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Influence of nitrogen speciation on the TDN measurement in fresh waters by High Temperature Catalytic Oxidation and Persulfate Digestion

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Abstract

Analytical methodologies for the determination of Total Dissolved Nitrogen (TDN) in waters are based on a conversion step able to transform selectively all the nitrogen species into a compound that is then quantified. A crucial requirement to meet accuracy is the quantitative recovery of all organic and inorganic nitrogen species during the conversion step. In this work, the N recoveries of two widely employed methodologies that use different conversion steps (High Temperature Catalytic Oxidation, HTCO, and Persulfate Digestion, PD) were assessed on a set of organic nitrogen compounds, representative of the structures of both DOM and anthropogenic contaminants. Low recoveries are due to poor selectivity during the conversion step, with the formation of nitrogen-compounds other than nitrogen oxide (HTCO) and nitrate (PD). The results show that in many instances the TDN measurements give systematically low results depending on N speciation. PD could give lower results than HTCO even for samples containing only DOM of biological origin. In particular *i*) low N recovery was always observed with compounds having two or more contiguous N atoms; *ii*) the HTCO method is very effective for TDN quantification in the presence of s-triazine rings while PD method did not yield satisfactory N recovery; *iii*) a full N

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recovery was observed with compounds having amido or amino groups or nitrogen atoms in imidazole, indole, pyrimidine rings; *iv*) the N recoveries for purine derivatives are almost complete with HTCO, but give systematically low results by PD.

Finally, the estimation of DON fluxes and pools from TDN measurements can be affected by uncertainties larger than previously thought as a consequence of *i*) the lower N recovery for some nitrogen-compounds and *ii*) the differences in the N recovery as a function of the adopted analytical methods.

Keywords: Total Dissolved Nitrogen, Dissolved Organic Nitrogen, High Temperature Catalytic Oxidation, Persulfate Oxidation, Total Organic Carbon, DON/DIN recovery.

1. Introduction

Total Dissolved Nitrogen (TDN) is a parameter of chief importance to assess nutrient cycling and the status of water systems. The nitrogen cycle defines the availability of the main nutrients in surface freshwaters and seawater, which directly affect both water quality and ecosystem biodiversity [1,2,3,4]. Pollution by organic compounds and eutrophication, caused by the presence of bioavailable nitrogen and phosphorus in excess, are among the most serious threats to the aquatic ecosystems. Accurate analytical determination of both Total Organic Carbon (TOC) and Dissolved Organic Nitrogen (DON) is essential for a correct evaluation of the status of a water body [5,6,7], as well as for the assessment of nitrogen budgets, fluxes and fixation at local and global level [8,9,10]. Moreover, the measurement of these parameters is widely employed in the monitoring of waste-water treatment processes, drinking water quality, as well as in industrial applications [11,12,13]. The formation of toxic nitrogenous disinfection byproducts, e.g. nitriles, N-nitrosoamines and haloamides in drinking water is linked to the presence of DON and emerging nitrogenous by-products formed from xenobiotic compounds were recently reported [14].

TDN is composed by two fractions: Dissolved Inorganic Nitrogen (DIN) and DON. DIN is the sum of the main inorganic N ions (nitrate, nitrite and ammonium). DON is mainly composed of the degradation products of biomolecules (proteins, nucleic acids, urea, aminopolysaccharides, humic and fulvic acids) and of compounds with unknown chemical composition. Besides biogenic compounds, in contaminated and waste water emerging and persistent anthropogenic contaminants can contribute significantly to DON. The complexity of dissolved organic matter (DOM) rules out any kind of rigorous analytical approach to individually quantify all contributing organic chemicals [15,16,17]. The simple spectrum of the inorganic nitrogen (DIN = $N–NH_4^+ + N–NO_3^- + N–NO_2^-$) allows the determination of its main components by accurate analytical methods. DON is evaluated by difference as DON = TDN – DIN. This approach can be problematic because the analytical performance of DON analysis have the combined uncertainty of at least three analyses (TDN, NH_4^+ and NO_3^-/NO_2^-). In highly eutrophic environments or in ocean deep waters DIN can be many times DON concentrations. Under these conditions DON estimates are inherently plagued by high uncertainties [18,19].

DON and TOC are compositional parameters related to the elemental composition of the sample. Their measurement is based on the quantitative conversion of all the species of an element into a single compound that is then determined. The absolute recovery of the conversion step should be carefully evaluated to have accurate measurements of the compositional parameter. Different conversion steps and different molecular structures could lead to different recoveries. Several approaches have been proposed and used to convert organic and inorganic nitrogen into a single and analytically quantifiable species. In principle the best approach should be the complete atomization of the sample, with spectroscopic or mass spectrometric determination of N atoms/ions [20]. TDN concentrations are commonly measured by UV photooxidation (UV-PO, possibly in the presence of hydrogen peroxide), Persulfate Digestion (PD), High Temperature Combustion (HTC) and High Temperature Catalytic Oxidation (HTCO) [18,21]. PD converts inorganic and organic nitrogen into nitrate ions through thermal oxidation in the presence of potassium persulfate [22,23]. Nitrate

concentration is measured by spectrophotometry, possibly after conversion to nitrite, or by ion chromatography. The HTCO approach is based on the injection of the sample into a furnace, where the high temperature and the presence of a catalyst (Pt on quartz wool, CeO_2, and CoCr) convert inorganic and organic nitrogen to nitric oxide (NO), which is then determined by chemiluminescence detector [23]. An important advantage related to the HTCO technique is the opportunity to carry out TDN and TOC determinations in a single analysis run. The introduction of HTCO spurred a big debate due to the systematic higher values of DON and DOC obtained with respect to wet chemical oxidation techniques, leading to a possible revision of the marine organic carbon and nitrogen pools. Extensive studies on the relative organic N recovery for different DON analytical methods (HTCO, PD, HTO and UV-PO) on standard solutions of organic nitrogen compounds were undertaken [24,25,26,27,28,29].

A careful analysis of the analytical procedures for the DON determinations highlighted the importance of: i – periodic evaluation of the reproducibility and accuracy of HTCO system on reference materials [24,30]; ii – correct evaluation of the analytical blanks. It was concluded that differences are limited to a 10% and mainly due to the low molecular weight fraction of the dissolved organic matter [31,32,33]. Therefore, now it is generally accepted that HTCO, PD and UV-PO give comparable and reproducible DON values when the analytical procedures are carried out properly with appropriate blank evaluation and reference material application [34,35,36].

Furthermore, concerning the dependence of absolute organic N recovery on organic N speciation, the DON subgroup of the 1991 Seattle DON/DOC workshop reported non quantitative and/or highly variable N recovery for some N containing heterocycles like guanosine, uridine and antipyrine with both HTCO and PD [24]. The seminal work by Nydal [37] underlined for the first time poor yields of N conversion by alkaline PD at 120 °C for some N-containing molecules such as hydrazinium sulphate, guanidinium carbonate, benzidine hydrochloride, creatinine, methyl orange, benzotriazole and antipyrine. Nydal reported the difficulty to obtain quantitative recovery for species containing nitrogen-to-nitrogen bond and the HN=C group. It is likely that organic

compounds containing N-N double bonds could be converted to N_2 gas. Also, cyanuric acid and nitriles are expected to be difficult to convert through oxidative processes. To our knowledge, the suggestion in [24] to test these classes of compounds with HTCO and PD in order to define the limits of these methods was not accomplished yet. Scattered examples of incomplete N recovery on specific molecules were reported. The choice of catalyst is critical to achieve complete recovery of N-NH_4^+ and N-NO_2^- by HTCO [32,33,34,38]. Walsh reported low recovery during the TDN determination of some proteins, amino acids, nucleic acids and bases by using photo-oxidation and HTO [25]. Low N recovery was reported for pirazole, indazole and azooxy compounds by HTO with chemiluminescence detection [39]. Some reports point out organic N-recovery with HTCO significantly lower than 100% for nitrogen heterocycles, specifically antipyrine [29]. The absence of a complete N recovery in some cases was related to the incomplete mineralization during the oxidation phase, in other cases to the formation of products other than NO (HTCO) or nitrate (PD) [40]. No clear relationship between the conversion and the chemical structure of the tested compounds was reported. Recently, Rus et al. reported a critical assessment of the precision and bias of some methods for the determination of total nitrogen, on the basis of the analysis of 900 geographically and compositionally diverse environmental fresh water samples [41]. The conclusion was that best estimates are obtained by determining TDN by alkaline PD and particulate N by HTCO and summing them to obtain total nitrogen.

Based on the above discussion, in this work our attention was focused on the evaluation of absolute N recovery for the two widely employed TDN determination methods (PD and HTCO) as a function of the organic N speciation. The choice of compounds reflect the suggestion of the cited DON subgroup report (e.g. molecules with triazole rings, s-triazine rings and azo groups [24]), also including molecules that represent emerging contaminants of water bodies [42,43,44,45]. The tested N-molecules chosen can be grouped as follows: nitrogen heterocycles of biological origin, aminoacids, ureas and amides, s-triazine compounds, azo compounds, triazole compounds of anthropogenic origin, and inorganic nitrogen compounds. Specific aims of the present study were: *i*)

to assess the role of the chemical structure of the selected compounds on the recovery of organic nitrogen; *ii*) to evaluate if the chosen techniques allow a quantitative recovery of the N atoms as NO_3^- or NO.

2. Experimental

Methods

Analytical Determinations

PD oxidation was carried out by alkaline digestion with potassium persulfate in borate buffer according to APHA AWWA WEF Standard Methods [22,46,47] and spectrophotometric determination of nitrate at 220 nm with a SAFAS Monaco UV mc2 spectrophotometer. HTCO measurements were accomplished by using a mixed catalyst made of CoCr and CeO_2 previously optimized for the analysis of fresh waters (lake, river, and rain water) with a Skalar Formacs^{HT} TOC/TN analyzer, controlled by the Skalar HT Access software (version 1.03) and interfaced via a Dionex UCI-100 device to a Dionex Chromeleon software (version 6.70). The detailed description of the adopted methods and the relative analytical conditions are reported in [34]. Both methods were calibrated with $NaNO_3$ (Aldrich, 99.9%, treated two hours at 120 °C) solutions, as a result the data reported are relative recoveries with respect to nitrate.

TDN analysis is routinely carried out on freshwater samples (lakes, rivers and atmospheric depositions) in the laboratory of the *Consiglio Nazionale delle Ricerche* (CNR) Institute of Ecosystem Study (CNR-ISE) since 1978 [8,47]. The PD method coupled with the spectrophotometric determination of nitrate at 220 nm (PD) has been used in the CNR-ISE laboratories since the 1980’s. From 2004 this methods was coupled with an automatic HTCO system. In this period the two methodologies were constantly checked by regular participation in intercomparison exercises and ring tests [48,49], which involve a constant check of the figures of

merit with the analysis of blanks, assessing the Limit of Detection (0.09 and 0.04 mg N L^{-1} for PD and HTCO, respectively) and Limit of Quantification (0.30 and 0.14 mg N L^{-1} for PD and HTCO, respectively) [34]. A regular use of Shewhard control charts was also made [50] and quantitative recovery (>99%) of N-NH_4^+ and N-NO_2^- has been previously reported with HTCO and PD in [34]. Measurement accuracy is routinely checked by the regular use of control charts representing different concentration ranges based on the analysis of standard solution containing different N-species (N-NO_3^-, N-NH_4^+ and N-NO_2^-) and Creatine as organic standard compound (for TOC analysis). When drifts or values outside the control ranges are observed, the catalyst is replaced.

The quality assurance procedures adopted allows the monitoring of systematic method drifts and method uncertainties. An assessment of systematic differences between TDN measures by PD and HTCO for fresh water and rain samples resulted in slightly (3%), but statistically significantly, higher values for PD than HTCO [8]. This difference can be due to a slightly lower conversion of N-NH_4^+ to nitric oxide by HTCO [8]. However, a study considering fresh and waste waters report no differences between the two methods [35]. In all the cases the determination of TOC by HTCO in the presence of only inorganic nitrogen (N-NO_3^-, N-NH_4^+ and N-NO_2^-) is below the LOD of the TOC determination by HTCO (LOD = 0.01 mg C/L).

Melamine and cyanuric acid were quantified by ion pair chromatography with a bonded phase octadecylsilica column (LiChrospher R100-CH 18/2 by Merck, 250 mm length, 10 mm i.d., 5 µm packing); the mobile phase was 0.01 M sodium hexane sulfonate (Aldrich 99+%) and 0.014 M H_3PO_4 dissolved in water/CH_3CN 95/5 at 1 mL min^{-1}. The detection was carried out at 200 nm [51].

Sample Preparation

The tested nitrogen containing compounds are reported in Table 1 and purchased from different vendors (Sigma-Aldrich, Fluka and Merck). They were used as received without further purification. All the tested solutions and the adopted standards were prepared in ultrapure water (18 MΩ cm, TOC < 2 ppb). The solutions to be analyzed were prepared at 1 and 5 mg N L^{-1}, a concentration range similar to the typical TDN values of common freshwater samples. [34,52,53]

Note that the choice of the two concentration levels tested was not done with the aim to simulate realistic concentrations of organic molecules (e.g. emerging pollutants).

Data Analysis

The nominal concentration of nitrogen and carbon (in mg C L^{-1}) was calculated from the molecular formula of each compound. The nitrogen (or carbon) recovery was defined as

$$R\% = \frac{C_m}{C_0} \times 100$$

where $R\%$ is the percentage recovery, C_0 the nominal nitrogen or carbon concentration, and C_m the measured nitrogen or carbon concentration against nitrate and potassium hydrogen phthalate standards respectively. The N or C recovery was expressed as the average value of at least 15 and 5 data for HTCO and PD, respectively. The carbon recovery, obviously measured with the HTCO method alone, is a direct measurement of the ability of the system to mineralize the organic compounds. It allows the discrimination, in the case of low N recovery, between a low mineralization during the conversion step and the conversion of nitrogen in species different from NO.

Results and discussion

Target Nitrogen Compounds

The present study was carried out on a selection of nitrogen compounds containing functionalities for which there are advanced suspects or it has been previously reported non quantitative recoveries for both HTCO and PD [24,25,29,39]. The tested compounds are summarized in Table 1 and their chemical structure is reported in Figure 1. They can be grouped on the basis of the chemical structure and the nature of the nitrogen atoms as follows:

1. nitrogen heterocycles of biological origin: purine type nucleobases adenine (Ade), guanine (Gua) and uric acid (UriAc); pirimidine type nucleobase uracil (Ura);
2. aminoacids: glycine (Gly), L-aspartic acid (Asp), L-tryptophan (Try) and L-histidine (His), the latter two containing a nitrogen heterocycle;
3. ureas and amides: urea (Ure), biuret (Biu) and creatinine (Cre);
4. s-triazine compounds: melamine (Mel) and cyanuric acid (CA);
5. azo compounds: orange II (OII), ethyl orange (EO) and tropaeolin O (TO);
6. triazole compounds of anthropogenic origin: 1H-benzotriazole (BTz), 5-methyl-1H-benzotriazole (5M-BTz), 1H-1,2,3-triazole (1,2,3-Tz), 1H-1,2,4-triazole (1,2,4-Tz), 1,2,4-triazole-3-carboxylic acid (1,2,4-Tz-3CA) and 3-amino-1,2,4-triazole (3A-1,2,4-Tz);
7. inorganic nitrogen compounds: sodium nitrate (used as primary standard), sodium azide (SA) and hydrazine sulfate (Hyd).

Compounds of the groups 1-3 encompass the structure of low molecular weight bioavailable DON, composed primarily by urea, free and bound aminoacids, nucleobases and their degradation products [54]. The N recovery with urea (Ure), biuret (Biu), uric acid (UriAc) the amino acids L-tryptophan (Try), L-aspartic acid (Asp), glycine (Gly) and L-histidine (His), the nucleobases uracil (Ura), adenine (Ade) and guanine (Gua) was evaluated with the aim of studying the behavior of compounds representative of the DON components of surface waters. Some of these derive from the decomposition of proteins and nucleic acid and from biological activity. L-histidine has an imidazole ring in its structure, and the N conversion ability of HTCO and PD toward this ring was also tested in the case of creatinine (Cre). Uracil has a pyrimidine ring, while adenine, guanine and uric acid have a purine ring.

1H-benzotriazole (BTz) and 5-methyl-1H-benzotriazole (5M-BTz, commercialized as Tolyltriazole) are two common emerging pollutants ubiquitously found in the aquatic ecosystems up to μM concentrations [44,55,56]. They are characterized by the presence of the 1,2,3-triazole ring. To gain

insight into the effect of the position of the nitrogen atoms in the triazole ring, we compared the nitrogen recovery of compounds having 1,2,3-triazole rings (1,2,3-Tz, BTz and 5M-BTz) with compounds having three nitrogen atoms in the ring but not all in a contiguous position: 1H-1,2,4-triazole (1,2,4-Tz), 1,2,4-triazole-3-carboxylic acid (1,2,4-Tz-3CA) and 3-amino-1,2,4-triazole (1,2,4-Tz-3CA), the latter commercialized as amitrol.

s-Triazine derivatives are well known to lead to an incomplete conversion of the organic carbon with wet chemical oxidation, also in the presence of strong oxidants [51,57,58]. The stability of the s-triazine ring toward different Advanced Oxidation Processes (AOPs) is noteworthy. As example, with photocatalysis over TiO_2 or H_2O_2 UV treatment the s-triazine derivatives have the unique property of easily lose the alkyl substituents. However, they retain the s-triazine ring with the final formation of CA, which is stable to further oxidation under photocatalytic conditions or other OH radical generating AOPs [51,57,58]. Therefore, it was possible to test the HTCO and PD oxidation steps toward a known nitrogen ring resistant to wet chemical oxidation. Furthermore, the study of the N recovery for melamine (Mel) and CA would highlight possible differences between the behavior of the amino groups linked to the 2,4,6 positions of the s-triazine ring (see the Mel structure) and the nitrogen atoms in position 1,3,5 of the ring. The study of the N recovery with orange II (OII), ethyl orange (EO) and tropaeolin O (TO) was focused on the role of azo-group. Finally, hydrazine (Hyd) and sodium azide (SA) were tested as they are two simple molecules with single and multiple N-N bonds.

N and C Recoveries

The histogram reported in Figure 2 shows the C recovery, while Figure 3 shows the N recovery with HTCO (Figure 3A) and PD (Figure 3B) at 1 and 5 mg N L^{-1}.

The TOC analysis with HTCO showed an almost complete C recovery for all the tested compounds, which indicates that the system was able to convert them to CO_2. For the samples at the lowest

concentration values a slight TOC overestimation and a higher associated error were observed. The relative standard deviation (RSD%) 1 mg N L^{-1} level ranged from 4 (5M-BTz) to 27 % (Ure), while in the case of 5 mg N L^{-1} it varied from 1.5 (BTz and 5M-BTz) to 11 % (Ure). These data are in perfect agreement with the analytical repeatability of the HTCO method at different TOC values, which is reported in Figure 4A and expressed as RSD%. Cyanuric acid and melamine that have s-triazine rings showed a complete C recovery. The TOC data suggest that the low nitrogen recoveries observed and commented later on cannot be accounted for by an incomplete mineralization of the organic structures because in this case the conversion of organic carbon to CO_2 would be only partial.

Figure 4B shows the analytical repeatability expressed as RSD% as a function of the TDN concentration for HTCO and PD. The repeatability of the two methods is quite similar and ranges in the 2-6% and 3-9% at low TDN concentration (1 mg N L^{-1}) for HTCO and PD, respectively, while in the 1-4% range at higher TDN concentration for both methods.

From Table 1 and Figure 3 it is apparent the low N recovery for a significant number of the studied compounds. In contrast, an almost complete N recovery with both HTCO and PD was observed with creatinine (Cre), urea (Ure), L-tryptophan (Try), L-aspartic acid (Asp), biuret (Biu), glycine (Gly) and L-histidine (His). The average N recovery of the latter compounds at the 5 mg N L^{-1} level was slightly lower with PD (87 %) than with HTCO (93 %).

Note that, from the comparison between the N (and C) Recovery % with both PD and HTCO it is not possible to notice significant differences between the two concentration levels. The conversions at 1 mg N L^{-1} are quite similar to those observed at 5 mg N L^{-1} without any systematic trends. From this analysis emerges that in the explored range, the concentration is not a key factor influencing the conversion level.

The compounds with triazole rings have a behavior dependent on the topology of the N atoms in the ring. The molecules having a 1,2,3-triazole ring showed a ~ 30 % N recovery with both methods.

PD showed an average N recovery of approximately 30 % also with compounds having 1,2,4-triazole rings. Conversely, the HTCO method showed an average value of roughly 60 % with 1H-1,2,4-triazole (1,2,4-Tz), 1,2,4-triazole-3-carboxylic acid (1,2,4-Tz-3CA) and 3-amino-1,2,4-triazole (3A-1,2,4-Tz). Interestingly, the shift of a nitrogen atom from the position 3 to 4 of the triazole ring doubled the N recovery with HTCO, while the recovery with PD did not change. The presence of at least two contiguous nitrogen atoms in the triazole ring could promote the release of molecular nitrogen not detected by either chemiluminescence detector or UV spectrophotometry. The HTCO seems to be more sensitive to the ring structure with respect to PD.

By comparing the N recovery of melamine (Mel) and cyanuric acid (CA) an asymmetry in the analytical behavior of the two tested methods was observed. The N recovery with HTCO for both Mel and CA was higher than 85 % (86–91 %), thereby suggesting that the HTCO conditions would be able to convert almost quantitatively the N atoms to NO. This would apply to both the s-triazine ring and the amino substituents of Mel. On the other hand, the N recovery of Mel and CA with PD was very low (57-58 % and 13–14 %, respectively). The higher recovery observed with Mel compared to CA can be explained considering that CA has all the N atoms in the s-triazine ring, while Mel also has three amino groups. Therefore, the higher recovery for Mel could be accounted for under the hypothesis that the PD oxidative step carries out an effective conversion to nitrate of the amino groups, but not of the ring N atoms. As pointed out in the discussion of the TOC data, it is well-known that s-triazine compounds under wet chemical oxidation conditions, even with strong oxidants like $^{\bullet}OH$ radicals, convert to cyanuric acid [24,25,26]. PD relies on the sulfate radicals as active oxidant species. These radicals are known to be less reactive than $^{\bullet}OH$, thus the low N recovery observed with PD can be attributed to an incomplete conversion of the tested compounds. The formation of nitrate from cyanuric acid with the PD method increase very sluggishly if the treatment is prolonged over 10 hours (around 10% recovery). This indicates a very slow hydrolysis of the cyanuric acid to ammonium and CO_2 in the adopted conditions. Chromatographic analysis of

the PD solutions after digestion confirmed this interpretation, revealing an almost quantitative conversion of Mel to CA and a decrease in CA around 10% after 10 hours of digestion.

Among the tested organic compounds, the lowest N recoveries were observed with the azo dyes orange II (OII), ethyl orange (EO) and tropaeolin O (TO). For instance, at 1 mg N L^{-1} levels the observed recoveries with HTCO were 11.5, 50 and 18 % for OII, EO and TO, respectively. The corresponding recoveries with PD were 10, 35 and 12%. Also in this case a low selectivity of the conversion step can be proposed to explain the low N recovery in the presence of azo groups. The presence of two contiguous N atoms might induce the formation of molecular nitrogen or otherwise different species than NO or nitrate. The formation of N_2 was observed during the treatment of azo-dyes with Advanced Oxidation Processes [59]. The significantly higher recovery observed with EO compared to TO and OII is consistent with the hypothesis of low conversion selectivity, because EO, in addition to the azo group, also bears a diethylamino group in its structure which undergoes efficient conversion.

The lowest N recoveries were observed with sodium azide (SA) and hydrazine (Hyd), which proves that the two adopted oxidation methods are unsuitable for the analysis of these compounds. Once more, the low N recovery is related to the presence in the molecular structure of two or more N atoms bound together. Also in this case the N recoveries observed with HTCO are significantly higher than those with PD.

The N recovery for compounds having only amino and amido groups (urea, biuret, L-aspartic acid, glycine) was complete within the analytical uncertainties. This finding is in agreement with the results reported above for tested N compounds having both “problematic” groups and amino substituents, which showed higher N recovery compared to compounds with similar structure but without the amino groups (e.g. comparison between CA and Mel). The N recovery was also very good for compounds having amino substituents as well as non-contiguous N atoms in rings: the amino acid L-histidine has an indole moiety, L-tryptophan and creatinine an imidazole group.

Concerning this group of compounds, the N recovery with HTCO (93–98 %) was slightly higher than with PD (87–93 %).

Finally, the study of the N recovery of the analyzed nucleobases (uracil, guanine and adenine) and of uric acid highlighted the behavior of the pyrimidine and purine derivatives. The N recovery observed with HTCO was always complete with these four compounds (average N recovery equal to 97 and 96 % at 1 and 5 mg N L^{-1}, respectively). Conversely, with PD a complete N recovery was observed only with uracil (96 and 94 % at 1 and 5 mg N L^{-1}, respectively) while with uric acid, adenine and guanine a partial recovery was observed. Thereby, the nitrogen conversion to nitrate ions by PD is completely selective for pyrimidine ring, while it is only partial with molecules containing purine rings. In the last case N recoveries ranged from 66 to 85 %, being systematically low. Also in this case the low recovery is related to the formation of cyanuric acid from the chemical oxidation of purine derivatives. It is long well-known that uric acid is partially oxidized to cyanuric acid in the presence of reactive oxygen species (ROS) leading to a partial conversion to cyanuric acid [60]. Analogously, in vivo and in vitro oxidation of guanine by ROS results in the formation of cyanuric acid, among other end products [61,62,63]. Also in this case, the chromatographic analysis of the PD solutions of guanine and uric acid after digestion revealed the presence of CA concentration levels equivalent to one third of the TDN initially present.

3. Conclusions

In this work we studied the N recovery with two widely employed analytical methods used for the determination of DON in fresh water samples (PD and HTCO), in the presence of different N-containing compounds. For a comparison of the major practical aspects regarding the use of the two methods please refer to [34]. The tested molecules were chosen with the aim to ascertain the role of the chemical structure on the conversion of organic nitrogen for a set of compounds representative of the DOM constituents and of the most common xenobiotic contaminants. The comparison was carried out on synthetic samples prepared with pure water and consequently our conclusion could

be extended to marine water only after a careful evaluation. The HTCO method allows the contemporary determination of TDN and TOC. A carbon recovery higher than 89% was observed with all the tested N-compounds and this emphasizes the complete and selective conversion of organic carbon to CO_2 for the studied compounds by HTCO. Conversely, the N recovery was unsatisfactory with both methods for a significant number of the studied compounds. In a number of cases this finding cannot be related to an incomplete mineralization of the organic molecules, but rather to a low selectivity of the conversion step of organic nitrogen to the detectable molecules (nitrate and NO).

In conclusion:

1) Low N recovery was always observed with compounds having two or more contiguous N atoms. Problems in N recovery can be foreseen for compounds with triazole rings (1,2,3- and 1,2,4-) as well as azo groups, and with sodium azide and hydrazine.
2) The HTCO method is very effective for DON quantification in the presence of s-triazine rings. In contrast, with the same s-triazine rings the PD method did not yield satisfactory N recovery. Final oxidation product of s-triazine ring under wet chemical conditions is the cyanuric acid ring. It is well-known that this compound is refractory with respect to oxidation by $^{\bullet}OH$ and $SO_4^{-\bullet}$ radicals, explaining the poor performance of PD toward the N conversion of s-triazines.
3) A full N recovery was observed with compounds having amido or amino groups or nitrogen atoms in imidazole, indole, pyrimidine rings. On the contrary, the N recoveries for purine derivatives are almost complete with HTCO, but give systematically low results by PD, being the recovery around 65-70%. Also in this case the reason is the formation of cyanuric acid among other oxidation products, depending on the relative kinetic of the various concurrent oxidation pathways of these molecules [61,62,63]. This is important considering that these structures are quite common in amino acids, nucleic acids and derived

compounds, which make up a large fraction of the N-containing organic compounds present in natural waters and derived from the biota activity.

The classes of compounds giving low N recovery here individuated are mainly of anthropogenic origin. As a consequence, oxidation of DON in contaminated waters can give systematically low results with both methods. Otherwise the compounds here tested representative of the biological DON gave satisfactory results with both methods, except for purine derivatives with the PD method. The results of this work give some suggestions to predict the possibility of systematically low TDN values obtained by PD and HTCO on the base of organic N speciation. As a final remark, the use of TDN as a proxy for the formation of toxic nitrogenous disinfection by-products in drinking water must be treated with great cautions in the presence of xenobiotic organic N.

Disclosure statement

The authors declare that they have no conflict of interest.

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Figures

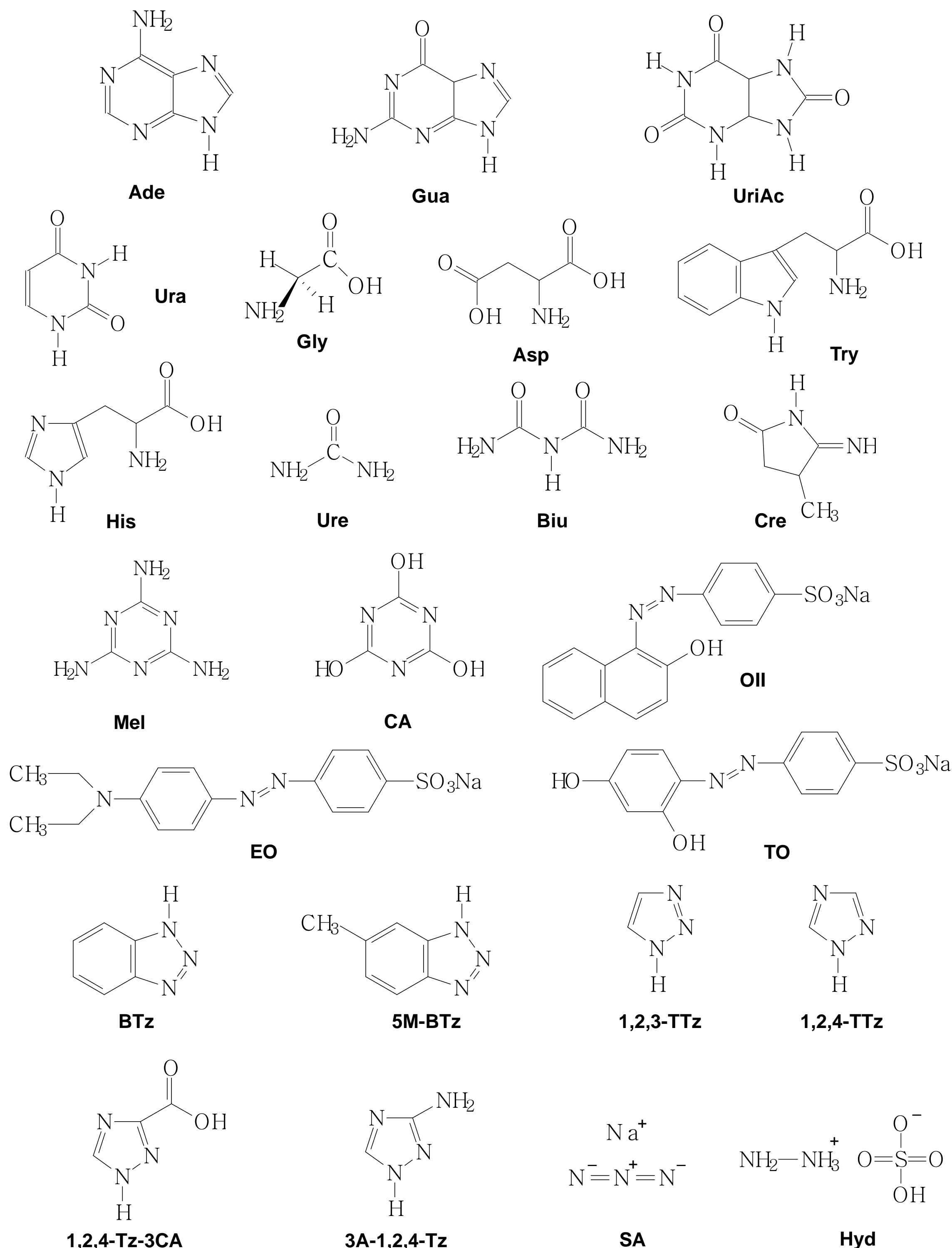

Figure 1. Chemical structures of the studied compounds. Adenine (Ade), Guanine (Gua), Uric Acid (UriAc), Uracil (Ura), Glycine (Gly), L-Aspartic acid (Asp), L-Tryptophan (Try), L-Histidine (His), Urea (Ure), Biuret (Biu), Creatinine (Cre), Melamine (Mel), Cyanuric acid (CA), Orange II (OII), Ethyl Orange (EO), Tropaeolin O (TO), 1H-benzotriazol (BTz), 5-methy-1H-benzotriazole (5M-BTz), 1H-1,2,3-triazole (1,2,3-Tz), 1H-1,2,4-triazole (1,2,4-Tz), 1,2,4-triazole-3-carboxylic acid (1,2,4-Tz-3CA), 3-amino-1,2,4-triazole (3A-1,2,4-Tz), , Sodium Azide (SA) and Hydrazine Sulfate (Hyd).

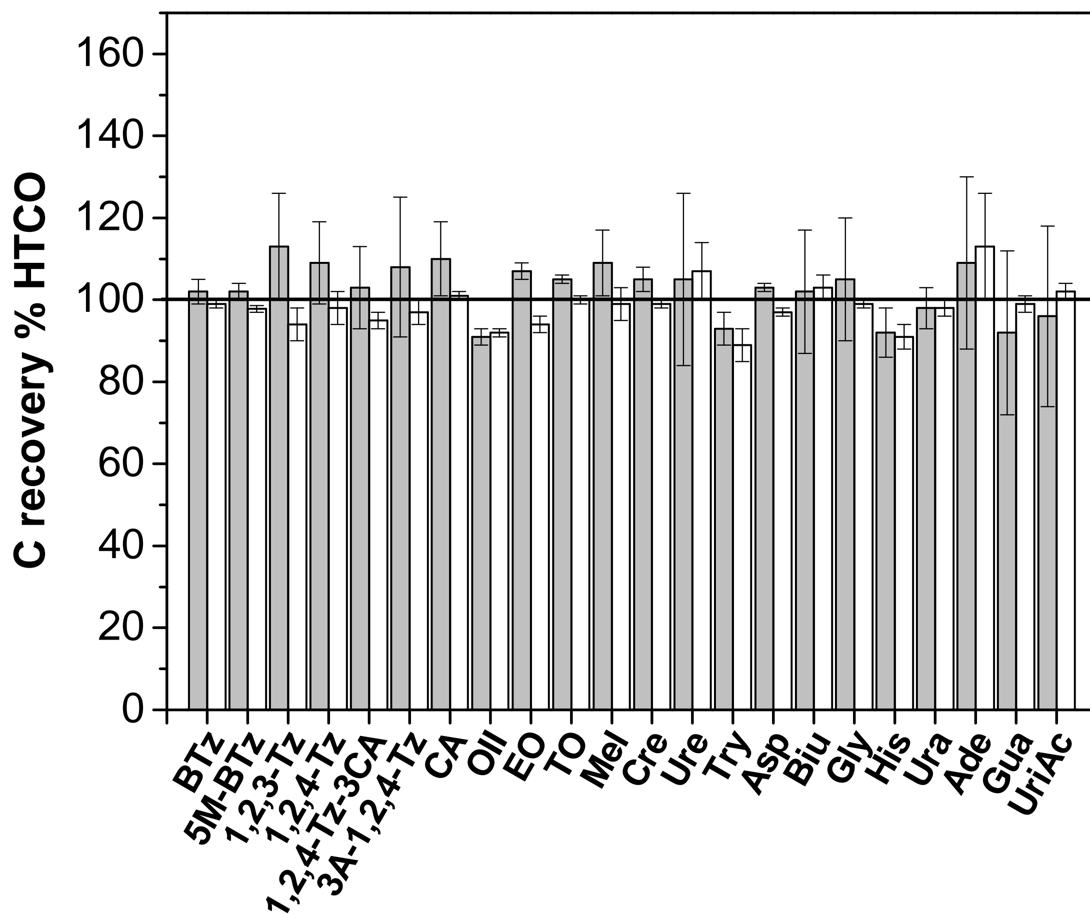

Figure 2. TOC recovery for each tested compounds with HTCO at 1 (grey) and 5 mg N L^{-1} (white). Error bars calculated considering a confidence level of 0.95.

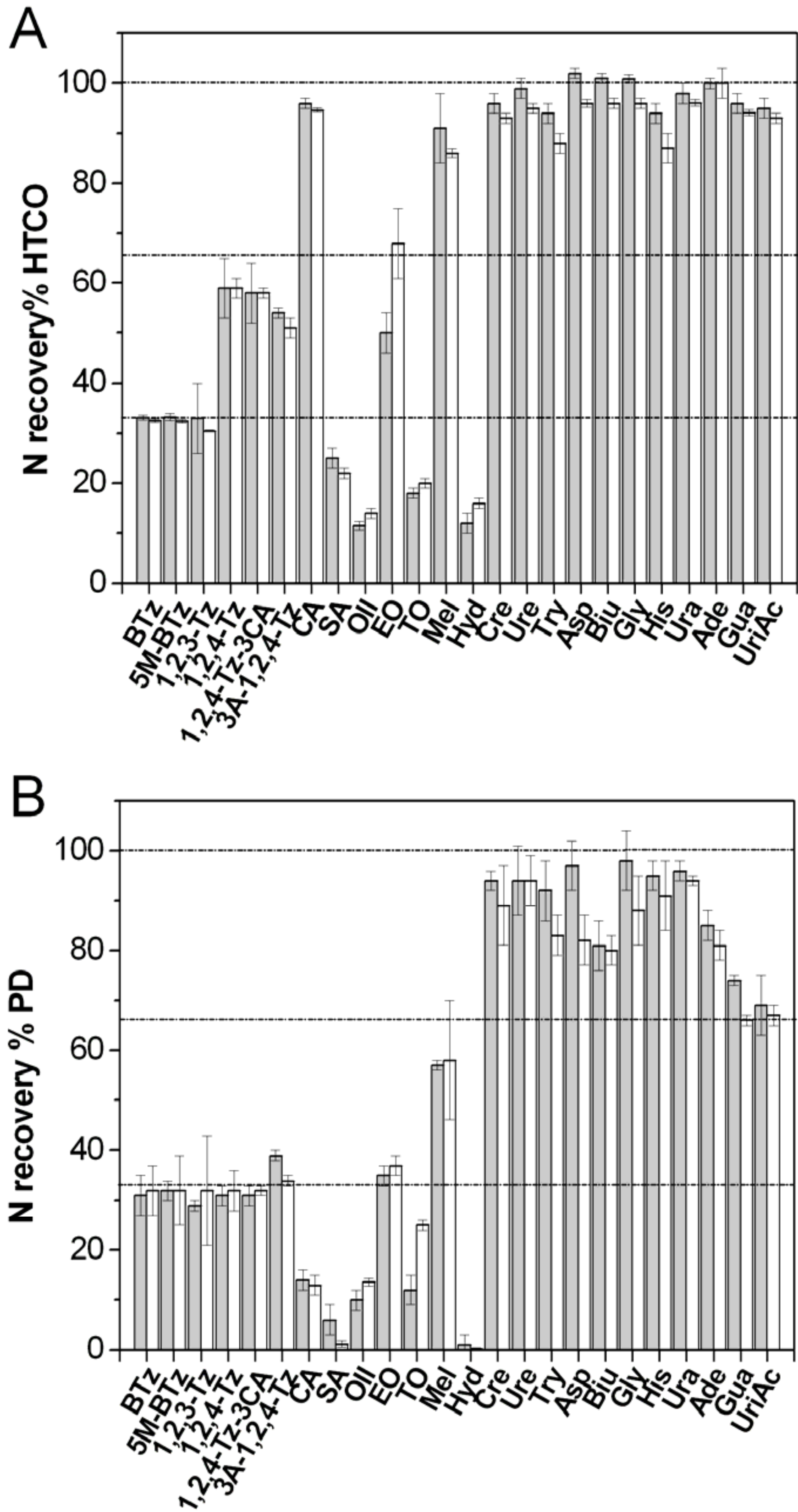

Figure 3. TDN recovery for each tested compounds with HTCO (A) and PD (B) at 1 (grey) and 5 mg N L^{-1} (white). Error bars calculated considering a confidence level of 0.95.

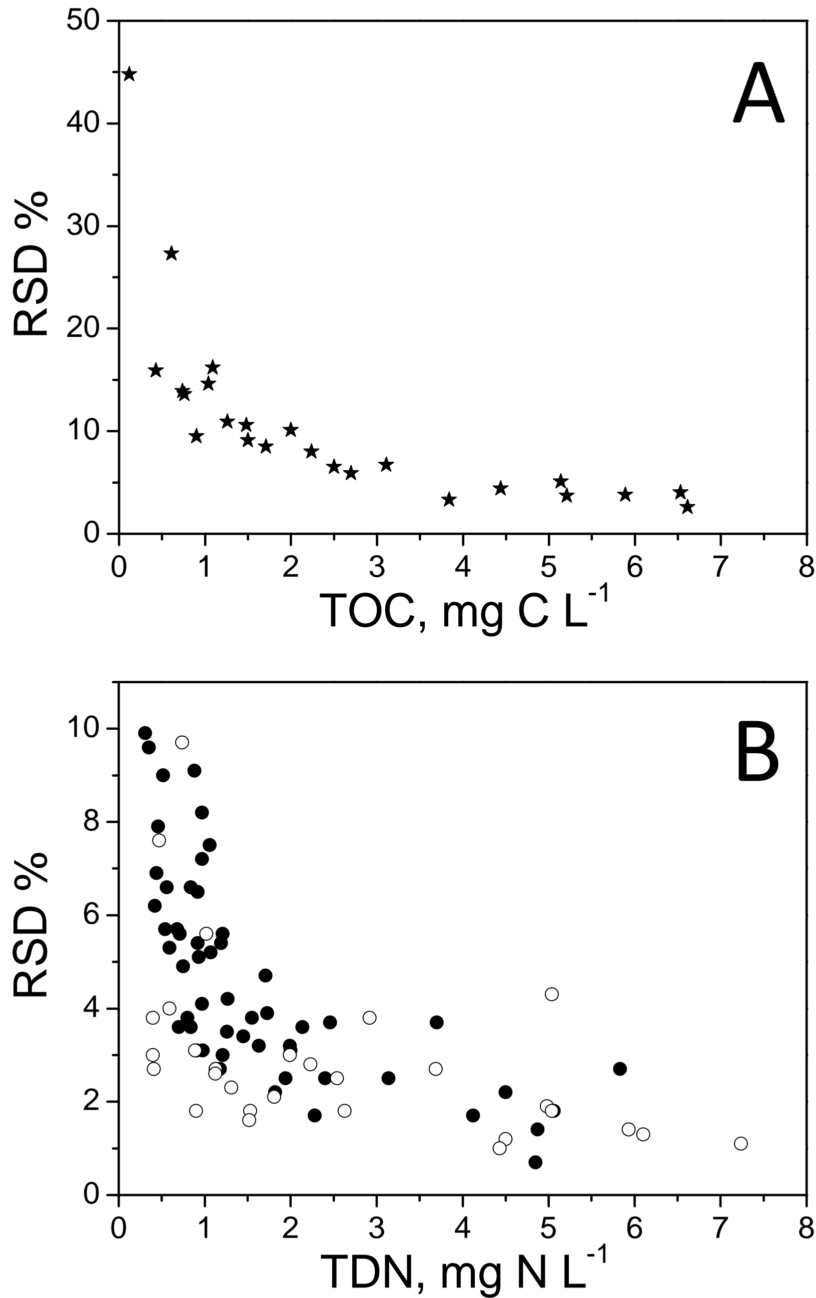

Figure 4. Analytical repeatability expressed as Relative Standard Deviation (%) for A) TOC analysis by HTCO; B) TDN analysis by PD (○) and HTCO (●). The RSD% was obtained by means of Shewhard control charts derived from the analysis of stable synthetic samples at different concentration values over quite prolonged periods (2-6 months).The data are related to control charts performed during the period 1984-2010 (PD) and 2004-2011 (HTCO).

Table 1. TOC and TDN recovery (%) for each tested compound by using HTCO and PD methods. ### = data not measurable

Substance	Abbreviation	Supplier	Purity(%)	Chemical Formula	Recovery					
					C HTCO	N HTCO	N PD	C HTCO	N HTCO	N PD
					1 mg L^{-1}			5 mg L^{-1}		
Adenine	Ade	Sigma-Aldrich	>99	$C_5H_5N_5$	109±21	100±1	85±3	113±13	100±3	81±3
Guanine	Gua	Fluka	99	$C_5H_5N_5O$	92±20	96±2	74±1	99±2	94.2±0.6	66±1
Uric Acid	UriAc	Sigma-Aldrich	99	$C_5H_4N_4O_3$	96±22	95±2	69±6	102±2	93±1	67±2
Uracil	Ura	Sigma-Aldrich	98	$C_4H_4N_2O_2$	98±5	98±2	96±2	98±2	96.1±0.7	94±1
Glycine	Gly	Sigma-Aldrich	98	$C_2H_5NO_2$	105±15	100.9±0.8	98±6	99±1	96±1	88±7
L-Aspartic acid	Asp	Sigma-Aldrich	≥98	$C_4H_7NO_4$	103±1	102±1	97±5	97±1	96.0±0.8	82±5
L-Tryptophan	Try	Aldrich	99	$C_{11}H_{12}N_2O_2$	93±4	94±2	92±6	89±4	88±2	83±4
L-Histidine	His	Sigma-Aldrich	≥99	$C_6H_9N_3O_2$	92±6	94±2	95±3	91±3	87±3	91±7
Urea	Ure	Carlo erba	99	CH_4N_2O	105±18	99±2	94±7	107±7	95±1	94±5
Biuret	Biu	Aldrich	97	$C_2H_5N_3O_2$	102±15	101±1	81±5	103±3	96±1	80±3
Creatinine	Cre	Sigma-Aldrich	98	$C_4H_7N_3O$	105±3	96±3	94±2	99±1	93±1	89±8
Melamine	Mel	Sigma-Aldrich	≥99	$C_3H_6N_6$	109±8	91±7	57±1	99±4	86±0.9	58±12
Cyanuric acid	CA	Mecrk	99	$C_3H_3N_3O_3$	110±9	96±1	14±2	101±1	94.7±0.4	13±2
Orange II	OII	Fluka	≥85	$C_{16}H_{11}N_2NaO_4S$	91±2	11.5±0.8	10±2	92±1	14±1	13.6±0.8
Ethyl Orange sodium salt	EO	Sigma-Aldrich	≥90	$C_{16}H_{18}N_3NaO_3S$	107±2	50±4	35±2	94±2	68±7	37±2
Tropaeolin O	TO	Sigma-Aldrich	≥90	$C_{12}H_9N_2NaO_5S$	105±1	18±1	12±3	100±1	20±1	25±1
1H-benzotriazole	BTz	Aldrich	99	$C_6H_5N_3$	102±3	33.1±0.5	31±4	99±1	32.5±0.4	32±5
5-methy-1H-benzotriazole	5M-BTz	Sigma-Aldrich	98	$C_7H_7N_3$	102±2	33.2±0.7	32±2	97.8±0.8	32.4±0.4	32±7
1H-1,2,3-triazole	1,2,3-Tz	Sigma-Aldrich	99	$C_2H_3N_3$	113±13	33±7	29±1	94±4	30.5±0.2	32±11
1H-1,2,4-triazole	1,2,4-Tz	Sigma-Aldrich	99	$C_2H_3N_3$	109±10	59±6	31±2	98±4	59±2	32±4
1,2,4-triazole-3-carboxylic acid	1,2,4-Tz-3CA	Sigma-Aldrich	99	$C_3H_3N_3O_2$	103±10	58±6	31±2	95±2	58±1	32±1
3-amino-1,2,4-triazole (Amitrol)	3A-1,2,4-Tz	Sigma-Aldrich	99	$C_2H_4N_4$	108±15	54±1	39±1	97±3	51±2	34±1
Sodium Azide	SA	Carlo Erba	99	NaN_3	###	25±2	6±3	###	22±1	1.2±0.7
Hydrazine sulfate	Hyd	Sigma-Aldrich	≥99	$H_4N_2.H_2O_4S$	###	12±2	1±2	###	16±1	0.1±0.3

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