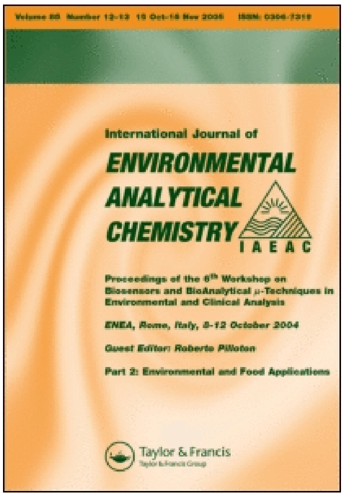

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A comparison between high-temperature catalytic oxidation and persulphate oxidation for the determination of total nitrogen in freshwater

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Total nitrogen (TN) in freshwater samples (lakes, rivers, and atmospheric deposition) has been routinely analysed at the laboratory of the CNR Institute of Ecosystem Study in Italy by persulphate oxidation (PO) with UV determination of nitrate at 220 nm since the 1980s. In 2004, high-temperature catalytic oxidation (HTCO) with a chemiluminescence detector (CLD) began to be used for TN analysis. A mixed catalyst of cobalt–chromium and cerium oxide was selected, as this gave the best recoveries for ammonium and organic N. To assess the comparability of the new method with the reference one, almost 800 samples were analysed for TN using both methods. The average difference between paired data was low, as both absolute and relative values (0.06 $\mathrm{mg\,N\,L^{-1}}$ and 3%, respectively, in the range of 0.1–7.0 $\mathrm{mg\,N\,L^{-1}}$). The results of our study confirmed HTCO as a reliable method for TN determination in freshwater samples. Compared with PO, HTCO proved to be faster and required less sample handling. From the point of view of linearity and repeatability, the HTCO results were comparable with those with PO UV 220. The LOD of HTCO calculated in this study was lower than that of PO UV 220 (0.04 with respect to 0.09 $\mathrm{mg\,N\,L^{-1}}$).

Keywords: Nitrogen; Freshwater; HTCO; Persulphate oxidation

1. Introduction

Total nitrogen (TN) is a parameter of utmost importance in assessing water status. The determination of nitrogen in its different forms is required in wastewater treatment, industrial applications, and monitoring of drinking water quality, and in studies of natural water bodies like rivers and lakes. Accurate determinations of TN in point sources, effluents, and surface water are extremely important in environmental monitoring.

Total nitrogen is a 'composite' parameter, i.e. the sum of the different nitrogen compounds, both organic and inorganic, in a sample. TN determination requires the conversion of these nitrogen species into a single product. For this reason, different analytical methods may give different recoveries depending on their capacity for

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converting nitrogen species to the desired product. This is often the case with natural water samples, which may have rather a complex chemical composition, and may include several nitrogen species not easily convertible into a single compound.

Along with inorganic nitrogen compounds, dissolved organic N (DON) is another important parameter in the monitoring of natural water bodies. Unlike the inorganic forms of nitrogen, DON cannot be quantified directly and is calculated as the difference between TN and dissolved inorganic nitrogen ($DIN = N\text{-}NH_4 + N\text{-}NO_3 + N\text{-}NO_2$). Hence, the precision of DON determination relies on the precision of the single methods used for the analysis of TN and inorganic nitrogen species. As it is calculated as the difference between two large numbers, TN and DIN, even small relative errors in TN or DIN measurements can lead to large errors in DON determination [1].

All the methods currently in use for TN analysis in freshwater have two distinct steps in common: (1) the conversion of all nitrogen species into a single form, usually nitrate and (2) the detection of this final product. For TN analysis, one of the following two approaches is commonly used:

(1) Wet chemical oxidation (WCO), in which all nitrogen species are converted to nitrate, then quantified by spectrophotometry or other relevant technique (e.g. ion chromatography). WCO methods include alkaline persulphate digestion and UV photo-oxidation.
(2) Direct aqueous injection of the sample into an oxidation column where nitrogen compounds are converted to nitrogen oxide (NO), successively determined by chemiluminescence (CLD). High-temperature oxidation of nitrogen compounds can be performed with (HTCO) or without a catalyst (HTO); the combustion temperatures are 680–850°C and 1100°C, respectively [2]. After combustion, the gas is introduced into a chemiluminescence detector, where nitric oxide reacts with ozone to produce nitrogen dioxide in an excited state that then returns to the ground state emitting chemiluminescence (590–2500 nm).

Along with these two methods, total Kjeldahl nitrogen (TKN) is also still employed as a method for TN analysis. TKN is the sum of organic N and ammonia but does not include nitrate/nitrite nitrogen. TKN proved to be a time-consuming method and has several drawbacks, such as the extensive use of sulphuric acid in the digestion process, the use of heavy-metal catalysts, the many interferences encountered, and the environmental precautions that have to be taken during the analysis.

Some methods for TN determination were introduced in the second half of the 1960s, the major aim of which was to replace the TKN analysis. These methods were based on the oxidation of organic nitrogen compounds through UV radiation and small amounts of oxidants (H_2O_2) [3, 4]. In the early 1970s, Koroleff proposed a strong chemical oxidation by potassium persulphate (PO) in an alkaline environment [5]. In the following years, the method underwent some modifications and was successfully used for the simultaneous determination of TN and TP in natural waters [6–8].

A new technique for TN determination in liquid samples was introduced in 1985 [9]. This was based on a combustion process at high temperature (680°C) under an oxygen atmosphere and in the presence of a Pt catalyst on Al_2O_3. The generated NO_2 was absorbed into a chromogenic reagent, and then spectrophotometrically determined at 545 nm. This technique, initially applied to the analysis of oceanic water in the North Pacific, systematically gave TN concentrations higher than those obtained with traditional methods [9]. Subsequently, applications of the HTCO technique with slight modifications showed how results comparable with those of the UV method could be obtained. For example, Walsh [10] adopted a high-temperature combustion system working at 1100°C and using a quartz wool layer above the catalyst. In this study, CLD was used to detect NO, and results were fairly comparable with those provided by the UV technique [10].

A boost to the application and improvement of the HTCO came in 1991 with the Seattle workshop on DOC/DON analysis [11]. After this workshop, HTCO was recognized as the most precise and efficient technique presently available for the conversion of organic nitrogen compounds in aqueous samples. Furthermore, several studies provided evidence of the comparability between HTCO and PO results in TN determination [12–14].

In the 1990s, a number of studies confirmed HTCO as a valid method for TN analysis and showed the progress that had been made in its application [15–17]. However, most of these studies were concerned with seawater analysis, while very few papers focused on the HTCO technique for monitoring TN in freshwaters [2].

In this paper, we have focused on the analysis of TN in freshwater samples (lakes, rivers, and atmospheric deposition). These types of samples are routinely analysed in the chemical laboratory of the Institute of Ecosystem Study of the National Research Council in Italy (CNR ISE) in the framework of national or EU research programmes designed to monitor surface water and atmospheric deposition [18, 19].

Nitrogen plays an important role in ecosystem dynamics. Its change in time in surface waters can be related to inputs from point or diffuse sources or may be an index of the response of the ecosystem to changes in environmental conditions. The assessment of long-term trends in TN concentrations is one of the key issues in the monitoring activities at CNR ISE. It follows that any change in the analytical methods must be carefully evaluated, to ensure the comparability of the data before and after the introduction of a new technique.

The method of persulphate oxidation with UV determination of nitrate at 220 nm (PO UV 220) has been in use at the CNR ISE laboratory since the 1980s for the determination of TN. In 2004, the HTCO technique with CLD began to be employed for TN determination using a SKALAR Formacs^{HT} TOC/TN analyser. TN analyses were performed using both the new and the traditional method (PO UV 220) for about 6 months. The main aims of this study were: (1) to validate the HTCO method by comparing it to the reference method for TN analysis; (2) to point out the major advantages and drawbacks of the two methods; and (3) to provide some guidelines for the application of HTCO to TN determination in freshwater samples.

2. Experimental

TN concentrations in surface water samples (lakes, rivers, and atmospheric deposition) have been measured in the CNR ISE laboratory since 1978 by alkaline hydrolysis and since 1982 by persulphate oxidation with pH varying between 9.7 and 5.0 [7] and spectrophotometric UV detection of nitrate at 220 nm [20]. This method has been constantly checked during over a period of more than 20 years, through regular participation in intercomparison exercises and ring-tests on surface water, atmospheric deposition and soil solution samples [21, 22]. Furthermore, the repeatability of the method has been assessed by the regular use of Shewhart control charts [23].

Method validation is the process used to ensure that a method is fit for its intended use [24]. Validation should be performed by analysing standard reference materials (SRM) with the same matrix of the samples to be analysed. Alternatively, validation can be done through a comparison with a reference method.

To validate the new method (HTCO) for TN analysis in our laboratory, we compared it with the reference method PO UV 220. A total of 793 samples (lake, river, and rain water) were analysed for TN by the two methods in the period June–December 2004. Thirty-six percent of the samples were surface water samples (rivers and lakes), and 64% were atmospheric deposition samples. Moreover, a subset of 75 samples was used for a further comparison: after PO, nitrate was determined both by spectrophotometry (UV 220) and by ion chromatography with UV detection at 215 nm (IC UV 215).

All the samples analysed were representative of dilute natural waters, with an ionic content between 0.01 and 20 meq L^{-1} and conductivity in the range 5–800 $\mu S\,cm^{-1}$. TN concentrations were between 0.1 and 7.0 mg N L^{-1}. Nitrate was the major form of N in surface water samples; organic N was between 10% and 20% of TN, and ammonium was usually below 10%. In atmospheric deposition samples, TN was mainly in inorganic form, with nitrate and ammonium in similar proportion.

TN concentrations in the samples were in the range 0.1–7.0 mg N L^{-1}. The lower limit was established as the limit of detection (LOD) of the PO UV 220 method (see below). The upper limit corresponded to the highest concentration of the standards used in the HTCO calibration.

All the samples were stored at 4°C during the analyses. The determinations of TN by PO UV 200 and HTCO were performed simultaneously or in close succession, and in any case they were completed in 2–3 days after the arrival of the samples in the laboratory.

The percentage difference between the two methods was calculated for each data pair as follows:

$$\text{Diff\%} = \left(\frac{X_{\text{PO}_i} - X_{\text{HTCO}_i}}{X_{\text{PO}_i}} \right) \times 100.$$

The absolute (M_{d}) and relative (M_{r}) average differences between paired data were calculated as follows:

$$M_{\text{d}} = \frac{\sum_{i=1}^{n} X_{\text{PO}_i} - X_{\text{HTCO}_i}}{n}$$

$$M_{\text{r}} = \frac{\left[\sum_{i=1}^{n} (X_{\text{PO}_i} - X_{\text{HTCO}_i}/X_{\text{PO}_i})\right]}{n}$$

where X_{PO_i} and X_{HTCO_i} are the TN values obtained by PO and HTCO, respectively, and n is the number of paired data.

2.1 *PO UV 220*

We determined TN by oxidation of all the nitrogen compounds to nitrate according to [7]. Ammonium, nitrite, and organic N in the sample were oxidized to nitrate using potassium peroxodisulphate in a boric acid–sodium hydroxide buffer. Oxidation of the nitrogen compounds was performed in an autoclave at 120°C and 2 bar pressure for 30 min, resulting in a buffer pH change from 9.7 to 5.0.

For autoclave oxidation, 25 mL aliquots of the sample were placed into a 50 mL screw cap with an o-ring gas proof and 3.5 mL of the oxidizing reagent added. To prepare the oxidizing reagent, 50 g of low-nitrogen (<0.001%) potassium persulphate, 30 g of boric acid, and 14 g of sodium hydroxide were dissolved in ultrapure water to a final volume of 1 L.

Nitrate was determined by spectrophotometry (SAFAS Monaco UV mc2) at 220 nm [20]. After oxidation, samples were acidified with 0.425 mL of low-nitrogen (0.0005%) H_2SO_4 98%. Silica cells (of 1 cm optical path) were used for spectrophotometric measurement. Absorbance of the sample was read against ultrapure water set at zero absorbance.

Two measuring ranges were used: 0.40–2.00 $\mathrm{mg\,N\,L^{-1}}$ and 0.40–6.00 $\mathrm{mg\,N\,L^{-1}}$. Calibration curves for the two ranges were prepared using five and nine standards, respectively, prepared from a stock $NaNO_3$ solution. Three blanks were included in each analytical sequence and the mean absorbance of the blanks subtracted from the absorbance values of the samples. Both standards and blanks were treated in the same manner as the samples.

The wavelength selected was 220 nm for nitrate determination according to standard methods [20]. The direct UV method for nitrate detection is suitable for uncontaminated water low in organic matter, because dissolved organic matter also absorbs at 220 nm. A wavelength of 275 nm should be used to determine the interference by organic matter: the absorbance read at 220 nm is corrected by subtracting twice the absorbance at 275 nm [20]. This interference is avoided in samples that have been treated with PO, because the mineralization fully decomposes dissolved organic matter. The absence of this interference was verified by analysing a series of samples high in organic matter (2–15 $\mathrm{mg\,C\,L^{-1}}$) before and after the PO.

To assess the accuracy of the PO UV 220 method, ion chromatography (Dionex series DX 320, columns Dionex AG17 and Dionex AS17) with UV detection (UV 215) was also used to determine nitrate after oxidation in a subgroup of samples ($n = 75$). This method avoids any interference on the conductometric detector that could result from the high amount of sulphate produced by PO.

A solution of KOH 9 mM was used as eluent at a flow rate of 1 $\mathrm{mL\,min^{-1}}$. Mineralized samples were injected using a 10 μL loop with electrochemical suppression of the eluent. Calibration was performed with standard KNO_3 solutions by diluting a stock nitrate solution with ultrapure water.

2.2 *HTCO CLD*

TN determinations by HTCO were performed using a stand-alone instrument for simultaneous analysis of TC and TN (SKALAR FORMACS^{HT} TOC/TN analyser). The TN measured in this way represents all the nitrogen in the sample, including elementary, organic, and inorganic nitrogen.

The operating conditions adopted in this study are summarized in table 1. We fixed the injection volume to 200 μL and the temperature at 850°C in all the analyses.

Table 1. Operating conditions for the Skalar FORMACS^{HT} TOC/TN adopted in this study.

Injection volume	200 μL
Carrier gas	Oxygen
Gas flux	$250\,\mathrm{mL\,min^{-1}}$
Catalyst	$CoCr + CeO_2$
Reactor temperature	850°C
Replicates for each sample	3
Standards	$NaNO_3$
TN analytical range	$0.1–7.0\,\mathrm{mg\,N\,L^{-1}}$
Carbon analytical range	$0.5–20.0\,\mathrm{mg\,C\,L^{-1}}$

Before selecting the catalyst to be used, we did a number of tests on recovery rates of different N compounds. As much of our interest focuses on atmospheric deposition samples, which usually have quite high concentrations of ammonium, we paid close attention to recoveries of this compound. Some authors reported a rather low recovery for NH_4^+ using HTCO [14]. In our study, we observed that some catalyst combinations (e.g. CoCr with a short layer of Pt at the top) gave low recoveries for N-NH_4; as a consequence, they were not suitable for analysing samples that are high in ammonium, such as atmospheric deposition. A mixed catalyst made of CoCr and CeO_2 in similar proportions proved to be most effective for N-NH_4 determination and was used in this study for all the analyses. The recoveries were regularly tested in the course of the study by including a few N-NH_4 standards ($5.0\,\mathrm{mg\,N\,L^{-1}}$) in each analytical sequence.

The ND10 detector of Skalar FORMACS^{HT} TOC/TN has two different measuring ranges: $0.05–4\,\mathrm{mg\,N\,L^{-1}}$ (low-range) and $4–300\,\mathrm{mg\,N\,L^{-1}}$ (high range). The TN concentration in the samples routinely analysed in the CNR ISE laboratory is rarely above $5.0\,\mathrm{mg\,N\,L^{-1}}$. Moreover, the linear regression performed by the standards proved to be linear in the range $0.1–7.0\,\mathrm{mg\,N\,L^{-1}}$ (see below). Hence, the low range option was selected for all the analyses.

Standards were prepared from a stock $NaNO_3$ solution. These covered the range $0.4–7.0\,\mathrm{mg\,N\,L^{-1}}$. Typically, two series of four to seven standards were positioned at the beginning and end of each analytical sequence. Three replicates were analysed for each sample.

3. Results and discussion

3.1 *Comparison of HTCO and PO UV 220 for TN analysis*

Figure 1 shows a scatter plot of the result obtained by the two methods. The regression line and the 1 : 1 line representing the best fit were imposed on this plot. The percentage differences calculated as above are shown in figure 2 as a function of TN concentration.

As a general result, the results by HTCO were lower than those produced by PO UV 220 in 72% of cases (table 2). Compared with the 1 : 1 line of best fit, the regression line had a slope of 0.9597 (figure 1). Relative differences between the two methods were positive for the majority of the samples. HTCO data tend towards higher values than PO UV 220 only at low TN concentrations (figure 2).

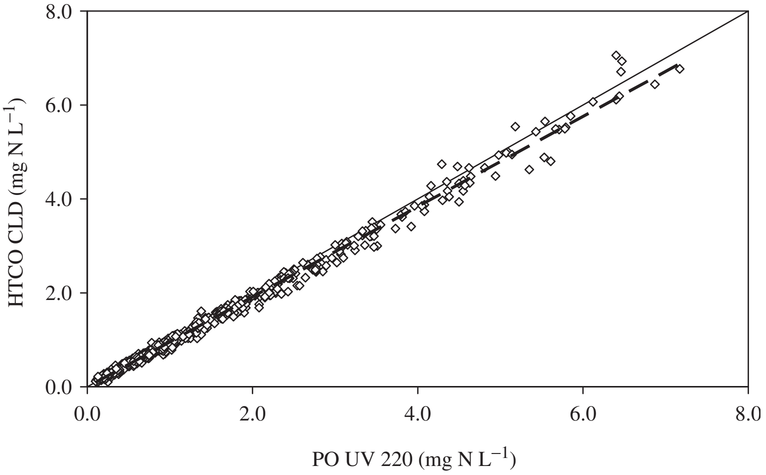

Figure 1. Scatter plot of the TN data analysed by high-temperature catalytic oxidation with a chemiluminescence detector (HTCO CLD) and persulphate oxidation followed by UV determination of nitrate at 220 nm (PO UV 220). Analytical range: 0.1–7.0 mg N L^{-1}; number of samples: 793. Regression line and 1 : 1 line of best fit imposed on the plot.

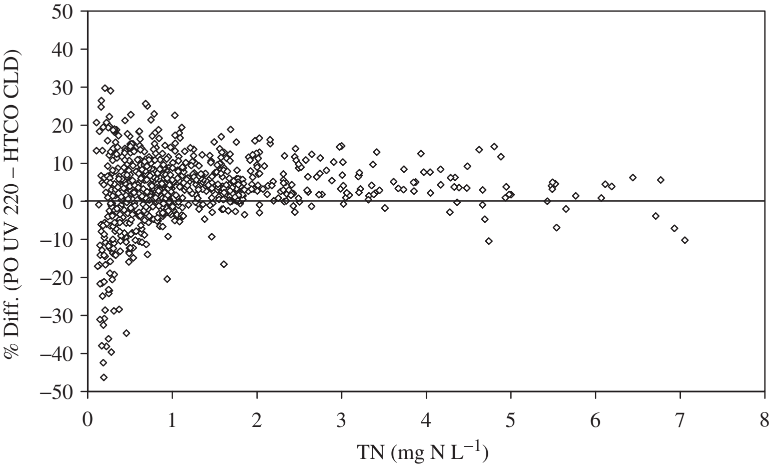

Figure 2. Relative difference between PO UV 220 and HTCO data as a function of TN concentration ($n = 793$). For method abbreviation, see legend to figure 1.

Relative differences above 20% were found for 41 samples. Of these samples, about half were characterized by low TN concentrations (0.10–0.20 mg N L^{-1}), close to the detection limit of the PO UV 220 method and below the lowest-concentration standard used in the HTCO calibration. Relative differences between the two methods were also rather high for some river and lake samples containing suspended solids (e.g. river samples collected during floods; lake samples taken close to the lake bottom). For these samples, TN concentrations measured by HTCO were 25–30% lower than those measured by PO UV 220.

On the basis of the median value of the data series, data were split into two groups, covering the range 0.1–1.0 and 1.0–7.0 mg N L^{-1}. Underestimation by HTCO is

Table 2. Descriptive statistics for the comparison between high-temperature catalytic oxidation with a chemiluminescence detector (HTCO CLD) and persulphate oxidation followed by UV determination of nitrate at 220 nm (PO UV 220).[a]

Paired data (n)	793
Rain water data	512 (65%)
Lake and stream water data	281 (35%)
Mean PO UV 220 (HTCO CLD)	1.34 (1.28) mg N L^{-1}
Median PO UV 220 (HTCO CLD)	0.96 (0.91) mg N L^{-1}

	Range 0.1–7.0 ($n = 793$)	Range 0.1–1.0 ($n = 396$)	Range 1.0–7.0 ($n = 397$)
Negative differences	222 (28%)	164 (41%)	58 (15%)
Positive differences	571 (72%)	232 (59%)	339 (85%)
M_d	0.06 mg N L^{-1}	0.02 mg N L^{-1}	0.10 mg N L^{-1}
M_r	2.9%	0.8%	5.0%
Min. difference (mg N L^{-1})	−0.211	−0.159	−0.211
Max. difference (mg N L^{-1})	0.416	0.150	0.416
Spearman correlation coeff.	0.9955	0.9820	0.9896
Linear regression slope	0.9597	0.9272	0.9735
Intercept (mg N L^{-1})	−0.001	0.025	−0.038

[a] The entire dataset as well as two subsets corresponding to the low range (0.1–1.0 mg N L^{-1}) and high range (1.0–7.0 mg N L^{-1}) were considered. n: number of data. M_d, M_r: absolute and relative average differences between paired data, respectively.

especially evident at medium-high concentrations, while a higher number of negative differences are found at low TN concentrations (below 1.0 mg N L^{-1}) (table 2; figure 2). The correlation coefficient (Spearman r) was slightly higher for the high-range subset, which also has the linear regression closer to the 1 : 1 line of best fit (table 2).

Since the data were not normally distributed (Kolmogorov–Smirnov test, $p < 0.01$), we tested the difference between the two data series using the Wilcoxon matched pair test, which is the nonparametric alternative for the paired t-test. Nevertheless, because t-tests work well with large samples even if the data distribution is only approximately Gaussian, we also applied the tests on the slope and intercept of the regression line.

The three tests were applied both to the entire dataset and to the two subsets corresponding to a low range (0.1–1.0 mg N L^{-1}) and high range (1.0–7.0 mg N L^{-1}). Results obtained with the two methods were significantly different ($p < 0.001$) according to all the tests considering the whole dataset as well as the two subsets. The only exception was the result of the test on the intercept of the linear regression. A significant difference between the results produced by the two methods was also found applying the Wilcoxon test to the dataset after exclusion of unfiltered samples ($n = 543$; $p < 0.001$).

Along with the results from the statistical tests, the average difference between paired data should also be considered to assess the comparability of the two methods. Both absolute and relative differences proved to be rather low (0.06 mg N L^{-1} and 3%, respectively; table 2). The mean difference is slightly higher for TN concentrations above 1.0 mg N L^{-1} (0.10 mg N L^{-1} or 5.0%), while it is 0.02 mg N L^{-1} or 0.8% for concentrations between 0.1 and 1.0 mg N L^{-1} (table 2).

A second assessment of the HTCO technique for TN determination was made on a subset of 75 samples. These were analysed for nitrate after PO with both spectrophotometry (UV 220) and ion chromatography with UV detection (IC UV 215)

Table 3. Descriptive statistic for TN results ($mg\,N\,L^{-1}$) from 75 samples analysed with high-temperature catalytic oxidation with a chemiluminescence detector (HTCO CLD) and persulphate oxidation (PO) with two different methods for nitrate determination: spectrophotometry (UV 220) and ion chromatography with UV detection (IC UV 215).

	Data (*n*)	Mean	Minimum	25-ile	Median	75-ile	Maximum
PO UV 220	75	1.53	0.17	0.80	1.13	1.93	5.99
HTCO CLD	75	1.52	0.24	0.78	1.16	1.81	6.06
PO IC UV 215	75	1.52	0.14	0.74	1.08	1.83	6.22

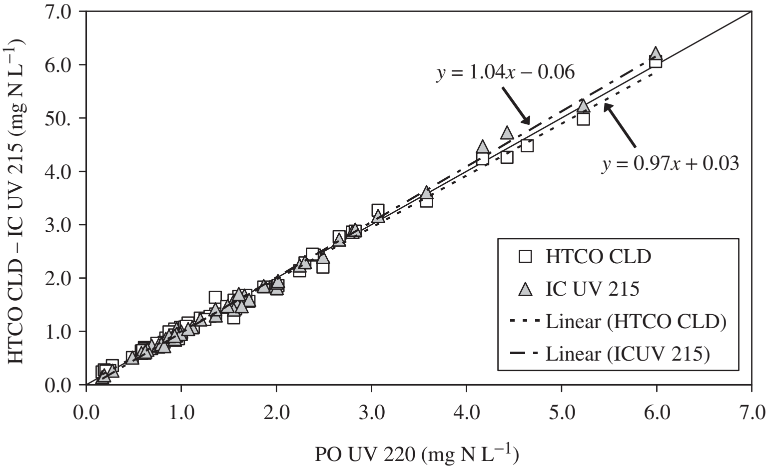

Figure 3. Scatter plot comparing the data produced by persulphate oxidation and UV determination of nitrate at 220 nm (PO UV 220) with those produced by high-temperature catalytic oxidation with a chemiluminescence detector (HTCO CLD) and by ion chromatography with UV detection (IC UV 215). Number of data: 75. Regression lines and 1 : 1 line of best fit imposed on the plot.

(table 3). The proportion of surface water and rain water samples in the subset was similar to that of the original dataset.

Results produced by the reference method (PO UV 220) were plotted against those produced by the alternative methods (HTCO and IC UV 215) in figure 3.

HTCO slightly overestimated TN at a low concentration (below $0.4\,mg\,N\,L^{-1}$), and underestimated it at a high concentration; viewed overall, positive and negative differences were almost equal (37 and 38, respectively). IC UV 215 data showed the opposite pattern, with a tendency towards overestimation of TN values compared with PO UV 220 (regression slope above 1; 49 positive differences out of 75 data) (figure 3). Regression coefficients (Spearman *r*) were very similar for paired data PO UV 220/HTCO and PO UV 220/IC UV 215 (0.9974 and 0.9963, respectively).

The differences among the three data series were tested with the Wilcoxon matched-pairs test. The results produced by the three methods did not show any significant differences ($p < 0.001$). This result is apparently in contrast with the previously obtained result, if HTCO and PO UV 220 results for all data are compared. The explanation may be related to the size of the dataset, which was much smaller in the second experiment ($n = 75$) than in the first ($n = 793$).

3.2 *Limit of detection (LOD) and limit of quantification (LOQ)*

The limit of detection (LOD) and the limit of quantification (LOQ) of an analytical method can be defined as a function of the blank signal and its standard deviation (SD):

$$\mathrm{LOD} = S_c - S_b \geq K_d \times \mathrm{SD}$$
$$\mathrm{LOQ} = S_c - S_b \geq K_q \times \mathrm{SD},$$

where S_c and S_b are the sample and the blank signal, respectively; SD is the standard deviation of the blank signal; and K_d and K_q are coefficients set to 3 and 10, respectively [25].

The blank should be determined by injection of 'zero' nitrogen water of the same matrix of the sample to be analysed (true blank). Since this zero water cannot actually be produced, the blank is the sum of 'instrument', 'reagent', and 'water' blanks [1], the first of which is probably the most important in TN determination by HTCO.

We analysed no fewer than five blanks for each analytical sequence, which usually included 50–80 samples. The average value of the blanks analysed in the study period was $0.03\,\mathrm{mg\,N\,L^{-1}}$ ($2.1\,\mu\mathrm{M}$). This agrees with previous findings [16], in which a combined blank of $2.37\,\mu\mathrm{M}$ was reported, while another study [2] estimated the system and ultrapure water blank as *c*.$1.0 \pm 0.1\,\mu\mathrm{M}$ and a detection limit of $1.0\,\mu\mathrm{M}$.

The LOD and LOQ had already been determined for PO UV 220, while they were calculated in this study for HTCO, by analysing a series of 184 blanks. HTCO has a lower LOD and LOQ than the reference method (table 4).

3.3 *Repeatability*

Repeatability can be defined as the closeness of agreement between independent results obtained in the normal and correct operation of the same method on identical test material, in a short space of time, and under the same test conditions [28].

Repeatability of HTCO and PO UV 220 was assessed in this study by means of Shewart control charts [23]. These were obtained from the analysis of synthetic samples over quite prolonged periods of time (2–6 months). For each synthetic sample, the relative standard deviation (RSD%) was calculated.

Relative repeatability (r%) is defined as 2.8 × RSD according to ISO 5725-1 [26]. In our study, as we wanted to have an index of repeatability dispersal, we simply used the RSD values of the control charts.

Six control charts were analysed for TN by HTCO from March 2004 (table 5). Three replicates for each control chart were included in all the analytical sequences. Control charts were representative of different TN concentration ranges, between 0.9 and $2.9\,\mathrm{mg\,N\,L^{-1}}$. The average composition of the control samples was as follows: 60%

Table 4. Limit of detection (LOD) and limit of quantification (LOQ) for the PO UV 220 and HTCO CLD methods.

	PO UV 220	HTCO CLD
Data (n)	1222	184
LOD $\mathrm{mg\,N\,L^{-1}}$	0.09	0.04
LOQ $\mathrm{mg\,N\,L^{-1}}$	0.30	0.14

N-NO$_3$, 20% N-NH$_4$, 20% organic N. The RSD of the control charts varied from a minimum of 1.8% to a maximum of 5.3% (table 5).

Forty control charts have been analysed for TN by PO UV 220 since the adoption of this method in 1982. The RSD values of both PO UV and HTCO control charts are shown in figure 4 as a function of TN concentration. The repeatability of PO UV was quite low (RSD 6–10%) at low TN concentrations ($<1.0\,\mathrm{mg\,L^{-1}}$), while it improved at higher concentrations. On the other hand the repeatability of HTCO was quite good at both low and medium TN concentrations. A slightly higher RSD (5.3%) was found in correspondence with the lowest concentration control chart ($0.48\,\mathrm{mg\,N\,L^{-1}}$). The better repeatability of HTCO compared with PO UV was confirmed by analysing one of the control charts (HTCO CLD 4) in parallel by the two methods: RSD was 5.6 and 2.7% for PO UV 220 and HTCO CLD, respectively (figure 4).

3.4 *Linearity of the regression line used in the calibration*

A further step in the validation of an analytical method is the assessment of the stability and linearity of the regression line used in the calibration. The regression is often

Table 5. Control charts for TN determination in use at the CNR ISE laboratory in 2004–2005. RSD: relative standard deviation.

Control chart	Data (*n*)	Mean value ($\mathrm{mg\,N\,L^{-1}}$)	RSD%
HTCO CLD 1 (01/03–14/04/04)	41	0.90	1.8
HTCO CLD 2 (19/04–14/07/04)	40	2.54	2.5
HTCO CLD 3 (20/07–20/10/04)	37	0.48	5.3
HTCO CLD 4 (24/03–12/05/04)	37	1.13	2.7
HTCO CLD 5 (10/06–20/10/04)	77	2.92	3.5
HTCO CLD 6 (04/11/04–27/01/05)	50	2.62	1.9

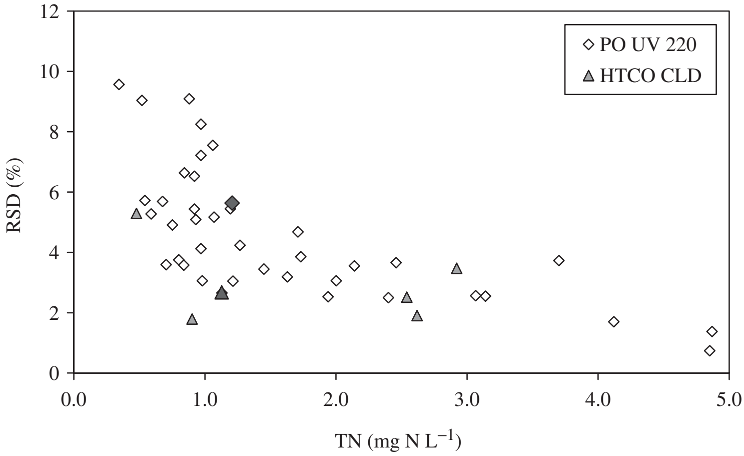

Figure 4. Relative standard deviation (RSD) of the control charts analysed with PO UV 220 (40 control charts in the period 1984–2004) and HTCO CLD (six control charts during March 2004–January 2005) as a function of TN concentration. Symbols in black show the RSD of the same control chart analysed in parallel by the two methods. For method abbreviations, see legend to figure 1.

Table 6. Number of data, mean value of the response factor (RF) and relative standard deviation (RSD) of the standards used in the calibration of HTCO for TN analysis over a period of 8 months.

Standard ($\mathrm{mg\,N\,L^{-1}}$)	0.4	0.8	1.5	3.0	5.0	7.0
Data (n)	76	72	64	68	70	52
Mean RF value $\times 10^6$	6.96	6.81	6.79	6.78	6.76	6.64
$\mathrm{RSD_{signal}}$ (%)	6.1	3.8	3.9	3.8	3.8	3.6

evaluated by means of the linear regression coefficient (r). Further approaches are the use of response factors (RFs) and the calculation of the residuals on the regression points [27, 28].

RF is defined as the ratio between the signal and the concentration of each standard. When the linear correlation is good, the RFs of the standards resemble each other. In contrast, if they significantly differ from the mean value, there is a poor correlation between the standard signals and concentrations. If the RFs tend to increase or decrease with increasing of standard concentration, a quadratic regression should be used instead of a linear regression [28].

The spectrophotometric PO UV 220 method consistently showed a very good linearity in the calibration range 0.2–6.0 $\mathrm{mg\,N\,L^{-1}}$. The parameters of the calibrations performed during the more than 20 years the method has been used showed a high stability in time (e.g. RSD of all the regression slopes: 4.6%).

The linearity of HTCO CLD was found to be in the range of 0.2–7.0 $\mathrm{mg\,N\,L^{-1}}$. Linearity of the calibration was very good (with an RF variation usually within 5%). Table 6 shows the RSD of the signal of the most frequently used standards over a period of about 8 months. The RSD values were rather low (3.1–6.1). Nevertheless, the stability was not so good as to allow the use of the same calibration for a long period of time, as was possible with the PO UV 220 method. In the case of HTCO, a new calibration was performed for each analysis. This was done by including a series of four to seven standards at the beginning and end of each analytical sequence, to check the stability of the signal during the analysis. Subsequently, one regression was performed using all the standards.

4. Conclusions

In this study, we tested the applicability of HTCO with a chemiluminescence detector for the analysis of TN in freshwater samples. We assessed the repeatability, linearity, and comparability of this method to that previously used for TN analysis at the CNR ISE laboratory (persulphate oxidation and spectrophotometric determination of nitrate at 220 nm) (table 7).

PO is a consolidated method, which has been widely applied to TN determination in freshwater [7, 8]. However, HTCO has recently been introduced as a technique for TN analysis. Compared with PO, HTCO is faster and automated, and requires less sample handling, thus avoiding any potential problems related to manipulation.

Some studies on HTCO application for TN analysis pointed out how the primary problem seems to be the catalysis/oxidation step, not the detection step [1]. Our experience with the HTCO confirmed the importance of the catalyst on the oxidation

Table 7. Table summarizing the characteristics of PO UV 220 and HTCO CLD for TN analysis.

	Persulphate oxidation and spectrophotometric determination of NO_3 at 220 nm (PO UV 220)	High temperature catalytic oxidation with chemiluminescence detector (HTCO CLD)
Analytical range	$0.1-6\,mg\,N\,L^{-1}$ (extensible to 10–12 $mg\,N\,L^{-1}$)	$0.1-6\,mg\,N\,L^{-1}$ (extensible to ~2000 $mg\,N\,L^{-1}$)
Calibration	Annual	Daily (two series of three to four standards for each analytical sequence)
Limit of detection	~0.09 $mg\,N\,L^{-1}$	~0.02 $mg\,N\,L^{-1}$
Approximate analysis time	3 h for oxidation	7 min per sample (three replicates)
	1 h for UV determination	4 h for 30 samples

efficiency of N compounds. Once the appropriate catalyst has been selected, a regular check in time of the oxidation efficiency is needed, for both organic and inorganic compounds. The yield of the catalyst can be checked by the regular use of control charts representing different concentration ranges. The standard analysis of different nitrogenous compounds (e.g. NH_4Cl, urea, EDTA) to determine oxidation efficiency is also recommended.

From the point of view of linearity and repeatability, the results with HTCO are comparable with those with PO UV 220. A calibration with three to four standards for each analytical sequence is recommended in the case of HTCO (table 7).

The LOD of HTCO calculated in this study proved to be lower than that of PO UV 220. The analytical range is also considerably wider, extending up to 2000 $mg\,N\,L^{-1}$ (table 7).

To see how far the new method was comparable with the reference method, we tested the difference between the paired data obtained with the two techniques. According to statistical tests, the results were significantly different. Nonetheless, the average difference between paired data was low, as both absolute and relative values (0.06 $mg\,N\,L^{-1}$ and 3%, respectively, in the range 0.1–7.0 $mg\,N\,L^{-1}$) and was even lower for concentrations below 1.0 $mg\,N\,L^{-1}$, which are typical of the samples we routinely analyse.

The results of our study confirmed HTCO as a reliable method for TN determination in water samples. Once the best operating conditions are selected and optimized for the type of samples to be analysed, HTCO can be regarded as a valid alternative to traditional techniques such as PO for TN analysis in freshwater. Routine intercalibration within laboratories using HTCO and the regular analysis of SRM, at present not available for TN, would be of great help in testing the analytical performance of this method.

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