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1 **Direct DOC and nitrate determination in water using**  
2 **dual pathlength and second derivative UV**  
3 **spectrophotometry**

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12 **Key words**

13 UV spectrophotometry, second derivative, nitrate, DOC, freshwaters, dual optical  
14 pathlength

15 **Summary**

16 UV spectrophotometry is largely used for water and wastewater quality monitoring. The  
17 measurement/estimation of specific and aggregate parameters such as nitrate and dissolved  
18 organic carbon (DOC) is possible with UV spectra exploitation, from 2 to multi wavelengths  
19 calibration. However, if nitrate determination from UV absorbance is known, major optical  
20 interferences linked to the presence of suspended solids, colloids or dissolved organic matter  
21 limit the relevance of UV measurement for DOC assessment. A new method based on UV

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22 spectrophotometric measurement of raw samples (without filtration) coupling a dual  
23 pathlength for spectra acquisition and the second derivative exploitation of the signal is  
24 proposed in this work. The determination of nitrate concentration is carried out from the  
25 second derivative of the absorbance at 226nm corresponding at the inflexion point of nitrate  
26 signal decrease. A short optical pathlength can be used considering the strong absorption of  
27 nitrate ion around 210nm. For DOC concentration determination the second derivative  
28 absorbance at 295nm is proposed after nitrate correction. Organic matter absorbing slightly in  
29 the 270-330nm window, a long optical pathlength must be selected in order to increase the  
30 sensitivity. The method was tested on several hundred of samples from small rivers of two  
31 agricultural watersheds located in Brittany, France, taken during dry and wet periods. The  
32 comparison between the proposed method and the standardised procedures for nitrate and  
33 DOC measurement gave a good adjustment for both parameters for ranges of 2-100 mg/L  
34 NO<sub>3</sub> and 1-30 mg/L DOC.

35

## 36 **1 Introduction**

37 Nutrient monitoring in water bodies is still a challenge. The knowledge of nutrient  
38 concentrations as nitrate and dissolved organic carbon (DOC) in freshwater bodies is  
39 important for the assessment of the quality impairment of water resources touched by  
40 eutrophication or harmful algal blooms for example. The export of these nutrients in  
41 freshwater is often characterized on one hand, by a high spatio-temporal variability regarding  
42 seasonal change, agricultural practices, hydrological regime, tourism and on the other hand,  
43 by the nature and mode of nutrient sources (punctual/diffuse, continuous/discontinuous)  
44 (Causse et al., 2015). In this context the monitoring of nitrate and DOC must be rapid and

45 easy to use on the field and UV spectrophotometry is certainly the best technique for that,  
46 given the great number of works, applications and systems proposed in the last decades.

47 Nitrate monitoring with UV sensing is a much more mature technique than DOC assessment  
48 by UV because nitrate ion has a specific and strong absorption. Several methods are available  
49 for drawing a relationship between UV absorbance and nitrate concentration using  
50 wavelength(s) around 200-220 nm, usually after sample filtration to eliminate interferences  
51 from suspended solids. Considering the presence of potential interferences such as dissolved  
52 organic matter (DOM) in real freshwater samples, the use of at least two wavelengths  
53 increases the quality of adjustment. The absorbance measurement at 205 and 300 nm was  
54 proposed by (Edwards et al., 2001) and the second derivative absorbance (SDA) calculated  
55 from three wavelengths was promoted by Suzuki and Kuroda (1987) and Crumpton et al.  
56 (1992). A comparison of the two methods (two wavelengths and SDA) carried out on almost  
57 100 freshwater samples of different stations in a 35 km<sup>2</sup> watershed, gave comparable data  
58 with ion chromatography analysis (Olsen, 2008). Other methods based on the exploitation of  
59 the whole UV spectrum were also proposed in the last decades, namely for wastewater and  
60 sea water, with the aim of a better treatment of interferences. Several multiwavelength  
61 methods were thus designed such as the polynomial modelisation of UV responses of organic  
62 matter and colloids (Thomas et al., 1990), a semi deterministic approach, including reference  
63 spectra (nitrate) and experimental spectra of organic matter, suspended solids and colloids  
64 (Thomas et al., 1993), or partial least square regression (PLSR) method built-into a field  
65 portable UV sensor (Langergraber et al., 2003). Kröckel et al. (2011) proposed a combined  
66 method of exploitation (multi component analysis (MCA) integrating reference spectra and a  
67 polynomial modelisation of humic acids), associated to a miniaturized spectrophotometer  
68 with a capillary flow cell. More recently, a comparison between two different commercials in  
69 situ spectrophotometers, a double wavelength spectrophotometer (DWS) and a

70 multiwavelength one (MWS) with PLSR resolution was carried out by Huebsch et al. (2015)  
71 for groundwater monitoring. The findings were that the MWS offers more possibilities for  
72 calibration and error detection, but requires more expertise compared with the DWS.

73 Contrary to UV measurement of nitrate in water, DOC is associated with a bulk of dissolved  
74 organic matter (DOM) with UV absorption properties less known and defined than nitrate.  
75 The study of the relation between absorbing DOM (chromophoric DOM or CDOM) and DOC  
76 has given numerous works on the characterisation of CDOM by UV spectrophotometry or  
77 fluorescence on one hand, and on the assessment of DOC concentration from the  
78 measurement of UV parameters on the other hand. Historically the absorbance at 254nm  
79 (A<sub>254</sub>), 254 nm being the emission wavelength of the low pressure mercury lamp used in the  
80 first UV systems, was the first proxy for the estimation of Total organic carbon (Dobbs et al.,  
81 1972), and was standardised in 1995 (Eaton, 1995). Then the specific UV absorbance, the  
82 ratio of the absorbance at 254 nm (A<sub>254</sub>) divided by the DOC value was also standardized ten  
83 years after (Potter and Wimsatt, 2005). Among the more recent works, Spencer et al. (2012)  
84 shown strong correlations between CDOM absorption (absorbance at 254 and 350 nm  
85 namely) and DOC for a lot of samples from 30 US watersheds. Carter et al. (2012) proposed a  
86 two component model, one absorbing strongly and representing aromatic chromophores and  
87 the other absorbing weakly and associated with hydrophilic substances. After calibration at  
88 270 and 350 nm, the validation of the model for DOC assessment was quite satisfactory for  
89 1700 filtered surface water samples from North America and the UK. This method was also  
90 used for waters draining upland catchments and it was found that both a single wavelength  
91 proxy (263 nm or 230 nm) and a two wavelengths model performed well for both pore water  
92 and surface water (Peacock et al., 2014). Besides these one or two wavelengths methods, the  
93 use of chemometric ones was also proposed at the same time as nitrate determination from a  
94 same spectrum acquisition (Thomas et al., 1993); (Rieger et al., 2004); (Etheridge et al.,

95 2014). A comparison of multiwavelengths exploitation methods for DOC determination  
96 including MLR/PLS was recently carried out by Avagyan et al. (2014) from the signal of a  
97 UV-vis submersible sensor with the recommendation to create site-specific calibration models  
98 to achieve the optimal accuracy of DOC quantification.

99 Among the above methods proposed for UV spectra exploitation, the second derivative of the  
100 absorbance (SDA) is rather few considered even if SDA is used in other application fields to  
101 enhance the signal and resolve the overlapping of peaks (Bosch Ojeda and Sanchez Rojas,  
102 2013). Applied to the exploitation of UV spectra of freshwaters SDA is able to suppress or  
103 reduce the signal linked to the physical diffuse absorbance of colloids and particles and slight  
104 shoulders can be revealed (Thomas and Burgess, 2007). If SDA was proposed for nitrate  
105 (Crumpton et al., 1992), its use for DOC has not been yet reported as well as a simultaneous  
106 SDA method for nitrate and DOC determination of raw sample (without filtration). This can  
107 be explained by the difficulty to obtain a specific response of organic matter and nitrate, in  
108 particular in the presence of high concentration of nitrate or high turbidity that cause spectra  
109 saturation and interferences. In this context, the aim of this work is to propose a new method  
110 to optimize the simultaneous measurement of DOC and nitrate using both dual optical  
111 pathlength and second derivative UV spectrophotometry.

## 112 **2 Material and methods**

### 113 ***2.1 Water samples***

114 Water samples were taken from the Ic and Frémur watersheds (Brittany, France) through very  
115 different conditions during the hydrological year 2013-2014. These two rural watersheds of  
116 86 km<sup>2</sup> and 77 km<sup>2</sup> respectively are concerned by water quality alteration with risks of green  
117 algae tides, closures of some beaches and contamination of seafood at their outlet. 580  
118 samples were taken from spot-sampling (342 samples) on 32 different subwatersheds by dry  
119 or wet weather (defined for 5 mm of rain or more, 24 h before sampling) and by auto-

120 sampling (233 samples) during flood events on 3 subwatersheds. For wet weather, sampling  
121 was planned by spot-sampling before the rain, or programmed according to the local weather  
122 forecast to ensure a sample collection proportional to the flow. Samples were collected in 1L  
123 polyethylene bottles (24 for auto-sampler ISCO 3700) following the best available practices.  
124 Samples were transported to the laboratory in a cooler and stored at  $5 \pm 3^\circ\text{C}$  (NF EN ISO  
125 5667-3, 2013).

## 126 **2.2 Data acquisition**

127 Nitrate concentration was analyzed according to NF EN ISO 13395 standard thanks to a  
128 continuous flow analyzer (Futura Alliance Instrument). Dissolved organic carbon (DOC) was  
129 determined by thermal oxidation coupled with infrared detection (Multi N/C 2100, Analytik  
130 Jena) following acidification with HCl (standard NF EN 1484). Samples were filtered prior to  
131 the measurement with  $0.45 \mu\text{m}$  HA Membrane Filters (Millipore®).

132 Turbidity (NF EN ISO 7027, 2000) was measured in situ for each sample, with a  
133 multiparameter probe (OTT Hydrolab MS5) for spot-sampling and with an Odeon probe  
134 (Neotek-Ponsel, France) for auto-sampling stations.

135

136 Finally discharge data at hydrological stations were retrieved from the database of the  
137 national program of discharge monitoring.

## 138 **2.3 UV measurement**

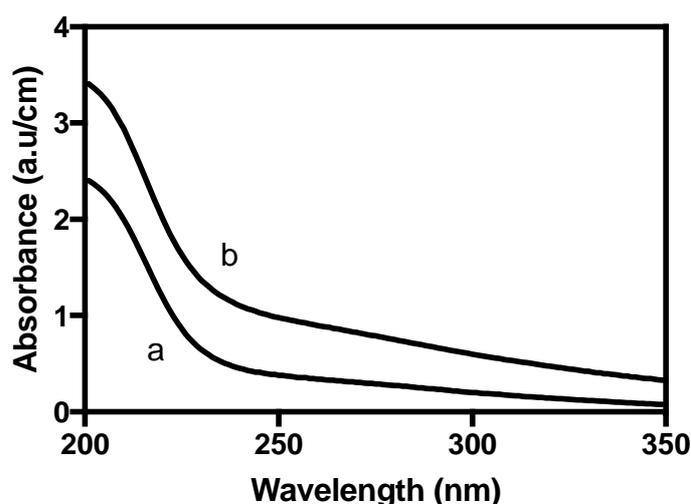
### 139 **2.3.1. Spectra acquisition**

140 UV spectra were acquired with a Perkin Elmer Lambda 35 UV/Vis spectrophotometer,  
141 between 200 and 400 nm with different Suprasil® quartz cells (acquisition step: 1 nm, scan  
142 speed: 1920 nm/min). Two types of quartz cells were used for each sample. A short path  
143 length cell of 2 mm was firstly used to avoid absorbance saturation in the wavelength domain  
144 strongly influenced by nitrate below 240 nm (linearity limited to 2.0 a.u.). On the contrary, a

145 longer pathlength cell (20 mm) was used in order to increase the signal for wavelengths  
146 outside of the influence of nitrates ( $> 240$  nm approx.). Regarding a classic UV  
147 spectrophotometer with a pathlength cell of 10 mm, these dual pathlength devices act as a  
148 spectrophotometric dilution/concentration system, adapted to a high range of variation of  
149 nitrate concentrations in particular.

### 150 *2.3.2 Preliminary observation*

151 Before explaining the proposed method, a qualitative relation between UV spectra shape and  
152 water quality can be reminded. Figure 1 shows two spectra of raw freshwaters with the same  
153 nitrate concentration (9.8 mgNO<sub>3</sub>/L) taken among samples of the present work. These spectra  
154 are quite typical of freshwaters. If the nitrate signal is well identified with the half Gaussian  
155 below 240 nm, the one of organic matter responsible for DOC is very weak with a very slight  
156 shoulder above 250 nm. In this context, the use of SDA already proposed for nitrate  
157 determination (Crumpton et al., 1992) and giving a maximum for any inflexion point in the  
158 decreasing part of the signal after a peak or a shoulder can be useful. However, given the  
159 absorbance values above 250 nm, the use of a longer optical pathlength is recommended in  
160 order to increase the sensitivity of the method.



161

162 Figure 1: Example of UV spectra of raw freshwaters with the same concentration of nitrate  
163 (9.8 mgNO<sub>3</sub>/L) and different DOC values (10.9 and 18.3 mgC/L for sample “a” and “b”  
164 respectively).

### 165 **2.3.3 Methodology**

166 The general methodology is presented in Figure 2. Firstly, a UV spectrum is obtained directly  
167 from a raw sample (without filtration nor pretreatment) with a 2mm pathlength (PL) cell. If  
168 the absorbance value at 210 nm (A<sub>210</sub>), is greater than 2 u.a., a dilution with distilled water  
169 must be carried out. If not, the second derivative of the absorbance (SDA) at 226 nm is used  
170 for nitrate determination. The SDA value at a given wavelength  $\lambda$  is calculated according to  
171 the equation 1 (Thomas and Burgess 2007):

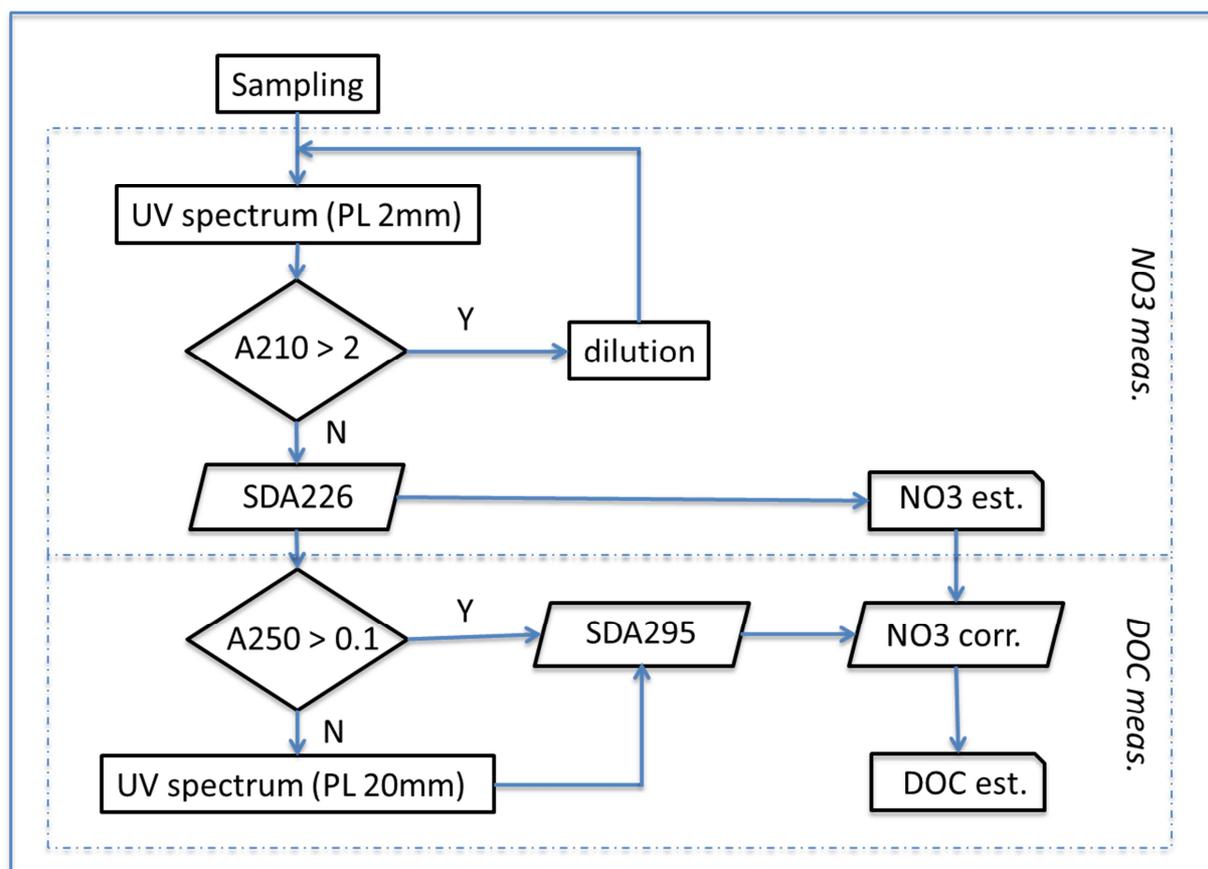
$$172 \quad SDA_{\lambda} = k * \frac{(A_{\lambda-h} + A_{\lambda+h} - 2 * A_{\lambda})}{h^2} \quad [1]$$

173 where A <sub>$\lambda$</sub>  is the absorbance value at wavelength  $\lambda$ ,  $k$  is an arbitrary constant (chosen here  
174 equal to 1000) and  $h$  is the derivative step (here set at 10 nm).

175  
176 Given the variability between successive SDA values linked to the electronic noise of the  
177 spectrophotometer, a smoothing step of the SDA spectrum is sometimes required, particularly  
178 when the initial absorbance values are low (< 0.1 a.u.). This smoothing step is based on the  
179 Stavitsky-Golay’s method (Stavitsky and Golay, 1964).

180 For DOC measurement, the SDA value at 295 nm is used if A<sub>250</sub> is greater than 0.1 a.u.. If  
181 A<sub>250</sub> is lower than 0.1, the intensity of absorbances must be increased with the use of a  
182 20mm pathlength cell. After the SDA<sub>295</sub> calculation, a correction from the value of SDA<sub>226</sub>  
183 linked to the interference of nitrate around 300nm is carried out. This point will be explained  
184 in the DOC calibration section.

185



186

187 Figure 2 : General methodology

188

189 **2.3.4 Nitrate Calibration**190 A mother solution of 100 mg NO<sub>3</sub><sup>-</sup>/L was prepared from a Nitrate Standard solution Certipur191 at 1g/L. Solutions of 2.5, 5.0, 10.0, 20.0, and 50.0 mg NO<sub>3</sub><sup>-</sup>/L were prepared with UHQ water.

192 Figure 3 shows spectra and second derivatives of standard nitrate solutions. Nitrate strongly

193 absorbs around 200-210 nm with a molar absorption coefficient of  $8.63 \cdot 10^5 \text{ m}^2/\text{mol}$  at 205.6

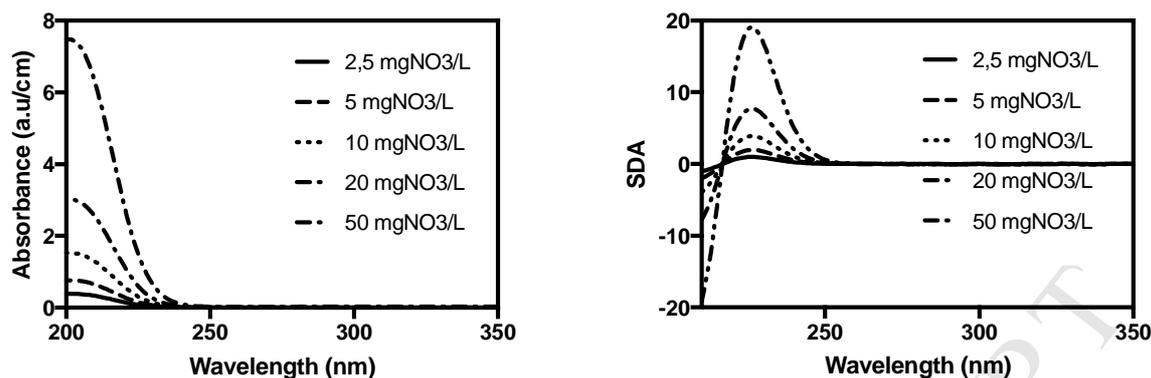
194 nm (Thomas and Burgess, 2007) and a maximum at 226 nm corresponding at the inflexion

195 point of absorbance spectrum, is observed for the second derivative signal. However if nitrate

196 strongly absorbs around 210nm for concentration usually found in freshwaters (below 100

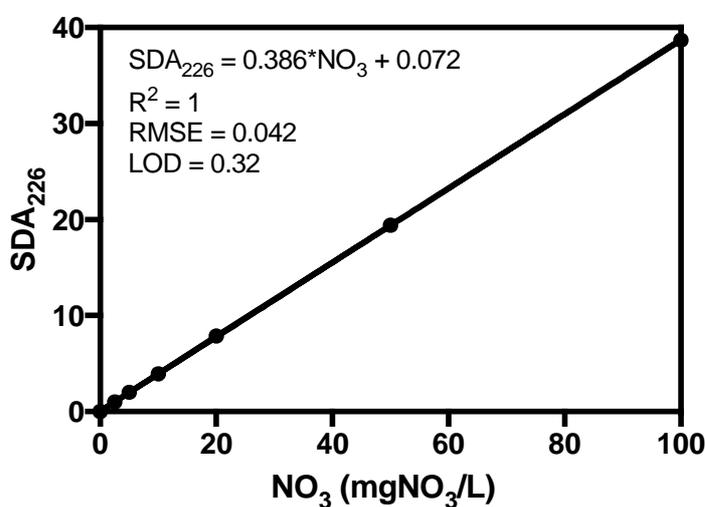
197 mgNO<sub>3</sub>/L), a peak of absorbance can also be observed for higher concentrations of nitrate198 with a much lower molar absorption coefficient of  $1.71 \cdot 10^3 \text{ m}^2/\text{mol}$  at 301nm (Thomas and

199 Burgess, 2007).



200  
201 Figure 3 : Spectra of standard solutions of nitrate (raw absorbances left, and SDA right)

202  
203 From the results of SDA values and the corresponding concentration of nitrate, a calibration is  
204 obtained for nitrate concentration ranging up to 100 mgNO<sub>3</sub>/L (Figure 4). This high value of  
205 nitrate concentration is possible thanks to the use of the 2 mm pathlength cell. Deduced from  
206 the calibration line, the R<sup>2</sup> value is very close to 1 and the limit of detection (LOD) is 0.32  
207 mgNO<sub>3</sub>/L.



208  
209 Figure 4 : Calibration line for nitrate determination from SDA at 226 nm (R<sup>2</sup> being equal to 1,  
210 99% prediction bands are overlapped with the best fit line)

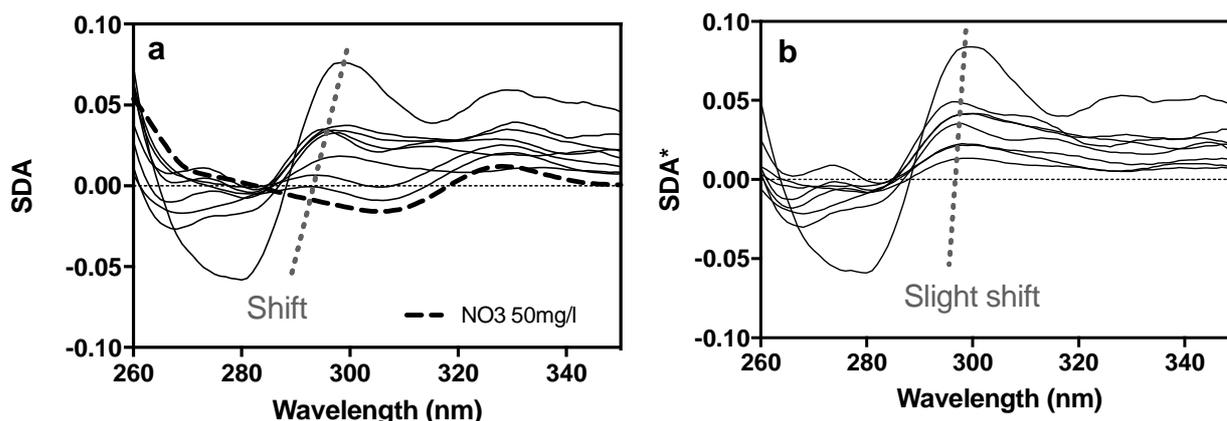
211  
212 **2.3.5 DOC Calibration**

213 For DOC calibration, the procedure is different from the one for nitrate given the absence of  
214 standard solution for DOC, covering the complexity of dissolved organic matter. A test set of  
215 49 samples was chosen among samples described hereafter, according to their DOC  
216 concentration up to 20 mgC/L. The choice of the SDA value at 295 nm is deduced from the  
217 examination of the second derivative spectra of some samples of the test set (Figure 5). Two  
218 peaks can be observed, the first one around 290 nm, and the second one less defined, around  
219 330 nm (Figure 5a). The maximum of the first peak is linked to the DOC content, but its  
220 position shifts between 290 and 300 nm, because of the relation between DOC and nitrate  
221 concentration, with relatively more important SDA values when DOC is low (Thomas et al.  
222 2014). Considering that the measurement is carried out with a long optical pathlength for  
223 DOC, and that nitrate also absorbs in this region, its presence must be taken into account. On  
224 Figure 5a the second derivative spectrum of a 50 mgNO<sub>3</sub>/L of nitrate presents a valley  
225 (negative peak) around 310 nm and a small but large peak around 330 nm. Based on this  
226 observation, a correction is proposed for the SDA of the different samples (equation 2):

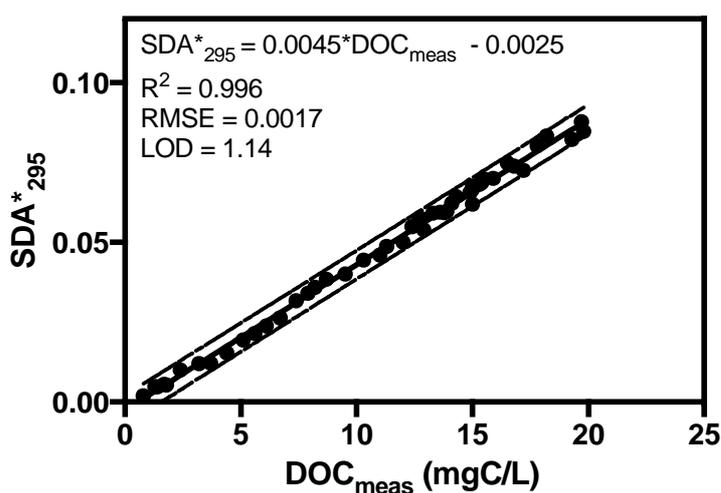
$$227 \quad SDA^* = SDA_{sample} - SDA_{nitrate} \quad [2]$$

228 Where SDA\* is the SDA corrected, SDA sample is the SDA calculated from the spectrum  
229 acquisition of a given sample and SDA nitrate is the SDA value corresponding to the nitrate  
230 concentration of the given sample.

231 After correction, the second derivative spectra show only a slight shift for the first peak  
232 around 300 nm and the peak around 330 nm is no more present (Figure 5b). From this  
233 observation, the SDA value at 295 nm is chosen for DOC assessment.



234  
 235 Figure 5: Second derivative spectra of water samples without nitrate correction (5a with a  
 236 spectrum of a 50 mgNO<sub>3</sub>/L nitrate solution, dotted line) and after nitrate correction (5b).  
 237 Nitrate, DOC and turbidity of samples vary respectively from 5.1 to 49.8 mgNO<sub>3</sub>/L, 2.9 to  
 238 15.4 mgC/L and 4 to 348 NTU.  
 239 Finally, Figure 6 displays the calibration relation between the SDA value at 295 nm corrected  
 240 by nitrate ( $SDA^*_{295}$ ) and the DOC concentration for the test set of 49 samples. The value of  
 241  $R^2$  is 0.996 and the LOD is 1.14 mgC/L.  
 242



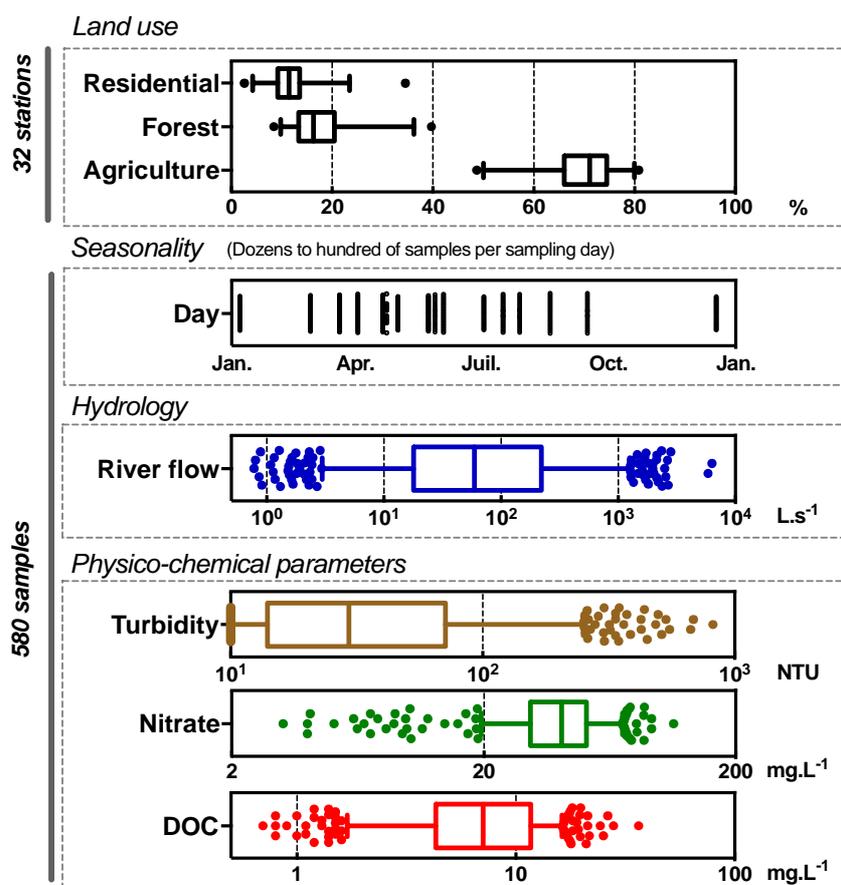
243  
 244 Fig 6: Calibration between SDA at 295nm corrected by nitrate ( $SDA^*_{295}$ ) and DOC  
 245 concentration (dotted lines define the 99% prediction band). DOC, nitrate, and turbidity of  
 246 samples vary respectively from 0.8 to 19.8 mgC/L, 2.9 to 65.7 mg NO<sub>3</sub>/L and 4 to 348 NTU

247

248 **3 Results**249 ***3.1 Samples characteristics***

250 For this work, a great number of samples were necessary for covering the different  
251 subwatersheds characteristics and the variability of hydrometeorological conditions all along  
252 the hydrological year. 580 samples were taken from 32 stations and the majority of samples  
253 were taken in spring and summer time with regard to the principal land use of the two  
254 watersheds and the corresponding agricultural practices, namely fertilization (Figure 7).  
255 Nitrate and DOC concentrations ranged respectively from 2.9 to 98.5 mgNO<sub>3</sub>/L and from 0.7  
256 to 28.9 mgC/L. The river flows were between 0.8 L/s and 6299 L/s and turbidity between 0.1  
257 and 821 NTU, after the rainy periods.

258

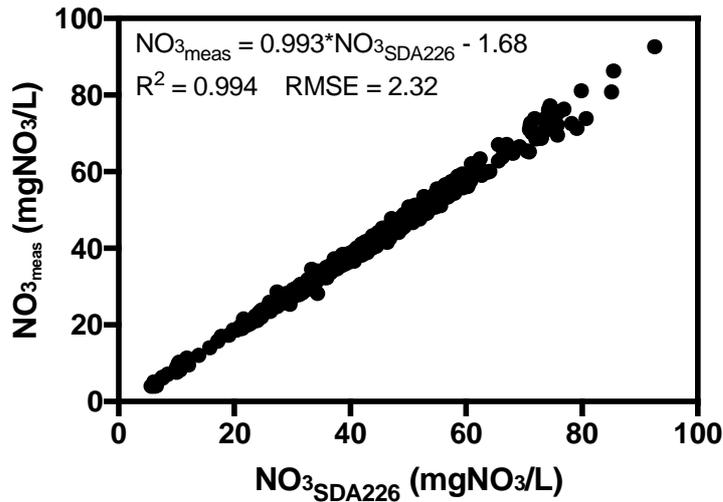


259

260 Figure 7: Relevance of samples in relation to land use, seasonality, land use and physico-  
 261 chemical parameters (box plots for inferior and superior quartile and median, whiskers for  
 262 minimum and maximum of 90 % of data, and points for the 5 % lower or greater  
 263 measurements)

### 264 3.2 Validation on freshwater samples

265 The validation of the method for nitrate determination was carried out on 580 samples (Figure  
 266 8). The adjustment between measured and estimated values of nitrate concentration gave a  $R^2$   
 267 greater than 0.99 and a RMSE of 2.32 mgNO<sub>3</sub>/L. The slope is close to 1 and the ordinate is  
 268 slightly negative (-1.68) which will be explained in the discussion section.



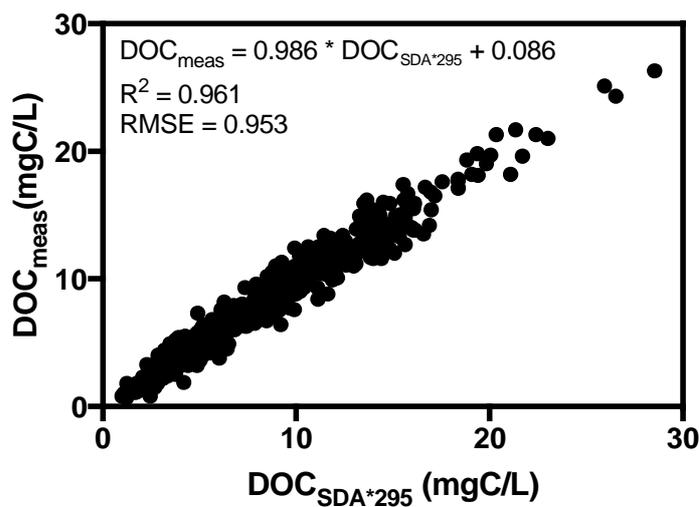
269

270 Fig 8: Relation between measured and estimated (from  $SDA_{226}$ )  $NO_3$  concentrations for 580  
 271 freshwater samples

272

273 The validation of the method for DOC determination was carried out on 580 samples (Figure  
 274 9). The adjustment between measured and estimated values of DOC concentration gave a  $R^2$   
 275 greater than 0.95 and a RMSE close to 1 mgC/L. The slope is close to 1 and the ordinate is  
 276 low (0.086 mgC/L).

277



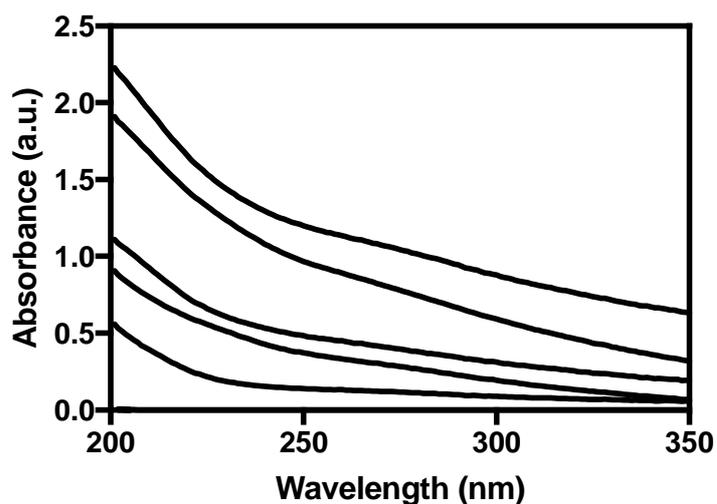
278

279 Fig 9: Relation between measured and estimated (by  $SDA_{295}$ ) DOC concentrations for 580  
 280 freshwater samples

## 281 **4 Discussion**

### 282 **4.1 Interferences**

283 Except for DOC assessment for which the value of SDA at 295 nm must be corrected by the  
284 presence of nitrate, different interferences have to be considered in nitrate measurement.  
285 Nitrate absorbing in the first exploitable window of UV spectrophotometry (measurement  
286 below 200 nm being quite impossible given the strong absorption of dioxygen) the presence  
287 of nitrite with a maximum of absorption at 213 nm could be a problem. However the molar  
288 absorption coefficient of nitrite is equal to the half of the value of nitrate (Thomas and  
289 Burgess, 2007) and usual concentrations of nitrite are much lower in freshwaters than nitrate  
290 ones (Raimonet et al., 2015). For other interferences linked to the presence of suspended  
291 solids or colloids (for raw samples) and organic matter for nitrate determination, Figure 10  
292 shows some example of spectral responses for some samples of the tests set taken under  
293 contrasted conditions (dry and wet weather) and corrected from nitrate absorption (i.e. the  
294 contribution of nitrate absorption is deduced from the initial spectrum of a each sample). The  
295 spectral shape is mainly explained by the combination of physical (suspended solids and  
296 colloids) and chemical (DOM) responses. Suspended solids are responsible for a linear  
297 decrease of absorbance up to 350 nm and more, colloids for an exponential decrease between  
298 200 and 240 nm and the main effect of the presence of DOM is the shoulder shape between  
299 250 and 300 nm the intensity of which being linked to DOC content. Thus the spectral shape  
300 is not linear around the inflexion point of the nitrate spectrum (226 nm) and the corresponding  
301 second derivative values being low at 226 nm give a theoretical concentration under 2  
302 mgNO<sub>3</sub>/L at maximum. This observation explains the slight negative ordinate of the  
303 validation curve (Figure 8).



304

305 Figure 10: Spectra of freshwater samples corrected from nitrate absorbance. Nitrate, DOC and  
 306 turbidity of samples vary respectively from 19.8 to 49.8 mgNO<sub>3</sub>/L, 4.9 to 15.4 mgC/L and 6  
 307 to 116 NTU.

308

309 In order to confirm the need of nitrate correction of SDA at 295 nm for DOC determination  
 310 the adjustment between DOC and SDA at 295nm without nitrate correction was carried out  
 311 for the same set of samples than for DOC calibration (Figure 6). Compared to the  
 312 characteristics of the corrected calibration line, the determination coefficient is lower (0.983  
 313 against 0.996) and the slope is greater (1.2 times) as well as the ordinate (7.9 times), the  
 314 RMSE (5.6 times) and the LOD (5.4 against 1.1 mgNO<sub>3</sub>/L. These observations can be  
 315 explained by the shift of the peak (around 290-295nm) and the hypochromic effect of nitrate  
 316 on the SDA value of the sample at 295 nm, (see Figure 5) showing the importance of the  
 317 nitrate correction for DOC determination.

318 Another interfering substance can be free residual chlorine absorbing almost equally at 200nm  
 319 and 291nm with a molar absorption coefficient of  $7.96 \times 10^4$  m<sup>2</sup>/mol at 291nm (Thomas O. and  
 320 Burgess C., 2007), preventing the use of the method for chlorinated drinking waters.

#### 321 **4.2 DOM absorption**

322 The peak around 295nm for the second derivative spectra reveals the existence of an inflexion  
323 point at the right part of the slight shoulder of the absorbance spectrum, between 250 and 300  
324 nm. This observation can be connected with the use of the spectral slope between 265 and 305  
325 nm (Galgani et al., 2011) to study the impact of photodegradation and mixing processes on  
326 the optical properties of dissolved organic matter (DOM) in the complement of fluorescence  
327 in two Argentine lakes. Fichot and Benner (2012) also used the spectral slope between 275  
328 and 295 nm for CDOM characterisation and its use as tracers of the percent terrigenous DOC  
329 in river-influenced ocean margins. Helms et al. (2008) propose to consider two distinct  
330 spectral slope regions (275–295 nm and 350–400 nm) within log-transformed absorption  
331 spectra in order to compare DOM from contrasting water types. The use of the log-  
332 transformed spectra was recently proposed by Roccaro et al. (2015) for raw and treated  
333 drinking water and the spectral slopes between 280 and 350nm were shown to be correlated to  
334 the reactivity of DOM and the formation of potential disinfection by-products. Finally a very  
335 recent study (Hansen et al., 2016) based on the use of DOM optical properties for the  
336 discrimination of DOM sources and processing (biodegradation, photodegradation), focused  
337 on the complexity of DOM nature made-up of a mixture of sources with variable degrees of  
338 microbial and photolytic processing and on the need for further studies on optical properties  
339 of DOM. Thus, despite the high number of samples considered for this work and the  
340 contrasted hydrological conditions covered, the relevance of DOM nature as representing all  
341 types of DOM existing in freshwaters is not ensured. The transposition of the method, at least  
342 for DOC assessment, supposes to verify the existence of the second derivative peak at 290-  
343 300 nm and the quality of the relation between the SDA value at 295 (after nitrate correction),  
344 and the DOC content.

### 345 **4.3 Optical pathlength influence for NO<sub>3</sub> and DOC**

346 Two optical pathlengths are proposed for the method, a short one (2mm) for nitrate  
347 determination and a longer one (20mm) for DOC (Figure 2). However, considering that the  
348 optimal spectrophotometric range UV spectrophotometers between 0.1 and 2.0 a.u. (O  
349 Thomas and Burgess, 2007) must be respected, other optical pathlengths can be chosen for  
350 some water samples depending on their UV response. If the absorbance value of a sample is  
351 lower than 0.1 a.u. at 200nm with the 2mm optical pathlength, a 20mm quartz cell must be  
352 used. Respectively, if the absorbance value is lower than 0.1 at 300nm with the proposed  
353 optical pathlength of 20mm, a 100 mm one must be used. This can be the case when nitrate or  
354 DOC concentration is very low given the inverse relationship often existing between these  
355 two parameters (Thomas et al., 2014). A comparison of the use of different optical pathlength  
356 for DOC estimation gives an R<sup>2</sup> value of 0.70 for the short pathlength (2mm) against 0.96 for  
357 the recommended one (20mm). Finally, the choice of a dual pathlength measurement was  
358 recently proposed by Chen et al. (2014) to improve successfully the chemical oxygen demand  
359 estimation in wastewater samples by using a PLS regression model applied to the two spectra.

### 360 **5 Conclusion**

361 A simple and rapid method for the UV determination of DOC and nitrate in raw freshwater  
362 samples, without filtration, is proposed in this work:

- 363 - Starting from the acquisition UV absorption spectra with 2 optical pathlengths (2 and  
364 20 mm), the second derivative values at 226 and 295 nm are respectively used for  
365 nitrate and DOC measurement.
- 366 - After a calibration step with standard solutions for nitrate and known DOC content  
367 samples for DOC, LODs of 0.3 mgNO<sub>3</sub>/L for nitrate and 1.1 mgC/L were obtained for  
368 ranges up to 100 mgNO<sub>3</sub>/L and 0-25 mgC/L.

369 - The method validation was carried out for around 580 freshwater samples representing  
370 different hydrological conditions in two agricultural watersheds.

371 - Given its simplicity, this method can be handled without chemometric expertise and  
372 adapted on site with field portable UV sensors or spectrophotometers.

373 It is the first UV procedure based on the use of the second derivative absorbance at 295nm for  
374 DOC determination, and calculated after correction of nitrate interference from the acquisition  
375 of the UV absorption spectrum with a long optical pathlength (20mm or more). This is a  
376 simple way to enhance the slight absorption shoulder around 280-300nm due to the presence  
377 of organic matter. Moreover the interferences of suspended matter and colloids being  
378 negligible on the second derivative signal, the measurement can be carried out for both  
379 parameters on raw freshwater samples without filtration. Finally, even if the validation of the  
380 method was carried out on a high number of freshwater samples covering different  
381 hydrological conditions, further experimentations should be envisaged in order to check the  
382 applicability of the method to the variability of DOM nature.

383

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- 481

**Highlights**

A new methodology for the simultaneous determination of DOC and nitrate in water using dual pathlength and second derivative UV is proposed:

- A dual pathlength is proposed to allow the simultaneous determination of DOC and NO<sub>3</sub>,
- The measurement can be carried out on raw samples, without prefiltration,
- Second derivative absorbance (SDA) at 226 nm is used for nitrate determination,
- SDA at 295 nm is used for DOC determination after nitrate correction,
- The method was tested on several hundred of freshwater samples.

