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1 **A highly-sensitive microplate fluorimetric method for the high-throughput**
2 **determination of nitrate ion in aqueous compost extracts.**

3
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8
9 **Abstract**

10 In this paper, a new spectrofluorimetric method for the determination of nitrate in aqueous
11 compost extracts is presented. The microplate procedure is based on the reduction of nitrate
12 to ammonium by means of Zn powder under acidic conditions and the following derivatization
13 of ammonium with o-phthalaldehyde (OPA) and N-acetylcysteine (NAC) to give a fluorescent
14 derivative. Optimization work allowed performing the reduction in 30 min on a small sample
15 volume (150 μL) and using a little quantity of metal (20 mg). The use of strong acids is also
16 avoided. Excellent limit of detection was achieved ($1.3 \mu\text{M} = 0.08 \text{ mg}\cdot\text{L}^{-1} \text{ NO}_3^-$) along with good
17 precision levels (4.2 and 14.3 % for 50 and 5 μM , respectively) and a satisfactory linear
18 dynamic range (4 - 100 μM). A good agreement between data coming from our method and
19 from ion-exchange chromatography was found on real compost samples, indicating a good
20 level of accuracy.

21
22
23 **Keywords:** Nitrate, microplate, compost extracts, spectrofluorimetric.
24
25

26 **1. Introduction**

27 Composting represents an useful way to recycle organic wastes into a fertilizing product. The
28 formation of compost occurs by means of an exothermic and aerobic process promoted by
29 micro-organisms who decompose the organic matter. The physical and chemical changes that
30 occur during the compost formation determine the loss of the putrescibility of the biomass
31 which also undergoes a partial mineralization [1-3]. The industrial importance of compost lies
32 in its capability to enhance the agronomic quality of soil. Its employment in the agricultural
33 industry has proved to be able to increase the yield of agronomic and horticultural crops and
34 to restore soil conditions after tree harvesting [4]. Moreover, it represents a valid alternative to

35 the traditional incineration and to landfills. In fact, thanks to the water loss and to the
36 mineralization of organic matter, the global volume and mass of the initial waste products can
37 be reduced up to 50% [5, 6].

38 The monitoring of the composting process is essential not only to pursue a correct waste
39 management but also to preserve the economic value of a commercial item. During the
40 composting process nitrogen present in the biomass goes through two main mineralization
41 phenomena: ammonification (production of NH_4^+) and nitrification (production of NO_3^-).
42 Nitrogen-based parameters (i.e. concentration of ion nitrate and ammonium, and $\text{NH}_4^+/\text{NO}_3^-$
43 ratio) have proved to be useful to monitor the correct development of the composting process.
44 In fact, while ammonium concentration tends to decrease during the compost stabilization,
45 formation of nitrate is facilitated in mature compost thanks to the lower respiration rates [7].
46 Monitoring of nitrate in various environmental samples in the frame of the 1991 European
47 Union Nitrates Directive [8] (which aims to protect water quality across Europe by preventing
48 nitrates from agricultural sources polluting ground and surface waters) can also benefit from
49 efficient analytical techniques applied to aqueous compost extracts. For these reasons, the
50 attention of researchers and enterprises has been focused on the assessment of reliable
51 analytical protocols aimed to quantify these chemical species in compost and compost
52 extracts. In the past, various methods have been proposed for the quantification of nitrate ion
53 in aqueous solutions including spectrophotometry [9, 10], fluorimetry [11, 12], ion
54 chromatography [13] and flow injection analysis [14]. Literature also offers some examples of
55 microplate-based procedures where nitrate is first reduced to nitrite which is later
56 spectrophotometrically revealed after derivatization [15, 16]. In spite of the strong contribution
57 given by these works for the determination of nitrate, the main problem related to the cited
58 procedures is given by the use of cadmium, which is renowned for his high toxicity, and also
59 by the strong interferences caused by complex samples such as compost extracts, when the
60 measurement is carried out by spectrophotometry.

61 The main goal of our work was to develop a new sensitive method for the determination of
62 nitrate by a microplate-based procedure in compost aqueous extracts. In the past, our research
63 group has developed various analytical methods based on the 96-wells technique [17-20]. In
64 particular, a fluorescence-based microplate procedure for the quantification of ammonium and
65 primary amines in compost water extracts and other matrices was recently assessed [17]. The
66 basic idea for the assessment of our method was to exploit the previous protocol, reducing first
67 the nitrate to ammonium and then quantifying the latter by means of the method already
68 assessed. An important advantage of this analytical strategy would be the easy determination
69 of both nitrate and ammonium ions with a single set of detection reagents (with or without the
70 reduction step), which would be highly valuable as the $\text{NH}_4^+/\text{NO}_3^-$ ratio is one of the principal
71 nitrogen-based indicators of compost maturity [7].

72 In the last years, metals have been employed for the reduction of nitrate to nitrite or ammonia
73 in aqueous solutions. Reducing properties of cadmium, aluminum, zinc, iron and also of some
74 types of alloys like Devarda's alloy and Arndt's alloy have been reviewed by Fanning [21].
75 Among all, Zn seems to represent a good candidate for the reduction of nitrate to ammonium.
76 Indeed, besides being less toxic than other metals (i.e. cadmium), it has been already
77 successfully used under mild reduction conditions for the quantification of nitrate in water
78 solutions and other matrices [22-24], but never for compost extracts so far. For this reason, we
79 present in this work a new fluorescence-based microplate method for the quantification of
80 nitrate in compost water extracts which includes the conversion to ammonium by means of Zn
81 powder, and which is developed as a simple analytical tool that can be used directly in
82 composting platforms in a non-specialized environment (no fume hoods for strong acids and
83 non-expert technicians).

84

85 **2. Experimental**

86

87 ***2.1 Samples and water extraction of organic matter***

88 Two type of compost samples were selected for this study. More specifically, the first type of
89 samples (A samples) was exclusively composed by green wastes deriving from activities of
90 gardening and maintenance of green public or private areas. Sampling sites were located in 3
91 private areas (agricultural lands) located in Provence Alpes-Côte d'Azur region (France, GPS
92 coordinates : near Gardanne : 43.466350N, 5.454541E ; near Rousset : 43.468935N,
93 5.634117E, and near Rougiers : 43.411657N, 5.841994E). Ages of the swaths were always
94 included between one week and twelve months. It should be noticed that studied swaths can
95 be very heterogeneous in their composition of plant species, size and height. The second type
96 of samples (B samples) consisted in sewage sludge mixed with green wastes (1/3-2/3, v/v). In
97 this case, samplings were performed on an industrial composting platform (Biotechna) located
98 in Ensûès-la-Redonne (Bouches-du-Rhône, France, GPS coordinates 43.376838N,
99 5.190849E), A single swath was studied in a period of time included between 1 week and 6
100 months, with a various frequency of sampling (samplings were performed every week during
101 the 2 first months, every two weeks the next month, and every month the three months
102 afterwards). For each type of compost, matter was collected in different places of the swath at
103 80 cm depth, in order to get a final composite sample of 2 kg representative of compost.

104 The water extractions were performed in PTFE Teflon tube (triplicates per soil sample) on an
105 orbital shaker (Fisher Scientific Bioblock SM30B) at 125 rpm and at room temperature. Organic
106 matter was extracted through shaking 6 g of fresh compost with 60 mL of ultrapure water
107 (resistivity of 18 M Ω cm⁻¹ at 25 °C, and total organic carbon < 20 μ g L⁻¹) with a soil/water ratio
108 of 1/2 (w/v) for 2 h under N₂. After extraction step, the tubes were centrifuged at 8000 rpm for

109 15 min (JP SELECTA, Medifriger BL-S), and the supernatants were collected and filtered
110 through 0.45 μm membrane filters (PES membrane, Millipore, France). The resulting solutions
111 were stored in the dark at $-18\text{ }^{\circ}\text{C}$ until analysis.

112

113 **2.2 Reagents and solutions**

114 All chemicals were of analytical reagent grade and used without further purification. Zn powder
115 (100 mesh) and Zn granules were purchased from Alfa Aesar (Germany) and Prolabo
116 (France), respectively. Copper granules were purchased by Fluka (France) while Fe powder
117 (puriss., $<212\text{ }\mu\text{M}$) and Al powder were provided by Sigma-Aldrich (France). O-phthaldehyde
118 (OPA) was obtained from Acros Organics (Belgium) and N-acetyl-L-cysteine (NAC) from
119 Sigma-Aldrich. Buffers solutions were prepared dissolving the proper amount of sodium
120 acetate (Sigma-Aldrich), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, Acros
121 organics), anhydrous sodium carbonate (Sigma-Aldrich) and sodium tetraborate decahydrate
122 (Sigma-Aldrich) in ultrapure water (Millipore, USA, resistivity $>18\text{ M}\Omega\text{ cm}$) and adjusting the pH
123 with sodium hydroxyde or hydrochloridic acid.

124 Stock standard nitrate solution (0.1 M) was prepared by dissolving appropriate amount of
125 sodium nitrate (Sigma-Aldrich) in ultrapure water. Working solutions were obtained by diluting
126 stock solutions to proper concentrations.

127

128 **2.3 Instruments**

129

130 **2.3.1 Microplate**

131 Microplate fluorescence measurements were carried out on a microplate reader (Infinite M200,
132 Tecan France SAS, France), operating at $30\text{ }^{\circ}\text{C}$ and controlled by i-control™ software (Tecan).
133 Detection was performed by top fluorescence reading at $\lambda_{\text{ex}} = 415\text{ nm}$ and $\lambda_{\text{em}} = 485\text{ nm}$. Other
134 parameters were as follows: gain: 80; number of flashes: 5; integration time: $20\text{ }\mu\text{s}$.
135 Fluorescence intensities were expressed in arbitrary units (a.u.). Polystyrene black 96 V-well
136 microplates (Fisher Scientific, France) were used.

137

138 **2.3.2 Ion chromatography analysis of nitrate**

139 Nitrate analysis was carried out by ion chromatography (EN ISO 10304-2:1996) on a ICS-3000
140 HPLC system (Dionex, USA), driven by Chromeleon® (6.80 version) equipped with a guard
141 column (Dionex AG11-HC), an analytic Dionex AS11-HC ($4 \times 250\text{ mm}$) column, a
142 conductimetric detector (Dionex CD-25) and using a $200\text{ }\mu\text{L}$ loop injection valve. Analysis were
143 performed in an isocratic mode (22.5 mM NaOH in helium sparged deionized water) at $30\text{ }^{\circ}\text{C}$,
144 with a flow rate set at 1.5 mL min^{-1} . To improve the signal-to-noise ratio of the conductivity

145 measurement, an external flow electrochemical suppressor system (ACRS 500 4 mm) was
146 added to the analytical system.

147

148 **2.4 Analytical protocol for nitrate determination**

149 Twenty mg of Zn powder (100 mesh) were dispensed into the wells of the microplate, 150 μL
150 of sample or standard solution were introduced and 20 μL of MES buffer (0.1 M, pH 6) were
151 added. The plate was shaken for 30 min at 30 °C. Then, 120 μL of the solution were transferred
152 into new wells where 20 μL of a solution of 20 mM NAC and 30 μL of 13 mM OPA in ethanol-
153 0.15 M carbonate buffer pH 10.5 (10:90, v/v) were subsequently added. The plate was shaken
154 for 10 min and fluorescence intensity was then recorded, with excitation and emission
155 wavelengths set at $\lambda_{\text{ex}}=415 \text{ nm}/\lambda_{\text{em}}=485 \text{ nm}$. Concentrations in unknown samples were
156 determined using the linear calibration curves obtained with standards. Nitrate concentration
157 was calculated after quantifying the ammonium by means of the microplate procedure
158 previously assessed by our research group [17].

159

160 **3. Results**

161

162 **3.1 Choice of the metal system for the reduction of nitrates**

163 The proposed analytical procedure is based on the reduction of nitrate ion to ammonium and
164 the subsequent determination of the latter by fluorescence (Figure 1). One of the main
165 challenges of this work was to find a metal for the effective reduction of nitrates under mild
166 conditions (moderate temperature to apply the developed protocol in polystyrene microplates
167 which don't withstand high temperatures, and also because the most affordable microplate
168 readers don't enable heating above 40°C), but which does not interfere with the second step
169 of the protocol (derivatization of ammonium with OPA/NAC and formation of isoindol adduct).
170 Alloys like Devarda's alloy generally provide efficient reduction of nitrates, but only under quite
171 harsh reaction conditions (i.e. boiling water). Several tests were performed in order to reduce
172 the nitrate to ammonium with various metals. Experiments conducted with aluminum (foil and
173 powder) and copper (granules) at various pH conditions did not provide any significant result
174 proving to be not efficient for the reduction of the analyte. On the other hand, tests performed
175 with iron (thread and powder) revealed that this metal is able to convert nitrate to ammonium
176 in acidic conditions (encouraging results were obtained at pH 5 and 6). Unfortunately, the
177 corrosion products formed during the reduction phase constituted a strong interference for the
178 revelation of ammonium preventing a precise and accurate determination. Attempts to
179 suppress the interference by adding EDTA at various concentration levels (0.1-1 M) were
180 unsuccessful. Finally, Zinc proved to be promising both for reduction of nitrate and ammonium

181 derivatization under mild conditions in our first experiments, so we decided to optimize our
182 method with this metal.

183

184 **3.2 Optimization of the analytical method with Zn**

185 Reduction of nitrate with Zn was performed in presence of various selected buffers in order to
186 identify the best pH conditions for the conversion of nitrate to ammonium and for the
187 derivatization. These first experiments were performed by means of Zn granules. A quantity of
188 metal granules large enough to entirely cover the bottom of the microplate wells was cut and
189 introduced. 100 μL of a water solution of NaNO_3 100 μM were introduced in the wells containing
190 the metal along with 20 μL of : i) acetate buffer (0.1 M, pH 5), ii) MES buffer (0.1 M, pH 6), iii)
191 HEPES buffer (0.1 M, pH 7), iv) carbonate buffer (150 mM, pH 10.5) , v) borate buffer (100
192 mM, pH 11.5). Also HCl 2.5 M and H_2SO_4 2.5 M were tested for the reduction. In this case,
193 NaOH 0.1 M was added to the wells until basic pH, before derivatization with NAC and OPA.
194 The suspensions were shaken for 1 h at 30 °C before addition of OPA and NAC reagents.
195 Experiments were also conducted on a solution of NH_4Cl 100 μM in order to detect possible
196 interferences of the reducing conditions on the ammonium derivatization. As shown in Figure
197 2, best results were obtained at pH 5 and pH 6. In fact, in both cases conversion of nitrate to
198 ammonium occurred and high values of fluorescence for ammonium were detected, resulting
199 in good sensitivity under these conditions. According to data obtained, reduction of nitrate is
200 much less efficient at pH 7 or when basic carbonate and borate buffers are employed, also
201 probably because of the evaporation of NH_3 which is predominant at neutral/basic pH.
202 Regarding the acidic conditions selected for the study, it appears that in the case of HCl 2.5 M
203 there is no substantial difference between the blank values and those of nitrate and
204 ammonium, while in the case of H_2SO_4 data related to ammonium derivatization are very low.
205 The MES buffer (0.1 M, pH 6) was selected for the following experiments, given the higher
206 value provided by the test on the ammonium solution revealing a higher sensitivity for this pH
207 level. The subsequent experiments were mainly aimed to optimise the conditions for the
208 enhancement of the conversion of nitrate to ammonium.

209 A kinetic test was carried out in order to select the best time condition for the reduction of
210 nitrate with zinc powder (more efficient than zinc granules). As shown in Figure 3, after 30 min
211 about 90% of the nitrate is reduced to ammonium indicating that this could represent a good
212 time condition for the proposed procedure. The influence of Zn powder quantity on the
213 reduction rate at the selected conditions (30 °C, 30 min) was also studied. Calibration curves
214 obtained with 10, 20 and 30 mg of Zn are shown in Figure 4. Tests performed with 10 mg of
215 powder showed that there is a loss of linearity at concentrations higher than 50 μM indicating
216 also that, in this case, contact surface is not large enough to provide efficient conversion of
217 nitrate to ammonium. At the same time, experiments carried out with 30 mg of powder, showed

218 a worsening of the R^2 value probably due to an interference caused by the excess of solid
219 particles in the suspension. For this reason, 20 mg was selected as the best compromise for
220 the procedure.

221 Finally, influence of an increase of the reaction temperature was also assessed (up to 40 °C,
222 the maximum heating capacity of most microplate readers/shakers), with no significant
223 improvements, and 30 °C was therefore selected for the optimized protocol.

224

225 **3.3 Interferences**

226 The interference of various major anions and cations that could be found in compost extract
227 samples was investigated. More specifically, K_2SO_4 , KH_2PO_4 , Na_2CO_3 , $MgCl_2$, $NaCl$, $NaBr$ and
228 $CaCl_2$ were selected for the study. Other less common redox active anions (low redox
229 potential/high reducing ability) such as HPO_3^{2-} , SO_3^{2-} and $C_2O_4^{2-}$ have also been included in
230 these interferences tests. The analytical protocol was applied to pure solutions of the cited
231 salts or anions at a concentration of 2 mM, giving a response not significantly different from
232 the blank. Moreover, tests were also performed on mixtures of $NaNO_3$ 100 μM and selected
233 interfering compounds in order to compare results with those given by the pure $NaNO_3$. Results
234 showed that the salts tested did not interfere up to at least 2 mM.

235 Experiments conducted on a standard solution of $NaNO_2$ 100 μM revealed that nitrite shows
236 the same behavior as nitrate, being reduced to ammonium by Zn at the same pH conditions.
237 Anyway, analysis performed by ion-exchange chromatography on the samples confirmed the
238 already expected absence of nitrite in the aqueous compost extracts. Interferences from nitrite
239 should therefore be negligible in this type of environmental samples, but has to be considered
240 (separate quantification by Griess reagent) if the method is applied to other samples with
241 significant nitrite concentrations.

242

243 **3.4 Analytical features**

244 The assessed procedure was validated in terms of sensitivity, linearity, precision and accuracy.
245 The limit of detection (LOD) was calculated from the residual standard deviation of the
246 regression (linearity study method). More specifically, LOD was evaluated as follows:

$$247 \text{ LOD} = 3s/b$$

248 where s is the standard deviation (SD) of the a-intercept and b the slope of the calibration
249 curve. LOD is 1.30 μM (0.08 $mg L^{-1}$). It is interesting to note that detection limit is almost an
250 order of magnitude smaller than the LOD estimated by Murray et al. [22], for their procedure
251 using zinc reduction and a spectrophotometric method (0.5 $mg L^{-1}$).

252 The calibration curve ($I = 11.10C + 316.2$; I = fluorescence intensity (arbitrary units), C = nitrate
253 concentration (μM)) was linear up to 100 μM with a correlation coefficient of 0.9941, resulting
254 in a large enough linear dynamic range for our analytical purposes (4.5-100 μM). Relative

255 standard deviations were assessed on a 5 μM (RSD = 14.3%) and on a 50 μM (RSD=4.2%)
256 nitrate standards (n=10 replicates) indicating a good repeatability for both concentration levels.

257

258 **3.5 Comparison with other microplate methods for nitrate determination**

259 The microplate format is an ideal tool for routine analysis when a large number of samples has
260 to be analyzed in a short time (typical case for ammonium or nitrate determination in aqueous
261 samples). All methods adapted in microplate are based on the reduction of nitrate to nitrite and
262 subsequent spectrophotometric measurements after reaction with Griess reagent. Reduction
263 of nitrate to nitrite has been performed by various methods: copperised cadmium granules [25]
264 or pins [15], vanadium (III) [26] or enzymatic reduction [27]. Global reaction times are a bit
265 longer with these methods (60-90 min at room temperature) compared to our method (40 min).
266 The main advantage of these spectrophotometric techniques is the low detection limits (12-20
267 $\mu\text{g L}^{-1}$) which are 4 to 6 times lower than our spectrofluorimetric method. However, these
268 methods also have limitations for compost analysis: i) the main reduction methods still use
269 cadmium which is highly toxic; ii) complex and colored samples such as compost extracts will
270 display strong matrix effects using spectrophotometric techniques [28], making fluorimetric
271 measurements highly desirable; iii) determination of nitrate and ammonium ($\text{NH}_4^+/\text{NO}_3^-$ ratio is
272 one of the principal nitrogen-based indicators of compost maturity) requires two sets of
273 detection reagents if the Griess reagent is used, while our methodology easily provides
274 quantification for both analytes (with or without reduction step).

275 Similarly, nitrate determination using nitration of resorcinol and spectrophotometric
276 measurement is not suitable for this type of analysis, as it uses pure sulfuric acid (not
277 compatible with microplates) and displays strong interferences due to sample color (own
278 experiments, data not shown).

279 Overall, our fluorimetric method seems more adapted to compost extract analysis in the
280 microplate format than methods based on spectrophotometry.

281

282 **3.6 Quantification of nitrate ion and method validation in aqueous compost extracts**

283 Nitrate was quantified by means of the assessed procedure in twenty-five aqueous extracts of
284 compost of various sources, using the external calibration method. Sample dilution represents
285 a critical point of the whole analytical protocol because the highly-colored matrix can heavily
286 disturb spectroscopic measurements. Moreover, dilution is required in order to match the
287 defined calibration range both for ammonium and nitrate. A comparison between the slope of
288 calibration curves obtained by means of external calibration and multiple standard addition
289 methods for some selected samples was exploited in order to select a dilution ratio able to
290 minimize the interferences given by matrix, but still able to detect nitrates and ammonium. A

291 5-fold dilution was adopted for A samples and a 50-fold one for B samples. Nitrate
292 concentration was quantified in a range included between 2.0 ± 0.1 and 90 ± 10 mg L⁻¹.
293 These twenty-five samples were also chosen for comparison purposes between ion-exchange
294 chromatography and microplate measurements with the developed procedure. As shown by
295 Figure 5, a good correlation ($R^2=0.9127$) was found between the two data sets, and a very
296 weak intercept value with regard to the studied concentration range sets. The slope reveals a
297 slight underestimation of data obtained by the microplate method, probably due to residual
298 matrix effects of these compost samples. A higher dilution rate would have improved these
299 results, but was not possible because most samples would have too low concentrations for
300 proper nitrate or ammonium quantification. Results are still satisfactory for a field test tool,
301 which is aimed to assess the evolution of nitrate concentration during the composting process
302 and which mainly requires a good correlation between the measurements and the actual
303 concentrations in the compost samples.

304

305 **4. Conclusions**

306 In this study, we presented the assessment and validation of a new analytical method using
307 the microplate format for the determination of nitrate ion in aqueous compost extracts.
308 Optimization work allowed to obtain fast and stable responses by employing a small quantity
309 of a non-toxic metal (zinc) for the reduction. Nitrate reduction with zinc (mainly studied for the
310 reduction of nitrate to nitrite, much less to the final ammonium reduction), was coupled with a
311 fluorimetric detection method, offering several advantages such as lower detection limits (an
312 order of magnitude lower than the spectrophotometric method described with zinc reduction
313 [22], especially using the microplate format) and less interferences due to complex and colored
314 samples such as compost extracts. It also potentially enables the easy determination of both
315 nitrate and ammonium ions with a single set of detection reagents (with or without the reduction
316 step), which is highly valuable as the $\text{NH}_4^+/\text{NO}_3^-$ ratio is one of the principal nitrogen-based
317 indicators of compost maturity. Besides, no strong acids are used in this method, on the
318 contrary of other frequently used analytical methods for nitrate [10,12]. Preventing the use of
319 strong acids or toxic metals and reagents is highly desirable for this type of analytical method
320 which is meant to be used directly on composting platforms by non-expert technicians. Only a
321 small volume of sample is required for the analysis and the whole procedure is completed in
322 about 40 min with the possibility to analyze several extracts at the same time thanks to the
323 microplate instrumentation. Validation showed that the proposed procedure is sensitive,
324 precise and with a good level of accuracy. For all these reasons, the method is a good
325 candidate for routine analysis of nitrate in aqueous compost extracts, either in a traditional
326 laboratory or directly in composting platforms. A simple on-site compost extraction with a

327 modified coffee machine is currently under evaluation in our laboratory in order to reach a full
328 on-site analytical procedure.

329

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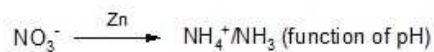
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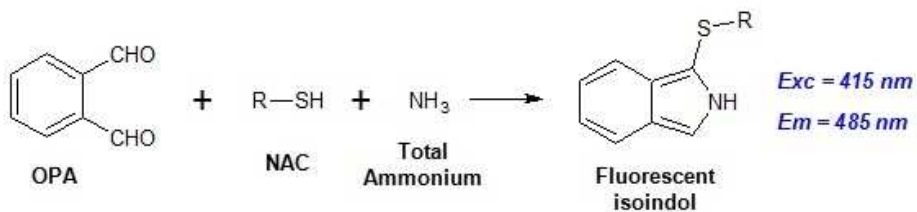
Figure captions

Figure 1. Proposed protocol for nitrate determination; **Figure 2.** Influence of the pH of the reduction step on the fluorescence intensity response of standard solutions after reduction and derivatization with OPA/NAC (zinc granules, 1h reduction time, n=2). **Figure 3.** Influence of the time of the reduction step on the fluorescence intensity response of standard solutions after reduction and derivatization with OPA/NAC (20 mg zinc powder, n=2). **Figure 4.** Calibration curves (0-100 µM) depending on the quantity of zinc powder used for the reduction step (30 min reduction time, n=2). **Figure 5.** Comparison between microplate method and ion-exchange chromatography (n=2).

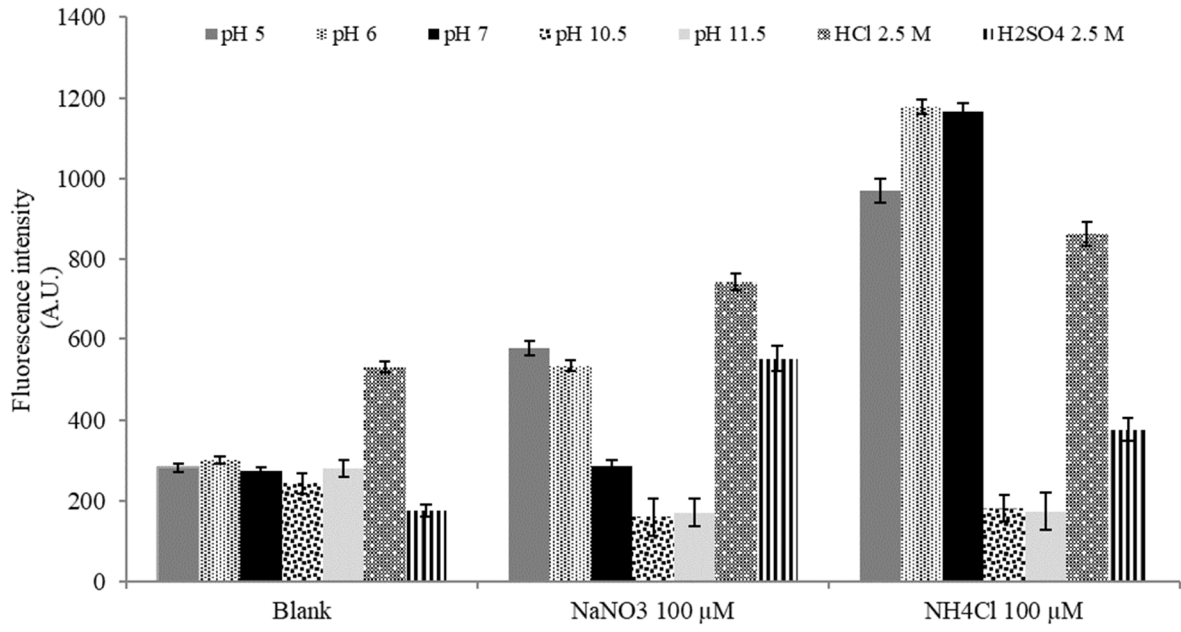
Step 1: reduction



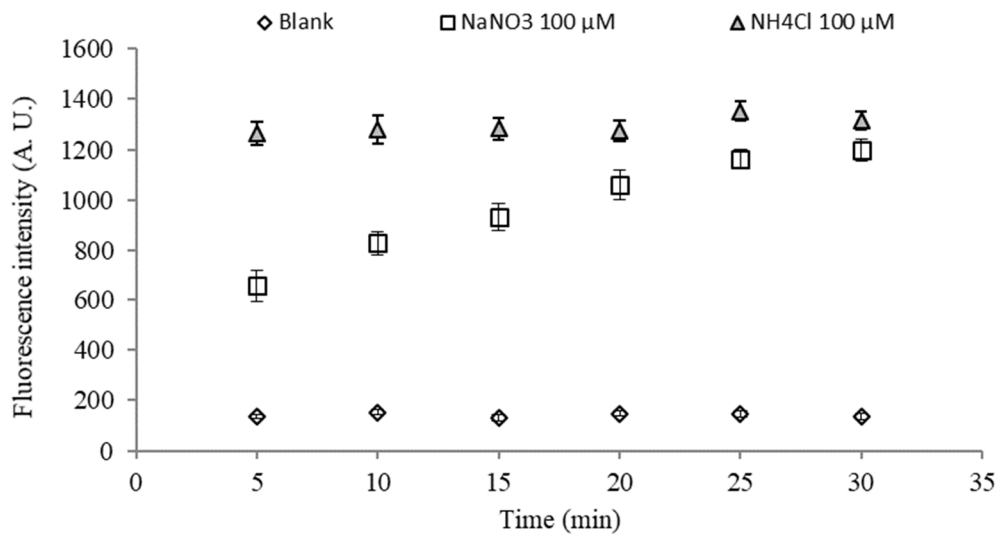
Step 2: derivatization



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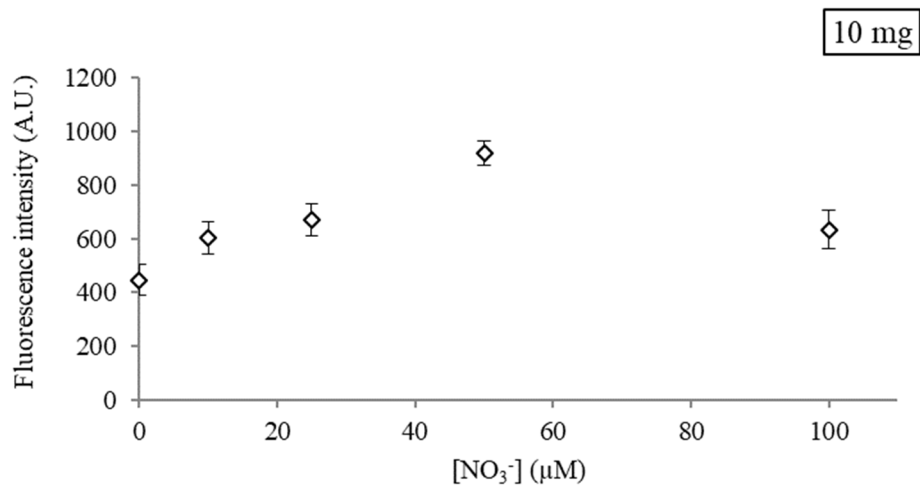


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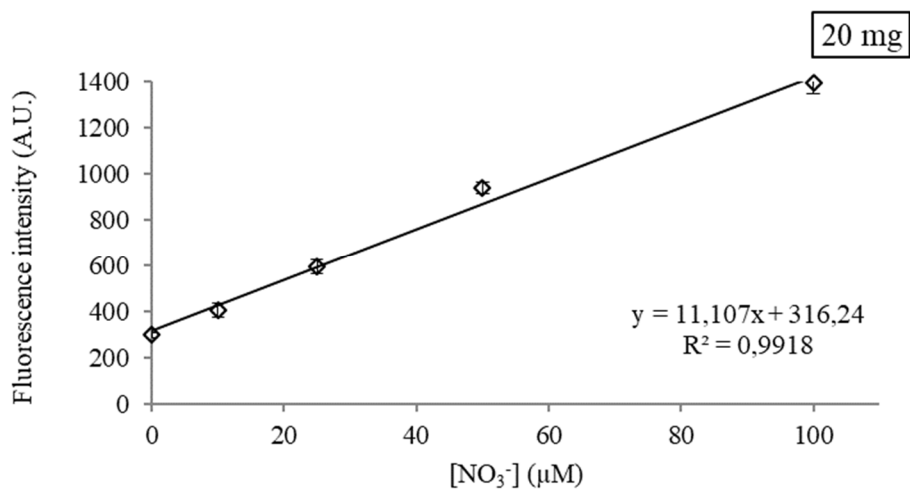


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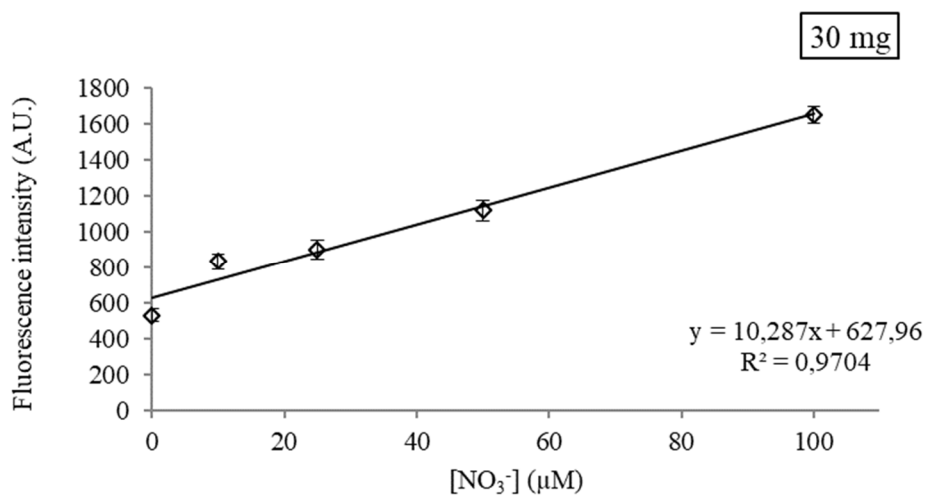
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