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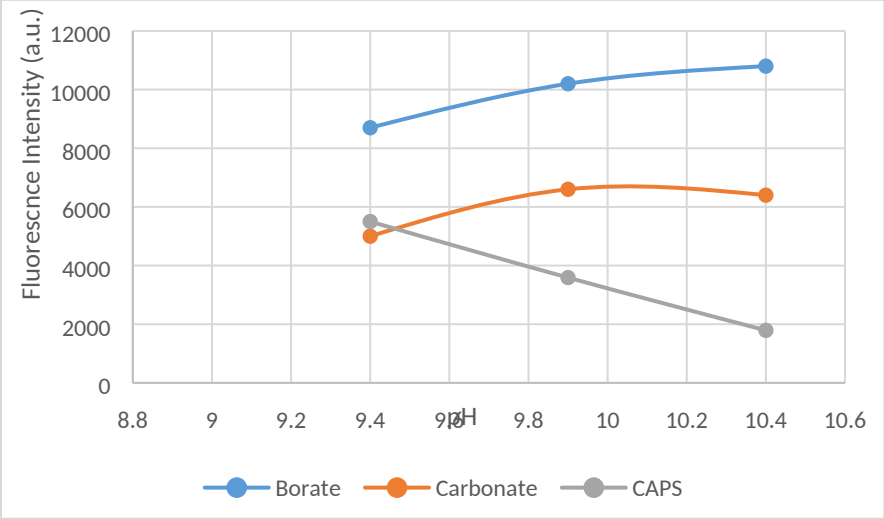
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High throughput determination of ammonium and primary amine compounds in environmental and food samples

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SUPPLEMENTARY INFORMATION



Sup. Fig. 1: Influence of buffer. [OPA]: 8 mM; [NAC]: 8 mM; [NH_4^+]: 100 µM; pH = 10.5

High throughput determination of ammonium and primary amine compounds in environmental and food samples

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Abstract

In this paper, an improved spectrofluorimetric method for the simultaneous and direct determination of ammonium and primary amine compounds is presented. The method is based on the derivatization with *o*-phthaldialdehyde (OPA) / *N*-acetylcysteine (NAC) reagent using high throughput microplates, and OPA/NAC ratio has been optimized in order to suppress interference of ammonium on primary amine determination. Direct measurement of these two parameters is therefore possible with a global procedure time that does not exceed ten minutes. Excellent limits of detection of 1.32 µM and 0.55 µM have been achieved for ammonium and primary amines, respectively. Reagent stability issues have also been addressed and formulation of reagents solution is described for improved reagents shelf life. The proposed protocol was finally applied and validated on real samples such as wine samples, compost extracts and wastewater.

Keywords: ammonium; primary amine; microplate; reagents stability.

1. Introduction

Amino compounds are widely distributed in the environment and essentially result from metabolic processes of degradation, hydrolysis and excretion at different levels of the food chains. Soil organic matter thus contains about 30% of its nitrogen pool as amino acids [1], and a significant proportion of NH_4^+ can be released from organic matter by microbial hydrolysis [2]. Anthropogenic activities also contribute to the presence of these compounds and ammonium in the environment, mainly attributed to the incineration of waste [3] and the discharges of waste waters from chemical industry and wastewater treatment plants [3,4].

Among the nitrogen pool, primary amino compounds determination is a significant parameter in the food processing or drinking-water treatment industry as it can react with nitrites or nitrates to form nitrosamines which are classified as "probably carcinogenic to human" [5,6].

Primary amino compounds (mainly primary amino acids) are also very important regarding the nitrogen management in some specific fields such as wine industry [7]. Indeed, assaying the total primary amino acids concentration is often considered to be the most convenient method to measure assimilable nitrogen, which is critical for wine flavour and style.

Regarding ammonium, its determination in environmental samples is highly relevant, due to its important micronutrient function in aquatic systems or as an important index in composting process studies. As example, high concentration in a water body can be an indicator of the environmental impact of human activities, with strong effects on microbiological activities that can potentially lead to eutrophication events [8]. In the composting field, low ammonium concentration coupled to low $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio has been proposed as indicators of a compost stability [9].

Several methods have been proposed for the analysis of ammonium [10], such as spectrophotometry [11,12,13], ion selective electrode [14], fluorimetry [15,16] or ion

chromatography [17]. Primary amines/amino acids are generally analyzed individually by liquid chromatography [18,19], but methods have also been developed to measure the total concentrations of these compounds in order to have a rapid and global assay of these important nitrogen compounds. This can be done for example by spectrophotometric measurements after reaction with ninhydrin [20] or by fluorimetry after reaction with *o*-phthaldialdehyde (OPA) and a thiol compound [21].

The numerous advantages of the combination of this OPA-thiol reagent (reaction with ammonium and amines, sensitivity, selectivity, low toxicity and price of reagents...) has led to the development of simultaneous determination methods for ammonium and primary amino compounds. Meseguer Lloret et al.[14] used solution derivatization with OPA-NAC reagent (NAC: N-acetylcysteine), two different excitation and emission fluorescence wavelengths and statistical analysis by multivariate Principal Component Regression in order to separate the responses of ammonium and amine under selected experimental conditions [15]. Darrouzet-Nardi et al. [22] developed a fluorescent assay with OPA and β-mercaptoethanol for analysis of primary amino compounds, by taking into account potential interference of ammonium. The main drawback of this method was the necessity to use a 1-h incubation time in order to reduce interference from ammonium.

The aim of the present study is to develop a fast, simple and efficient method for ammonium and primary amino compounds analysis, with no need of statistical analysis, direct measurement from calibration curves and a global procedure time that does not exceed ten minutes. Moreover, this method has been developed as a potential routine analytical method applicable to the large number of samples that an analytical laboratory typically has to deal with (especially for ammonium). Therefore, a high-throughput microplate method based on OPA-NAC reagent was used, with special care on reagents stability which is a key point for routine analysis development (shelf life of at least 3 months without deterioration of analytical

76 performances). The final goal of this study was to conduct a strong validation on complex
77 samples like compost extracts or wastewaters by comparison with a chromatographic
78 reference method, in order to have a robust method for routine analysis of ammonium and
79 primary amino compounds.
80
81 2. Experimental
82 2.1 Reagents and solutions
83 All chemicals were of analytical reagent grade and used without further purification. OPA
84 was obtained from Acros Organics and *N*-acetyl-L-cysteine (NAC) and tris(2-carboxyethyl)
85 phosphine hydrochloride (TCEP) from Sigma-Aldrich. OPA solutions were prepared by
86 dissolving pure compound in appropriate buffer and adjusted at pH=10.5 with sodium
87 hydroxide or hydrochloric acid. CAPS, borate and carbonate buffers were prepared by
88 dissolving *N*-cyclohexyl-3-aminopropanesulfonic acid (Acros Organics), sodium tetraborate
89 decahydrate (Sigma-Aldrich) and anhydrous sodium carbonate (Sigma-Aldrich) respectively
90 in ultrapure water (Millipore, resistivity >18 MΩ cm). Stock solutions of individual amino
91 compounds (10 mM each) were prepared by dissolving appropriate amounts of pure
92 compound (Sigma-Aldrich) in ultrapure water. Stock standard ammonium solution (55.5 mM)
93 was prepared by dissolving appropriate amount of ammonium chloride in deionized water.
94 Working solutions were obtained by diluting stock solutions to proper concentrations.
95
96 2.2 Instruments
97 2.2.1 Microplate
98 Microplate fluorescence measurements were carried out on a microplate reader (Infinite
99 M200, Tecan France SAS, Lyon, France), operated at 30 °C and controlled by i-control™
100 software (Tecan). Detection was performed by top fluorescence reading at λ_{ex} = 335 nm and

λ_{em} = 455 nm for total primary amines quantification and at λ_{ex} = 415 nm and λ_{em} = 485 nm for ammonium determination. Other parameters were as follows: gain: 80; number of flashes: 5; integration time: 20 µs. Fluorescence intensities were expressed in arbitrary units (a.u.). Polystyrene black 96 V-well microplates (Fisher Scientific, Illkirch, France) were used.

2.2.2 Ion chromatography analysis of ammonium

The ion chromatographic system consisted of an IonPac CS12A 4x250 mm column (ThermoScientific), a CSRS-Ultra 4 mm self-regenerating suppressor, an AS40 auto-sampler, an ED40 electrochemical detector operated in the conductivity mode and a GP40 gradient pump operating at a flow-rate of 1.0 mL/min (Dionex). The injection loop was 50 µl. Elution was carried out in isocratic mode by 18 mM methanesulfonic acid solution. System control, data collection and data processing was performed with PeakNet 5.1 Chromatography Workstation software (Dionex).

2.2.3 Ion exchange chromatography for primary amines determination

Primary amino acid compounds in selected wines were determined by an external laboratory using an automatic amino acid analyzer (Biochrom 30+, Cambridge, England). Wine samples were initially diluted in a sodium citrate buffer (pH 2.2). All amino acids were spectrophotometrically detected after post-column derivatization with ninhydrin reagent at 570 nm. Concentrations of amino acid compounds in unknown samples were determined by comparison with standard peak areas (Sigma-Aldrich amino acid standard kit) and by using norleucine as internal standard. Ion-exchange chromatography analyses on real wine samples were performed on the same day as microplate analyses for validation purposes.

2.3 Analytical protocol for primary amines and ammonium determination

100 µL of sample or standard solution were dispensed into the wells of the microplate, where 30 µL of 13 mM OPA in ethanol-0.15 M carbonate buffer pH 10.5 (10:90, v/v) and 20 µL of a solution of 20 mM NAC and 1.5 mM TCEP in 0.1 M HCl were added. The plate was shaken for 10 min and fluorescence intensity was then recorded, with excitation and emission wavelengths set at at λ_{ex}=335 nm / λ_{em}=455 nm and at λ_{ex}=415 nm / λ_{em}=485 nm for total primary amines and ammonium determination respectively. Concentrations in unknown samples were determined using the linear calibration curves obtained with standards. All experiments were performed in duplicate.

3. Results

3.1 Optimization of analytical method

3.1.1 OPA/NAC concentration and pH

Initially derivatization of amino acids by OPA-NAC reagent developed by Aswad [23] was carried out with an OPA/NAC ratio of 1:2. Even if the reaction rate and the fluorescence yield are not dependent on the OPA/NAC ratio [24], it is nevertheless necessary to use an OPA concentration higher than targeted amino acid concentration [25]. In past years OPA/NAC 1:1 ratio solution has often been used as pre-column derivatization reagent for HPLC or capillary electrophoresis separation of amino acids or biological amines.

More recently, this method was adapted for the determination of total primary amine compounds and ammonium in environmental samples by Meseguer Lloret et al. [15] with a fluorescence measurement at two $\lambda_{ex}/\lambda_{em}$ couples of wavelengths to separate the response of the primary amines with that of ammonium. However, this method was based on the use of a statistical calibration model to avoid cross interferences of the analytes during fluorescence measurement. Optimization of the concentration of OPA/NAC 1:1 ratio reagent may reduce

150 decrease of the fluorescence response of ammonium adduct at the wavelengths used for amino
151 compounds determination.
152 Fig. 1 displays the evolution of fluorescence intensity of OPA-NAC-ammonium adduct at
153 amino compounds wavelengths as a function of reaction time. The increase in OPA and NAC
154 concentration can reduce ammonium interference during primary amines determination. For a
155 concentration greater than 8 mM of OPA and NAC, a reaction time of 600 seconds allows to
156 fully eliminate the cross-interference of ammonium over amino compounds determination.
157 This reaction time is longer than the one used by Meseguer-Lloret et al. [15] (120 and 300
158 seconds respectively for amines and ammonia) but greatly simplifies the calibration process
159 by avoiding the use of statistical tools. The reaction time also depends on the pH used for the
160 derivatization reaction. Fig. 2 shows the fluorescence intensity of the OPA-NAC-ammonium
161 adduct as a function of pH and reaction time. The reaction time previously set at 600 seconds
162 for the simultaneous measurement of ammonium and primary amines is sufficient to obtain a
163 high and stable fluorescence signal for ammonium derivatization by OPA/NAC 8mM/8mM at
164 pH = 10.5. This reaction time is significantly lower than that proposed by Darrouzet-Nardi et
165 al. (60 minutes) for a OPA/mercaptoethanol (ME) procedure developed in order to limit
166 interference of ammonium on the measurement of primary amines in soils [22].
167
168 3.1.2 Buffer
169 Sodium or potassium tetraborate are certainly the most commonly used buffers for
170 derivatization of ammonia or amines with OPA and NAC or ME in alkaline conditions
171 [14,15,21-25]. However, tetraborate salts have been classified as toxic for reproduction
172 (category 1B) by European regulations since 2008 [26]. Substitution of these products is
173 therefore recommended, especially for analytical procedures that are developed as potential
174 routine methods with high frequency of use for the reagent solutions.

175 In this study, we replaced the borate buffer solution with a carbonate or CAPS buffer which
176 have pKa values compatible with the pH used in the derivatization reaction. Experiments
177 showed that a carbonate buffer could replace the borate buffer but the fluorescence signal
178 obtained decreased by 40%. Nevertheless, despite this significant decrease of fluorescence
179 intensity, the analytical features obtained with the carbonate buffer fit with expected values of
180 ammonium and amines in environmental samples (see 3.3). CAPS, on the other hand, lead to
181 a very significant decrease in the fluorescence signal by 90% (Sup. Fig. 1).
182
183 3.2 Conservation of reagents
184 3.2.1 Reducing agent
185 It is well known that thiol group can easily be oxidized and form disulfide bond. This
186 oxidation reaction will quickly limit NAC reactivity and therefore inhibit derivatization of
187 ammonium or amines by OPA. This usually leads the authors to prepare OPA/NAC solutions
188 daily. However, it may be interesting in an analytical laboratory to keep OPA and NAC
189 solutions for several days, several weeks or even a few months, again especially for analytical
190 methods that are used routinely.
191 Three reducing agents conventionally used in analytical procedures have been studied: 2,2-
192 thiodiethanol (TDE), ascorbic acid and tris(2-carboxyethyl) phosphine hydrochloride (TCEP).
193 Fluorescence intensity of an ammonium standard adduct (100 µM) was measured over a
194 period of 60 days (Fig. 3). Derivatization was carried out with OPA/NAC solution comprising
195 one of the reducing agent mentioned above at a concentration of 1 mM (TCEP) or 10 mM
196 (ascorbic acid, TDE).
197 We can observe with data of Fig. 3 that ascorbic acid did not enable good preservation of
198 OPA/NAC solutions. TDE and TCEP reduced degradation of OPA/NAC solution with
199 approximately 14% decrease in fluorescence intensity after 30 days in both cases. TCEP was

preferred for subsequent experiments because TDE is unpleasant to use due to its malodorous properties. The optimization of the concentration of TCEP was then carried out (fig .4). Data showed that TCEP concentration of 1 mM was sufficient to improve the conservation of OPA/NAC reagent. Higher concentrations lead to an increase in the blank signal. Similar results have been obtained for amino compounds derivatization (glycine as a reference).

3.2.2 Conservation mode of reagents

The optimization of the preservation of reagents was finally optimized by studying the conservation mode of the reagents: OPA and NAC solutions can be stored in a mixture or separately. It is well known that a formulation at low pH can be used to prevent oxidation of *N*-acetylcysteine [27]. We have thus studied the evolution of the slope of calibration curves over time (up to 6 months) either with the two liquid reagents prepared together (condition called ‘mixed reagents’ on Fig. 5A) or with the two liquid reagents stored separately (condition called ‘separated liquid reagents’ on Fig. 5B), and also with solid NAC deposited in microplate wells and liquid OPA reagent stored separately (condition called ‘solid NAC + liquid OPA’ on Fig. 5C). For this last preservation condition of the reagents, NAC was solubilized in acetone and then introduced into microplate wells. Acetone was evaporated at room temperature, then the plate was sealed with a polyethylene film. The OPA reagent was similar to the previous condition (in carbonate buffer at pH 10.5). All the solutions or microplates used in this study were stored in the dark at 4 °C.

For each experimental condition, confidence interval (CI) of slope value was determined at initial time (0 day) from the standard deviation of the b-slope value of the calibration curve s(b), depending on the residual standard deviation of the regression (n=5, P=0.05) [28]. Calculated values of (b±UM), with UM the uncertainty of measurement, for separated liquid reagents, mixed reagents and solid NAC/liquid OPA were 58.1±8.1, 54.6±1.6 and 49.1±9.2,

225 respectively. On figure 5, CI limits are noticed by dotted lines. We can observe that the slope
226 remained inside CI for separated liquid reagents up to 4 months, on contrary to mixed
227 reagents only after 7 days. For solid NAC/liquid OPA condition, a great variability of slope
228 value during experimental period was noticed and one data was outside CI after only 4 weeks.
229 These results prompted us to use separated liquid reagents condition.
230
231 3.3 Analytical features
232 3.3.1 Screening of primary amines
233 Screening of some biogenic amines and primary amino acids (25 µM) that could be likely
234 present in environmental or food samples was performed using OPA/NAC optimized
235 analytical protocol (Fig. 6). The fluorescence intensity of the different adducts was
236 standardized over that of the glycine adduct (reference 100).
237 A great diversity of amines and primary amino acids can therefore be detected by the
238 developed method, although their responses are not all similar. Secondary amines (proline)
239 and primary amines involved in a conjugated system (creatine, urea, creatinine) do not react.
240 The method allows for example the control of amino compounds in raw waters at the inlet of
241 treatment plants. Brosillon et al. [6] found that alanine, valine and tyrosine were the main
242 amino acids at the origin of disinfection by-products in drinking water during chlorination.
243 These 3 amino acids exhibit responses of 103, 82 and 78% compared to glycine. Although
244 individual concentrations of amino acids in raw waters range from 0.2 to 0.9 nM depending
245 on the season, the method is sensitive enough to quantify the total amount of amino
246 compounds. Likewise, the most frequently measured primary amino acids in wine samples
247 (alanine, glutamic acid, arginine) all lead to responses comparable with that of glycine, and a
248 global measurement based on a glycine standard is therefore relevant for this study.
249

3.3.2 Cross-interference and other interferences

In this section, a compound was considered as interferent when its presence resulted in more than 5% modification of the pure ammonium (100 µM) or glycine (as reference amino acid; 100 µM) response.

The cross-interference of amines over ammonium (λ_{ex}=415 nm / λ_{em}=485 nm) and inversely ammonium over amines (λ_{ex}=335 nm / λ_{em}=455 nm) was evaluated. The results showed that ammonium could be quantified in the presence of a 1000-fold excess of glycine and that primary amines could be quantified in the presence of a 100-fold excess of ammonium.

The interference of various metal cations has also been studied. The presence of Fe^{3+}or Cu^{2+} at 15 µM resulted in an interference of more than 5%. The addition of 50 mM EDTA to the reaction mixture reduced this interference. Ammonium could thus be quantified in the presence of 750 µM of Fe^{3+} or Cu^{2+} and the amines could be quantified in the presence of 180 µM of Fe^{3+} and 450 µM of Cu^{2+}. The other metallic elements tested (Al^{3+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) did not interfere up to 1 mM.

3.3.3 Performance and validation of the analytical method

The developed method was characterized and validated according to the AFNOR procedure XP T 90–210 [28]. Regarding analytical features, calibration curves for ammoniacal nitrogen and amine compounds were constructed with several standards (respectively, n=6 and n=5) with triplicate measurements for each, and have been obtained for each compound by linear regression of the fluorescence intensity against the concentrations of standards. The calibration range lies between the limit of quantification and a maximum concentration depending on the concentration range allowing to keep linearity between instrumental signal and standard concentrations. The limit of detection (LOD) of the analytical procedure is defined as the lowest amount of analyte in a sample that can be detected and considered as

different from the blank value but not necessarily quantified as an exact value, whereas the limit of quantification (LOQ) is the lowest amount of analyte in a sample which can be quantitatively determined with the analytical procedure with a defined variability. The LOD and LOQ were evaluated from the residual standard deviation of the regression (linearity study method) as LOD= 3.s(a)/b and LOQ= 10.s(a)/b, where s(a) is the standard deviation of the a-intercept and b is the slope of the calibration curve.

The accuracy of an analytical procedure is defined as the closeness of agreement between the conventional “true” value (obtained by the reference analytical procedure) and the measured value. The accuracy of our microplate procedure with fluorescence detection was assessed by analyzing a great number of samples from various origins. The calculation of the existing difference (d) between the measured value and the value issue from cationic chromatography, for each sample, and the calculation of the absolute value of their mean ($|\bar{d}|$) and the standard deviation (s) of all calculated data, is used to evaluate the good accuracy by check of the normal distribution of these data around zero ($w=|\bar{d}| \prec s3$; P=0.01) [28].

The repeatability, i.e. the precision, of analytical procedures was assessed by calculating the standard deviation of repeatability taking into account each replicate measurement (n=3) for each sample. A comparison of variance of repeatability obtained with the two analytical methods on the same multiple samples as previously is realized in order to determine their potential significative difference or not (Fisher-test, P=0.01) [28].

3.3.3.1 Ammonium

The calibration curve (y=57.076x+623.96) was linear up to 100 µM with a correlation coefficient of 0.997 (n=6). A LOD of 1.32 µM and a LOQ of 4.41 µM have been obtained. The working range of this analytical method is then 4.4-100 µM. The relative standard deviation evaluated from a sample containing 50 µM (n=6) was 1.58%.

Validation of the analytical method requires satisfactory results for the accuracy and the repeatability. No significant differences (Fisher-test, n=54, P=0.01) were noticed between variance of repeatability of our new analytical method and cationic chromatography, and the closeness of agreement of results proved a good accuracy (w=0.16 < 3; P=0.01). Figure 7 shows regression line obtained by comparison of results of 54 samples (surface water, wastewater, water extracts of compost). Slope of regression line is 0.96±0.05 and intercept were 1.13±1.88. Result was satisfactory for the intercept and slope as confidence intervals of their value included 0 and 1 respectively, in accordance with previous statistical test for accuracy. The analytical range and the low limit of quantification allow the determination of ammonium in various types of samples as natural waters, raw or treated wastewaters, aqueous extracts of soils or wastes.

3.3.3.2 Primary amines

For primary amines, the calibration curve (y=153.94x+374.89) was linear up to 100 µM with a correlation coefficient of 0.995 (n=5). A LOD of 0.55 µM and a LOQ of 1.84 µM have been obtained. The working range of this analytical method is then 0.55-100 µM. The relative standard deviation evaluated from a sample containing 50 µM (n=6) was 1.25%.

As for ammoniacal nitrogen, the accuracy and the repeatability for amine compounds gave satisfactory results. No significant difference (Fisher-test, n=13, P=0.01) was noticed between variance of repeatability of the developed analytical method and cationic chromatography, and the closeness of agreement of results proved a good accuracy (w=0.22 < 3; P=0.01). Figure 8 shows regression line obtained by comparison of results of 13 samples (various samples of wines). Slope of regression line is 0.94±0.23 and intercept were 178.7±535.2. Confidence intervals for the intercept and slope were relatively wide, essentially due to higher residual standard deviation of the regression, including however 0 and 1 respectively, equally

in accordance with previous statistical test for accuracy. The analytical range and the low limit of quantification allow the determination of primary amines in various types of samples as natural waters or food samples for example.

4. Conclusion

In this study, we presented the development and validation of a method for the determination of ammonium and primary amine compounds in various environmental and food samples. Optimization of reagents ratio and pH buffer enables fast and stable responses with no cross interferences between these two structurally close analytes. Reagent storage conditions were also evaluated in order to improve reagents shelf life, and we showed that separated storage of OPA and NAC solutions was the best option. Regarding analytical features, excellent limits of detection of 1.32 µM and 0.55 µM have been achieved for ammonium and primary amines, respectively, with RSD below 2%. Validation on various real samples by comparison with reference methods resulted in very good accuracies, proving the efficiency of this methodology for routine analysis of these nitrogen compounds.

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Figure captions

Fig. 1: Fluorescence intensity of OPA-NAC-ammonium adduct at λ_{ex}=335 nm / λ_{em}=455 nm as a function of reaction time and OPA/NAC concentration. [NH_4^+]: 50 µM; borate buffer pH=10.5.

Fig. 2: Fluorescence intensity of OPA-NAC-ammonium adduct at λ_{ex}=415 nm / λ_{em}=485 nm as a function of pH and reaction time. [OPA]: 8 mM; [NAC]: 8 mM; [NH_4^+]: 100 µM; borate buffer.

Fig. 3: Fluorescence intensity (λ_{ex}=415 nm / λ_{em}=485 nm) of an ammonium standard adduct (100 µM) over a period of 60 days depending on the reducing agent used. [OPA]: 8 mM; [NAC]: 8 mM; carbonate buffer pH=10.5.

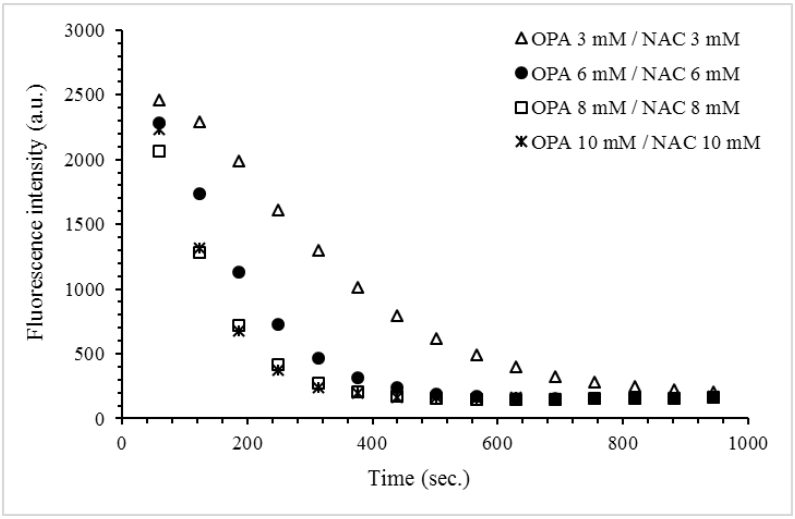
Fig. 4: Evolution of fluorescence intensity (λ_{ex}=415 nm / λ_{em}=485 nm) of an ammonium standard adduct (100 µM) over a period of 70 days as a function of TCEP concentration. [OPA]: 8 mM; [NAC]: 8 mM; carbonate buffer pH=10.5.

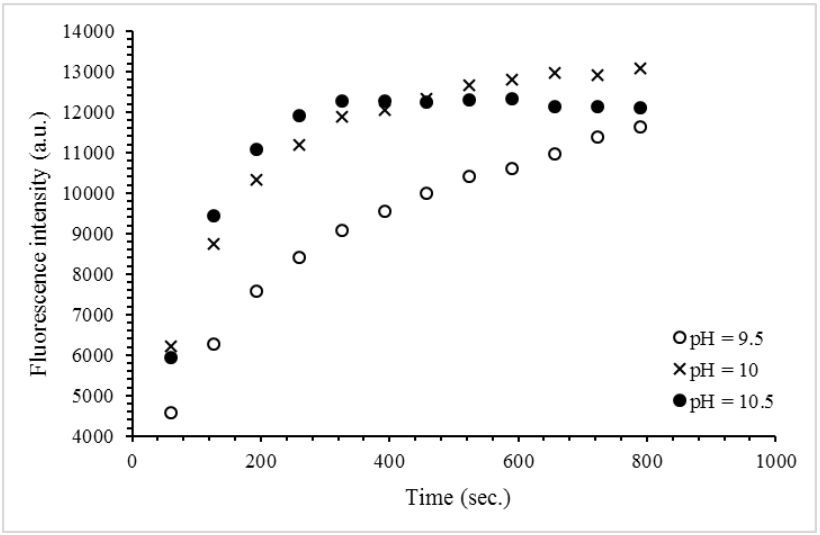
Fig. 5: Evolution of the slope of calibration curves over time depending on conservation mode of reagents. A: OPA and NAC mixed reagent; B: OPA and NAC separated liquid reagents; C: solid NAC and liquid OPA. Confidence intervals (CI) are represented in dotted lines. [OPA]: 8 mM; [NAC]: 8 mM; [TCEP]: 1 mM; carbonate buffer pH=10.5.

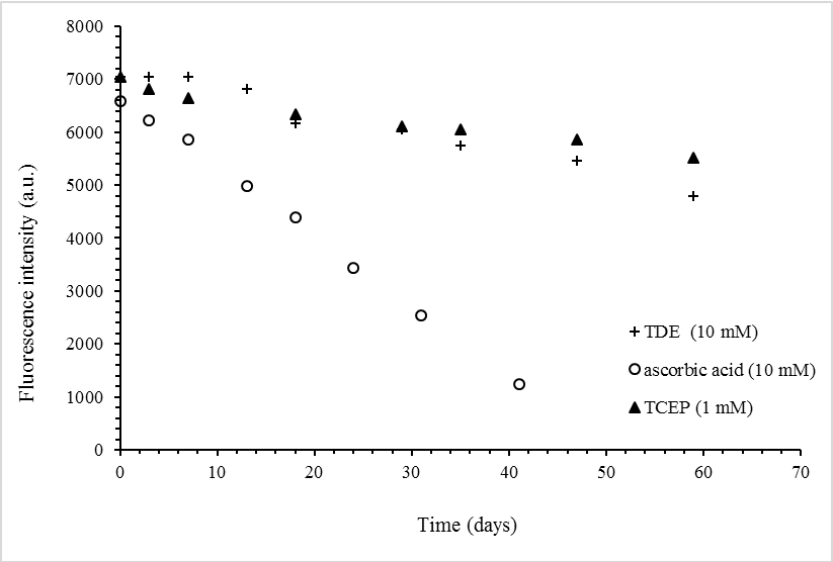
Fig. 6: Screening of biogenic amines and primary amino acids (25 µM). [OPA]: 8 mM; [NAC]: 8 mM; [TCEP]: 1 mM; carbonate buffer pH=10.5.

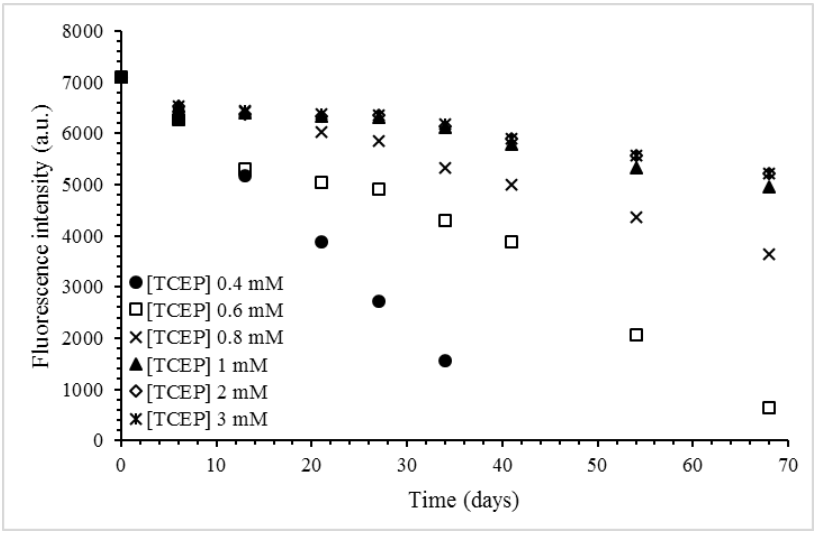
Fig. 7 : Regression line for comparison of analytical results between Microplate Assay and Ion Chromatography for ammonium determination.

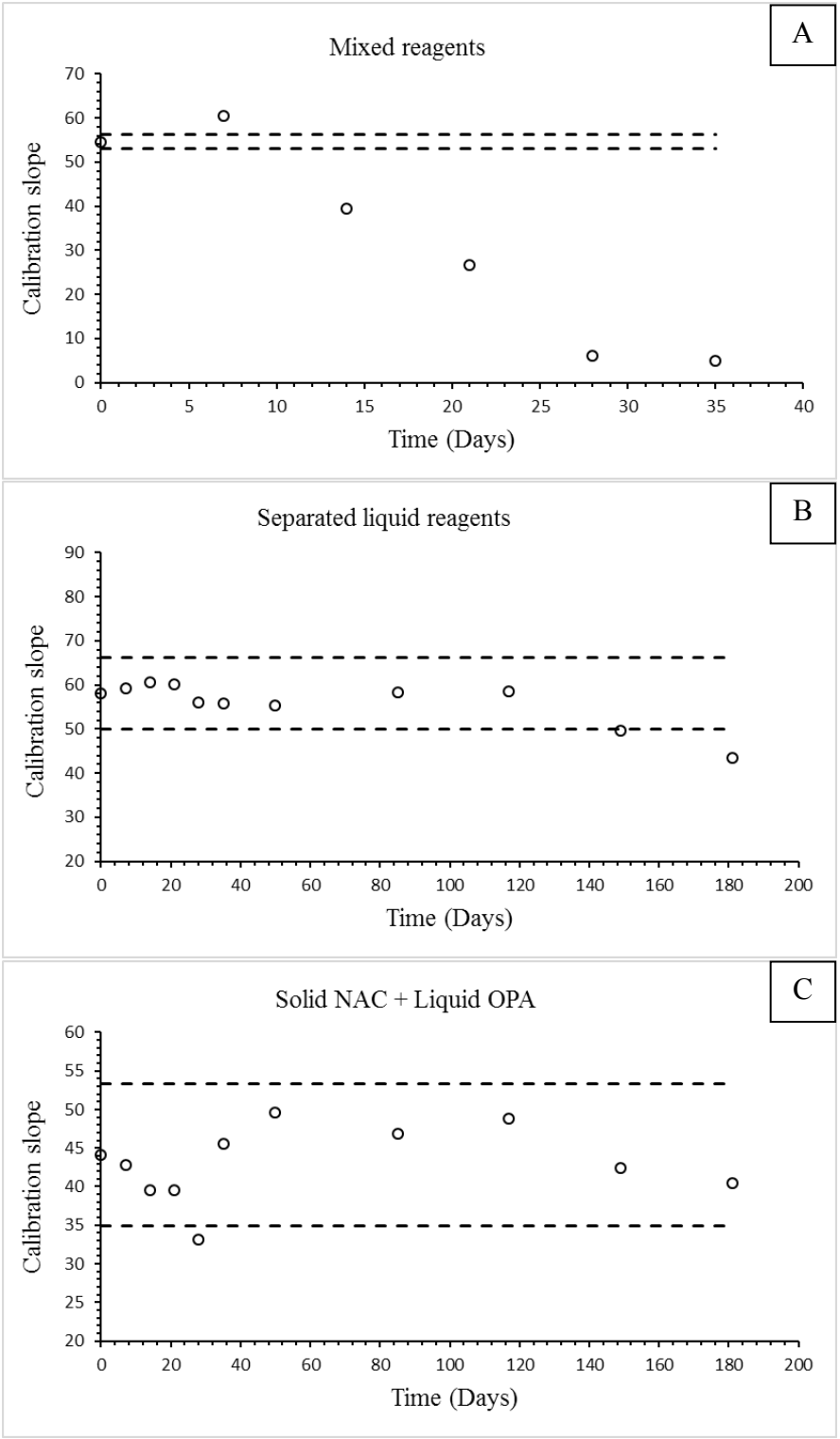
Fig. 8 : Regression line for comparison of analytical results between Microplate Assay and Ionic Exchange Chromatography for primary amines determination.

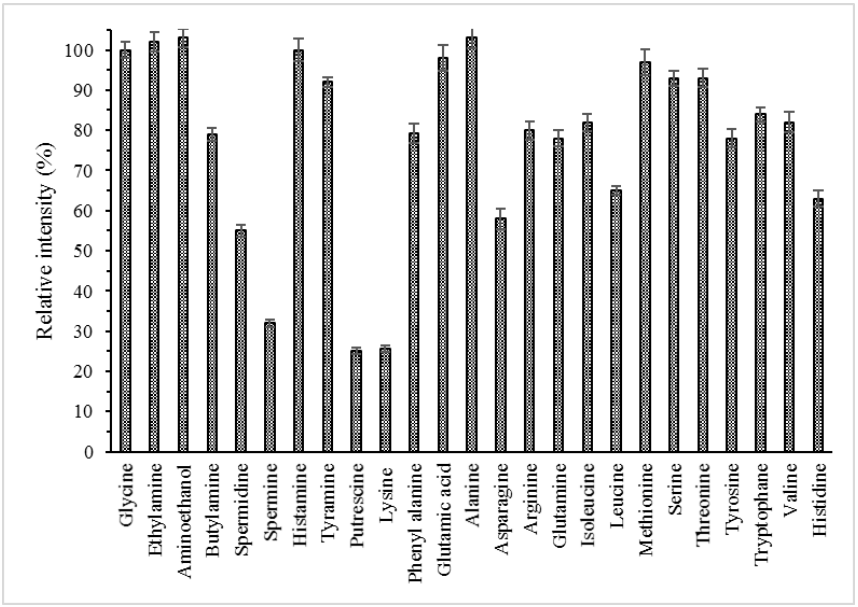

Fluorescence intensity (a.u.)
3000
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
Time (sec.)
OPA 3 mM / NAC 3 mM
OPA 6 mM / NAC 6 mM
OPA 8 mM / NAC 8 mM
OPA 10 mM / NAC 10 mM

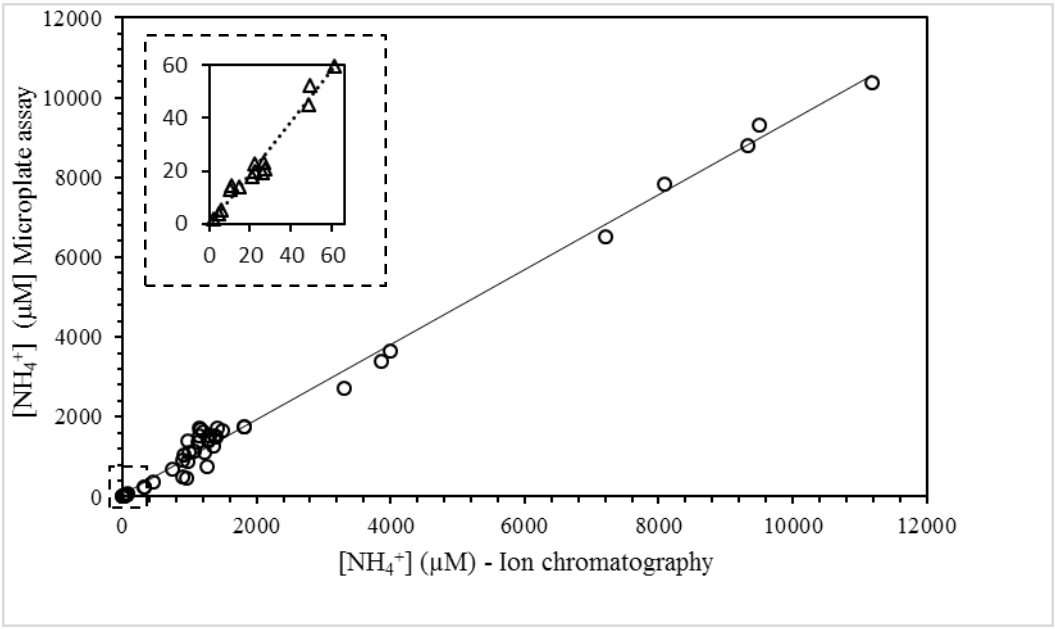

Fluorescence intensity (a.u.)
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
0
200
400
600
800
1000
Time (sec.)
pH = 9.5
pH = 10
pH = 10.5

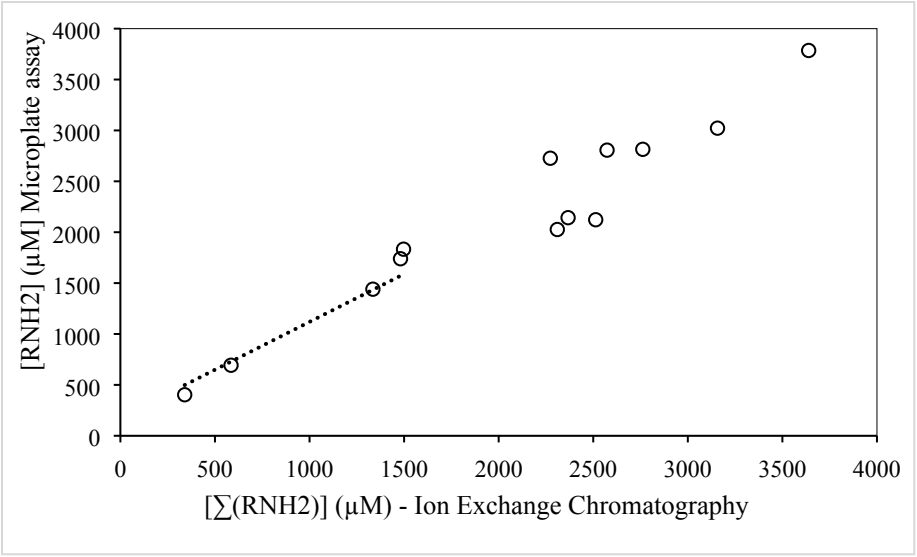

Fluorescence intensity (a.u.)
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
Time (days)
TDE (10 mM)
ascorbic acid (10 mM)
TCEP (1 mM)


Fluorescence intensity (a.u.)
Time (days)
[TCEP] 0.4 mM
[TCEP] 0.6 mM
[TCEP] 0.8 mM
[TCEP] 1 mM
[TCEP] 2 mM
[TCEP] 3 mM
0
1000
2000
3000
4000
5000
6000
7000
8000
0
10
20
30
40
50
60
70


A
Mixed reagents
Calibration slope
Time (Days)
B
Separated liquid reagents
Calibration slope
Time (Days)
C
Solid NAC + Liquid OPA
Calibration slope
Time (Days)


Relative intensity (%)
0
10
20
30
40
50
60
70
80
90
100
Glycine
Ethylamine
Aminoethanol
Butylamine
Spermidine
Spermine
Histamine
Tyramine
Putrescine
Lysine
Phenyl alanine
Glutamic acid
Alanine
Asparagine
Arginine
Glutamine
Isoleucine
Leucine
Methionine
Serine
Threonine
Tyrosine
Tryptophane
Valine
Histidine


12000
10000
8000
6000
4000
2000
0
[NH4+] (µM) Microplate assay
0
2000
4000
6000
8000
10000
12000
[NH4+] (µM) - Ion chromatography
60
40
20
0
0 20 40 60


[RNH2] (µM] Microplate assay
4000
3500
3000
2500
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
3000
3500
4000
[∑(RNH2)] (µM) - Ion Exchange Chromatography

HIGHLIGHTS

- An improved spectrofluorimetric method for the simultaneous and direct determination of ammonium and primary amine compounds is presented.
- Reagents ratio has been optimized in order to suppress known cross-interferences.
- Optimization of reagents formulation led to high reagents stability for routine analysis.
- The method was validated on real samples (wine samples, compost extracts or wastewater).