQ water and spring water, respectively (**Table 1.**).

Degradation profiles of HCQ obtained in MilliQ and spring water fortified with HCQ are shown on **Fig. 1**.

Table 1. Photodegradation rates and half-lives of HCQ

	Spring water	**MilliQ**	**MilliQ water**		
			pH 4	**pH 7**	**pH 9**
k (min^{-1})	0.0021	0.0010	0.0005	0.0040	0.1272
$t_{1/2}$	5.5 h	11.6 h	23.1 h	2.9 h	5.5 min

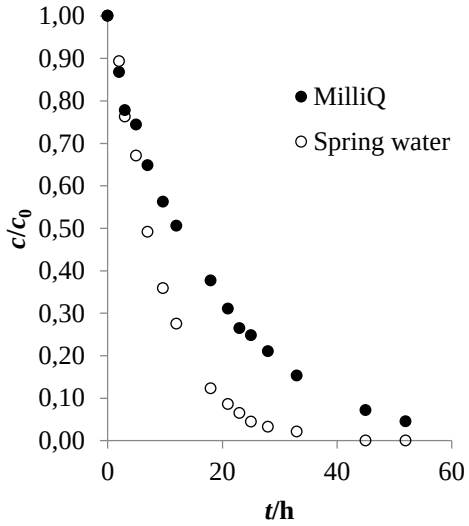

Figure 1. Photolytic degradation of HCQ in MilliQ and spring water

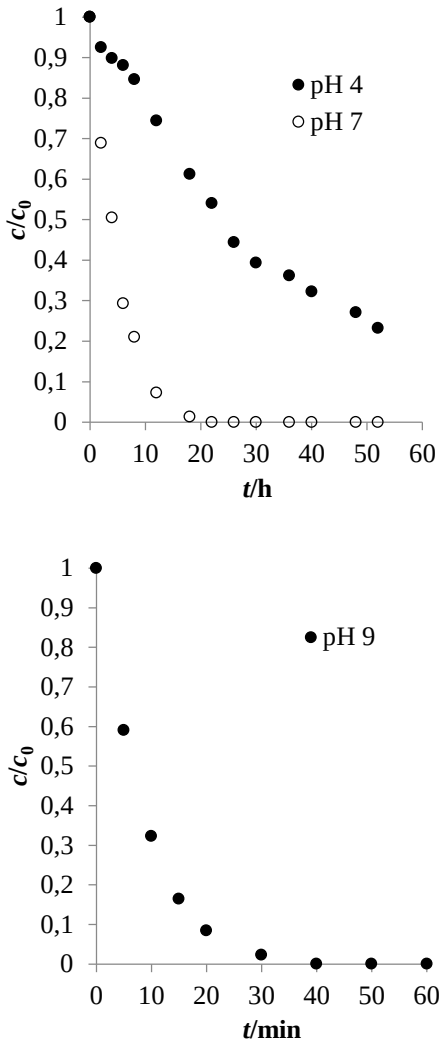

Figure 2. Effect of pH on HCQ photolytic degradation

In both experiments linear regression of $\ln(c/c_0)$ versus time (t) showed that photodegradation followed first order kinetics with r^2 higher than 0.99. As can be seen from obtained results matrix components present in spring water accelerated the photodegradation of HCQ by double.

3.2.1. The effect of pH

The influence of water pH value on the photo-degradation rate under simulated solar irradiation was investigated in experiments at pH 4, 7 and 9 using HCQ solution with initial concentration of 10 mg L^{-1} at 25 °C. Effects of pH value on the rate of photolytic degradation of HCQ are shown on **Fig 2**.

The photolysis is directly proportional to the water pH value; significantly faster degradation occurs at higher pH values. HCQ is completely degraded after 40 min and 22 h at pH 9 and 7, respectively. At pH 4 complete degradation of HCQ was not obtained even after 52 hours of irradiation. At all three pH values, first-order reaction kinetics can be used to describe the photodegradation of HCQ. The corresponding photodegradation rate constants (k) and half-lives ($t_{1/2}$) are shown in **Table 1** ($r^2 > 0.99$).

The faster kinetics at higher pH values can be explained by the different ionization form of HCQ at different pH values. **Fig 3.** shows the dissociation of HCQ and structures of HCQ species[3] under different pH values.

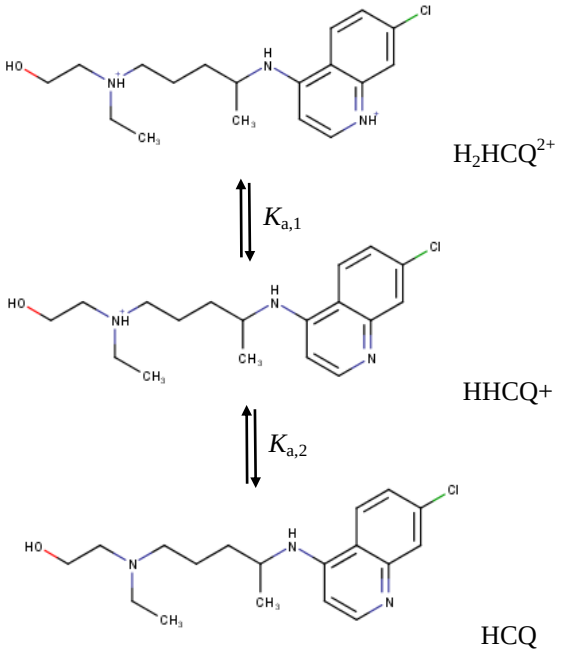

Figure 3. Dissociation equilibrium of HCQ

HCQ is a basic substance with calculated pK_a values of 7.28 and 9.76[4]. At pH 4 HCQ is fully protonated as H_2HCQ^{2+} (**Fig. 3**). In neutral solution HCQ is mostly protonated as H_2HCQ^{2+} (around 66%) and partly as $HHCQ^{+}$ (around 34%). At higher pH values, in alkaline

[3] Structures obtained by Marvin Sketch 17.2.13.

[4] Values obtained from Chemicalize.com.

solution, proportion of protonated form $HHCQ^+$ increased (around 83%) and HCQ is also present in around 15% in its neutral form. At higher pH values, HCQ is mostly present in $HHCQ^+$ and neutral forms which might be more photoreactive species than H_2HCQ^{2+}.

3.2.2. Photodegradation products

The appearance of new peaks on chromatograms for samples obtained at different irradiation times is indicative of possible degradation products. **Fig. 4** shows the chromatograms obtained for standard HCQ solution and after 30 min of irradiation at pH 4 where two new peaks can be observed (DP−1 and DP−2).

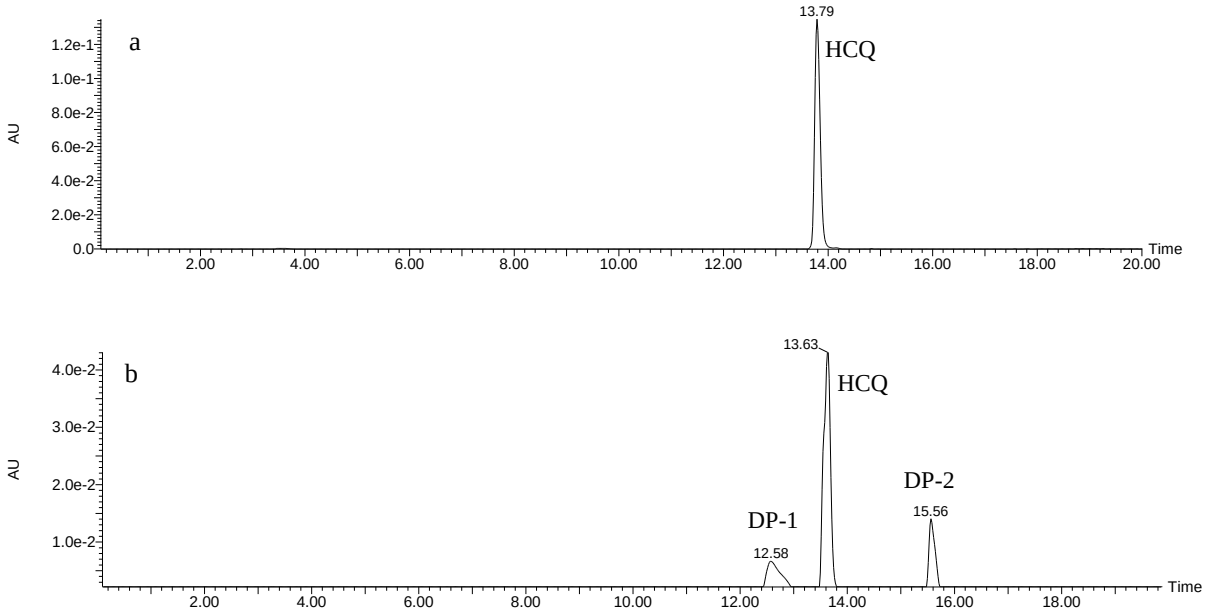

Figure 4. Chromatograms of HCQ at *t*=0 (a) and at *t*=30 h in buffer

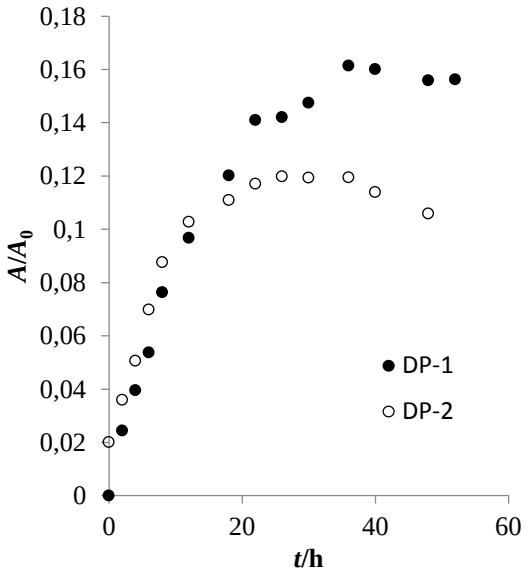

Figure 5. Formation and degradation profiles of HCQ degradation products in buffer solution pH 4

Time curves of the relative peak area A/A_0 of the photodegradation products during photolysis of HCQ at pH 4 (A is the peak area of the degradation product at a specific time and A_0 is the peak area of HCQ at 0 min) are shown on **Fig. 5**. Since response factors in the HPLC-DAD analysis are dependent on the chemical structure of the analyte, no conclusions on the absolute concentrations can be done without appropriate standards. As can be seen, both products reached their maximum concentration upon irradiation (26 h for DP-2 and 36 h for DP-1), dropping down slowly onwards.

4. Conclusions

Since hydroxychloroqione are identified as high volume production pharmaceutical, potentially persistent and bioacumulative, understanding of its environmental fate and behavior is an important issue. In the present study abiotic, hydrolysis and photolysis, processes were investigated. It has been demonstrated that HCQ is resistant to hydrolytic degradation. On the contrary, photodegradation under solar irradiation could be an important elimination process for hydroxychloroquine in the environment. Photodegradation followed pseudo-first order kinetics and the degradation rate is highly dependent on the matrices and pH value.

To obtain a complete picture on hydroxychloroquine fate in the aquatic environment further investigations are needed. During photolytic degradation two degradation products are formed. Currently, work on identification of degradation products is in progress in order to propose their structural formulae and degradation pathways. Also, fate of HCQ metabolites should be investigated.

Acknowledgement

This work has been fully supported by Croatian Science Foundation under the project IP-2014-09-2353: Fate of pharmaceuticals in the environment and during advanced wastewater treatment (PharmaFate).

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