AMMONIUM DETERMINATION FROM MUNICIPAL WASTEWATERS. METHOD VALIDATION AND UNCERTAINTY ESTIMATION

Camelia DRAGHICI[1]*, Domnica COMAN[2],
Mihaela SICA[2], Carmen DIMA[2]

Abstract.
The analytical method for ammonium ion measurements in aquatic media was subject to validation procedure, based on the standard method. The following method performance criteria were investigated: selectivity, linearity domain, precision, accuracy, limit of detection, limit of quantification and robustness. The sources of uncertainties were identified, and the extended standard uncertainty was estimated. The method was demonstrated to be suitable for ammonium determination and was further used to determine ammonium content in domestic and industrial wastewaters.

Key words: chemiluminescence, chromatography, method selectivity, validation.

Introduction

Ammonium in wastewaters is mostly produced by the decomposition of organic compounds containing nitrogen, or by urea hydrolysis, being one of the primary forms of nitrogen in wastewater. When high concentrations of nitrogen compounds are released in running waters and lakes, eutrophication (excessive plants growth) can occur. Due to the restrictive nitrogen presence in water, a good control of ammonium concentration is imposed. To fulfil this aim, reliable analytical results are necessary.

In accredited laboratories the method validation is compulsory for the quality control system, but the international scientific community recognises more and more the method validation as a meaningful tool to demonstrate that the results are trustful and reliable. Thus, the method performance criteria like specificity/selectivity, precision, trueness, linearity range, limit of detection (LOD), limit of quantification (LOQ), and robustness are required for methods validation (EURACHEM Guide, 1998; Taverniers et al., 2004). Moreover, even if not yet imposed by all standard methods, measurement uncertainty is a statistical parameter, which describes the possible dispersion of the values reasonably attributed to the measurand, that is determined by the addition of

---

[1] Transilvania University of Brasov, Department of Product Design, Mechatronics and Environment, Brasov, Romania, c.draghici@unitbv.ro
[2] Compania Apa Brasov (Water Company), Wastewater Laboratory, Brasov, Romania

the variances of the individual steps of the test procedure (Meyer, 2007; Ellison and Williams, 2012).

In accredited laboratories, the most applied methods for ammonium determination are based on titrimetric or spectrometric measurements, given in different standards EPA-Method-3502 (1974), or ISO 7150-1:1984 (1984). Table 1 gives a survey during the last ten years of the analytical methods applied for ammonium determination in different aqueous matrixes, that also included method performances criteria. The exemplified research studies used spectrophotometric/colorimetric and florometric techniques, electrochemic methods (amperometric, conductometric, potentiometric), reflectance spectroscopy, or separation methods (chromatography and capillary electrophoresis) applicable to ionic compounds, using different detection systems (UV, fluorescence, chemiluminescence, MS tandem).

Table 1. Applications of method validation for ammonium ion determination from different matrixes

	Matrix	*Analytical methods*	*Method performance criteria*								*Reference*
			1	2	3	4	5	6	7	8	
1	seawater	IC-FLD	√	√		√	√	√			Kuo et al., 2005
2	**wastewater**	biosensor – amperometric	√				√	√			Kwan et al., 2005
3	seawater	colorimetric	√				√	√			Li et al., 2005
4	irrigation ditch, **residual** and fountain waters	fluorometric (Roth's method)		√		√	√	√			Meseguer Llore et al., 2005a
5	bottled, tap, lake, fountain, irrigation, **waste,** sea waters	HPLC-CLD	√	√	√	√	√	√		√	Meseguer Llore et al., 2005b
6	river water	reflectance spectroscopy	√	√	√		√	√			Moliner-Martinez et al., 2005
7	river water and sewage	CZE-UV	√	√			√	√			Fukushi et al., 2006

8	irrigation ditch, **residual**, fountain, lake, river, dam, sea, treated waters	Nessler; indophenol; Roth's; luminal; indothymol; bis(2,4,6 trichlorophe-nyl) oxalate; ion-selective electrode	√	√			√	√			Molins-Legua et al., 2006
9	rain water	IC-DAD	√	√		√	√	√			Niedzielski et al., 2006
10	irrigation ditch; dam, field, river lake, fog waters	capillary LC-UV	√	√	√	√	√	√	√		Moliner-Martinez et al., 2007
11	**wastewater**, river, **industrial effluent**	spectrophotometric	√	√		√	√	√			Oliveira et al., 2007
12	river waters	fluorometric	√	√				√			Poulin and Pelletier, 2007
13	water from agricultural area	biosensor	√	√			√	√			Azmi et al., 2009
14	river waters	spectrophotometric		√			√	√			Almeida et al., 2011
15	seawater	LC-MS		√		√		√			Bassarab et al., 2011
16	tap, mineral, lake waters	spectrophotometric	√	√		√	√	√			Eskandari and Shariati, 2011
17	river, lake and tap waters	LC-FLD	√	√		√	√	√			Muniraj et al., 2012
18	tap, rain, lake waters	fluorescence	√	√			√	√			Xue et al., 2012
19	seawater	fluorescence	√	√		√	√	√			Zhu et al., 2013
20	seawater	spectrophotometric		√		√	√	√			Zhu et al., 2014

			1	2	3	4	5	6	7	8	
21	**water treatment plant effluents**	potentiometric		√	√		√	√			Calvo-López et al., 2015
22	river, pond waters	conductometric	√	√			√	√			Jaikang et al., 2015

where: 1 – selectivity / matrix effect; 2 – repeatability; 3 – interim precision / reproducibility; 4 – accuracy / recovery; 5 – calibration / linearity; 6 – LOD; 7 – LOQ; 8 – robustness; capillary LC-UV – capillary liquid chromatography with UV detection; CZE-UV – capillary zone electrophoresis with direct UV detection; HPLC-CLD – high performance liquid chromatography with chemiluminescence detection; IC-DAD – ion chromatography with UV diode array detection; IC-FLD – ion chromatography with fluorometric detection; LC-FLD – liquid chromatography with fluorescence detection; LC-MS – liquid chromatography with mass spectrometry; Roth's method – derivatization with *o*-Phthaldialdehyde/*N*-acetyl-cisteine (OPA/NAC);

According to the examples given in Table 1, most of the selected publications present information about calibration and limit of detection (as sensitivity related criteria), repeatability or interferences (such as selectivity criteria). It is worth noticing that the performance criteria like interim precision/reproducibility are found only in 4 scientific studies, the limit of quantification and robustness are found as applicable in only one paper (each), and none of the selected papers presents uncertainty estimation.

The present study gives a validation procedure for the ammonium determination method, based on the European standard (transposed in the Romanian legislation). The validation criteria were selectivity, calibration curve, LOD, LOQ, precision, accuracy, including robustness (EURAGHEM Guide, 1998). Even if not yet imposed by all standard methods, the sources of uncertainties were identified, and the extended standard uncertainty was estimated for the measurements on standard samples (Ellison and Williams, 2012). The method is used to determine the ammonium content in domestic and industrial wastewaters.

Reagents and method

All the reagents were of analytical grade and the used glassware was A class. The validation procedure was performed according to the EU standard (ISO 7150-1/1984), also transposed as the Romanian one (SR ISO 7150-1/2001), based on spectrometric measurements. Ammonium reacts with salicilate and hypochloride ions in the presence of sodium nitrozo-pentacyanoferrate (III), sodium nitroprussiate, forming a green complex. Spectrophotometric measurements were performed using Precisa Dynamica DB 20 spectrophotometer, at 650 nm, using 10 mm cuvettes. The method performance criteria and the uncertainty were evaluated using equations 1-11.

$$x_{LOD} = \bar{x}_{blank} + 3s_{blank} \qquad (1)$$

$$x_{LOQ} = \bar{x}_{blank} + 10s_{blank} \qquad (2)$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad (3)$$

$$RSD = \frac{s}{x} \cdot 100 \qquad (4)$$

$$s_x = \frac{s}{\sqrt{n}} \qquad (5)$$

$$\bar{x} \pm t \cdot s_x \qquad (6)$$

$$R = \frac{C_F - C_I}{C_A} 100 \qquad (7)$$

$$u_x = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad (8)$$

$$u_r = \frac{u_x}{x} \qquad (9)$$

$$u_c = \sqrt{\sum u_r^2} \qquad (10)$$

$$U = u_c \cdot k \cdot 100 \text{; for } (k=2, P=95\%) \qquad (11)$$

where: C_A – added concentration; C_I – initial concentration; n – number of discrete measurements; C_F – final concentration; R – recovery; RSD – relative standard deviation; s – standard deviation; s_{blank} – standard deviation of the measurements with blank solution; s_x – standard deviation of the mean; t – normal distribution factor; U – expanded standard uncertainty; u_c – combined relative standard uncertainty; u_r – relative standard uncertainty, u_x – standard uncertainty; $\bar{X}$ – average value of n repeated measurements; $\bar{X}_{blank}$ – average value registered with the blank solution; x_{LOD} – limit of detection; x_{LOQ} – limit of quantification; X_i – discrete measured values.

Results and discussions

Selectivity

For the evaluation of the method selectivity, Na_2SO_4, $CaCO_3$ and $MgSO_4$ were added in the NH_4^+ reference samples then the ammonium concentration was compared in the absence and presence of the interfering reagents (Table 2).

Table 2 shows that in the presence of interferer reagents (IR) the ammonium concentrations were significantly different from the ones in the absence of the IR. The results require that these interfering compounds should be removed from the wastewater samples before measurements.

Table 2. Method selectivity for ammonium measurements

Interferer reagent	Ammonium concentration		Observation
	Initial (mg/L)	In the presence of IR (mg/L)	
Na_2SO_4	20.00	0.003	interferer
$CaCO_3$	20.00	35.33	interferer
$MgSO_4$	20.00	34.27	interferer

Calibration curve, LOD and LOQ

The calibration curve was determined with ammonium chloride solutions used as reference material (RM) of 6 different concentrations. LOD and LOQ were calculated based on equations 1-2, using the absorbance measured on 10 blanks. The results are presented in Table 3.

Table 3. Correlation coefficient, LOD and LOQ for ammonium measurements.

R^2	$\overline{X}_{blank}$ (nm)	Lowest NH_4^+ measurement (nm)	LOD		LOQ	
			(nm)	(mg/L)	(nm)	(mg/L)
0.9999	0.020	0.079	0.027	0.019	0.042	0.029

The results show a very good correlation coefficient. LOD and LOQ values (evaluated in nm) are situated between the blank measurement and the lowest value measured with NH_4^+ RM solution ($0.020 < 0.027 < 0.042 < 0.079$). The standard method requires an applicability concentration domain of 0.064 mg/L − 1.288 mg/L, while the estimated LOD and LOQ are lower than the lower limit of the accepted concentration interval.

Precision

The precision was evaluated by repeatability (RSD_r) for 10 replicates, and interim precision (RSD_{ip}) for 10 replicates measured by 3 different analysts, based on relative standard deviations (RSD%) calculated using equations 3-4. The result was expressed considering the confidence interval, calculated with equations 5-6. The method is precise, with repeatability lower than the interim precision (Table 4).

Accuracy

The accuracy was evaluated based on the recovery test, using equation 7, applied for measurements of three replicates, for three different solution

volumes. The recovery is included in the required interval (85% < R% < 105%) shoving that the method is accurate (Table 5.).

Table 4. Method precision and trust interval

Precision	n	Average $[NH_4^+]$ (mg/L)	s	RSD (%)	Tolerance (t=2)	
					$[NH_4^+]_{real}$ (mg/L)	trust interval
RSD_r	10	20.009	0.0277	0.1386	20.009 ±	0.0175
RSD_{ip}	30	20.014	0.0476	0.2349	20.014 ±	0.0243

Table 5. Accuracy by recovery tests for ammonium measurements

Volume (mL)	C_I (mg/L)	C_A (mg/L)	C_F (mg/L)	Recovery (%)
1	19.82	5	24.72	97.93
2	19.82	10	29.71	98.93
3	19.82	15	34.69	99.13

Robustness

The robustness evaluation was based on the RSD% parameter and was tested against two different working conditions that might influence the method: the volume of the colouring reagent (4 mL required) and the reaction time (60 minutes required). The results are given in Tables 6 and 7 and they revealed that the method is not robust against the volume of the MB reagent added, but is robust against the reaction time.

Table 6. Robustness evaluation by volume of colouring reagent

$V_{reagent}$ (mL)	$[RM]_{theor}$ (mg/L)	$[RM]_{real}$ (mg/L)	Evaluation parameter
2.0	20.00	13.78	average=18.38
4.0	20.00	20.08	s=10.290
6.0	20.00	22.94	s_x=4.201
8.0	20.00	24.82	RSD%=55.994
10.0	20.00	28.64	trust interval = 8.40

Uncertainty estimation for the ammonia determination in standard solutions

Uncertainty sources identification

The ammonium content was measured in standard solutions, expressed in mg/L, and the following sources of uncertainty were identified: reference material, analytical balance, repeatability (n=10), recovery, glassware of different volumes (V1, V2, V3, V4), calibration curve (CC) and equipment.

Table 7. Robustness evaluation by reaction time

Reaction time (min)	[RM] *theor* (mg/L)	[RM] *real* (mg/L)	Evaluation parameter
30	20.00	19.20	average=20.12
60	20.00	20.01	RSD%=2.34
90	20.00	20.22	trust interval=0.002

Uncertainty estimation for the ammonia determination in standard solutions

The uncertainty was estimated based on calculations of standard uncertainty (u_x), relative standard uncertainty (u_r), combined relative standard uncertainty (u_c) and expanded standard uncertainty (U), according to equations 8-11, and the results are presented in Tables 7 and 8.

Table 7. Standard uncertainty and relative standard uncertainty

	RM (mg/L)	Balance (mg)	RSD$_r$ (mg/L)	R (%)	V$_1$ (mL)	V$_2$ (mL)	V$_3$ (mL)	V$_4$ (mL)	CC (mg/L)	Equipment
x	1	381.9	0.644	0.7205	10	4	4	50	40.00	650.00
u$_x$	0.0020	0.3000	0.0033	0.0024	0.0290	0.0130	0.0130	0.0520	0.0717	0.0080
u$_r$	0.0035	0.0007	0.0051	0.0024	0.0029	0.0033	0.0033	0.0010	0.0018	0.000012

Table 8. Combined relative standard uncertainty and expanded standard uncertainty (k = 2, P = 95%)

u$_c$	u$_c$ (%)	U	U (%)	Result (mg/L)
0.0270	4.19	0.0540	8.38	0.644 ± 0.054

The higher contribution to the combined relative standard uncertainty was given by repeatability (u_r = 0.0051), while the smaller contribution was given by the equipment (u_r = 0.000012). As expected, the expanded standard uncertainty (U%) is higher than repeatability and interim precision.

Conclusions

The analytical method for ammonia measurements in aquatic media was validated, the method being selective, sensitive, precise, accurate and robust against the reaction time. Based on the identified sources of uncertainty, different types of uncertainties were estimated. Further works will be performed for comparative studies. The method is applied for ammonium determination in wastewaters, with repeatability lower than 1%.

References

Almeida M.I.G.S., Estela J.M., Segundo M.A., Cerdà V., (2011), A membraneless gas-diffusion unit – multisyringe flow injection spectrophotometric method for ammonium determination in untreated environmental samples, Talanta, 84, 1244–1252.

Azmi N.E., Ahmad M., Abdullah J., Sidek H., Heng L.Y., Karuppiah N., (2009), Biosensor based on glutamate dehydrogenase immobilized in chitosan for the determination of ammonium in water samples, Analytical Biochemistry, 388, 28–32.

Bassarab P., Williams D., Dean J.R., Ludkin E., Perry J.J., (2011), Determination of quaternary ammonium compounds in seawater samples by solid-phase extraction and liquid chromatography–mass spectrometry, Journal of Chromatography A, 1218, 673–677.

Calvo-López A., Ymbern O., Puyol M., Casalta J.M., Alonso-Chamarro J., (2015), Potentiometric analytical microsystem based on the integration of a gas-diffusion step for on-line ammonium determination in water recycling processes in manned space missions, Analytica Chimica Acta, 874, 26–32.

Ellison S.L.R., Williams, A., (Eds), (2012), EURACHEM/CITAC Guide CG 4, Quantifying Uncertainty in Analytical Measurement, third edition.

Eskandari H., Shariati M.R., (2011), Dodecylbenzene sulfonate-coated magnetite nanoparticles as a new adsorbent for solid phase extraction-spectrophotometric determination of ultra trace amounts of ammonium in water samples, Analytica Chimica Acta, 704, 146– 153.

Fukushi K., Ito H., Kimura K., Yokota K., Saito K., Chayama K., Takeda S., Wakida S.-I., (2006), Determination of ammonium in river water and sewage samples by capillary zone electrophoresis with direct UV detection, Journal of Chromatography A, 1106, 61–66.

Jaikang P., Grudpan K., Kanyanee T., (2015), Conductometric determination of ammonium ion with a mobile drop, Talanta, 132, 884–888.

Kuo C.-T., Wang P.-Y., Wu C.-H., (2005), Fluorometric determination of ammonium ion by ion chromatography using postcolumn derivatization with o-phthaldialdehyde, Journal of Chromatography A, 1085, 91–97.

Kwan R.C.H., Hon P.Y.T., Renneberg R, (2005), Amperometric determination of ammonium with bienzyme/poly(carbamoyl) sulfonate hydrogel-based biosensor, Sensors and Actuators B, 107, 616–622.

Li Q.P., Zhang J.-Z., Millero F.J., Hansell D.A., (2005), Continuous colorimetric determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell, Marine Chemistry, 96, 73– 85.

Meseguer Lloret, S., Verdu Andres, J., Molins Legua, C., Campins Falco, P., (2005a), Determination of ammonia and primary amine compounds and Kjeldahl nitrogen in water samples with a modified Roth's fluorimetric method, Talanta, 65, 869–875.

Meseguer Lloret S., Molins Legua C., Campins Falco P., (2005b), Selective determination of ammonium in water based on HPLC and chemiluminescence detection, Analytica Chimica Acta, 536, 121–127.

Meyer V.R., (2007), Measurement uncertainty, J. Chromatogr. A, 1158, 15-24.

Moliner-Martinez Y., Herraez-Hernandez R., Campins-Falco P., (2005), Improved detection limit for ammonium/ammonia achieved by Berthelot's reaction by use of solid-phase extraction coupled to diffuse reflectance spectroscopy, Analytica Chimica Acta, 534, 327–334.

Moliner-Martinez Y., Herraez-Hernandez R., Campins-Falco P., (2007), A microanalytical method for ammonium and short-chain primary aliphatic amines using precolumn derivatization and capillary liquid chromatography, Journal of Chromatography A, 1164, 329–333.

Molins-Legua C., Meseguer-Lloret S., Moliner-Martinez Y., Campins-Falco P., (2006), A guide for selecting the most appropriate method for ammonium determination in water analysis, Trends in Analytical Chemistry, 25, 282- 290.

Muniraj S., Yan C.-T., Shih H.-K., Ponnusamy V.K., Jen J.-F., (2012), Determination of ammonium in aqueous samples using new headspace dynamic in-syringe liquid-phase microextraction with in situ derivatization coupled with liquid chromatography–fluorescence detection, Analytica Chimica Acta, 754, 54– 60.

Niedzielski P., Kurzyca I., Siepak J., (2006), A new tool for inorganic nitrogen speciation study: Simultaneous determination of ammonium ion, nitrite and nitrate by ion chromatography with post-column ammonium derivatization by Nessler reagent and diode-array detection in rain water samples, Analytica Chimica Acta, 577, 220–224.

Oliveira S.M., Lopes T.I.M.S., Toth I.V., Rangel A.O.S.S., (2007), A multi-commuted flow injection system with a multi-channel propulsion unit placed before detection: Spectrophotometric determination of ammonium, Analytica chimica acta, 600, 29–34.

Poulin P., Pelletier E., (2007), Determination of ammonium using a microplate-based fluorometric technique, Talanta, 71, 1500–1506.

Taverniers I., de Loose M., van Bockstaele E., (2004), Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, Trends in Analytical Chemistry, 23, 535-552.

Xue S., Uchiyama K., Li H.-f., (2012), Determination of ammonium on an integrated microchip with LED-induced fluorescence detection, Journal of Environmental Sciences, 24, 564–570.

Zhu Y., Yuan D., Huang Y., Ma J., Feng, S., (2013), A sensitive flow-batch system for on board determination of ultra-trace ammonium in seawater: Method development and shipboard application, Analytica Chimica Acta, 794, 47– 54.

Zhu Y., Yuan D., Huang Y., Ma J., Feng S., Lin K., (2014), A modified method for on-line determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell and spectrophotometric detection, Marine Chemistry, 162, 114–121.

*** EPA-Method-3502, (1974), Nitrogen, Ammonia (Colorimetric, Titrimetric, Potentiometric Distillation Procedure).

*** EURAGHEM Guide, (1998), The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics.

*** ISO 7150-1/1984, (1984), Spectrophotometric determination of ammonium ion.

*** SR ISO 7150-1/2001, (2001), Calitatea apei. Determinarea conţinutului de amoniu. Partea 1: Metoda spectrometrică manuală (in Romanian).