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1 Photodegradation of novel oral anticoagulants under sunlight irradiation in
2 aqueous matrices

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24

25 **Abstract**

26 Kinetics of photodegradation of novel oral anticoagulants dabigatran, rivaroxaban, and
27 apixaban were studied under simulated solar light irradiation in purified, mineral, and river
28 waters. Dabigatran and rivaroxaban underwent direct photolysis with polychromatic quantum
29 yields of 2.2×10^{-4} and 4.4×10^{-2} , respectively. The direct photodegradation of apixaban was not
30 observed after 19 hours of irradiation. Kinetics of degradation of rivaroxaban was not
31 impacted by the nature of the aqueous matrix while photosensitization from nitrate ions was
32 observed for dabigatran and apixaban dissolved in a mineral water. The photosensitized
33 reactions were limited in the tested river water (Isle River, Périgueux, France) certainly due to
34 the hydroxyl radical scavenging effect of the dissolved organic matter. The study of
35 photoproduct structures allowed to identify two compounds for dabigatran. One of them is the
36 4-aminobenzamidine while the second one is a cyclisation product. In the case of rivaroxaban,
37 as studied by very high field NMR, only one photoproduct was observed *i.e.* a photoisomer.
38 Finally, seven photoproducts were clearly identified from the degradation of apixaban under
39 simulated solar light.

40

41 **Keywords:** photolysis; solar light irradiation; surface water; apixaban; dabigatran;

42 rivaroxaban

43

44

45 **1. Introduction**

46 Until recently, oral anticoagulants were synonyms of vitamin K antagonists (VKAs) such as
47 warfarin. These medicines are used for venous thromboembolism, stroke, atrial fibrillation
48 treatment, and most generally in medicinal conditions that require chronic anticoagulation
49 (Hanslik et al., 2004). VKAs may not be appropriate for many patients especially due to the
50 necessity of dietary precautions and frequent laboratory monitoring (El-Helou et al., 2013). In
51 fact, if warfarin concentration is too low the anticoagulation effect does not act efficiently; a
52 too high concentration causes excessive bleeding. In this context, novel oral anticoagulants
53 (NOACs) were developed (Spyropoulos, 2008; Beyer-Westendorf et al., 2014). The U.S.
54 Food and Drug Administration (F.D.A.) recently approved the use of three new active
55 ingredients, namely dabigatran etexilate (2010), rivaroxaban (2011), and apixaban (2012)
56 (Wanat, 2013). Dabigatran etexilate is a prodrug that is converted to dabigatran and acts as a
57 direct thrombin inhibitor (Khoo et al., 2010). Rivaroxaban and apixaban are direct factor Xa
58 inhibitors that do not require cofactors for activity (Gulseth et al., 2008; Frost et al., 2013).
59 These three compounds are also increasingly used in Europe. In particular, these NOACs
60 currently represent 30% of ingested anticoagulants in France with an increase in their
61 consumption by a factor of 10 between the second trimester 2012 and the third trimester 2013
62 (ANSM, 2013). The main limitation for their use was the absence of antidote. In 2015, the
63 F.D.A. approved the idarucizumab as dabigatran antidote while rivaroxaban and apixaban
64 antidote (andexanet alpha) is about to be validated (Pollack et al., 2015; Siegal et al., 2015).
65 Thus, their prescriptions are expected to continue to rise in the near future.

66 To our knowledge, information reported in the literature about the occurrence of NOACs in
67 the aquatic compartment, their fates, and their toxicity, as well as their degradation products
68 are very scarce (Kasad, 2013; Ramiseti et al., 2014; Secrétan et al., 2015; Swain et al., 2016;
69 Tantawy et al., 2016; Wingert et al., 2016). This is especially worrying since human

70 metabolism studies have shown that NOACs are eliminated in urines as unchanged forms at
71 levels between 27 and 85% of the delivered dose (eVidal, 2017). In fact, organic pollutants
72 are known to undergo degradation processes in environmental waters which must be taken
73 into consideration. Photolysis can be one of the major ways of degradation for pharmaceutical
74 compounds absorbing solar light as well as for those degraded by photosensitization (Szabo et
75 al., 2011; Zuo et al., 2013; Carlson et al., 2015; Zhang et al., 2016; Chen et al., 2017).
76 This study has for objective to bring information on the environmental fate of NOACs. In
77 particular, direct and indirect photolysis of NOACs was studied in different aqueous matrices.
78 Results on molar absorption coefficients, rate constants and polychromatic quantum yields of
79 photodegradation are discussed. Rate constants were obtained from simulated solar light
80 irradiation in purified, mineral, and river waters to evaluate the effect of ions and dissolved
81 organic matter naturally present in surface waters. Finally, the elucidation of structures of
82 photoproducts was performed using LC-HRMS, and 700 and 800 MHz NMR spectrometers.
83

84 2. Materials and methods

85 **2.1. Chemicals.** The novel oral anticoagulants (NOACs) dabigatran (APIChem Technology,
86 CAS 211914-51-1, 99%), rivaroxaban (AKSci, CAS 366789-02-8, 98%), and apixaban
87 (APIChem Technology, CAS 503612-47-3, 99%), as well as 4-aminobenzamidine
88 dihydrochloride ($\geq 99.0\%$), methanol (CHROMASOLV®, $\geq 99.9\%$), and sodium bicarbonate
89 salt ($\geq 99.5\%$) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Sodium
90 nitrate salt (analytical reagent grade) was provided by Fisher Chemical. All chemicals were
91 used as received. Purified water was produced using a Milli-Q® Direct-Q® 5 system
92 (Millipore, resistivity 18.2 M Ω .cm). Mineral water (Volvic) contains 7.2 and 74.0 mg.L⁻¹ of
93 nitrate and hydrogen carbonate ions, respectively. River water was collected from the Isle
94 River (Périgueux, France). This surface water contained 7.7 and 140-200 mg.L⁻¹ of nitrate and
95 hydrogen carbonate ions, respectively. Before use, the river water was filtered on a 0.45 μ m
96 hydrophilic PVDF Durapore® membrane (Millipore). Stock solutions of NOACs were
97 prepared in purified water at concentrations of 5.0×10^{-5} , 1.0×10^{-5} , and 3.0×10^{-6} mol.L⁻¹ for
98 dabigatran, rivaroxaban, and apixaban, respectively. During the preparation, volumetric flasks
99 were covered with aluminum foils and solutions were left under magnetic stirring during one
100 week. Then, stock solutions were stored in amber glass bottles at room temperature until use
101 to prevent photodegradation and solubility problems, respectively. The stability of the three
102 compounds was checked.

103

104 **2.2. UV-Visible spectroscopy.** UV-Visible absorption spectra were recorded on a UV-1800
105 spectrophotometer (Shimadzu, Japan). A 1-nm resolution and a “medium” scan rate were
106 used to record the reference spectrum and spectra in the range 200-400 nm (NOACs do not
107 absorb visible light) using either 1 or 5 cm quartz cells (QS, Hellma Analytics).

108 Molar absorption coefficients of dabigatran and rivaroxaban in purified water were calculated
109 from the absorbance of five solutions with concentrations included in the range 10^{-6} - 10^{-5}
110 mol.L⁻¹. Due to the particularly low solubility of apixaban in water, a stock solution was
111 prepared in methanol at a concentration of 9.6×10^{-6} mol.L⁻¹. Then, this stock solution was
112 used to prepare five solutions at concentrations between 1.0×10^{-6} and 4.8×10^{-6} mol.L⁻¹ by
113 adding purified water (*i.e.* the maximal proportion of methanol was of 50%).
114 The solar light emission spectrum at the Earth surface was recorded using a USB2000+
115 radiometer coupled to an optical fiber (Ocean Optics, Florida, USA).

116

117 **2.3. Irradiation experiments.** Solar radiations at the Earth surface were simulated using a
118 SUNTEST CPS apparatus from Atlas Material Testing Solutions (Illinois, USA). The
119 irradiance was set to 250 W.m². In the case of kinetic studies, purified, mineral, and river
120 waters were spiked with the stock solutions prepared in purified water to obtain final
121 concentrations of 3.5×10^{-7} , 3.5×10^{-7} , and 4.5×10^{-7} mol.L⁻¹ for dabigatran, rivaroxaban, and
122 apixaban, respectively. For the elucidation of photoproduct structures, non-irradiated and
123 irradiated stock solutions were analyzed without pre-treatment.

124 Before irradiation, samples were placed into 12 mL transparent glass tubes (optical
125 pathlength: 1.5 cm) and were sealed to avoid water evaporation. NOACs were irradiated in
126 purified water alone and doped with nitrate and bicarbonate ions (same concentrations as in
127 mineral water), in mineral water, and in river water. For each aqueous matrix, two tubes were
128 filled with 10 mL of solution and one of them was wrapped in an aluminum foil for dark
129 control. To obtain kinetic data, aliquots of each sample were collected before irradiation and
130 after scheduled irradiation time. For each kinetics, at least five samplings were performed and
131 the analysis was repeated in triplicate. The maximum irradiation time was of 10 hours for
132 dabigatran, 4 hours for rivaroxaban, and 19 hours for apixaban. To calculate the

133 polychromatic quantum yields of photodegradation, photon fluence rates were measured with
134 a one nanometer interval in the range 290-350 nm using a USB2000+ radiometer coupled to
135 an optical fiber (Ocean Optics, Florida, USA).

136

137 **2.4. HPLC and LC-QToF-MS analyses.** For kinetics studies, HPLC analyses were carried
138 out using an Agilent 1100 series chromatograph (Agilent Technologies) equipped with a
139 variable wavelength detector (Zuo, 2014). For dabigatran and rivaroxaban, the column was a
140 Nucleosil® C₁₈ Nautilus, 250 mm x 4.6 mm, with a particle size of 5 µm (Macherey-Nagel)
141 while for apixaban the column was a Nucleodur® 100-5 C₁₈ ec, 250 mm x 4.6 mm, with a
142 particle size of 5 µm (Macherey-Nagel). Solvent A was methanol and solvent B purified
143 water. Analyses were carried out in isocratic mode with a ratio of 60:40 (A:B, v/v) for
144 rivaroxaban and apixaban, and a ratio of 30:70 (A:B, v/v) for dabigatran. The UV detection
145 was performed at 303, 248, and 277 nm for dabigatran, rivaroxaban, and apixaban,
146 respectively.

147 UV absorption spectra of photoproducts were recorded with an HPLC Spectra System P1000
148 coupled with a diode array detector model UV6000LP (Thermo Separation® Products). For
149 each NOAC, elution conditions described above were used. With the goal to check the elution
150 of all the products formed after irradiation, other analyses were performed in gradient mode.
151 The gradient elution started with 10% of A and reached 100% in 25 minutes linearly. Then, it
152 was kept stable during 5 minutes. No new photoproducts were observed compared to isocratic
153 mode analyses.

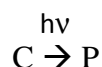
154 LC-HRMS analyses were carried out using an Agilent 1290 HPLC system coupled to an
155 Agilent 6540 QToF mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA)
156 equipped with an Agilent Jet Stream electrospray ionization source (ESI). The
157 chromatographic separation of the parent compound and of its photoproducts was performed

158 on a Kinetex C₁₈ column (100 x 2.1 mm, 1.7 μm; Phenomenex®, Agilent Technologies, Les
159 Ulis, France) regulated at 40°C. Gradient LC elution was performed at a flow rate of 0.3
160 mL.min⁻¹ using 0.1% formic acid in purified water as mobile phase A and 0.1% formic acid in
161 acetonitrile as mobile phase B. The gradient started with 10% of B and was linearly increased
162 to 35% in 13 minutes. The fraction B was then immediately increased and held at 100% for 1
163 min, before returning to the starting conditions for 3 min. Sample injection volume was set at
164 5 μL. Non-irradiated and irradiated stock solutions in purified water were analyzed without
165 pre-treatment.

166 After elution, the analytes were detected using the QToF operating in positive ion mode
167 (ESI+) with the following parameters: capillary voltage, 3000 V; nebulizer pressure, 40 psig;
168 drying gas, 8 L/min; gas temperature, 300 °C; sheath gas flow, 11 L/min; sheath gas
169 temperature, 400 C; nozzle voltage, 0 V; fragmentor voltage, 190 V. LC-MS accurate mass
170 spectra were recorded across the range m/z 70 – 1000 at 2 GHz. The data recorded were
171 processed with MassHunter Qualitative software (version B.06.00, Agilent Technologies,
172 Santa Clara, CA, USA). Accurate mass measurements of each peak from the extracted ion
173 chromatograms were obtained by means of a calibrant solution delivered by an external
174 quaternary pump, allowing system to continuously correct for any mass drift by using the
175 reference mass ion HP-921 [hexakis-(1H,1H,3H-tetrafluoropropoxy)-phosphazine] at m/z
176 922.0098. Stability of mass accuracy was checked before, and if values went above 0.2 ppm
177 error, then the instrument was recalibrated. The instrument was operated in single MS with
178 full spectra (1 spectrum/s) and in MS/MS (2 spectra/s) using an isolation width set at medium
179 (m/z ~ 4), and collision energies of 10, 20, and 40 eV. A standard solution containing 36
180 compounds was injected before the samples to check the retention time of compounds and the
181 instrumental sensitivity.

182

183 **2.5. Rate constants and polychromatic quantum yields.** Following the absorption of a
 184 photon, an organic contaminant (C) can be degraded and one or several photoproducts (P) are
 185 formed. The equation of the reaction is:



187 Under specific experimental conditions (*i.e.* low absorbance), this reaction generally follows
 188 an apparent first-order kinetics modeled by equation 1:

$$189 \quad \ln \left(\frac{[C]_0}{[C]_t} \right) = k_{poly} \cdot t \quad \text{eq. (1)}$$

190 where $[C]_0$ and $[C]_t$ are the organic contaminant concentrations (mol.L^{-1}) respectively before
 191 irradiation and at the instant t , k_{poly} is the apparent first-order rate constant under
 192 polychromatic irradiation (s^{-1}), and t is the irradiation time (s).

193 In this framework, the rate of photolysis of C is given by equation 2:

$$194 \quad v(C) = k_{poly} \cdot [C]_t = \sum_{\lambda_1}^{\lambda_n} I_{0,\lambda_i} \cdot (1 - e^{-2.303 \cdot A_{\lambda_i}}) \cdot \phi_{\lambda_i} \quad \text{eq. (2)}$$

195 where I_{0,λ_i} is the photon fluence rate of the irradiation source ($\text{E.L}^{-1} \cdot \text{s}^{-1}$), A_{λ_i} is the absorbance
 196 of C and ϕ_{λ_i} is the quantum yield of photolysis of C (mol.E^{-1}) at the λ_i wavelength. λ_1 - λ_n is
 197 the overlapping wavelength range between the UV-Vis absorptions of the organic
 198 contaminant and the emission spectrum of the radiation source.

199 In environmental conditions, the absorbance of C is generally low ($A < 0.02$, hyper dilute
 200 medium). Based on this hypothesis, the expression of polychromatic quantum yield of
 201 photolysis (ϕ_{poly} , mol.E^{-1}) of C is given by equation 3:

$$202 \quad \phi_{poly} = \frac{k_{poly}}{2.303 \cdot l} \cdot \sum_{\lambda_1}^{\lambda_n} \frac{1}{\varepsilon_{\lambda_i} \cdot I_{0,\lambda_i}} \quad \text{eq. (3)}$$

203 where l is the sample's optical pathlength (cm) and ε_{λ_i} is the molar absorption coefficient
 204 ($\text{L.mol}^{-1} \cdot \text{cm}^{-1}$) at the λ_i wavelength.

205

206 **2.6. Nuclear magnetic resonance spectroscopy.** LC-QToF-MS analyses were not enough to
207 identify the structure of the rivaroxaban photoproduct due to the same fragmentation pattern
208 (see supplementary materials). The main limitation for NMR analyses was the low solubility
209 of rivaroxaban in water and thus the low concentration of its degradation product. In order to
210 concentrate both compounds, a solution of rivaroxaban was prepared in methanol at an initial
211 concentration of 52.9 mg.L^{-1} and was irradiated with a SUNTEST CPS+ (250 W.m^{-2}) during 8
212 hours. A volume of 8 mL of this solution was evaporated and the residue dissolved in 500 μL
213 of methanol- d_3 (Eurisotop, France) for NMR experiments. The photochemical reactivity of
214 rivaroxaban was the same in purified water and in methanol. A second sample of pure
215 rivaroxaban (few mg of powder were dissolved in methanol- d_3) was prepared.
216 1D and 2D NMR spectra of non-irradiated and irradiated rivaroxaban were recorded at room
217 temperature on Bruker Avance III HD SB 700 MHz and 800 MHz spectrometers. The 700
218 MHz spectrometer was equipped with a 5 mm ^1H - ^{13}C / ^{15}N /D TXI probe with Z-gradients
219 operating at 176.07 MHz and 700.15 MHz for ^{13}C and ^1H , respectively. The 800 MHz
220 spectrometer was equipped with a 5 mm ^1H - ^{13}C / ^{15}N /D TCI cryoprobe with Z-gradients
221 operating at 81,08 MHz, 201.21 MHz and 800.23 MHz for ^{15}N , ^{13}C and ^1H , respectively. In
222 order to assign the 2 structures, the following experiments were performed: 1D- ^1H and 1D-
223 ^{13}C experiments and, HSQC ^1H - ^{13}C , HMBC ^1H - ^{13}C , COSY, TOCSY, NOESY, and SOFAST-
224 HMQC ^1H - ^{15}N , two-dimensional experiments. Data treatment was accomplished using the
225 TopSpin Bruker software.

226

227

228 3. Results and discussion

229 **3.1. Ultraviolet absorption spectra.** UV absorption spectra of dabigatran, rivaroxaban, and
230 apixaban, are presented in Fig. 1. Dabigatran dissolved in deionized water displays two
231 maxima located at 302 nm ($\epsilon_{302} = 24\,180\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$) and 220 nm ($\epsilon_{220} = 40\,940\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$).
232 In the case of rivaroxaban, the spectrum recorded in the aqueous medium shows a
233 maximum at 248 nm ($\epsilon = 23\,820\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$). Moreover, a shoulder was observed at 273 nm
234 ($\epsilon = 14\,930\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$). UV absorptions of apixaban have been studied in a mixture of
235 methanol and water due to the very low solubility of the drug into the aqueous solvent. A
236 maximum of absorption is observed at 278 nm ($\epsilon = 14\,320\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$). This maximum was
237 found at the same wavelength in pure methanol ($9.6\times 10^{-6}\text{ mol}\cdot\text{L}^{-1}$) and in pure water (3.0×10^{-6}
238 $\text{mol}\cdot\text{L}^{-1}$). The calculated molar absorption coefficient in pure methanol was of $14\,700\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$.
239

240
241 **Please insert Fig. 1.**

242
243 The three novel oral anticoagulants (NOACs) absorb photons above 300 nm. Therefore, they
244 absorb the solar light reaching the Earth surface and they can undergo direct photolysis (Fig.
245 1). The study of this abiotic degradation pathway is of main concern from the environmental
246 point of view. Moreover, the role of ions and dissolved organic matter (DOM) naturally
247 present in surface waters is also discussed further.

248
249 **3.2. Rate constants and quantum yields of photolysis.** Dabigatran, rivaroxaban, and
250 apixaban were irradiated under simulated solar light in unspiked and spiked purified water
251 with nitrate (NO_3^-) and hydrogen carbonate (HCO_3^-) ions, in mineral and in river waters.
252 Calculated rate constants, coefficients of determination, half-lives ($t_{1/2}$), and polychromatic

253 quantum yields of photolysis are compiled in Table 1. In each case, NOACs photolysis
254 followed apparent first-order kinetics. From dark control samples no thermal degradation and
255 no hydrolysis were observed at the same time as the irradiation experiments.

256 Kinetics of photodegradation of dabigatran was dependent on the nature of the aqueous
257 matrix. The calculated half-life was of 19.9 hours in deionized water and decreased by a
258 factor of 2.7 to reach 7.4 hours in the mineral water. Afterwards, dabigatran was irradiated in
259 purified water spiked with nitrate ions at a concentration of 7.2 mg.L^{-1} *i.e.*, the same
260 concentration occurring in mineral water. The obtained half-life was of 6.8 hours, which is
261 almost the same value as that obtained in mineral water. In river water (nitrate ions
262 concentration: 7.7 mg.L^{-1}), 50% of dabigatran was degraded in 24.1 hours; therefore, in this
263 aqueous medium the degradation induced by light is less efficient by a factor 3.3 compared to
264 the mineral water.

265 For rivaroxaban, kinetics of photodegradation were not significantly dependent on the nature
266 of the aqueous matrix. In fact, half-lives of 2.3, 2.1, and 2.2 hours were obtained in purified,
267 mineral, and river waters, respectively. Photolysis of rivaroxaban in purified water spiked
268 with nitrate ions led to a half-life of 2.0 hours.

269 The behavior of apixaban under simulated solar light irradiation is a function of the aqueous
270 medium. In purified water no degradation of the drug was observed after 19 hours of
271 irradiation. At the opposite, in mineral water, a degradation was observed with a calculated
272 half-life of 23.6 hours. This half-life decreased by a factor of 2.3 (10.4 hours) when apixaban
273 was irradiated in purified water in the presence of nitrate ions. When both nitrate and
274 hydrogen carbonate (74.0 mg.L^{-1}) ions were present in the medium, the half-life increased to
275 26.9 hours. In the river water, 50% of degradation would be reached after 96.3 hours, clearly
276 showing a lowering of the photodegradation efficiency.

277 **Please insert Table 1.**

278
279 From these results, we can state that among the three NOACs, dabigatran and rivaroxaban are
280 subject to direct photolysis under simulated sunlight irradiation, *i.e.*, degradation occurs in
281 purified water. At the opposite, direct photodegradation of apixaban is not significant. In
282 addition to direct photolysis, photosensitized reactions are clearly observed for dabigatran and
283 apixaban. Ions naturally present in the mineral water can be at the origin of this processes.
284 The photosensitizing potential of nitrate ions was particularly reviewed by Mack and Bolton
285 (1999). These ions absorb solar light and then react with water to produce highly reactive
286 hydroxyl radicals (Zuo et al., 2006; Chen et al., 2013). The photodegradation kinetics of
287 dabigatran is accelerated in mineral water and in purified water spiked with nitrate ions. Both
288 aqueous media led to a half-life close to 7.0 hours. We can thus conclude that the ion mainly
289 responsible for the acceleration of dabigatran photodegradation was NO_3^- . In the case of
290 apixaban, circumstances are slightly different. Nitrate ions are also proved to accelerate the
291 drug photolysis but this result is not sufficient to explain the obtained half-life in mineral
292 water. In fact, 50% of degradation would be reached after 23.6 and 10.4 hours in mineral
293 water and deionized water spiked with NO_3^- , respectively. Addition of hydrogen carbonate
294 ions to water spiked with nitrate ions induced an increase of the half-life by a factor of 2.6 ($t_{1/2}$
295 = 26.9 h). Hydrogen carbonate ions are among the most widespread anions in environmental
296 waters and they are known to act as scavengers of hydroxyl radicals (Nakatani et al., 2007;
297 Tercero Espinoza et al., 2007). Therefore, in our case the increase of apixaban half-life is
298 mainly due to the reaction of HCO_3^- ions with hydroxyl radicals produced by nitrate ions
299 under simulated sunlight irradiation. Finally, rivaroxaban photolysis seems not to be
300 significantly impacted by ions present in the mineral water. This result can be explained by
301 the very fast direct phototransformation of rivaroxaban. As a consequence, indirect
302 photochemistry is unable to affect the phototransformation rate significantly.

303 In the river water, the concentration of nitrate ions (7.7 mg.L^{-1}) is close to that measured in
304 mineral water (7.2 mg.L^{-1}). The concentration of hydrogen carbonate ions is included between
305 200 and 140 mg.L^{-1} , *i.e.*, at least twice as high as in mineral water. Thus, the HCO_3^- ions
306 scavenging effect is expected to be more efficient in river water than in mineral water. In
307 surface waters, the ions effects are added to those of DOM that can act as photosensitizer, as
308 screen through competitive light absorption, as inhibitor of triplet-sensitized transformation
309 by reducing partially oxidized intermediates to the starting compounds, and as radical
310 scavenger (Walse et al., 2004; Wenk et al., 2011, Chen et al., 2013; Liang et al., 2015; Zhang
311 et al., 2016). In the case of dabigatran, the half-life in river water increases by a factor of 3.3
312 in comparison with the mineral water. This increase cannot be assigned only to the
313 scavenging effect of HCO_3^- ions. In fact, the comparison of results in mineral water and in
314 purified water spiked with NO_3^- ions shows no clear effect of HCO_3^- ions on the rate constants
315 for photolysis of dabigatran (Table 1). In the same way, apixaban half-life increases by a
316 factor of 4.1. Thus, the increase of half-lives of dabigatran and apixaban cannot be explained
317 only by the concentration increase of HCO_3^- ions in the river water but by the effect of DOM.
318 Results obtained with rivaroxaban help to understand its role. In fact, rivaroxaban
319 phototransformation is apparently not impacted by the presence of DOM. Thus, no significant
320 screen effect is observed from DOM (see SF1 of supplementary materials). This result leads
321 to the conclusion that the observed slowing down of the degradation of dabigatran and
322 apixaban in river water is mainly due to a scavenging of hydroxyl radicals. The effect of
323 DOM is emphasized with apixaban, for which the degradation occurs only by means of
324 photosensitization.

325 Finally, the efficiency of NOACs direct photolysis was evaluated from polychromatic
326 quantum yields of photodegradation. Values of 2.2×10^{-4} and $4.4 \times 10^{-2} \text{ mol.E}^{-1}$ were calculated
327 for dabigatran and rivaroxaban, respectively. Dabigatran absorbs sunlight to a higher extent

328 than rivaroxaban (Fig. 1), which partially offset the strong difference in the photolysis
329 quantum yields. In the case of apixaban, no quantum yield for photolysis was determined due
330 to the absence of degradation after 19 hours of simulated sunlight irradiation.

331

332 **3.3. Structures of NOACs photoproducts.** For each NOAC, non-irradiated and irradiated
333 solutions were analyzed by means of LC-QToF-MS. Three products (D1, D2, and D3) were
334 observed for dabigatran and one product (R1) for rivaroxaban, both irradiated in purified
335 water. For apixaban, seven products (A1 to A7) were observed in mineral water. Results are
336 gathered in Table 2.

337 In the case of dabigatran, among the three photoproducts observed two of them were
338 associated to the drug photodegradation (Fig. 2). These compounds have m/z of 136.0874
339 (D1, $C_7H_{10}N_3$) and 323.1145 (D2, $C_{17}H_{15}N_4O_3$). D1 fragmentation spectrum displays m/z at
340 119 (-17) associated to the loss of a NH_3 group located on the amidine part, and at 92 (-27)
341 associated to the loss of the $C=NH$ fragment (see SF4 of supplementary materials).

342

343 **Please insert Table 2.**

344

345 D1 was identified as the 4-aminobenzamidine (4-AB). This hypothesis was confirmed from
346 LC-UV (Fig. 3a) and LC-QToF (see SF4 of supplementary materials) analyses of the
347 standard. 4-AB is known to act as inhibitor of trypsin, an enzyme involved in the digestion of
348 proteins (Safarik et al., 2002). D2 structure is formed from the other moiety of dabigatran for
349 which an intramolecular cyclization and a demethylation occurred (Fig. 2). Only one fragment
350 ($m/z = 251$) is clearly observed for D2. This fragment is associated to a decarboxylation and a
351 CO elimination. The UV spectrum of D2 is displayed in Fig. 3a.

352

Please insert Fig. 2.

353 Finally, a last photoproduct (D3) with a m/z of 279.1248 ($C_{16}H_{15}N_4O$) was observed. This
354 compound is not directly formed from dabigatran but from D2. In fact, D2 and D3 have a m/z
355 difference of 44 (CO_2 group) and produce the same fragment at $m/z = 251$. Therefore, we can
356 assume that D3 is formed from the photo-decarboxylation of D2.

357

358 **Please insert Fig. 3.**

359

360 Rivaroxaban degradation induced by simulated solar light irradiation in purified water
361 produces only one main photoproduct with molecular ion at m/z 436.0732 (named R1) in
362 ESI+ mode (Table 2). R1 has the same m/z as rivaroxaban, *i.e.* a photoisomerization occurred.
363 The UV absorption spectrum of R1 displays one band at 245 nm while that of rivaroxaban
364 presents a maximum at 248 nm with a shoulder at 273 nm (Fig. 3b). The substituted thiophene
365 moiety is responsible for the maximum of absorption around 245 nm (Boig et al., 1953); this
366 part of the parent compound is unchanged in R1. This result is supported by the presence of a
367 fragment at m/z 145 assigned to the substituted thiophene ring and observed on both spectra
368 of isomers (see SF8 of supplementary materials).

369

370 **Please insert Fig. 4.**

371

372 Processes of photoisomerization involving an intramolecular cyclisation as photo-Fries
373 (Sandner et al., 1968) and Norrish type II reaction (Norrish et al., 1937) were considered. The
374 two processes lead to structures in which the substituted thiophene ring is unchanged as
375 validated using high field NMR. Indeed, 1H and ^{13}C NMR spectra show the same splitting
376 patterns for both compounds (see SF9 and SF10 of supplementary materials). The SOFAST-
377 HMQC 1H - ^{15}N 2D map undoubtedly shows the presence of a NH group in the R1 structure

378 (Fig. 4a) and the TOCSY sequence clearly exhibits two correlation pathways between NH
379 proton (8.93 and 8.75 for R and R1 structures, respectively) and protons at 3.74 (J3), 4.92
380 (J4), and 4.24/3.95 (J5) ppm for R, 4.24/3.95 (J5) ppm for R and 3.74/3.84 ppm (J3), 4.96
381 (J4), and 4.00/4.26 (J5) ppm for R1 (Fig. 4b). On this basis, the proposed structure for R1 is
382 presented in Fig. 5.

383

384

Please insert Fig. 5.

385

386 The simulated sunlight irradiation of apixaban in mineral water results in the clear observation
387 of seven photoproducts (Table 2). Assuming that LC-MS/MS structure responses are the same
388 for each compound, four degradation products can be considered as major (A3, A5, A6, and
389 A7). Their structures are presented in Fig. 6. Structures of minor photoproducts have also
390 been determined (A1, A2, and A4; see SS2, SS3, and SS5 of supplementary materials).

391

392

Please insert Fig. 6.

393

394 A1 ($m/z = 191.1186$) and A2 ($m/z = 287.1146$) are assigned to $C_{11}H_{15}N_2O$ and $C_{14}H_{15}N_4O_3$,
395 chemical formula, respectively. Concerning the main photoproducts, A3 (354.1581,
396 $C_{18}H_{20}N_5O_3$) is formed from the release of methoxybenzene, A4 and A5 (476.1939,
397 $C_{25}H_{26}N_5O_5$) are obtained by hydroxylation of the phenyl group, A6 (446.1818, $C_{24}H_{24}N_5O_4$)
398 is formed from the substitution of the methoxy group by a hydroxy group, and A7 (458.1820,
399 $C_{25}H_{24}N_5O_4$) is obtained from the formation of an unsaturation into the 2-oxo-1-piperidinyl
400 ring linked to the pyrazole ring. This latter structure is in agreement with a polarity lowering
401 and thus a retention time increase. The fragmentation pattern of each photoproduct is
402 described in SS4, SS6, SS7, and SS8 of supplementary materials.

403 **4. Conclusion**

404 Kinetics of photodegradation show that photolysis can be one of the main ways of
405 degradation for NOACs present in surface waters. Rivaroxaban degradation is not
406 significantly impacted by the nature of the aqueous matrix. This compound seems only to
407 undergo direct photolysis leading to a photoisomerization. The structure of the photoisomer
408 has been clearly identified from NMR spectra. Dabigatran is photolyzed by direct and indirect
409 routes. Nitrate ions are mainly responsible for the acceleration of dabigatran degradation.
410 Results in river water show that dissolved organic matter slows down the degradation of
411 dabigatran probably related to its action as a scavenger of hydroxyl radicals. Two
412 photoproducts are formed, *i.e.* the 4-aminobenzamidine and a cyclization product. Apixaban
413 is degraded only by photosensitization which is induced by nitrate ions. At the opposite,
414 hydrogen carbonate ions partially block the degradation of apixaban. This effect is also
415 observed in the presence of dissolved organic matter. Seven photoproducts were clearly
416 identified under simulated solar light irradiation of apixaban.
417 Following these results, it will be important to evaluate the ecotoxicological impact of
418 NOACs photoproducts. Compounds of potential concern are especially the isomer of
419 rivaroxaban and the 4-aminobenzamidine, which is a known inhibitor of trypsin and could be
420 at the origin of toxic effects.

421

422 **ACKNOWLEDGEMENTS**

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430 this study.

431

432 **SUPPLEMENTARY MATERIALS**

433 Supplementary information related to this article can be found at

ACCEPTED MANUSCRIPT

434 **REFERENCES**

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436 V11.02 (© 1994-2016 ACD/Labs).
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1 Photodegradation of novel oral anticoagulants under sunlight irradiation in
2 aqueous matrices

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Tables

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Table 1

Apparent first-order rate constants (k , min^{-1}), coefficients of determination (R^2), half-lives ($t_{1/2}$, h), and polychromatic quantum yields of photolysis (ϕ , $\text{mol}\cdot\text{E}^{-1}$) obtained from Suntest irradiation of NOACs in aqueous matrices. ND: not degraded. Error bar on first-order rate constants: $\pm 10\%$.

NOAC	Aqueous matrix	$k \times 10^4$	R^2	$t_{1/2}$	ϕ
Dabigatran	purified water	6.0	0.9944	19.9	2.2×10^{-4}
	mineral water	14.1	0.9778	7.4	-
	purified water + NO_3^-	17.1	0.9953	6.8	-
	river water	4.7	0.9633	24.1	-
Rivaroxaban	purified water	49.4	0.9975	2.3	4.4×10^{-2}
	mineral water	55.9	0.9943	2.1	-
	purified water + NO_3^-	57.5	0.9808	2.0	-
	river water	51.6	0.9960	2.2	-
Apixaban	purified water	ND	-	-	ND
	mineral water	4.9	0.9924	23.6	-
	purified water + NO_3^-	11.1	0.9892	10.4	-
	purified water + NO_3^- + HCO_3^-	4.3	0.9823	26.9	-
	river water	1.2	0.9875	96.3	-

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38

39 **Table 2**

40 LC-MS/MS (ESI+) analyses of NOACs and of their photoproducts. RT: retention time.

NOAC	Name	m/z (RT in min)	Proposed chemical formula	$\Delta(m/z)$ (ppm)	Fragments (relative intensity in %)
Dabigatran	D	472.2116 (2.2)	$C_{25}H_{26}N_7O_3$	-4.02	-
	D1	136.0874 (1.0)	$C_7H_{10}N_3$	0.73	119 (100), 92 (17)
	D2	323.1145 (2.3)	$C_{17}H_{15}N_4O_3$	-0.31	251 (100)
	D3	279.1248 (3.2)	$C_{16}H_{15}N_4O$	-0.72	251 (100)
Rivaroxaban	R	436.0739 (11.3)	$C_{19}H_{19}ClN_3O_5S$	-1.15	-
	R1	436.0732 (8.2)	$C_{19}H_{19}ClN_3O_5S$	0.46	436 (73), 231 (63), 145 (100)
Apixaban	A	460.2012 (11.7)	$C_{25}H_{26}N_5O_4$	-5.87	-
	A1	191.1186 (1.5)	$C_{11}H_{15}N_2O$	-1.05	191 (100), 147 (16), 107 (13)
	A2	287.1146 (5.3)	$C_{14}H_{15}N_4O_3$	-0.70	270 (100), 242 (9)
	A3	354.1581 (5.5)	$C_{18}H_{20}N_5O_3$	-4.24	337 (100)
	A4	476.1939 (8.0)	$C_{25}H_{26}N_5O_5$	-1.05	459 (100), 241 (13), 199 (74)
	A5	476.1939 (8.5)	$C_{25}H_{26}N_5O_5$	-1.05	459 (100), 361 (11), 241 (18), 200 (13), 199 (70)
	A6	446.1818 (8.8)	$C_{24}H_{24}N_5O_4$	2.24	430 (23), 429 (83), 282 (13), 227 (15), 186 (13), 185 (100), 171 (12), 93 (11)
	A7	458.1820 (12.3)	$C_{25}H_{24}N_5O_4$	1.75	458 (42), 441 (100), 385 (64), 342 (10), 277 (15), 199 (36)

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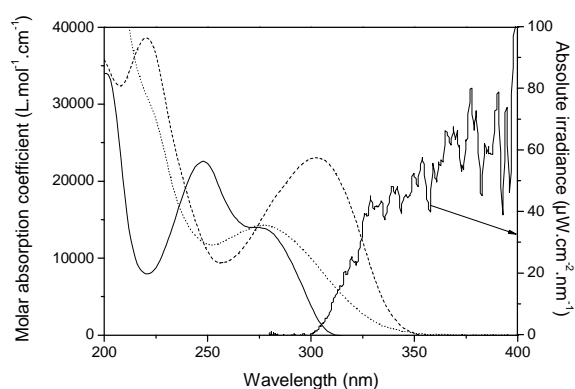
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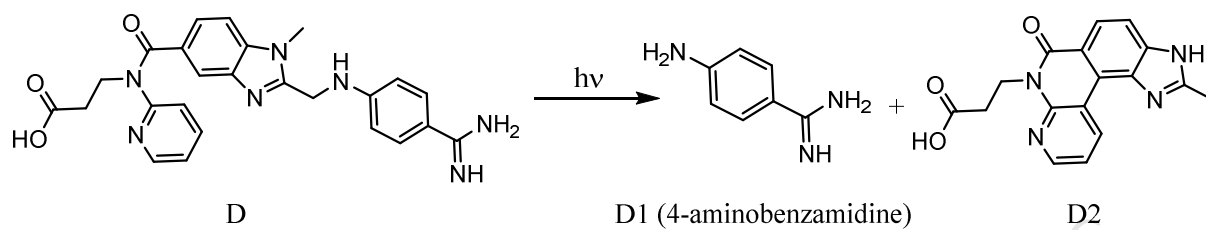


31

32 **Fig. 1.** UV absorption spectra of dabigatran (dashed line) and rivaroxaban (solid line)
33 dissolved in purified water, and of apixaban (dotted line) dissolved in a methanol-water
34 mixture. Solar light emission spectrum at the Earth surface on May 5, 2016 at coordinates
35 (45.195510, 0.718811).

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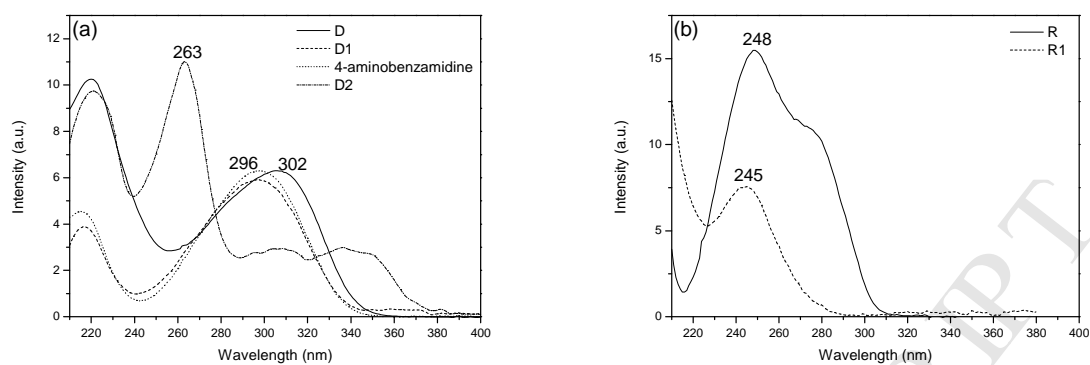
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40 **Fig. 2.** Photodegradation of dabigatran under simulated solar light irradiation in purified

41 water.

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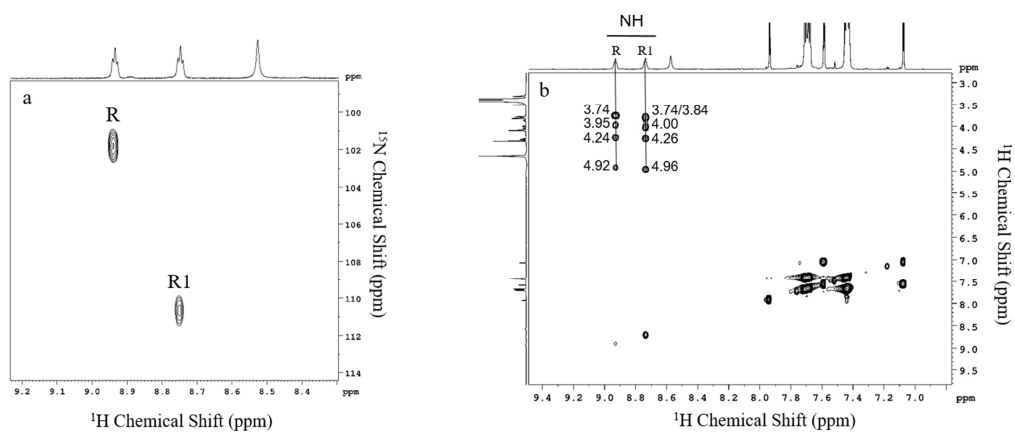


44

45 **Fig. 3.** UV absorption spectra of (a) dabigatran (solid line), D1 (dashed line), 4-
46 aminobenzamidine (dotted line), and D2 (dashed-dotted line); (b) rivaroxaban (solid line) and
47 of its photoisomer (R1, dashed line).

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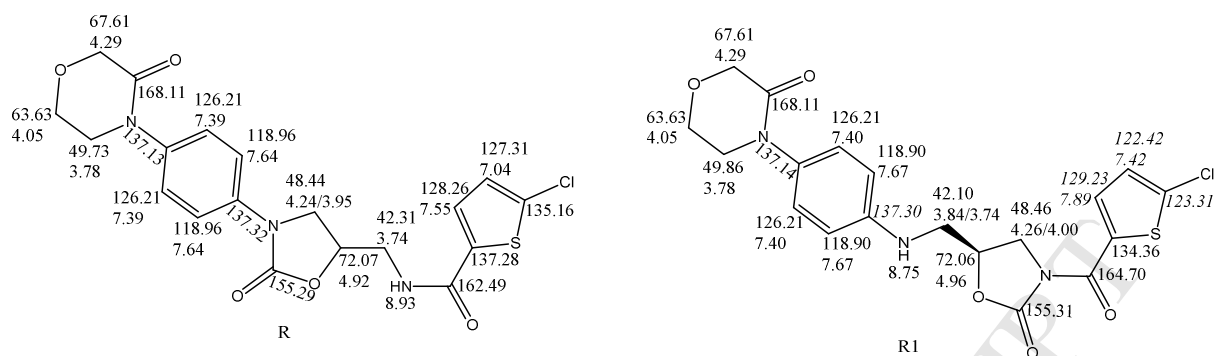


50

51 **Fig. 4.** Selected expansions of 2D NMR spectra of rivaroxaban (R) and of its photoisomer
52 (R1) in MeOH- d_3 : (a) SOFAST-HMQC ^1H - ^{15}N and (b) TOCSY NMR spectra.

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57 **Fig. 5.** ^{13}C and ^1H chemical shifts (ppm) of rivaroxaban (R) and its photoisomer (R1).

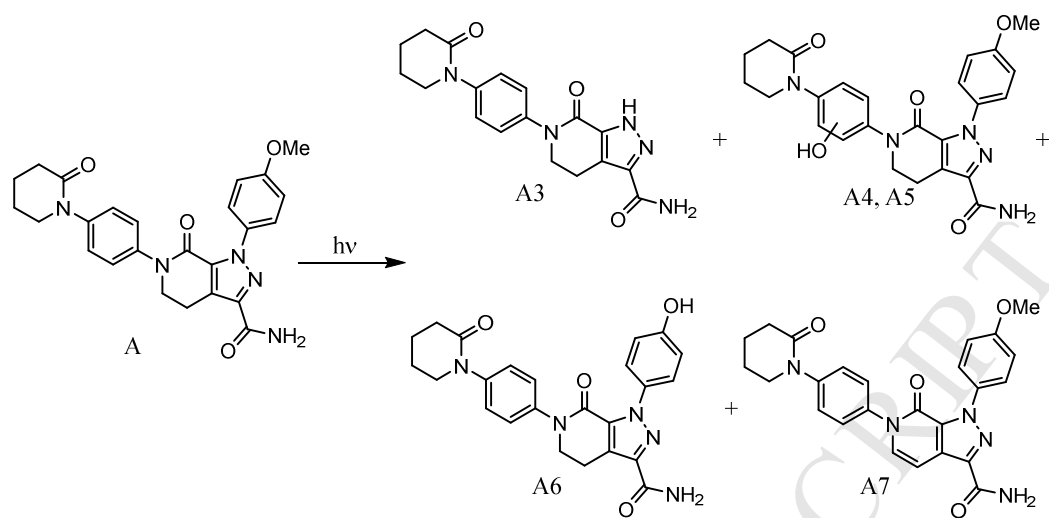
58 Chemical shifts in italics: the similarity of the two structures (R and R1) as well as the low

59 concentration of the samples did not allow, despite the use of a very high magnetic field, to

60 remove the ambiguity on the assignment of chemical shifts.

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64 **Fig. 6.** Major photoproducts of apixaban formed under simulated solar light irradiation in
65 mineral water.

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Photodegradation of novel oral anticoagulants under sunlight irradiation in
aqueous matrices

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Highlights

- Dabigatran undergoes direct and indirect photolysis, $t_{1/2}$ is of 24h in river water.
- Direct photolysis is predominant for rivaroxaban, $t_{1/2}$ is of 2.2h in river water.
- Argatroban is mainly photodegraded by photosensitization.
- Structures of main photoproducts have been identified.