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Water and Wastewater Analysis

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Dear readers,

In times of scarce resources and a steadily growing world population, environmental monitoring is of particular importance. It contributes to improving quality of life and public health by providing a valid data base for political and technological decisions. The analysis of water and wastewater, as a subarea of environmental analysis, plays a central role in this context. Although three quarters of the earth are covered with water, only less than one percent can be used as drinking water. Growing pollution and the climate change are further reducing the amount of clean, easily accessible drinking water. Water and wastewater analyses are therefore key measures taken by municipalities and states to improve water recycling continuously and to make available water resources safer.
Our mission is to support customers worldwide with Analytik Jena equipment and solutions to improve their services and products and thus sustainably increase environmental quality and the quality of life. This e-book follows this intention.
We aim to present ways, both methodologically and technologically, to meet the challenges of water analysis in laboratories around the world more easily, reliably, and effectively. Today, environmental analysis faces major challenges: Ever-increasing numbers of samples must often be processed with dwindling financial and human resources. We are addressing these demands with the application examples and solutions demonstrated in this document.
The e-book focuses on central applications in water analysis. In addition to elemental analysis, sum parameter analysis of TOC/TN_b and AOX, and UV/Vis spectroscopy, we also present a solution for molecular biological testing: the detection of water pathogens. Get to know flexibly applicable methods and technologies that can be individually tailored to your particular application.

We hope you will get many valuable insights. In case of any questions about the methods and solutions presented in this e-book, please do not hesitate to get in touch with us. Our experts will be happy to support you with any issues regarding environmental monitoring solutions.

Enjoy reading,

Bernd Bletzinger, Team Leader Environment

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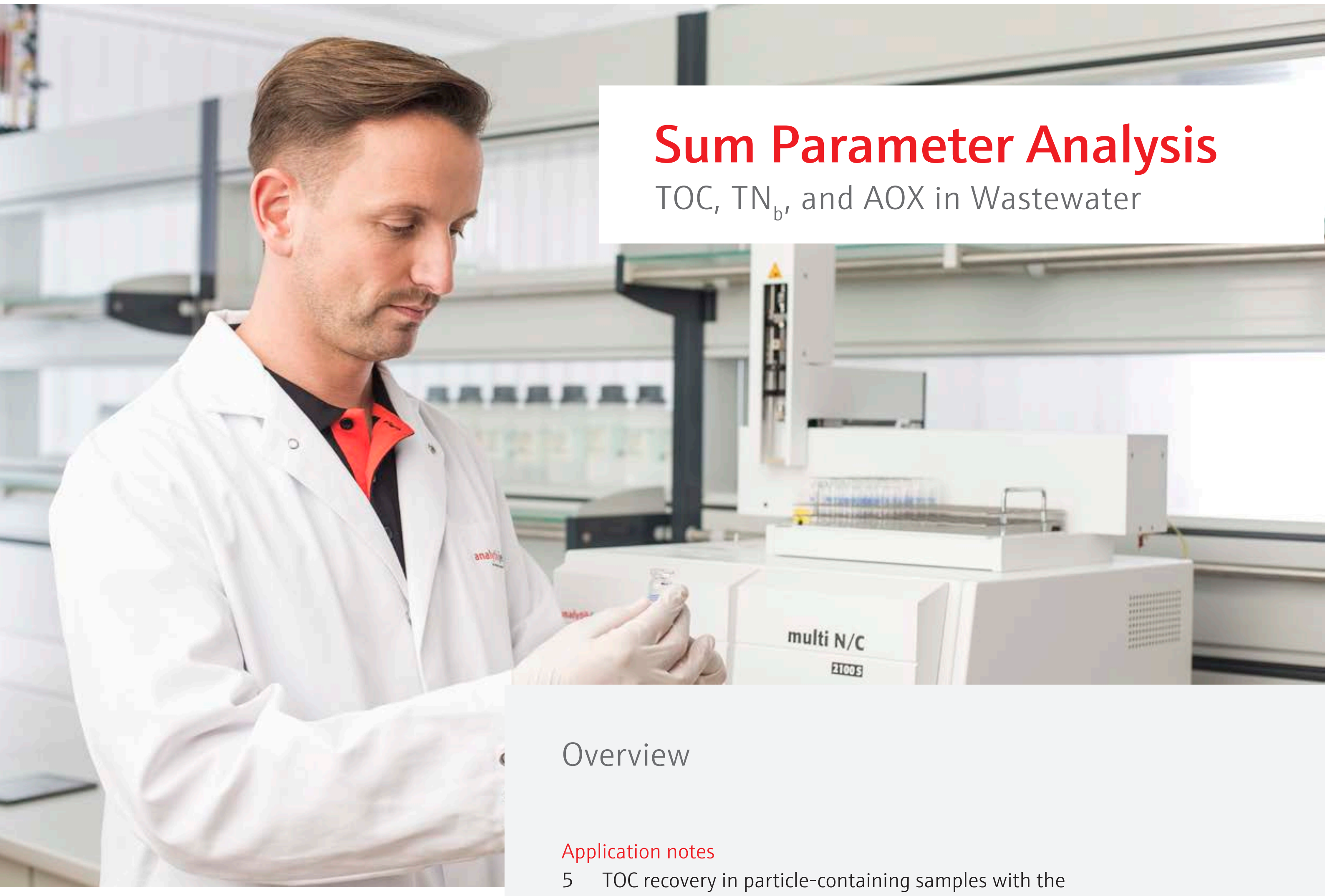
Sum Parameter Analysis

TOC, TN_b, and AOX in Wastewater



Overview

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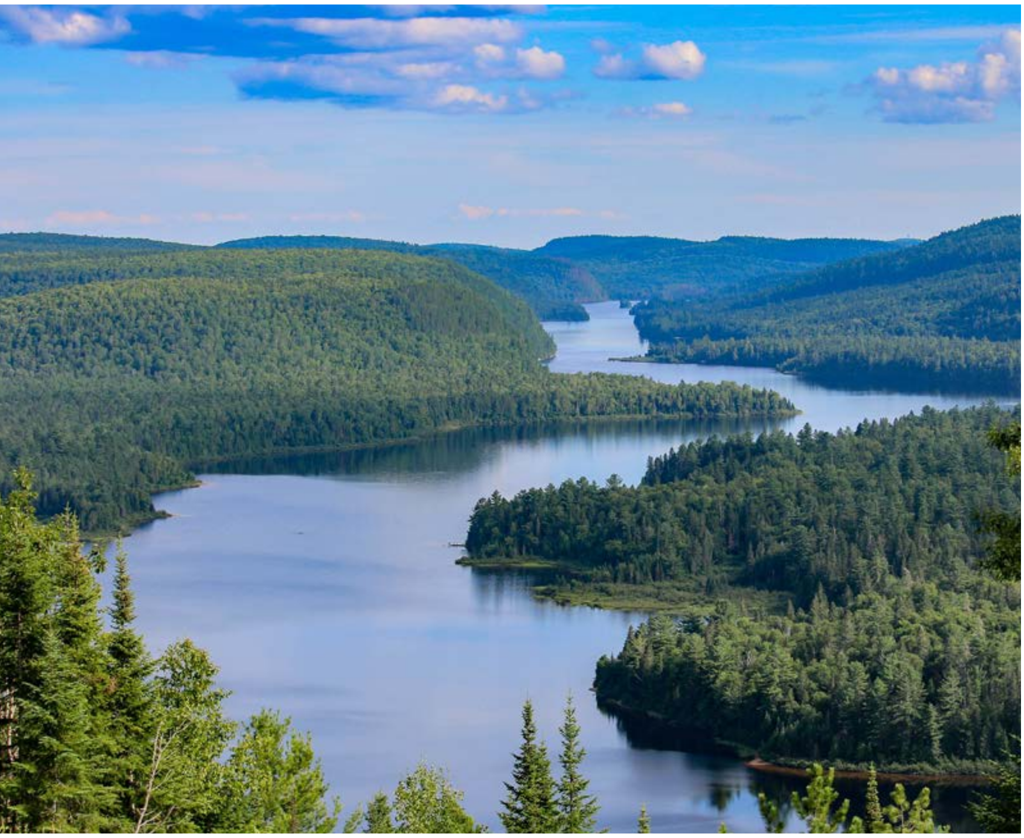
Private households and many industries produce large volumes of wastewater that need to be treated before this water can be reused or discharged into natural waters. The sum parameters total organic carbon (TOC) and total bound nitrogen (TN_b) have to be measured routinely at the influent and effluent of a sewage treatment plant, both to operate the plant in the best possible manner and to meet legal limits. These pollutants can lead to eutrophication of surface water recourses, endanger aquatic life, and contaminate groundwater supplies. A sub-fraction of TOC belongs to a more toxic and persistent substance class of the halogenated hydrocarbons, which can be determined by the sum parameter AOX (adsorbable organically bound halogens).

With the multi N/C series, the multi X 2500, and the associated sample preparation units (APU series and AFU 3) Analytik Jena offers a large range of analyzers for powerful TOC/TN_b as well as AOX/TOX, EOX, and POX analysis in wastewater. Whether routine analysis of moderate sample quantities or high throughput analysis, we offer customized automation solutions. The analyzers of the multi N/C series and the multi X 2500 are especially designed for particulate water samples and are characterized by simple operation, high robustness, and easy maintenance.



Challenge

TOC determination in cellulose suspension with undissolved particles at a size of up to 100 µm according to DIN EN 1484 or ISO 20236.

Solution

Fast, reliable, and cost-efficient measurement in highly particle loaded samples thanks to the excellent particle handling of the combustion-based analyzers of the multi N/C series.

TOC Recovery in Particle-Containing Samples with the Cellulose Test According to DIN EN 1484

Introduction

When monitoring total organic carbon (TOC) and total bound nitrogen (TN_b) in environmental samples like surface or waste water, by definition not only the dissolved organic carbon and nitrogen compounds have to be measured, but also the particle-bound fraction of these compounds have to be fully detected. According to DIN EN 1484 and ISO 20236, the TOC is defined as the sum of all organically bound carbon present in water, both in dissolved and suspended matter.
On the other hand, the dissolved organic carbon (DOC) is defined as the sum of the organically bound carbon in water originating from compounds passing through a membrane filter of 0.45 µm pore size. Accordingly, a TOC/ TN_b analyzer used for the determination of particulate environmental samples has to prove that both sample homogenization and sample handling in the analyzer from sample vial up to the combustion process ensures complete transfer and oxidation of a representative aliquot of the particulate sample.

To verify this, Annex C of DIN EN 1484 and chapter 8.2 of ISO 20236 include a specific test to determine the recovery and variation of replicate determination for particle processing of a cellulose suspension with up to 100 µm particle size at a 100 mg/L TOC concentration. The mean value from a triplicate measurement must not exceed ±10% of the theoretical value. The repeatability variation coefficient must be ≤ 10%.
In addition to the necessary sample homogenization and sample processing preconditions of a TOC/TN_b system, the oxidation power of the analyzer is also critical to pass this so-called cellulose test. At this point it has to be mentioned that conventional wet-chemical and/or UV-based TOC analyzers show only low recovery rates on this cellulose test and are therefore not suitable for the TOC measurement of typical particle-containing environmental water samples.

Materials and Methods

Samples and reagents

To prepare a 100 mg/L C suspension solution, 225 mg cellulose (particle size between 20 µm and 100 µm) is placed in a 1000 mL volumetric flask and made up to the mark with water. For proper homogenization, the solution should then be stirred on a magnetic stirring plate, possibly overnight to allow the micro cellulose to swell. Before each use, the suspension must be stirred again in order to prepare for a homogeneous solution. The filling of the respective sample vessel plays a very important role. It is absolutely necessary that the suspension solution is shaken vigorously immediately before filling and then transferred into the vessel relatively quickly with a large volume surge until the vessel overflows. Alternatively, a large 5 or 10 mL micropipette can be used to transfer the thoroughly mixed suspension into sampler vials.

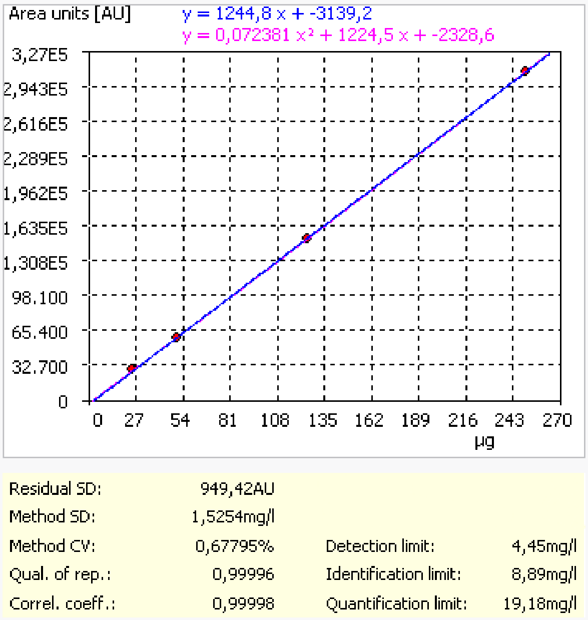
- Wastewater samples were taken from the inlet as well as from an intermediate treatment stage of a sewage plant
- 2 M HCl was used for automatic sample acidification to a pH value < 2

Sample preparation and measurement

The samples were stored in a refrigerator at 4 °C until analysis and then transferred into suitable autosampler vials after appropriate stirring. The wastewater samples and the cellulose test suspensions were analyzed in direct mode applying an NPOC method. The samples were adjusted to pH < 2 using 2 M HCl, and subsequently purged for a period of 5 minutes. Using an autosampler, representative sample aliquots were transferred into the combustion tube. The samples were catalytically oxidized at a furnace temperature of 800 °C in an oxygen-rich atmosphere. A 16 mm combustion tube filled with platinum catalyst was used. The CO_2 formed was quantitatively detected by the focus radiation non-dispersive infrared detector (FR-NDIR). Optionally, the formed nitrogen oxides can be detected by means of a chemiluminescence detector (CLD) or an electrochemical detector (ChD).

Calibration

For the determination of the cellulose suspensions and the samples, the multi N/C analyzer was calibrated with potassium hydrogen phthalate according to EN 1484. Standards with the following concentrations were prepared and measured: 50 mg/L, 100 mg/L, 250 mg/L, and 500 mg/L. Each calibration point was injected with three replicates as agreed in the method. The method characteristics resulting from the linear regression are shown in the following diagram.



Residual SD:	949,42AU		
Method SD:	1,5254mg/l		
Method CV:	0,67795%	Detection limit:	4,45mg/l
Qual. of rep.:	0,99996	Identification limit:	8,89mg/l
Correl. coeff.:	0,99998	Quantification limit:	19,18mg/l

Figure 1: Calibration curve and method characteristics

Sample preparation and measurement

The TOC measurements were performed on multi N/C 2100S and multi N/C 3100. The method settings shown in Table 1 were used to determine the TOC content.

The proper alignment of the sample aspiration needle on the autosampler is important for a representative sample aspiration of particle-containing samples, as magnetically stirred water sample forms a stirring funnel. Sample take-up is optimized when the sample aspiration needle is located outside the center of the vial and, depending on the vial size, at least 5 to 7 mm above the magnetic stirring bar. It is also important to set the correct stirring speed in the method in order to achieve good homogenization on the one hand, but not to create a large stirring funnel at the same time. Therefore, depending on the sample vessel size, the settings recommended in the table above should be used.

	multi N/C 2100S	multi N/C 3100
Parameter	NPOC (direct TOC measurement)	NPOC (direct TOC measurement)
Digestion	High temperature combustion with Pt catalyst at 800 °C	High temperature combustion with Pt catalyst at 800 °C
Number of repetitions	Min. 3, max. 3	Min. 3, max. 3
Autosampler and vial sizes	AS 60, 8 mL vials, 250 µL syringe	AS vario ER, 72 pos. rack, 40 mL vials
Sample rinses before injection	3	3
Reverse rinses	-	1
Sample injection volume	250 µL	500 µL
Stirring speed	5	7
NPOC purge time	300 s	300 s

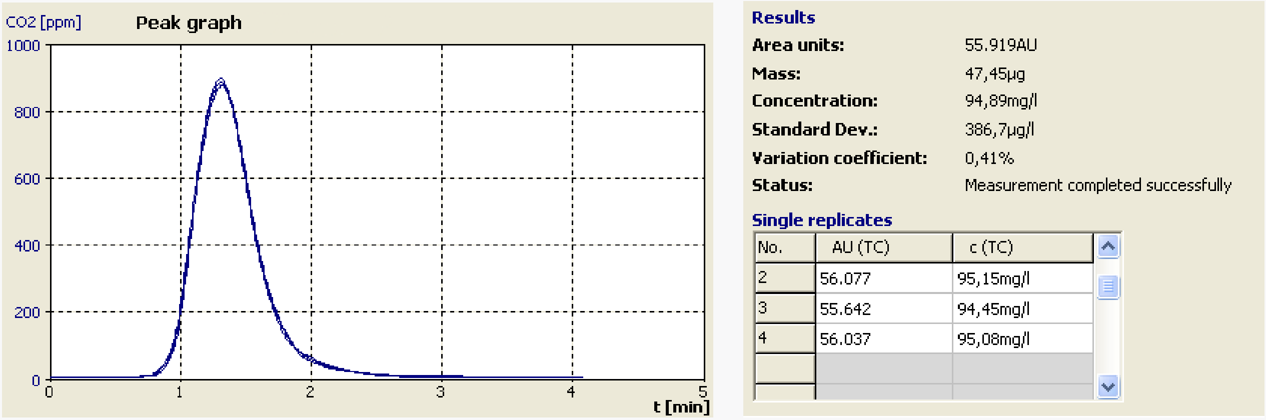
Table 1: Method settings

Results and Discussion

Table 2 shows blank value corrected mean values from at least two repeat measurements for different real samples and recoveries for TOC and TN_b reference solutions. Dilution ratios are also reported. For informative purposes, the COD contents provided for the respective samples and the TOC-COD correlation factors calculated from them were also given.

Sample ID	Result: NPOC [mg/L]	RSD [%]
Sewage plant inflow	284.7	0.86
1. Cellulose test at 100 mg/L	94.9	0.41
Intermediate treatment	125.2	1.44
2. Cellulose test at 100 mg/L	99.3	0.48
KHP check standard at 150 mg/L	150.3	0.10

Table 2: Results



Results

Area units: 55.919AU
Mass: 47,45µg
Concentration: 94,89mg/l
Standard Dev.: 386,7µg/l
Variation coefficient: 0,41%
Status: Measurement completed successfully

Single replicates

No.	AU (TC)	c (TC)
2	56.077	95,15mg/l
3	55.642	94,45mg/l
4	56.037	95,08mg/l

Figure 2: Typical measurement curve and replicate results for the investigated cellulose suspension

Conclusion

The measurement results of the cellulose suspensions exceed the required expectations of a recovery in the range from 90 to 110% with results of 95% and 99%. Likewise, the coefficients of variation at 0.41% and 0.48% exceed the requirements of DIN EN 1484 or ISO 20236, respectively. Decisive for these good results are the excellent particle handling capabilities of the multi N/C analyzers. With the multi N/C 2100 S, this is achieved by the 0.7 mm inner diameter of the aspiration needle in combination with the ultra-short sample dosage line. The sample transfer needles, tubing, and the rotary valve of the multi N/C 3100 with an inner diameter of 0.8 mm and, in combination with the proven, fast sample loop injection principle, are perfectly suited for samples containing particles. Additionally, the high furnace temperatures guarantee 100% sample oxidation. The use of large sample injection volumes and the routine filling of the sample vessels further support a successful cellulose particle test.

Real wastewater samples taken from the sewage treatment process also show excellent reproducibility. A fully automated measurement process for large sample sequences is supported by a wide range of available autosamplers with integrated sample homogenization, ranging from 21 sample positions (AS 21hp) to 146 sample positions with the AS vario and AS vario ER for the multi N/C 3100. The AS 60 autosampler for the multi N/C 2100S also offers a very good level of automation for sewage plant testing laboratories.

Figure 3: multi N/C 3100 with AS vario ER autosampler

References:

DIN EN 1484 Water analysis – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)

ISO 20236: Water quality – Determination of total organic carbon (TOC), dissolved organic carbon (DOC) total bound nitrogen (TNb) and dissolved bound nitrogen (DNb) after high temperature catalytic oxidative combustion

Challenge

Reproducible and reliable determination of TOC and TN_b contents in samples with high particle loads/wastewater.

Solution

Fully automated and simultaneous TOC/TN_b measurement using direct injection technology and catalytic high-temperature combustion, which allow optimum particle handling and a minimized carry-over risk.

TOC/TN_b Determination in Municipal Sewage Plants

Introduction

In wastewater treatment plants, the organic and nitrogen load have to be measured in the untreated inflow, the pretreated effluent, and the final effluent after the completed treatment process. In many cases, the chemical oxygen demand (COD) and total nitrogen contents are measured with separate methods. This is a labor- and time-consuming process often associated with the formation of chromium-VI-contaminated waste. Through correlation studies, an empirical conversion factor for TOC to COD conversion can be established. Hence, a fully automated analytical process for TOC/TN_b determination according to EN 1484 and EN 12260 can be applied to save resources and time.

In order to guarantee accurate and efficient analysis, it is recommendable to use a catalytic high-temperature combustion TOC analyzer for simultaneous determination of NPOC and TN_b from one single injection. According to these standards, TOC is defined as the sum of dissolved and particle-bound organic carbon compounds. Thus, the challenge is to assure a representative sample transfer, including all the particles, into the combustion system and at the same time to guarantee a complete oxidation of both, difficult-to-oxidize substances and particle-bound organics. This requires an effective sample homogenization on the autosampler rack and a sample introduction technique that ensures no particles get lost on the way to the combustion tube or cause any blockages, which may lead to system downtime or to extended wear and tear on sensitive Teflon parts inside the dosing system. A combustion system capable of providing sufficiently high furnace temperatures to assure a complete sample digestion is required as well. This application note describes a method using the multi N/C 2100S TOC/TN_b analyzer. The samples were measured at a customer's site, a wastewater treatment plant in Germany, using a direct injection system for optimized particle handling.

Materials and Methods

Samples and reagents

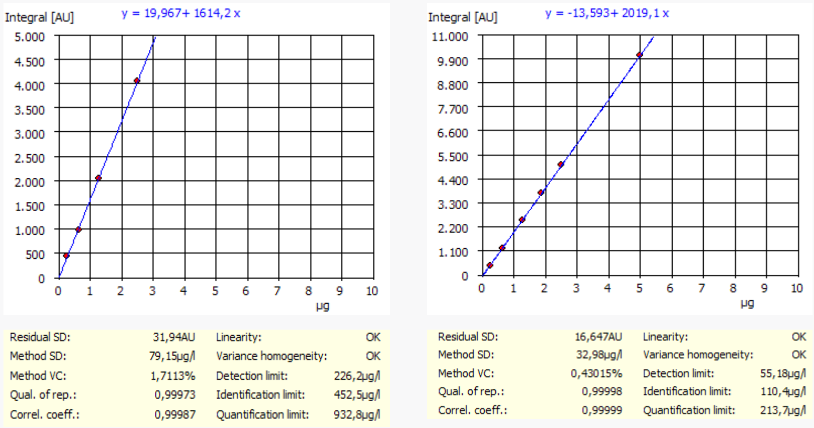
- Wastewater samples were taken from the inflow into the sewage plant as well as intermediate treatment stages
- 2 M HCL was used for automatic sample acidification to a pH < 2

Sample preparation

The samples were stored in a refrigerator at 4 °C until analysis and then transferred into suitable autosampler vials. The wastewater samples were analyzed in direct mode applying an NPOC/TN$_b$ method. The samples were adjusted to pH < 2 using 2 M HCl, and subsequently purged for a period of 5 minutes. An injection volume of 250 µL was used for these measurement sequences. The samples were catalytically oxidized at a temperature of 800 °C in an oxygen flow. The 16 mm combustion tube, filled with platinum catalyst, was used. The formed nitrogen oxides are detected by means of a chemiluminescence detector (alternatively a ChD detector can be utilized), CO$_2$ detection was done by the Focus Radiation NDIR detector.

Calibration

The multi N/C analyzer was calibrated between 1 and 1000 mg/L for total organic carbon with a potassium hydrogen phthalate standard solution. A multipoint calibration was used to evaluate the results of the NPOC measurement. For total bound nitrogen a calibration was carried out from 1 to 100 mg/L with an ammonium sulfate and potassium nitrate solution (50:50 mix) according to EN 12260.

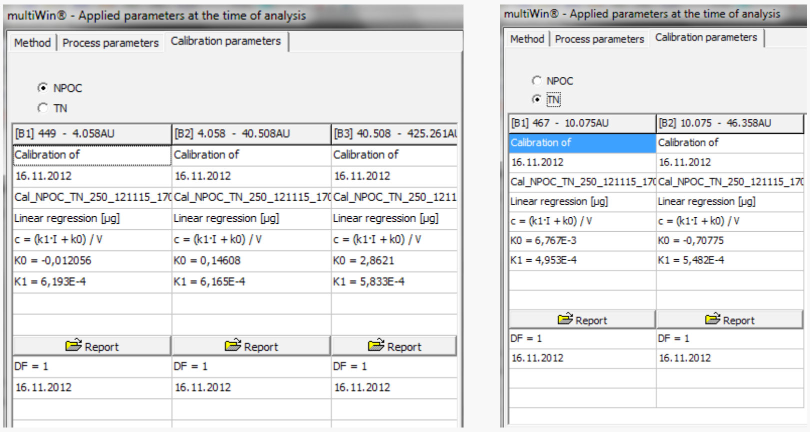


Residual SD:	31,94AU	Linearity:	OK
Method SD:	79,15µg/l	Variance homogeneity:	OK
Method VC:	1,7113%	Detection limit:	226,2µg/l
Qual. of rep.:	0,99973	Identification limit:	452,5µg/l
Correl. coeff.:	0,99987	Quantification limit:	932,8µg/l

Residual SD:	16,647AU	Linearity:	OK
Method SD:	32,98µg/l	Variance homogeneity:	OK
Method VC:	0,43015%	Detection limit:	55,18µg/l
Qual. of rep.:	0,99998	Identification limit:	110,4µg/l
Correl. coeff.:	0,99999	Quantification limit:	213,7µg/l

Figure 1 and 2: Example of TC and TN calibration and method characteristics

Within the method up to three calibration ranges can be linked to each parameter in order to cover an over-all working range of up to three magnitudes. With the NPOC/TN$_b$ method three NPOC and two TN$_b$ multi-point calibration ranges were linked as follows:



● NPOC
○ TN

[B1] 449 - 4.058AU	[B2] 4.058 - 40.508AU	[B3] 40.508 - 425.261AU
Calibration of	Calibration of	Calibration of
16.11.2012	16.11.2012	16.11.2012
Cal_NPOC_TN_250_121115_170	Cal_NPOC_TN_250_121115_170	Cal_NPOC_TN_250_1211
Linear regression [µg]	Linear regression [µg]	Linear regression [µg]
c = (k1·I + k0) / V	c = (k1·I + k0) / V	c = (k1·I + k0) / V
K0 = -0,012056	K0 = 0,14608	K0 = 2,8621
K1 = 6,193E-4	K1 = 6,165E-4	K1 = 5,833E-4
DF = 1	DF = 1	DF = 1
16.11.2012	16.11.2012	16.11.2012

○ NPOC
● TN

[B1] 467 - 10.075AU	[B2] 10.075 - 46.358AU
Calibration of	Calibration of
16.11.2012	16.11.2012
Cal_NPOC_TN_250_121115_170	Cal_NPOC_TN_250_121115_170
Linear regression [µg]	Linear regression [µg]
c = (k1·I + k0) / V	c = (k1·I + k0) / V
K0 = 6,767E-3	K0 = -0,70775
K1 = 4,953E-4	K1 = 5,482E-4
DF = 1	DF = 1
16.11.2012	16.11.2012

Figure 3/4: NPOC and TN$_b$ calibration ranges used with the combined NPOC/TN method

Instrumentation

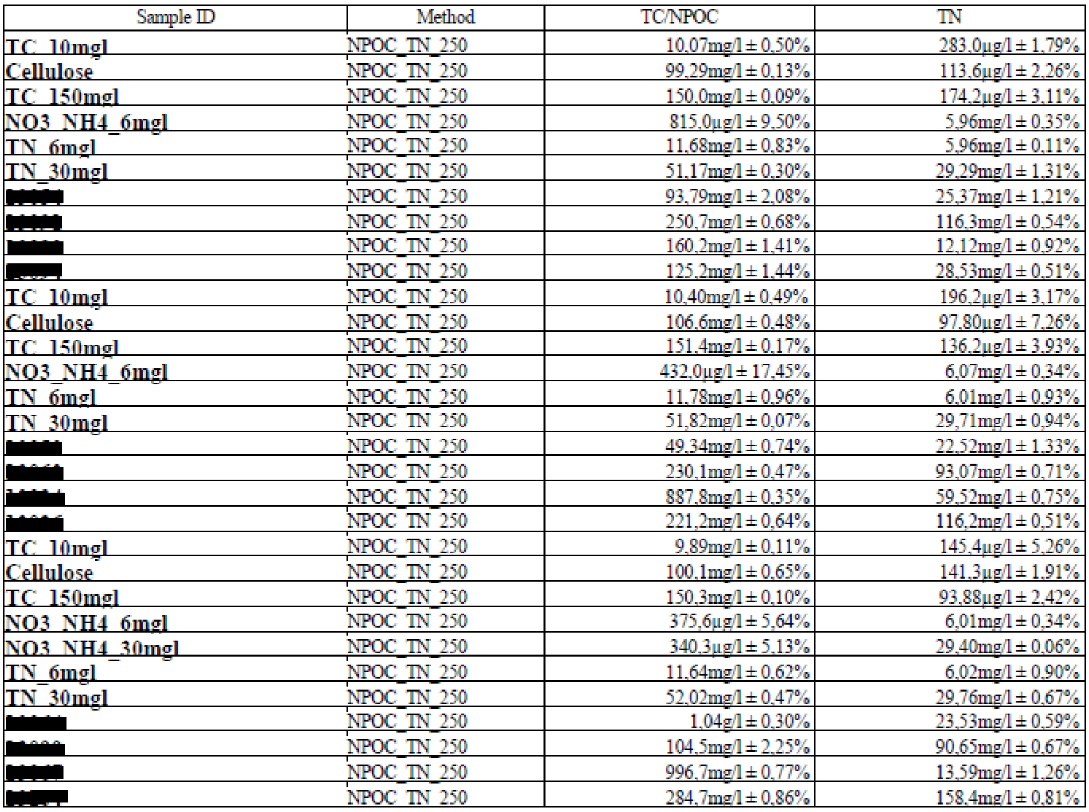
The following method settings were used to determine the NPOC and TN_b contents:

Parameter	multi N/C 2100S
Measurement parameter	NPOC/TN_b
Digestion	High-temperature digestion at 800 °C with platinum catalyst
Number of repetitions	min. 3, max. 4
Rinse with sample before injection	3 times
Sample purge time	300 sec.
Injection volume	250 µL

Table 1: Method settings

Results and Discussion

The table below shows the mean values of three replicate injections with relative standard deviations for different real samples (anonymized) and various recovery checks for different TN, TOC and particle suspension reference solutions (cellulose according to EN 1484, annex C), respectively.



Sample ID	Method	TC/NPOC	TN
TC_10mgl	NPOC_TN_250	10,07mg/l ± 0,50%	283,0µg/l ± 1,79%
Cellulose	NPOC_TN_250	99,29mg/l ± 0,13%	113,6µg/l ± 2,26%
TC_150mgl	NPOC_TN_250	150,0mg/l ± 0,09%	174,2µg/l ± 3,11%
NO3_NH4_6mgl	NPOC_TN_250	815,0µg/l ± 9,50%	5,96mg/l ± 0,35%
TN_6mgl	NPOC_TN_250	11,68mg/l ± 0,83%	5,96mg/l ± 0,11%
TN_30mgl	NPOC_TN_250	51,17mg/l ± 0,30%	29,29mg/l ± 1,31%
[redacted]	NPOC_TN_250	93,79mg/l ± 2,08%	25,37mg/l ± 1,21%
[redacted]	NPOC_TN_250	250,7mg/l ± 0,68%	116,3mg/l ± 0,54%
[redacted]	NPOC_TN_250	160,2mg/l ± 1,41%	12,12mg/l ± 0,92%
[redacted]	NPOC_TN_250	125,2mg/l ± 1,44%	28,53mg/l ± 0,51%
TC_10mgl	NPOC_TN_250	10,40mg/l ± 0,49%	196,2µg/l ± 3,17%
Cellulose	NPOC_TN_250	106,6mg/l ± 0,48%	97,80µg/l ± 7,26%
TC_150mgl	NPOC_TN_250	151,4mg/l ± 0,17%	136,2µg/l ± 3,93%
NO3_NH4_6mgl	NPOC_TN_250	432,0µg/l ± 17,45%	6,07mg/l ± 0,34%
TN_6mgl	NPOC_TN_250	11,78mg/l ± 0,96%	6,01mg/l ± 0,93%
TN_30mgl	NPOC_TN_250	51,82mg/l ± 0,07%	29,71mg/l ± 0,94%
[redacted]	NPOC_TN_250	49,34mg/l ± 0,74%	22,52mg/l ± 1,33%
[redacted]	NPOC_TN_250	230,1mg/l ± 0,47%	93,07mg/l ± 0,71%
[redacted]	NPOC_TN_250	887,8mg/l ± 0,35%	59,52mg/l ± 0,75%
[redacted]	NPOC_TN_250	221,2mg/l ± 0,64%	116,2mg/l ± 0,51%
TC_10mgl	NPOC_TN_250	9,89mg/l ± 0,11%	145,4µg/l ± 5,26%
Cellulose	NPOC_TN_250	100,1mg/l ± 0,65%	141,3µg/l ± 1,91%
TC_150mgl	NPOC_TN_250	150,3mg/l ± 0,10%	93,88µg/l ± 2,42%
NO3_NH4_6mgl	NPOC_TN_250	375,6µg/l ± 5,64%	6,01mg/l ± 0,34%
NO3_NH4_30mgl	NPOC_TN_250	340,3µg/l ± 5,13%	29,40mg/l ± 0,06%
TN_6mgl	NPOC_TN_250	11,64mg/l ± 0,62%	6,02mg/l ± 0,90%
TN_30mgl	NPOC_TN_250	52,02mg/l ± 0,47%	29,76mg/l ± 0,67%
[redacted]	NPOC_TN_250	1,04g/l ± 0,30%	23,53mg/l ± 0,59%
[redacted]	NPOC_TN_250	104,5mg/l ± 2,25%	90,65mg/l ± 0,67%
[redacted]	NPOC_TN_250	996,7mg/l ± 0,77%	13,59mg/l ± 1,26%
[redacted]	NPOC_TN_250	284,7mg/l ± 0,86%	158,4mg/l ± 0,81%

Table 2: Results

Conclusion

The measurements covered the analysis of undiluted wastewater samples with TOC concentrations up to almost 1 g/L and TN_b concentrations up to 160 mg/L. The analyses were performed with outstanding accuracy and precision. The cellulose test to check for particle handling according to EN 1484, as well as measurements of analytical quality assurance (AQA) standards for TOC and TN_b were performed frequently and showed convincing recovery rates. Especially the recoveries for 6 mg/L and 30 mg/L nicotinic acid TN_b standards prove a high performance for organically bound nitrogen.

The outstanding performance of multi N/C analyzers for such matrices like wastewater is based on the optimized combustion process with freely selectable combustion temperatures up to 950 °C. The direct injection with a septum-free pneumatic injection head in combination with a wide-bore needle of 0.7 mm inner diameter, as well as proper sample homogenization on the auto sampler rack and the valve- and tubing-free sample transfer into the combustion system further contribute to this performance. An operation mode keeping the stainless-steel injection needle in the oven head at elevated temperatures during peak integration time to assure complete evaporation of TOC components and a clean needle for further sample processing in combination with an effective rinsing of the microliter injection syringe minimize carry-over effects.

A high degree of automation combined with the well-proven Self Check System for trouble free unattended system operation make light work of TOC/TN_b analyses even in challenging samples. In addition, the patented VITA flow management system compensates flow fluctuations inside the system caused by sample evaporation, providing TOC calibration stability for up to one year and saving valuable measurement time.

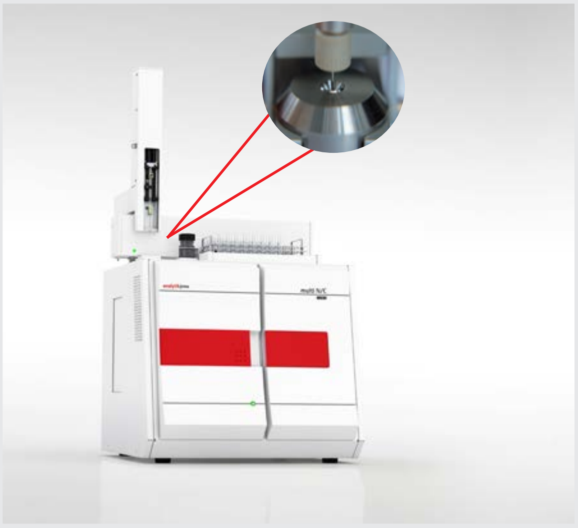


Figure 5: multi N/C 2100 S with APG60 and direct injection

References:

1) EN 1484 Water analysis – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)

2) EN 12260 Water quality – Determination of nitrogen Determination of bound nitrogen (TN_b) following oxidation to nitrogen oxides

3) ISO 20236 Water quality - Determination of total organic carbon (TOC), dissolved organic carbon (DOC), total bound nitrogen (TN_b) and dissolved bound nitrogen (DN_b) after high temperature catalytic oxidative combustion

Challenge

Reproducible and reliable determination of TOC and TN_b contents in demanding wastewater samples.

Solution

Fully automated and simultaneous TOC/TN_b measurement using catalytic high-temperature combustion and direct injection technology providing optimum particle handling and minimized carry-over.

TOC/TN_b Determination in Refinery Effluents

Introduction

The petrochemical industry generates large volumes of wastewater or effluents that need to be treated before they can be reused or released into natural waterways. Total organic carbon (TOC) and the total nitrogen bound (TN_b) content is routinely determined, as these contaminants lead to eutrophication of surface water recourses, endangering aquatic life and groundwater supplies.
According to the European Industrial Emissions Directive (IED)$^{1)}$, Best Available Techniques (BAT) have to be implemented within the EU for direct wastewater discharges from the refining of mineral oil and gas.$^{2)}$ The BAT reference document (BREF) indicates that, among other parameters, TOC and TN_b are of growing importance. They need to be monitored on a daily basis. Furthermore, a preference for the parameter TOC instead of COD is given, as TOC does not require the use of highly toxic compounds, such as dichromate (Cr VI) and mercury.

In many cases, the COD and TN contents are still measured using separate methods. This is a labor- and time-consuming process and is often associated with the formation of chromium-VI-contaminated waste. Through correlation studies, an empirical conversion factor for TOC to COD conversion can be established. Hence, a fully automated analytical process for TOC/TN_b determination according to EN 1484 and EN 12260 (also the planned new ISO 20236 for both parameters) can be applied to save resources and time.

Effluent waters from refining processes usually represent demanding samples for TOC/TN_b analyzers, since tubing and valve technique feeding the sample into the combustion process are prone to carry-over. Direct injection as applied in the multi N/C 2100S using a septum-free injection port and a wide-bore needle for optimum particle handling can overcome this problem. Direct injection ensures a sample transfer without particle losses and prevents blockages, hence increasing system uptime and reducing wear and tear on sensitive Teflon parts inside the dosing system.
In addition, the injection needle is fully thermally cleaned before injecting the next sample, since it stays in the hot furnace inlet zone during analysis time, thus reliably avoiding carry-over. multi N/C 2100S thus offers a robust solution for simultaneous TOC/TN_b analysis of particulate or oily samples.

Materials and Methods

Samples and reagents

Samples from different process streams and clean-up stages were collected and measured together with a reference standard.

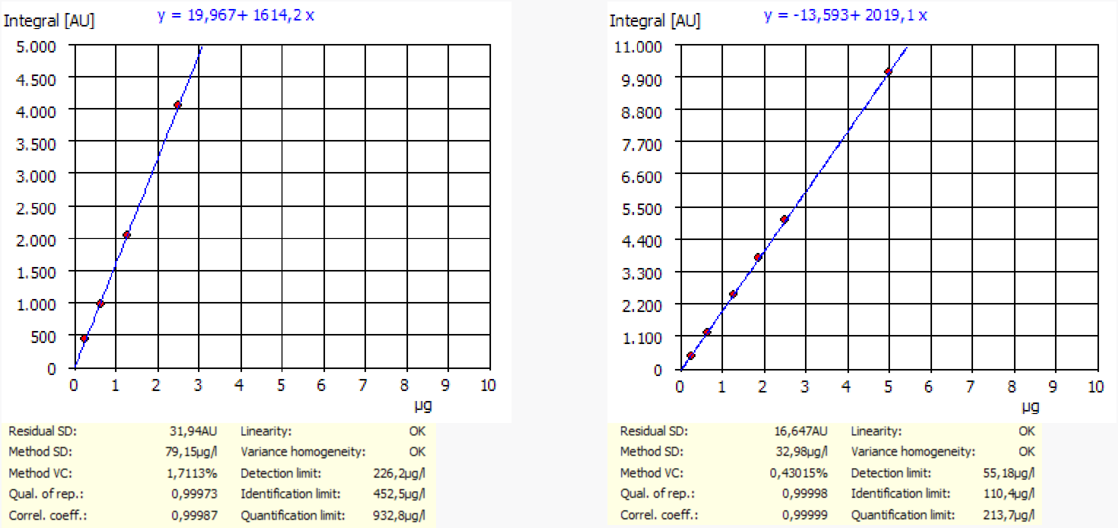
Sample preparation

The samples were stored in a refridgerator at 4 °C until analysis. For measurement, the samples were transferred into suitable autosampler vials. The wastewater samples were analyzed in direct mode using an NPOC/TN method.
Using 2 M HCl the samples were automatically acidified in the autosampler run and then automatically purged for 5 minutes for complete TIC removal. An injection volume of 250 µL was used for these measurement sequences. The samples were catalytically oxidized at a temperature of 800 °C in an oxygen-rich atmosphere. A combustion tube filled with platinum catalyst was used for complete sample oxidation. The formed nitrogen oxides were detected by means of a chemiluminescence detector (alternatively ChD detector can be utilized), CO_2 detection was done by focus radiation none-dispersive infrared detection (FR-NDIR).

Calibration

The multi N/C analyzer was calibrated between 1 and 500 mg/L with a potassium hydrogen phthalate standard solution for TOC determination. A multi-point calibration was used to evaluate the NPOC measurement results. For total bound nitrogen a calibration was carried out from 1 to 50 mg/L using an ammonium sulfate and a potassium nitrate (50:50 mix) solution according to the standard EN 12260.

Within the method up to three calibration ranges can be linked to each parameter in order to cover an over-all working range of up to three magnitudes. Detection limits and limits of quantification are depending on the selected working range and can be derived from the method characteristics given above.



Residual SD:	31,94AU	Linearity:	OK
Method SD:	79,15µg/l	Variance homogeneity:	OK
Method VC:	1,7113%	Detection limit:	226,2µg/l
Qual. of rep.:	0,99973	Identification limit:	452,5µg/l
Correl. coeff.:	0,99987	Quantification limit:	932,8µg/l

Residual SD:	16,647AU	Linearity:	OK
Method SD:	32,98µg/l	Variance homogeneity:	OK
Method VC:	0,43015%	Detection limit:	55,18µg/l
Qual. of rep.:	0,99998	Identification limit:	110,4µg/l
Correl. coeff.:	0,99999	Quantification limit:	213,7µg/l

Figure 1 and 2: Example of NPOC and TN calibration and method characteristics

Method parameters

The following method settings were used to determine the NPOC and TN_b contents:

Parameter	multi N/C 2100S
Measurement parameter	NPOC / TN_b
Digestion	High temperature digestion at 800 °C with platinum catalyst
Number of repetitions	min. 3, max. 4
Rinse with sample before injection	3 times
Sample purge time	300 sec.
Injection volume	250 µL

Table 1: Method parameters

Results and Discussion

The table on the right shows the mean values of three replicate injections with relative standard deviations for different real samples (anonymized) and recoveries of nicotinic acid used as TOC and TN_b reference solutions. According to the Best Available Techniques (BAT) reference document issued under the Industrial emission directive 2010 / 75 / EU1), the associated average emission levels (BAT-AEL) for direct waste water discharges from refining processes can be expected as in the following ranges:

COD: 30–125 mg/L equals to:
TOC: 7–32 mg/L
TN_b: 1–25 mg/L

Sample ID	NPOC Average [mg/L]	TOC RSD [%]	TN_b [mg/L]	TN_b RSD [%]
Sample 1	26.2	2.1	19.5	1.4
Sample 2	161	0.9	41.3	1.1
Sample 3	12.9	1.2	5.38	0.8
Check Standard Nicotinic Acid (TOC 20.0 / TN_b 3.88)	19.8	0.6	4.06	0.7

Table 3: Results

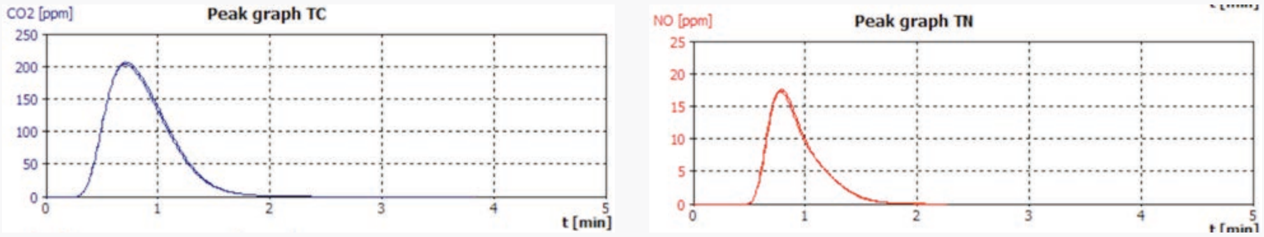


Figure 3a/b: Example for a TOC and TN_b measurement curve of sample 3

Conclusion

The measurements covered undiluted wastewater samples from different sampling points in the wastewater treatment process and with varying TOC and TN_b concentrations. All samples were measured with outstanding accuracy and precision. Nicotinic acid was used as an analytical quality-assurance standard (AQA) to simultaneously check for TOC and TN_b recoveries. Very good recoveries were achieved for the reference material for organically bound nitrogen.

This outstanding performance of multi N/C analyzers for such demanding wastewater matrices is based on the optimized combustion process with freely selectable combustion temperatures up to 950 °C. The direct injection with a septum-free pneumatic injection head in combination with a wide-bore needle of 0.7 mm inner diameter, as well as proper sample homogenization on the auto sampler rack and the valve- and tubing-free sample transfer into the combustion system, contribute to this performance. An operation mode keeping the stainless-steel injection needle in the oven head at elevated temperatures during peak integration time to assure complete evaporation of TOC components and a clean needle for further sample processing in combination with an effective rinsing of the microliter injection syringe minimize carry-over effects.

The high degree of automation due to the applied AS 60 autosampler combined with the well-proven Self Check System for trouble-free unattended system operation make light work of TOC/TN_b analyses, even in challenging samples. In addition, the patented VITA flow-management system compensates flow fluctuations inside the system caused by sample evaporation, providing TOC calibration stability for up to one year and saving valuable measurement time.

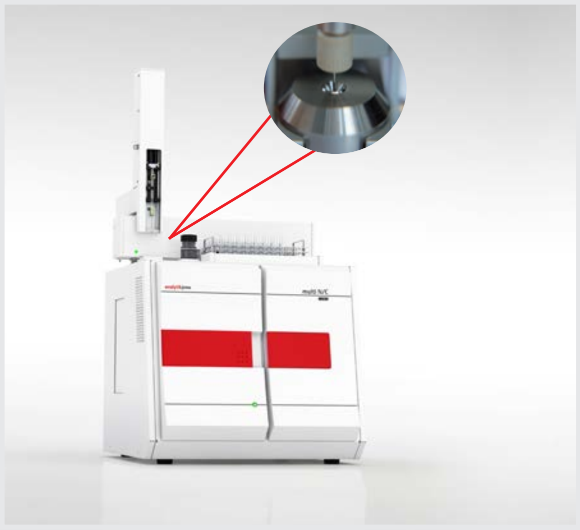


Figure 4: multi N/C 2100 S with APG60 and direct injection

References

1) DIRECTIVE 2010/75/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 24 November 2010 on industrial emissions (integrated pollution prevention and control)

2) Official Journal of the European Union, L 307/38, 28.10.2014, Commission Implementing Decision of October 9, 2014 "Establishing best available techniques (BAT) conclusions, under Directive 2010/75/EU of the European Parliament and of the Council on industrial emissions, for the refining of mineral oil and gas"

3) EN 1484 Water analysis – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)

4) EN 12260 Water quality – Determination of nitrogen Determination of bound nitrogen (TN_b) following oxidation to nitrogen oxides

5) ISO 20236: Water quality – Determination of total organic carbon (TOC), dissolved organic carbon (DOC) total bound nitrogen (TN_b) and dissolved bound nitrogen (DN_b) after high temperature catalytic oxidative combustion

Challenge

Reproducible and reliable determination of TOC and TN_b contents in particle-loaded wastewater samples.

Solution

Fully automated and simultaneous TOC/TN_b measurement using direct injection technology providing optimum particle handling and minimized carry-over.

TOC/TN_b Determination in Pulp and Paper Process Effluents

Introduction

The pulp and paper industry generates large volumes of wastewater or effluents that need to be treated before they can be reused or released into natural waterways. According to the European Industrial Emissions Directive (IED) Best Available Techniques (BAT) have to be implemented within the EU for direct wastewater discharges from pulp, paper, and card production1). The BAT reference document (BREF) indicates that among other parameters, TOC (total organic carbon) and TN_b (total bound nitrogen) are of growing importance. They need to be monitored on a daily or weekly basis. Furthermore, for economic and environmental reasons a preference for the parameter TOC instead of chemical oxygen demand (COD) is given as it does not require the use of highly toxic compounds like dichromate (Cr VI) and mercury.

In many cases the COD and TN contents are still measured using separate methods. This is a labour and time-consuming process. The process is also associated with the formation of chromium VI and mercury-contaminated waste. By correlation studies an empirical conversion factor for TOC to COD conversion can be established for specific emission sources and wastewater treatment steps. This allows a fully automated analysis process for TOC/TN_b determination according to EN 14842) and EN 122603) (or ISO 202364) for both parameters) to be applied, which saves resources and time.

Since process effluents from pulp and paper production often contain a large amount of solids (cellulose fibers), direct injection technology has proven particularly effective for TOC/TN_b analysis. The multi N/C 2100S does not require hoses or valve technology, but doses the sample directly into the combustion chamber via a septum-free injection port and a wide bore needle using a microliter syringe. The direct injection technique provides a safe and reproducible particle transfer without the risk of clogging or carry-over. This increases instrument availability, reduces wear and tear, and increases sample throughput and efficiency.

Materials and Methods

Samples and reagents

- Samples from different product processing and clean-up stages as well as raw water used in production were analyzed
- 2 M HCl was used for automatic acidification in NPOC mode

Samples preparation and measurement

The samples were stored in a refrigerator at 4 °C until analysis. For the measurement, the samples were transferred into 8 mL autosampler vials. The wastewater samples were analyzed in direct mode using an NPOC/TN method. The sampler AS 60 with 60 sample positions was used for automated measurement.

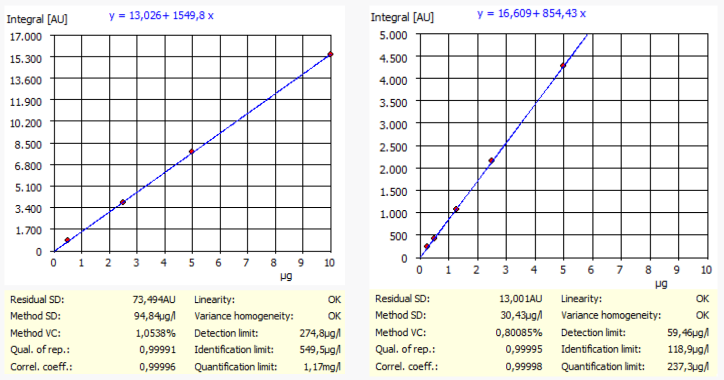
The samples were automatically acidified to a pH <2 using 2 M HCl, and subsequently purged to remove any inorganic carbon (TIC). The complete TIC elimination from the sample can be checked by TIC control measurement. A representative injection volume of 500 µL was transferred into the combustion tube for measurement. The samples were completely oxidized at a temperature of 800 °C in an oxygen-rich atmosphere using a platinum catalyst. The formed CO_2 was quantitatively determined by focus radiation non-dispersive infrared detection (FR-NDIR). Simultaneously the nitrogen oxides were detected by means of an electrochemical chemiluminescence detector (alternatively, the ChD detector can be used for total bound nitrogen (TN_b) determination.

Calibration

Within the method up to three calibration ranges can be linked to each parameter in order to cover an over-all working range of up to three magnitudes. Detection limits and limits of quantification are depending on the selected working range and can be derived from the method characteristics given above.

Instrumentation

The analysis was performed on a multi N/C 2100S using an NPOC/TN method.



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Residual SD:        73,494AU  Linearity:                   OK
Method SD:          94,84µg/l  Variance homogeneity:        OK
Method VC:          1,0538%   Detection limit:       274,8µg/l
Qual. of rep.:      0,99991   Identification limit:  549,5µg/l
Correl. coeff.:     0,99996   Quantification limit:   1,17mg/l
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Residual SD:        13,001AU  Linearity:                   OK
Method SD:          30,43µg/l  Variance homogeneity:        OK
Method VC:          0,80085%  Detection limit:        59,46µg/l
Qual. of rep.:      0,99995   Identification limit:   118,9µg/l
Correl. coeff.:     0,99998   Quantification limit:   237,3µg/l
```

Figure 1/2: Example of NPOC and TN calibration and method characteristics

Method Parameters

The following method settings were used to determine the NPOC and TN_b contents:

Parameter	multi N/C 2100S
Measurement parameter	NPOC / TN_b
Digestion	High temperature digestion at 800 °C with platinum catalyst
Number of repetitions	min. 2, max. 3
Rinse with sample before injection	3 times
Sample purge time	300 sec.
Injection volume	500 µL

Table 1: Method settings

Results and Discussion

Table 2 shows blank value corrected mean values from at least two repeat measurements for different real samples and recoveries for TOC and TN_b reference solutions. Dilution ratios are also reported. For informative purposes, the COD contents provided for the respective samples and the TOC-COD correlation factors calculated from them were also given.

Sample ID	COD	NPOC ± RSD [mg/L] ± [%]	TN_b ± RSD [mg/L] ± [%]	TOC-COD correlation factor	Dilution factor
Raw water	8,0	2.79 ± 1.84	3.50 ± 1.02	2.86	-
EVII	8,9	2.99 ± 0.71	3.49 ± 0.68	2.98	-
EX	365	120.5 ± 0.18	4.60 ± 0.26	3.04	-
ZF	4302	1480 ± 0.08	15.36 ± 1.89	2.91	1:10
Sedimat	4450	1580 ± 0.47	15.50 ± 0.08	2.82	1:10
ZA	365	1600 ± 0.04	39.63 ± 1.33	3.17	1:10
AA	4302	956.5 ± 2.01	55.05 ± 1.66	3.27	1:10
BK	4450	3230 ± 0.10	11.97 ± 2.18	2.55	1:100
Check Standard Nicotinic Acid (TOC 20.0 / TN_b 3.88)	365	20.2 ± 0.9	3.97 ± 0.8	-	-

Table 2: Results

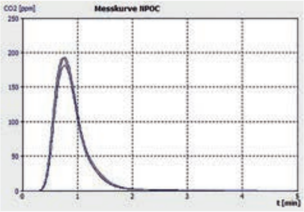


Fig. 3: Peak graph NPOC BK

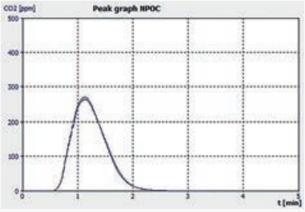


Fig. 4: Peak graph NPOC EX

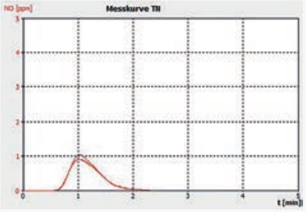


Fig. 5: Peak graph TN_b BK

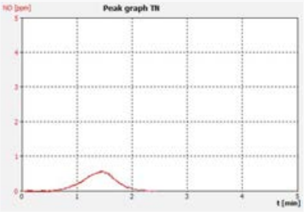


Fig. 6: Peak graph TN_b EX

Conclusion

The analyzed samples represent a typical range of process effluents from different process stages and sampling points in the wastewater treatment process, as well as raw water for paper production. Despite the very different TOC and TN_b concentrations, all samples were measured with outstanding accuracy and precision. Nicotinic acid was used as an analytical quality assurance standard (AQA) to simultaneously check for TOC and TN_b recoveries. Very good recoveries were achieved with this reference material for organically bound nitrogen.

This exellent performance of multi N/C analyzers for such demanding wastewater matrices is based on the optimized combustion process with freely selectable combustion temperatures up to 950 °C. The direct injection with a septum-free pneumatic injection head in combination with a wide-bore needle of 0.7 µm inner diameter, as well as proper sample homogenization on the auto sampler rack and the valve- and tubing-free sample transfer into the combustion system contribute to this. An operation mode keeping the stainless steel injection needle in the oven head at elevated temperatures during peak integration time to assure complete evaporation of TOC components and a clean needle for further sample processing in combination with an effective rinsing of the microliter injection syringe minimize carry-over effects.

A high degree of automation combined with the well-proven self-check system for trouble-free unattended system operation make light work of TOC/TN_b analyses, even in challenging samples. In addition, the patented VITA flow management system compensates flow fluctuations inside the system caused by sample evaporation, providing TOC calibration stability for up to one year and saving valuable measurement time.

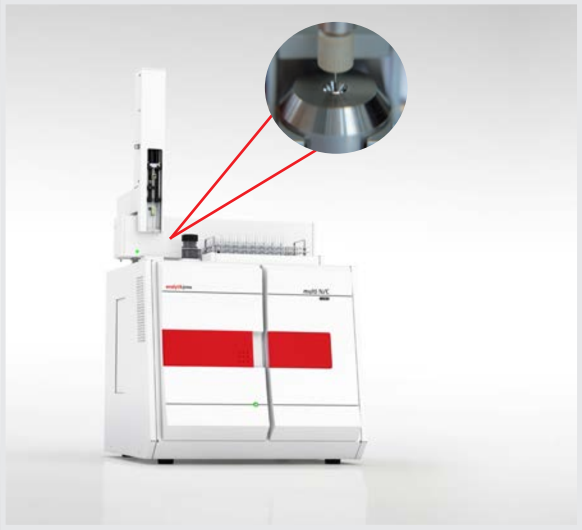


Figure 7: multi N/C 2100 S with APG60 and direct injection

References

[1] Official Journal of the European Union, L 284/76, 30.09.2014, Commission Implementing Decision of 26. September 2014 "Establishing best available techniques (BAT) conclusions, under Directive 2010/75/EU of the European Parliament and of the Council, for the production of pulp, paper and board"

[2] DIN EN 1484 – Water analysis - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC); German version EN 1484:1997

[3] DIN EN 12260 – Water quality - Determination of nitrogen - Determination of bound nitrogen (TN_b), following oxidation to nitrogen oxides; German version EN 12260:200

[4] ISO 20236 – Water quality — Determination of total organic carbon (TOC), dissolved organic carbon (DOC), total bound nitrogen (TN_b) and dissolved bound nitrogen (DN_b) after high temperature catalytic oxidative combustion

Challenge

Passing the linearity test according to DIN EN ISO 9562.

Solution

Fast and cost-effective procedure applying adsorption of the AOX samples on an automatic preparation unit followed by analysis with an AOX analyzer.

AOX Linearity Test According to DIN EN ISO 9562 (Column Method)

Introduction

AOX (AOX - adsorbable organically bound halogens) is an analytical convention which represents the sum of all organically bound halogens (except fluorine), mainly used in water and waste water analysis. Solid samples like sludge or sediments can also contain AOX. The solid AOX however has to be determined by the batch method which is not part of this application. The compounds containing halogens are adsorbed onto the surface of activated carbon (charcoal) in dissolved or suspended form. The activated carbon is washed afterwards with aqueous nitrate washing solution to remove inorganic halides. During the combustion of the loaded charcoal in pure oxygen at high temperatures hydrogen halides are formed. After absorption of the halides the final detection takes place inside a coulometric cell by means of argentometric titration.

Materials and Methods

Instrumentation

A multi X 2500 AOX analyzer equipped with an autoX36 auto sampler was used in combination with an automatic sample preparation unit of the APU series. Thanks to the combination of sample preparation and analysis idle time is minimized and sample throughputvis increased. The multiWin software allows fast sample analysis and data evaluation simultaneously. In combination with the Self Check System the multi X 2500 ensures trouble-free and fully automated operation.

Samples and reagents

The following reagents were used for sample and standard preparation

- Deionized water/ ultra-pure grade
- Nitric acid >65% p.a.
- p-chlorophenol aqueous stock solution; 1 mg/L Cl; (Bernd Kraft GmbH)

Samples preparation and measurement

The p-chlorophenol stock solution was diluted with ultra-pure water according the ISO 9562 to obtain different concentration as follows: 10, 50, 100, 200, and 250 µg/L. 100 mL of these solutions was adjusted to pH 2 with concentrated nitric acid. The low pH value inhibits bacterial growing and enables the storage of the samples at 0 to 4 °C.

The determination was carried out using a multi X 2500 AOX analyzer. The adsorption of the AOX samples was performed on an automatic preparation unit of the APU series. p-chlorophenol was used as organic standard solution to evaluate the system and method in terms of sample recovery. For this a linearity test was performed. According to the column method, two quartz glass containers filled with activated carbon were used. Both are positioned vertically, one behind the other. The different standard solutions were adsorbed with a flow rate of 3 mL/min. After rinsing with 25 mL washing solution at the same flow rate the columns with the loaded charcoal were combusted at 950 °C in pure oxygen stream.

Results and Discussion

According to DIN EN ISO 9562 the results are acceptable if the correlation coefficient is ≤ 0.999. The slope of the linearity should be within 0.95 and 1.05. The results of the determinations are shown in Table 1.

Standard concentration [µg/L]	Abs. value 1st column [µg Cl]	Abs. value 2nd column [µg Cl]	Blank value [µg Cl]	Recovery [µg/L]	Average [µg/L]
10	1.08	0.06	0.265	8.75	**9.65**
10	1.08	0.24	0.265	10.55	
50	4.86	0.09	0.265	46.85	**47.7**
50	5.01	0.11	0.265	48.55	
100	9.63	0.17	0.265	95.35	**96.0**
100	9.79	0.14	0.265	96.65	
200	19.38	0.27	0.265	193.85	**194.1**
200	19.35	0.35	0.265	194.35	
250	24.02	0.33	0.265	240.85	**242.65**
250	24.43	0.28	0.265	244.45	

Table 1: Results of the linearity test of the AOX determination of p-chlorophenol in water

The recovery rates, correlation coefficient, and slope of the linearity test fulfill the specifications of the DIN EN ISO 9562. This confirms a correct adsorption behavior of the APU system and the excellent analytical performance of the analyzer.

Min. value [µg/L]	Theoretical AOX value [µg/L]	Max. value [µg/L]	Obtained AOX value [µg/L]
9	10	11	9.7
45	50	55	47.7
90	100	110	96.0
180	200	220	194.1
225	250	275	242.7

Table 2: Standard recovery of p-chlorophenole

The slope of the recovery function is rounded 0.97 and the correlation coefficient is > 0.999.

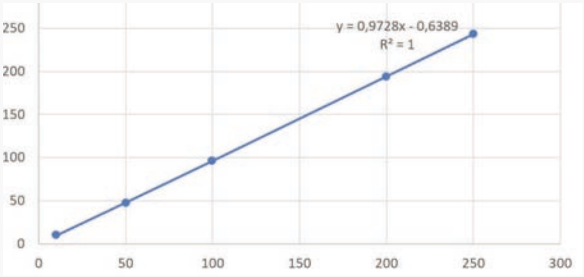


Figure 1: Regression linearity test

Conclusion

This linearity test shows that the multi X 2500 AOX analyzer and the APU sample preparation unit provide accurate standard recovery. This enables fast and reliable analysis for water quality monitoring. Due to the high automation level, the APU sample preparation unit allows fast sample preparation without any manual handling. An overnight sample treatment followed by analysis with multi X 2500 during day time is extremely timesaving. The AOX analysis by column method is cost-effective compared to the time intensive batch method.

Figure 2: multi X 2500 with autoX autosampler

Challenge

Quick and reliable adsorption on activated carbon using column method to save time for sample preparation.

Solution

With the APU sample preparation units series even challenging wastewater samples can be adsorbed very fast onto activated carbon columns, fully compliant with the relevant international AOX regulations.

Determination of AOX in Wastewater Samples by Column Method According to DIN EN ISO 9562

Introduction

The parameter AOX (adsorbable organically bound halogens) is mainly used for monitoring water quality, but it also plays an important role in the evaluation of solid waste (e.g., in aqueous waste eluates) and sewage sludge. It is of interest for municipal sewage treatment plants, environmental surveillance authorities, but also for industrial companies, which directly and indirectly discharge their wastewaters. Industrial dischargers are obliged to check that the wastewater they discharge into the environment resp. into a public sewage treatment plant does not exceed the industry-specific AOX limit value. Sewage treatment plants must not only monitor compliance with the limit values but also the effectiveness of their treatment process by comparing the AOX content before and after treatment. Finally, state authorities ensure that the pollution of surface waters with organically bound halogens is kept as low as possible.

The determination of AOX is an analytical convention that describes the sum of organically bound chlorine, bromine, and iodine (but not fluorine), which can be adsorbed on activated carbon under precisely defined conditions. In case of unfiltered water samples, also AOX contents adsorbed on suspended solids are included in the result. The parameter is used internationally and therefore standardized, e.g., according to ISO 9562, which is applied in many countries. The ISO 9562 describes three adsorption procedures with activated carbon for AOX determination: the stirring, the shaking and the column method. The choice of the adsorption method is determined by laboratory or administrative regulations. In many countries the determination of AOX in wastewater samples using the column method is mandatory.

The *stirring method*, also called carbodisc method, requires very experienced lab personnel and high manual effort. For these reasons it is rarely used in routine labs. Furthermore, it is not very suitable for water samples containing particles, like typical wastewater samples do. The shaking or *batch method*, for example, is mandatory for sewage sludge and other solid samples, but also often applied to water samples. However, the major disadvantages of this method are its limited degree of automation, a higher risk of contamination from the lab environment, and last, but not least, a missing simple control possibility regarding the completeness of the AOX adsorption step. The batch method is not recommended to be used for water samples where (inorganic) chloride concentration cannot be reduced to a value ≤ 500 mg/L Cl^- by dilution.

The *column method* enables the adsorption not only of particle-free, but also of particle-containing samples and offers a lot of advantages for the user especially for challenging wastewater samples. Besides a simple possibility to control the completeness of the AOX adsorption step, it is more robust for samples with high chloride concentrations (up to 1 mg/l Cl^-), because of a more effective charcoal rinse procedure by the nitrate washing solution. Furthermore, it allows for a high degree of automation, which results in a high sample throughput and minimal maintenance effort. The risks of contamination and handling errors are reduced by using prefilled AOX columns for adsorption, which minimizes interaction with the lab atmosphere. For particle-containing samples there is the possibility of fitting a filter column upstream of the columns filled with activated carbon. This ensures that the particles are separated and thus also prevents the activated carbon columns from potentially becoming clogged.

Materials and Methods

Samples and reagents

- Wastewater samples of different origin
- Standard solution p-chlorophenol cont. 100 µg/L AOX
- Prefilled columns with 50 mg activated carbon
- Concentrated HNO_3
- $NaNO_3$ stock and washing solution
- 0.01 N HCl for performance check of the AOX analyzer

Sample preparation: APU sim	Settings
Sample volume	100 mL
Nitrate washing solution	25 mL
Flow rate	3 mL/min

Table 1: Column method settings

Sample preparation

Three different water samples were analyzed, each in two different dilutions. One sample was obtained from an industrial water treatment plant (effluent) with a DOC (dissolved organic carbon) content of approx. 50 mg/L. Another sample with a high particle load and a high DOC content (about 1 g/L) was a raw sewage from a chemical plant. This wastewater with a complex matrix contained approx. 10 g/L of inorganic chloride. The third wastewater was a sample from a German round robin test, containing a small particle load. All samples were diluted in two different ratios to demonstrate the advantages of the column method when samples with difficult matrices (high particle load, high content of chloride, high amount of DOC) are adsorbed. According to DIN EN ISO 9562 results for two different dilutions of one and the same sample should not differ by more than 10%.

The wastewater sample with high particle load was homogenized prior to adsorption by using a high-speed stirrer (disperser). After dilution all samples were adjusted to a pH value of 2 with concentrated nitric acid. Finally, 5 mL of nitrate stock solution was added to 100 mL of each diluted and acidified sample. Prepared samples were filled into syringes and placed onto the APU sim sample preparation unit. Adsorption was carried out automatically by passing the samples through the activated carbon columns (filled with approx. 50 mg activated carbon each) at a flow rate of 3 mL/min, followed by automatic rinsing with nitrate washing solution at the same flow rate. All sample preparation steps were compliant to the procedure for column method as regulated by the DIN EN ISO 9562.

Instrumentation

Sample preparation with the APU sim sample preparation unit was completed within approximately 45 minutes for all six diluted samples due to its simultaneous operation mode. The final AOX determination was carried out using the multi X 2500 AOX analyzer in vertical operation mode. For the automated sample supply the autoX 36 sampler was used. The prepared AOX columns were placed into the tray of the autoX 36. This sampling system automatically introduces the loaded columns into the vertically arranged open AOX combustion tube. The furnace temperature of 950 °C was chosen in compliance with DIN EN ISO 9562. During combustion the organic halogen compounds were converted to HCl gas. After drying with concentrated sulfuric acid, the HCl gas was transferred into the micro coulometric measuring cell. The cell has a wide working range from 1 µg to 100 µg chlorine absolute. The chlorine detection was carried out by means of argentometric titration. Proper operation of the analysis system was confirmed by analysis of a commercial AOX standard solution (4-chlorophenol in water). As micro coulometric chlorine determination is an absolute method, no calibration of the analyzer is needed.

multi X 2500	Specification
Furnace temperature	> 950 °C
Titration delay	240 s
Maximum titration time	1200 s
Cell temperature	18–35 °C
Carrier gas	Oxygen 99.995% (4.5)
Working range coulometric cell “sensitive”	1 µg–100 µg Cl abs.
Entire working range	10 ng–1000 µg Cl abs.
Sample supply	autoX 36 resp. autoX 112

Table 2: Process and detection parameter settings

Results and Discussion

The three different wastewater samples were each analyzed twice in two different dilutions. The results and the measurements of a commercial AOX standard are summarized in Table 3. Typical measuring curves are shown in Table 4. The different dilution ratios deliver comparable results with deviations far less than 10%, thus demonstrating the general suitability of the column method for each single wastewater sample.

Sample Name	Adsorption Volume [mL]	Dilution ratio	Column 1 [µg] Cl abs.	Column 2 [µg] Cl abs.	Blank Value [µg] Cl abs.	Result AOX [µg/L]
Effluent	100	1 in 2	4.91	0.63	0.30	104.8
	100	1 in 5	2.10	0.41	0.30	110.5
Industrial wastewater	100	1 in 10	9.05	4.22	0.30	1297
	100	1 in 50	2.42	0.54	0.30	1330
Wastewater round robin test, 583.3 µg/L		1in 5	11.68	0.20	0.30	579
		1 in 10	6.03	0.11	0.30	584
AOX standard 100 µg/L	100	none	10.40	0.23	0.30	103.3

Table 3: AOX results of different water samples in different dilutions

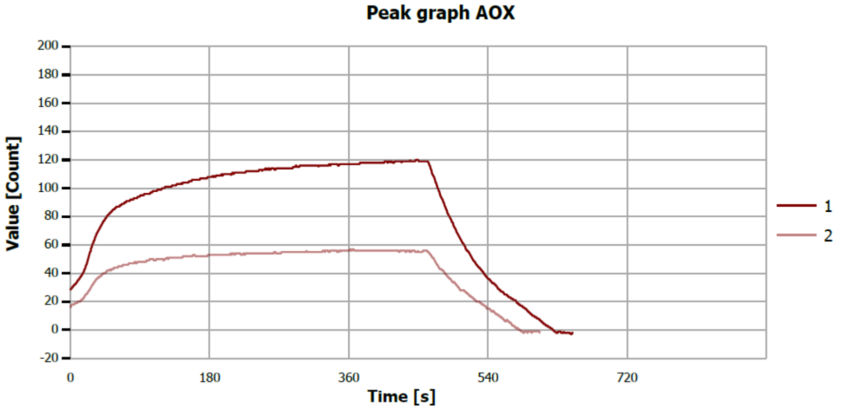
Sample Name	Measuring curve showing column 1 and column 2
Industrial wastewater, dilution ratio 1 in 10 Column 1 (9.05 µg Cl abs.) Column 2 (4.22 µg Cl abs.)	

Table 4: Example of a measuring curve

The curves illustrate quite clearly the analytical advantage of the column method compared with the batch method. Both curves represent an absolute amount of AOX (expressed as Cl), which has been adsorbed on the surface of the activated carbon. The quality of the adsorption can be assessed through the ratio of the absolute contents to each other. As a general rule, the first column contains several times more AOX than the second column, which means that the first curve is "higher" than the second one. This is called a complete adsorption. Ideally, the absolute content of the second column is in the blank value range of the activated carbon. Depending on the type of the water sample, specific matrix components might interfere with the adsorption process. In such a case, the absolute amounts of Cl of the individual columns can be equal or the second column can be even higher than the first. This is called a „breakthrough" of the AOX on the activated carbon.

The example curves of the industrial wastewater sample are showing the influence of the complex matrix of this sample: column 2 contains nearly 50% of the content of column 1, so a „breakthrough" can be assumed. A second analysis with the same sample but in a higher dilution ratio helps to verify whether the adsorption process was complete or if an AOX "breakthrough" has occurred. The result of the 1 in 50 diluted sample (1330 µg/L AOX), where only approx. 20% of column 1's content can be found on column 2, is nearly the same as for the less diluted sample (1297 µg/L AOX). This finally confirms that no AOX "breakthrough" took place during the sample adsorption step. Experienced users can easily judge the quality of AOX results by evaluating the curves of the two columns. Often there is no need for a repeated analysis of the same sample in a higher dilution ratio. When applying the batch method to such a sample, no statements can be made about the completeness of the adsorption step, since only one aliquot of activated carbon is used for AOX adsorption and measurement by the batch method. Therefore, it is a must to run replicates with different dilution ratios to avoid falsified results.

Figure 1: APU sim

Figure 2: APU 28

Conclusion

The provided measurement results show that sample preparation by column method provides reproducible AOX results for different types of challenging wastewater samples. Even samples with high particle loads and complex matrices (high salt and DOC levels) could be adsorbed onto activated carbon quickly and reliably. The APU sim sample preparation unit enables to process six samples simultaneously in less than one hour without manual intervention. Compared to the time and labor-intensive batch method, the AOX analysis according to the column method is faster and cost-effective.

For larger sample series or higher throughput demand, the versatile systems of the APU 28 series are recommended. They enable automatic AOX sample preparation according to the column method for up to 28 samples. The sequential mode of operation, with overnight adsorption of the samples by APU 28 and subsequent analysis of the enriched AOX columns with the multi X 2500 during the day, is not only extremely time-saving, but also guarantees a high sample throughput around the clock.

Figure 3: multi X 2500 with autoX autosampler

References

1) DIN EN ISO 9562:2004 "Water quality – Determination of adsorbable organically bound halogens (AOX)"

Challenge

Fast and trouble-free filtration of activated carbon / solid sample mixtures after the adsorption step of the shaking procedure in accordance with the requirements of EN 16166.

Solution

Significant increase of the sample throughput by using the automatic filtration unit AFU 3.

Determination of AOX in Sewage Sludge Samples by Batch Method According to EN 16166

Introduction

Sewage sludge is a type of waste that is produced after wastewater treatment. It is composed of organic and mineral components, which are present in both dissolved and solid form. There are two basic types of sewage sludge: the raw sewage sludge and the treated sewage sludge, whereby the treatment serves the further utilization or disposal of the sewage sludge. Here procedures like anaerobe decomposition (in digestion towers), thickening, drainage, or drying are used.
The forms of utilization for treated sewage sludge include its use as fertilizer, landfilling, and incineration, which can also be used to generate energy. Sewage sludge incineration (or co-incineration) represents the largest share of the individual types of recovery/disposal. All forms of recycling are subject to country-specific laws and regulations.

In addition to toxic heavy metals, such as chromium (VI), sewage sludge can contain a variety of organic pollutants that can be toxic, carcinogenic, or mutagenic. These substances include, among others, the substance classes of halogenated hydrocarbons (determined as sum parameters AOX – adsorbable organically bound halogens), PCBs (polychlorinated biphenyls), and PCDD/PCDFs (polychlorinated dibenzodioxins and dibenzofurans). Even if these substances are initially present in wastewater in low concentrations, they can be concentrated by processing the sewage sludge and reintroduced into the food chain by using it as fertilizer on arable land, for example. By setting limit values for certain elements (e.g., heavy metals) and organic compounds (e.g., AOX), sewage sludge and its recycling is well monitored by regulations in many countries. In the European Union, the directive 86/278/EEC applies and in Germany, the so-called sewage sludge ordinance (AbfKlärV) ensures that both, humans and the environment are protected. AOX determination in sewage sludge is therefore one of the standard analytical procedures in environmental and agricultural laboratories as well as in laboratories of surveillance authorities.

EN 16166 describes a method for the determination of AOX in sludge, treated biowaste, soil, and sediments, which is widely used for the determination of AOX in sewage sludge. The AOX in this standard method is defined as the amount of chlorine, bromine, and iodine (not fluorine) present as organic compounds in the sample matrix and determined under the specified conditions. The result is expressed as chloride. The basic principle of the method is based on an enrichment step of the targeted analytes on activated carbon, followed by the combustion of the sample / activated carbon mixture in an oxygen stream at high temperatures. The hydrogen halides formed in this process are absorbed and determined e.g., by microcoulometry. The enrichment step of activated carbon is carried out by the shaking method. Here, activated carbon and the solid sample are brought into intensive contact, with the addition of a nitrate solution they are shaken for one hour in a glass vessel. This first sample preparation step can be very well automated with the help of so-called orbital shakers where many samples can be processed in parallel or "in a batch". The shaking is followed by the filtration of each individual sample in order to bring it into a feeding form suitable for the analyzer. Different filter materials can be used for the filtration step. In addition to commercially available membrane filters (here polycarbonate 0.45 µm), suitable other filter materials made of ceramic or quartz can also be used. During filtration, it is essential to avoid dry suction of the samples in order to reliably prevent contamination by the ambient air. This fact alone makes it clear that automation of the filtration step is difficult to achieve; the user must filter each individual sample in a controlled manner. Here, the use of the automatic filtration unit AFU 3 has proven well suited, which allows to filter three samples almost simultaneously but controlled by the user at any time.

Materials and Methods

Samples and reagents

- Sewage sludge samples, dried and homogenized
- Standard solution p-chlorophenol containing 100 µg/L AOX
- Activated carbon, grain size approx. 10 to 50 µm
- $NaNO_3$ stock and washing solution
- 0.01 N HCl for the performance check of the AOX analyzer

Sample preparation

Three different sewage sludge samples were examined for their AOX content, two of which were samples from a German interlaboratory test. All samples were available in dried and homogenized condition. According to DIN 16166, 10 to 100 mg of the sample material were weighed into an Erlenmeyer flask with a nominal volume of 25 mL. Approximately 20 mg activated carbon and 10 mL nitrate stock solution were then added to each sample vessel. The prepared samples were then placed on a mechanical shaker (orbital shaker) and shaken intensively for one hour. Subsequently, the samples were filtered with the automatic filtration unit AFU 3. The sludge / activated carbon mixture was completely transferred into a quartz container filled with ceramic filter material. The filter cake was rinsed in portions with nitrate washing solution to completely remove inorganic chloride from the sample / carbon mixture. A total of 25 mL nitrate solution was used for each. The filtration was carried out by means of overpressure generated by an integrated powerful pump. All sample preparation steps were compliant to the procedure for the shaking method described in DIN EN 16166.

Instrumentation

The three sewage sludge samples, each shaken with three different weights (approx. 10 mg, approx. 50 mg and approx. 100 mg), were available for analysis on the AOX analyzer multi X 2500 within a few minutes thanks to the simultaneous filtration with the AFU 3. The multi X 2500 was operated in vertical mode, the sample containers were automatically fed into the combustion system of the analyzer by means of the autoX 36 sampler. In order to achieve a quantitative conversion of all analytes, a so-called pre-combustion adapter was used for the incineration of the sewage sludge samples (which usually have a high organic load). This adapter allows the controlled drying and pre-digestion of the sample first at low temperature in the upper part of the vertical furnace. After a defined pre-combustion time (here two minutes) the sample is automatically transferred to the hot zone of the furnace. According to DIN EN 16166, a combustion temperature of 950 °C was set on the analyzer. Under these conditions the organically bound halogens are completely converted to hydrogen halides. These in turn are first transferred by the carrier gas to the drying section (concentrated sulfuric acid) and finally introduced into the coulometer cell. Here the halide ions are detected by argentometric titration. The result is given as chloride and represents the AOX content of the sample. It is not necessary to calibrate the analyzer because coulometric titration is one of the absolute methods. To prove the functionality of the AOX instrument, control standards and blank values are measured every working day.

AOX analyzer multi X 2500	Specification
Combustion temperature	> 950 °C
Pre-combustion time	2 min
Titration delay	240 s
Maximum titration time	1200 s
Cell temperature	18–35 °C
Carrier gas	Oxygen 99.995% (4.5)
Working range coulometric cell "sensitive"	1 µg–100 µg Cl abs.
Extended working range	10 ng–1000 µg Cl abs.
Automation	autoX 36

Table 1: Process parameters

Results and Discussion

The three different sewage sludge samples were each analyzed three times, using three different weights. The results as well as the test of a commercial AOX standard are summarized in Table 2. A typical measurement curve is shown in Figure 1.

Sample ID	Sample weight/ Volume	Amount inside the quartz container [µg] Cl abs.	Blank Value [µg] Cl abs.	Result AOX	Mean Value AOX ± Standard Deviation	RSD [%]
Sewage sludge 1 Interlaboratory test sample 75.2 mg/kg	13.2 mg	1.60	0.56	78.8 mg/kg	77.4 ± 1.2 mg/kg	1.6
	50.7 mg	4.44	0.56	76.5 mg/kg		
	98.3 mg	8.13	0.56	77.0 mg/kg		
Sewage sludge 2 Interlaboratory test sample 256 mg/kg	12.1 mg	3.72	0.56	261 mg/lg	258 ± 3.1 mg/kg	1.2
	51.6 mg	13.9	0.56	259 mg/kg		
	102.0 mg	26.6	0.56	255 mg/kg		
Sewage sludge 3	11.6 mg	5.18	0.56	398 mg/kg	404 ± 6.5 mg/kg	1.6
	48.6 mg	20.2	0.56	404 mg/kg		
	101.3 mg	42.2	0.56	411 mg/kg		
AOX standard 100 µg/L	100 mL	10.47	0.56	99.1 µg/L	-	-

Table 2: AOX results of different sewage sludge samples

The different weights delivered comparable results with a relative standard deviation of well below 2%, which underlines the excellent handling of the shaking method by means of AFU 3. The main advantage in handling with the AFU 3 is that the mixture of sample and activated carbon is transferred directly into a quartz container. If the filtration is carried out via a conventional vacuum filtration apparatus on a membrane filter, e.g., made of polycarbonate, there is the danger of dry suction, because very moist membrane filters can only be transferred without loss into a sample boat for the AOX analyzer with difficulty. As a result, the risk of contamination of the sample by ambient air is significantly higher. The overpressure filtration at the AFU 3 additionally minimizes the contamination risk by the possibility of an inert gas connection. The filtration of up to three samples can run almost simultaneously, so that the filtering of the samples is no longer the speed-determining step of the sample preparation.

The shape of the measurement curve clearly shows that the combustion process using the pre-combustion adapter and the introduction of HX into the coulometric measuring cell is very controlled and fast (rapid rise and reaching a signal plateau after a short time). The subsequent titration (descending signal curve) is carried out with adapted, dynamic titration currents, so that over-titration and thus false high AOX values are reliably prevented. Even with high contents, the titration progress is fast and the special cell electrolyte ensures that even unexpectedly high AOX loads or long sample series can be measured reliably and without interference.

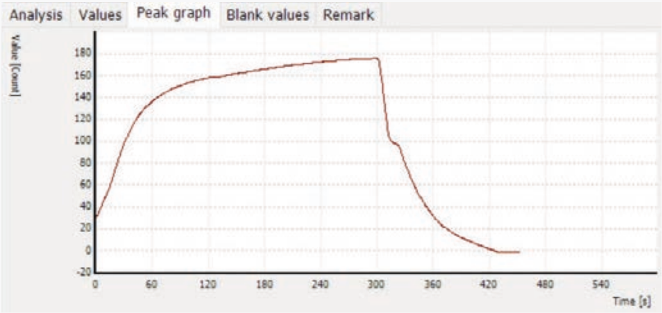


Figure 1: Typical measurement curve of sewage sludge sample 3 with a high amount of AOX

Figure 2: multi X 2500 vertical with autoX 36

Figure 3: AFU 3

Conclusion

The measurement results prove that correct and precise results for the AOX content in sewage sludge samples according to DIN EN 16166 can be achieved with the aid of the automatic filtration unit AFU 3 and the multi X 2500. The risks of using the conventional shaking method with membrane filters (e.g., loss of AOX during transfer or contamination by ambient air during dry suction) are significantly minimized by using the AFU 3. Furthermore, the sample throughput can be increased by the simultaneous filtration of three samples. This makes the AFU 3 an ideal tool even for inexperienced laboratory personnel.

References:

EN 16166:2012- "Sludge, treated biowaste, soil and sediments – Determination of adsorbed organically bound halogens (AOX)"

Challenge

Measuring the AOX content properly in the presence of more than 1g/L inorganic bound Cl.

Solution

Using the smart solid phase extraction autosampler APU 28 SPE offers a fast, effortless, and fully automated SPE-AOX sample preparation.

Determination of AOX in Highly Saline Water Samples after Solid Phase Extraction (SPE-AOX)

Introduction

An easy and fully automated method for the correct determination of AOX contents in the presence of extremely high (c > 1 g/L) quantities of inorganic halides is often needed when analyzing marine water samples, industrial wastewater, and brine applications (SPE-AOX according to ISO 9562:2004). The presence of high amounts of inorganic halogen compounds negativetely affects the determination of AOX. When such samples are analyzed directly by means of common AOX methods (adsorption on charcoal and combustion), results will be falsified and too high. To avoid this, the interfering compounds have to be removed before the adsorption. Solid phase extraction (SPE) has proven to be the most efficient and easy method to do this.

For the measurement, the samples were enriched on SPE cartridges. To achieve an easy and effective segregation between organic and inorganic matter, a polymer resin was used for separation. The resin captures only the organic halogen compounds while letting the inorganic material pass through. The AOX was then eluted with methanol and diluted in ultrapure water. This mixture was adsorbed on prefilled charcoal columns, followed by a final washing process to ensure a complete separation of inorganic and organic halogen compounds.

Since this is a time-consuming process, laboratories facing increasing time and work pressure strive to facilitate the workflow. The sample preparation unit APU 28 SPE enables fast and unattended, fully automated preparation of SPE-AOX samples, thus highly shortening processing time. Combined with the multi X 2500 AOX analyzer, the APU 28 SPE ensures fast, precise, and reliable results, which are mandatory for laboratories.

Materials and Methods

Samples and reagents

- Water samples with high load of inorganic chlorides (> 1 g/L)
- Standard solution: 100 µg/L p-chlorophenol
- NaNo$_3$ washing solution
- 0.01 M HCl

As micro coulometric chlorine determination is an absolute method, no calibration was needed.

Instrumentation

The samples were prepared automatically using an APU 28 SPE system. For the combustion of samples, a multi X 2500 analyzer in vertical operation mode with AOX combustion tube and the standard sensitive cell was used. Sample introduction was carried out fully automated by means of the autoX 36 autosampler.

Method parameters

The parameter and method settings for the combustion process are summarized below. Standard method settings from the method library were applied. The parameter settings for the combustion process and the sample transfer are summarized in Table 1. The evaluation parameters for the detection of chlorine are given in Table 2.

Parameter APU 28 SPE Sample Preparation	Specification
Sample volume (SPE cartridge)	100 mL
Sample volume (AOX column)	100 mL
Washing volume (SPE and AOX step)	25 mL
Methanol volume (conditioning)	10 mL
Methanol volume (elution)	5 mL
Adsorption speed	3 mL/min

Table 2: Settings for sample preparation (ISO 9562:2004)

Parameter multi X 2500 AOX determination	Specification
Furnace temperature	> 950 °C
Titration delay	approx. 240 s
Max. titration time	1200 s
Cell temperature	18–35 °C
Quality oxidation/carrier gas	Oxygen 4.5
Working range coloumetric cell (AOX)	1 µg–100 µg Cl abs.
Working range chlorine module	10 ng–1000 µg Cl abs.
Automation	autoX 36 or autoX 112

Table 2: Settings for AOX determination (acc. to ISO 9562:2004)

Results and Discussion

The column method was used according to ISO 9562:2004. Before being enriched on activated carbon, the sample underwent an SPE process to separate inorganic from organic bound halides (Figure 1). Figure 2 shows the APU 28 SPE during the automatic elution of the SPE cartridges with methanol. The SPE procedure, which works without any manual intervention, and the adsorption step itself were performed automatically by the APU 28 SPE sample preparation unit.

The treated water samples ran through two adsorption columns (prefilled charcoal containers) with a flow rate of 3 mL/min. The charcoal-filled containers were then rinsed with 25 mL of nitrate washing solution to remove the inorganic salt load at the same flow rate.

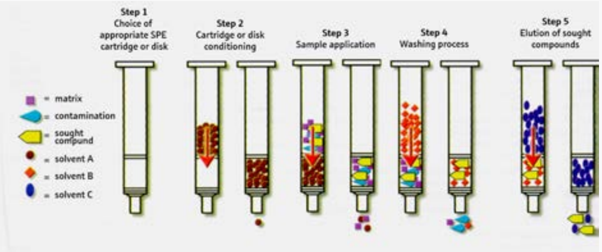


Figure 1: SPE process, which takes place in SPE cartridges

The prepared charcoal containers were placed into the autoX 36 sample plate. This sampling system automatically introduces the loaded columns into the vertically arranged open AOX combustion tube. The furnace temperature of 950 °C was chosen in compliance with DIN EN ISO 9562:2004. The organic halogen compounds were converted in the presence of a surplus of oxygen to HCl gas. After sufficient drying with concentrated sulphuric acid, the HCl gas was transferred into the sensitive cell. The cell has a wide working range from 1 µg to 100 µg chlorine absolute. The chlorine detection was carried out by means of microcoulometric titration. Proper operation of the analysis system was confirmed by analysis of a commercial standard solution (4-chloro-phenole in water).

Figure 2: APU 28 SPE sample rack

Sample ID	Adsorption Volume [mL]	Dilution ratio	abs. Cl value Column 1 [µg]	abs. Cl value Column 2 [µg]	Blank value [µg abs.]	Result [µg/L]
Standard	100	1:1	10.5	0.23	0.3	104.3
1	100	50:100	1.51	0.18	0.3	27.8
2	100	20:100	2.67	0.62	0.3	149.5
3	50	50:100	0.64	0.51	0.3	34.0
Standard	100	1:1	9.67	0.52	0.3	9.89

Table 3: Settings for AOX determination (acc. ISO 9562:2004)

Conclusion

The APU 28 SPE system is fully suited for the automatic preparation of AOX and SPE-AOX samples. Due to its workflow automation, including all adsorption and rinsing steps, the APU 28 SPE is an ideal partner for high sample throughput. In combination with its continuous flow principle, a fast, efficient and accurate analysis is achieved. The multi X 2500 is an excellent instrument for AOX (EOX, POX, and TX/TOX) determination in all kinds of water, sludge, and soil samples, as well as other organic liquids and solids (e.g., used oil). Due to its enormous flexibility, changing between different parameters is fast and easy. This makes the multi X 2500 very efficient, saving valuable time and costs.

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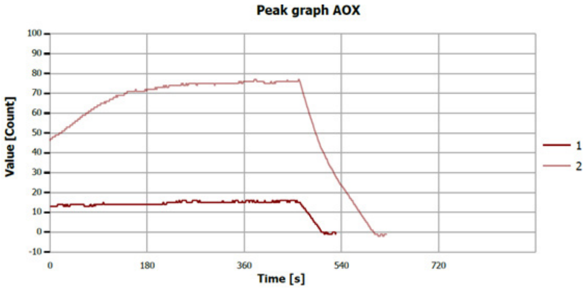
p-chloro-phenole</td><td>
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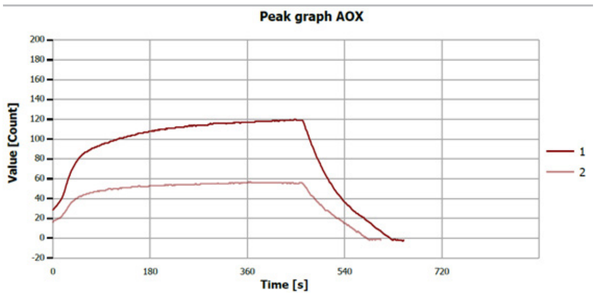
<tr><td>Sample 2</td><td>
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Table 4: Signal profile of a para-chloro-phenole standard solution and of sample 2

UV/Vis Spectroscopy

Double-beam UV/Vis Photometry

Many parameters in wastewater, which have to be monitored regularly, such as ammonia, nitrate, nitrite, phosphate, turbidity, SAC, COD, and BOD can be analyzed with UV/Vis spectrophotometers. Herewith not only qualitative and quantitative analyses of the parameters are possible, but also substance-specific information can be obtained. The measurement results by means of UV/Vis spectrometry are quickly available, reliable, and cost-effective. They are suitable for optimization and control of processes in wastewater treatment plants and for checking the reference and limit values.

In addition to robust and durable hardware, Analytik Jena's SPECORD PLUS series offers a wide range of accessories such as holders for cuvettes up to 100 mm, cell changers, sipper, and autosamplers. In addition, standard test kits for water analysis can be used in the round cell holder. With the Quick Start module, the ASpect UV software offers a simple and efficient way of recording and evaluating data.

Overview

Application Notes

Challenge

Standard-compliant determination of various parameters including anions (group D), cations (group E) and substance group analysis (group F) in wastewater.

Solution

Spectrophotometric determination of nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) and phosphate (PO_4) ions and total phosphor (P_{tot}) in wastewater using the SPECORD 50 PLUS.

Spectrophotometric Determination of Standard Parameters in Wastewater According to Standard Methods

Introduction

In July 2010, the United Nations General Assembly declared the access to clean water and sanitation to be a human right.[1] This emphasizes the significance of clean drinking water as one of the most vital resources with ever increasing importance. To ensure future access to this vital resource, it is essential to effectively purify polluted wastewater. One important step for the purification procedure is the precise qualitative and quantitative analysis of the pollutants.

Among other impurities, total phosphorus (P_{tot}), phosphate (PO_4), ammonium (NH_4), nitrate (NO_3) and nitrite (NO_2) ions are not only present in industrial and household wastewater in varying concentrations, but are also found in drinking water, ground water, and surface water. The first step in the purification procedure of wastewater in sewage treatment plants is the removal of solids with physical methods. Bacterial and yeast oxidation processes occur in the following biological stage. The mainly organically bound nitrogen (as proteins, nucleic acids, and urea) is then oxidized to NO_3 and NO_2. During the third step, the chemical purification, PO_4 is precipitated.

The spectrophotometric determination of the above mentioned compounds is regulated in the German standard methods for examination of water, wastewater and sludge (DIN 38405) and other standards.[2,3] Detailed instructions for the analysis including sample preparation, interfering ions, as well as detection limits are given. The SPECORD 50 PLUS spectrophotometer with its high-resolution optics and robust construction ensures precise and reliable data acquisition. The software ASpect UV supports fast and standard-compliant measurements and data evaluation through the straightforward method for the creation of calibration curves. The use of accessories for large sample throughput, such as the cell carousel with 15 positions and the 6-fold cell changer, additionally supports the simple and fast handling of large sample quantities.

Materials and Methods

Instrumentation and software settings

All measurements were performed using a SPECORD 50 PLUS spectrophotometer equipped with the 15-position cell carousel for the analysis of NH_4, NO_2, and NO_3 or the 6-fold cell changer for the analysis of PO_4 and 10 mm glass cuvettes. Using the ASpect UV software 1.4.4 (other versions of ASpect UV give the same results), the absorbance for each parameter was measured at one defined wavelength. The software settings are shown in Table 1.
The measurement settings were made using the photometry module of the software ASpect UV. Required evaluations can be set in the method. Concomitantly, a report in PDF format can be saved directly after the measurement. The set and saved method for each parameter was stored in the Quick Start menu.

Samples and reagents

Standard and sample preparation were carried out according to the DIN standards[3]. The conditions for the calibration curves according to standards are shown in Table 2.

	NH_4	NO_2	NO_3	PO_4
Measurement mode	Absorbance	Absorbance	Absorbance	Absorbance
Wavelength [nm]	655	540	338	880
Integration time [s]	0.2	0.2	0.2	0.2
Regression	y = B*x	y = B*x	y = B*x	y = B*x

Table 1: Software settings

Measurement

The prepared samples were measured within the following times:

- NH_4: 1–3 h
- NO_3: 1 h
- NO_2: 20 min
- PO_4 / P_{tot}: 10–30 min

Reference, blank and samples were measured sequentially. The reference was measured against air. For the blank distilled water was used and identically prepared as the wastewater samples. The absorbance of the blank was automatically subtracted from the subsequent samples.

Results and Discussion

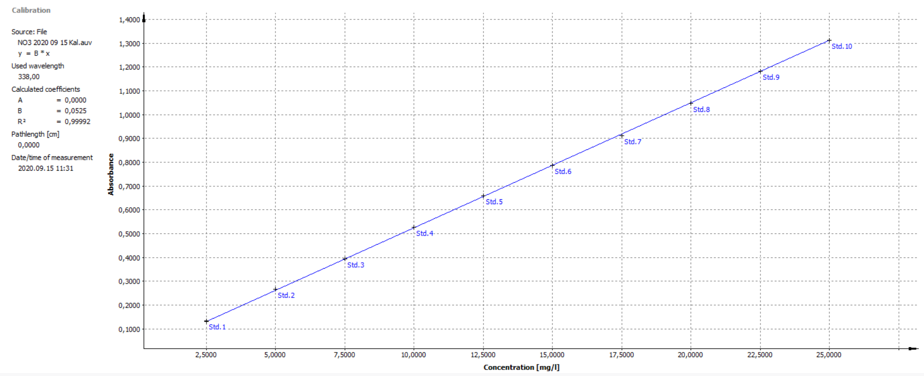
Creation of calibration curves

To determine the concentration of NH_4, NO_3, NO_2, PO_4 and P_{tot}, the first step is the creation of the calibration curve according to the referenced standards.[3] According to literature, ten distinct solutions with varying reference concentration were prepared. Table 2 shows the preparation conditions for the calibration standards and subsequent plotting of calibration curves for each parameter according to the referenced standards.

Standard	Parameter	Concentration range	Reference compound	Wavelength
DIN 38406-05	NH_4	0.03–1 mg/L	Ammonium sulfate ($(NH_4)_2SO_4$)	655 nm
DIN 38405-9	NO_3	0.5–25.0 mg/L	Potassium nitrate (KNO_3)	338 nm
DIN EN 26777	NO_2	up to 0.25 mg/L	Sodium nitrite ($NaNO_2$)	540 nm
DIN EN ISO 6878	PO_4 / P_{tot}	0.005–0.8 mg/L	Potassium dihydrogen phosphate (KH_2PO_4)	880 nm

Table 2: Conditions for the creation of calibration curves according to standards

As an example, the calibration curve of NO_3 is shown in Figure 1. The absorbance of the blank measurement with distilled water is automatically subtracted in the ASpect UV software from the absorbances of the standard measurements. The calculated absorbance of the standard solutions is plotted as a function (y-axis) of the standard solution concentration (x-axis). The later one is given in milligrams per liter (mg/L). As observed in Figure 1, correlation between the absorbance and the concentration within the given ranges, clearly indicates a linear dependency. The ASpect UV software automatically calculates the slope and R^2-value of the calibration curve. The unknown concentration of any sample is then automatically derived upon absorbance measurement. In addition, the concentration is also automatically calculated if information about dilution and initial weight (initweight) of the sample is given. Therefore, calculation errors can be excluded, and the user saves valuable analysis times.



Calibration

Source: File
NO3 2020 09 15 Kal.auv
y = B * x
Used wavelength
338,00
Calculated coefficients
A = 0,0000
B = 0,0525
R² = 0,99992
Pathlength [cm]
0,0000
Date/time of measurement
2020.09.15 11:31

Figure 1: Calibration curve for the determination of concentration of NO_3

Measurements of wastewater samples

It is important to note that the impact of other substances on the absorbance needs to be verified prior to estimating any parameter result, as interfering ions (e.g., NO_3 and NO_2: chlorid; PO_4: arsenat) can significantly affect the test results. If the presence of interfering ions can be excluded, the wastewater samples are treated with the detection reagents as described in the standards. Subsequently, the absorbance of the wastewater samples is measured, and the concentration is determined using the calibration curve. If the selected parameters are frequently measured, it is recommended to save the methods and calibration curves in the Quick Start function (see Figure 2). This enables fast and precise measurement as well as rapid evaluation of further samples. After opening the software ASpect UV, the method starts and the results are automatically recorded and evaluated within just four clicks (sample sequence including number of samples and sample names can additionally be adjusted). Five water samples had been measured after the purification procedure. The results are shown in Table 3. Due to the continuous monitoring of the concentration of different parameters, the purification process of wastewater in the sewage plants can be reviewed. The measurement's data are recorded and stored for review of the authorities to check if the values are within the threshold. Additionally, a continuous improvement and optimization of the purification process can be achieved.

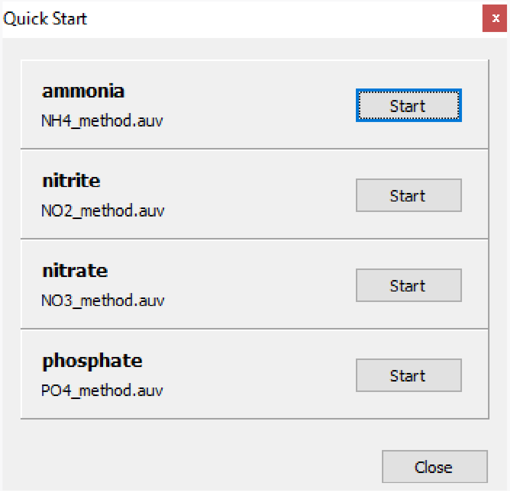


Figure 2: ASpect UV Quick Start menu

Sample	NH_4	NO_2	NO_3	P_{tot}	PO_4
1	0.5035	0.4839	12.2777	0.2066	-
2	0.0237	0.0079	5.8712	0.1991	0.0666
3	0.0813	0.0305	6.7569	0.2237	0.0724
4	0.0194	0.0157	5.0116	0.0246	-
5	0.0105	0.0139	3.8557	0.0267	-

Table 3: Concentration of the selected parameters exemplified for five wastewater samples from a sewage treatment plant

Depending on the pH value, ammonia can be present in wastewaster samples in different forms (as ammonium ion (NH_4^+), ammonia (NH_3), or ammonium hydroxide (NH_4OH)). In order to detect all possible ammonia species and therefore get reliable results independently of the source and pH value of wastewater, severe alkaline conditions are required. Conversely, in order to catalyze the formation of the chromatic target compounds and thus alllowing detection at the characteristic wavelength, stronger acidic solutions for the detection of NO_3 and NO_2 ions are required.
Due to extreme conditions of the solutions, the handling of the samples is complicated. Additionally, the use of large quantities of solvent should be avoided in order to reduce the amount of hazardous waste. Therefore, the measurements are preferable performed in 10 mm cuvettes, although 40 mm or 50 mm cuvettes are also permitted according to the standards. Another advantage of the use of the 10 mm cuvettes over 40 mm or 50 mm cuvettes is a higher suitability for sample automation. Whereas 40 mm or 50 mm cuvettes can only be treated individually or in a 6-fold cell changer, the 10 mm cuvettes can additionally be placed in a 15-position carousel or an 8-fold cell changer. Further automation procedures can be established using the APG autosampler and sipper system.
The SPECORD PLUS spectrophotometers are particularly suitable for the analysis of wastewater samples, as a special cuvette position for turbid samples is included. The cuvette can be closely positioned to the detector and therefore the amount of scattered light is reduced. The SPECORD 50 PLUS spectrophotometer with the split-beam-technology provides a high energy flux from the light sources, which is especially suitable for the analysis of highly concentrated wastewater samples. The use of standardized test kits for water analysis parameters such as SAC, turbidity, COD, BOD, sulfate, and many more is possible with the use of the round cuvette holder. Low concentrated samples can easily be analysed with the use of 100 mm cuvette holder to increase the path length.

Conclusion

The SPECORD 50 PLUS (Figure 3) spectrophotometer allows fast, precise, and simple determination of the concentration of important water parameters in wastewater samples.
The calibration curves can be created with good R^2-values, with the help of the high precision optics. The automatic calculation of the concentration depending on dilution and initial weight in the software ASpect UV supports the fast and error-free calculation of the concentration of different pollutants in the wastewater samples.
The use of accessories such as the autosampler, cell changer, or cell carousel support high-throughput measurements. Additional equipment enables the analysis of turbid and low concentrated samples.

Figure 3: SPECORD 50 PLUS

References

[1] United Nations – Water (https://www.un.org/en/sections/issues-depth/water/index.html)

[2] Verordnung über Anforderungen an das Einleiten von Abwasser in Gewässer (Abwasserverordnung - AbwV)

[3] DIN 38406-05:1983-10 (E5-1); DIN 38405-09:2011-09 (D9); DIN EN 26777:1993-04 (D10); DIN EN ISO 6878:2004-09 (D11, part 4); DIN EN ISO 6878:2004-09 (D11, part 7)

Challenge

Evaluation of the rinsing effectiveness of household and commercial washing machines and washer-dryer combos as described in technical specification PD CLC/TS 50677:2019.

Solution

Quick and precise determination of the amount of residual surfactant through UV/Vis measurements with SPECORD 210 PLUS.

Determination of Concentration by Means of a Calibration Curve – Exemplified by Surfactant Measurements According to PD CLC/TS 50677:2019

Introduction

A new technical specification for determination of the rinsing effectiveness of clothes washing machines and washer-dryer combos for household use was released in 2020 by the European Committee for Electronical Standardization (PD CLC/TS 50677:2019). It applies to all washing machine manufacturers which are producing for the European market. Herein, the amount of residual linear alkylbenzene sulfonate surfactant (LAS), which was extracted from unstained test swatches after the washing performance test, is determined. LAS is used as a reference material for the total amount of base powder detergent IEC-A*, assuming a fixed linear relation between the quantity of surfactant in total and LAS. This simplification is made due to the easy detection of LAS in UV/Vis measurements at 223 nm and 330 nm with 1 nm spectral bandwidth. The characteristic absorbance of LAS at 223 nm is based on a π-π^* transition of the aromatic group of LAS and enables the determination of the concentration of the detergent.

Initially, a calibration curve is created and the absorbance values are plotted against the concentration of known standards. This calibration curve is then used to determine the concentration of the unknown samples, based on their absorbance values. The challenge is the very low concentration of LAS which is remaining in the samples after the washing procedure – therefore, a very low limit of detection is required for the measurement. The SPECORD 210 PLUS instruments have a very low deviation of the baseline and high reproducibility, which is vital for the accurate creation of a calibration curve with low concentration values.

Materials and Methods

All measurements were performed using a SPECORD 210 PLUS spectrophotometer equipped with a standard cell holder and 10 mm quartz cuvettes. Using the latest ASpect UV software version (1.4.4, other versions of ASpect UV give the same results), absorbance spectra were recorded in the range of 200–350 nm (preliminary studies). For measurements according to the PD CLC/TS 50677:2019 standard, the absorbance of the dissolved reference detergent IEC-A* (base powder, as described in EN 60456:2016) under different concentration was measured (further details see below). The calibration curve was created using the photometry module.

Preliminary studies

In order to exclude any potential effect derived by the cuvettes, preliminary tests were performed. Herein, ten quartz cuvettes (QS) with the same specifications were compared by measuring the absorbance spectrum of each. All cuvettes were cleaned using HELLMANEX, rinsed with distilled water and ethanol and gently dried under a continuous air flow. Spectra of cuvettes filled with degassed distilled water were measured against air (empty chamber). Upon detailed evaluation, only the cuvettes with the smallest deviation (ΔA) from each other were selected for further measurements.

Standard preparation

The reference detergent IEC-A* was taken as described in Annex A of the technical specification. The distilled water, which was used for the stock and standard preparation, was filled into a volumetric flask at room temperature for at least 24 hours to ensure that it was degassed. All further experiments were performed using the degassed distilled water.

Preparation of Stock 1 (St1):
Approximately 10 g of IEC-A* were weighed (mass was recorded up to 3 decimals), transferred to a 1 L volumetric flask and filled up to 1 L with distilled water. The flask was stirred for about 45 minutes.
Preparation of Stock 2 (St2):
Approximately 10 mL of stock solution 1 were taken while stirring and transferred to a 100 mL volumetric flask. The mass was recorded up to 3 decimals. Then the flask was filled up to 100 mL with distilled water. The flask was stirred for about 15 minutes.
Preparation of Working Standards (WS):
For all working standards stock solution 2 was transferred into a 100 mL volumetric flask while stirring, the mass was recorded to 3 decimals and filled up with distilled water. The volume of stock 2 which was used is shown in Table 1.

Working standard	Target volume of stock 2 in mL (approx.)	Target concentration in mg/L (approx.)	Mass of stock 2 added in g	Concentration calculated in mg/L*
WS000	0	0	0	0
WS010	1	7.7	0.980	7.616
WS020	2	15.4	1.980	15.387
WS050	5	38.5	4.943	38.412
WS100	10	77.0	9.910	77.011
WS250	25	192.5	24.838	193.016

* calculated as described in PD CLC/TS 50677:2019

Table 1: Working standard data

The standards were measured with the settings below. As reference air was used, the blank was measured with distilled water.

Instrumentation

To meet the conditions required by PD CLC/TS 50677:2019 the measurements were performed by using a SPECORD 210 PLUS spectrophotometer, because it is a double beam instrument with the possibility to set a spectral bandwidth of 1 nm.

Accessories
- 10 mm quartz cuvettes
- Standard cell holder

Instrument and software settings

The instrument warmed up for about one hour before the measurement started. Following method settings of photometry module in the SPECORD PLUS software ASpect UV 1.4.4 were used for the measurements:

Measurement mode:	Absorbance
Wavelength [nm]:	223 and 330
Integration time [s]:	0.1
Spectral bandwidth [nm]:	1.0
Multiple measurement:	3
Regression:	y = A+B*x

Measurement

The standard shall be measured within 24 hours after the preparation. The reference was measured against air. For the blank and standard measurement two matching quartz cuvettes were used. To start with, the cuvettes were filled with distilled water for the blank measurement, one was placed in the measurement beam of the instrument, the other one in the reference beam.
The cuvette in the reference beam stayed in the sample compartment. The other cuvette was taken out and rinsed three times with the selected working standard. After rinsing, the cuvette was filled with the working standard and the absorbance was recorded. The rinsing and filling steps were repeated for each working standard.

Results and Discussion

Preliminary studies

In order to achieve an R^2-value of at least 0.9995, as required by the technical specification (PD CLC/TS 50677:2019), several preliminary tests were performed. Initially, the cuvettes and the degassed distilled water were checked for impurities. Therefore, all cuvettes were initially cleaned and subsequently filled with distilled water. It is important to check the purity of the cuvettes, which is a time-saving procedure prior to every measurement. Additionally, it is important to check the cuvettes with the solvent which is also used for the measurements, as some impurities or deviations of the cuvettes are weakened or intensified depending on the polarity of the solvent. Therefore, reference spectra of all cuvettes were recorded, checking for deviations and significant absorbance exceeding 0.002 A between 220 nm and 350 nm.
For all following tests, the cuvettes with the smallest deviation (ΔA) from each other were used and it was made sure that the orientation of the individual cuvettes remained the same. Under the above determined conditions, a spectrum of the reference detergent IEC-A* was recorded in the highest concentration of the working standard (WS250) with a spectral bandwidth of 1.0 nm, as shown in Figure 1. Additionally, WS250 was also measured with different spectral bandwidths, and the difference of the absorbance values at a bandwidth of 1.0 nm and 2.0 nm are within the tolerance of the cuvettes. Consequently, the use of SPECORD 200 PLUS with a spectral bandwidth of 1.4 nm also provides reliable results.

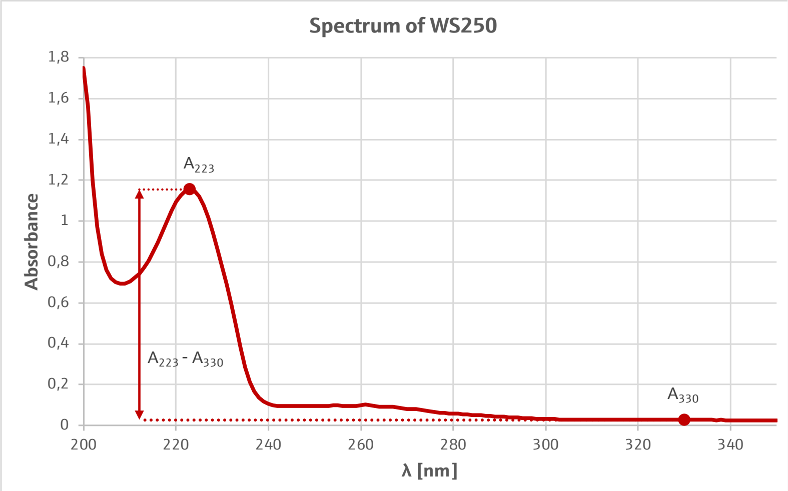
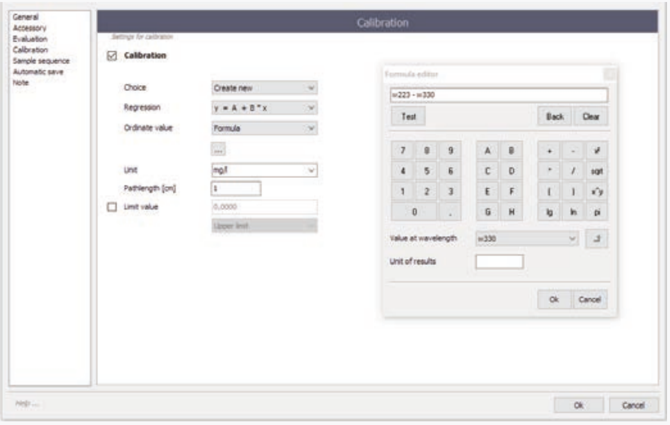


Figure 1: UV/Vis spectrum of WS250 with 1.0 nm spectral bandwidth and degassed distilled water as reference

Generating the calibration curve

After the above described preliminary studies to minimize sources of error, the working standards were measured. As described in the technical specification, the calibration curve was created with the net absorbance which is calculated by $A_{223} - A_{330}$. The software ASpect UV can automatically create calibration curves according to customized formulas (Figure 2). The calibration curve was generated in the photometry module with the shown calibration settings.
All working standards had been measured three times at 223 nm and 330 nm. The results can be seen in Table 2.



☑ Calibration

Choice: Create new
Regression: y = A + B * x
Ordinate value: Formula
Unit: mg/l
Pathlength [cm]: 1
☐ Limit value: 0,0000
Upper limit

Formula: w223 - w330
Value at wavelength: w330
Unit of results:

Figure 2: Calibration curve settings in ASpect UV 1.4.4

Working standard	223 nm 1st value	223 nm 2nd value	223 nm 3rd value	330 nm 1st value	330 nm 2nd value	330 nm 3rd value
WS000	0.0001	−0.0003	0.0001	−0.0001	−0.0001	0.0002
WS010	0.0495	0.0491	0.0494	0.0032	0.0029	0.0033
WS020	0.1005	0.1003	0.1009	0.0065	0.0063	0.0070
WS050	0.2460	0.2463	0.2475	0.0149	0.0151	0.0152
WS100	0.5043	0.5036	0.5041	0.0341	0.0339	0.0340
WS250	1.2402	1.2414	1.2441	0.0807	0.0804	0.0809

Table 2: Repetitive measurement of the working standards at 223 nm and 330 nm for the calibration curve

The values of the net absorbance ($A_{223} - A_{330}$) are shown in Table 3. Additionally, the average values and standard deviations were calculated.

Working standard	1st value	2nd value	3rd value	Average	Standard deviation
WS000	0.0002	−0.0002	−0.0001	0.0000	± 0.0002
WS010	0.0463	0.0462	0.0461	0.0462	± 0.0001
WS020	0.0940	0.0940	0.0939	0.0940	± 0.0001
WS050	0.2311	0.2312	0.2323	0.2315	± 0.0007
WS100	0.4702	0.4697	0.4701	0.4700	± 0.0003
WS250	1.1595	1.1610	1.1632	1.1612	± 0.0019

Table 3: Net absorbance of the working standards for the calibration curve

Additionally, the tolerance of the cuvettes had been calculated and compared to the standard deviations of the triple measurements. The results are depicted in Table 4. It is apparent that the standard deviation is in the range of the tolerance of the 10 mm cuvettes. The low deviation of the individual measurements is due to the low baseline noise of the SPECORD 210 PLUS. This helps to avoid time consuming multiple measurements, as the individual measurements are highly precise.

	223 nm	330 nm	Net absorbance
Absorbance	1.2419	0.0807	1.1612
Standard deviation measurement	± 0.0016	± 0.0002	± 0.0019
Tolerance of the cuvettes	± 0.0012	± 0.0001	± 0.0012

Table 4: Comparison of the standard deviation of the measurements and the tolerance of the cuvettes for WS250

The software ASpect UV 1.4.4 automatically creates the calibration curve of the net absorbance by adding the formula ($A_{223} - A_{330}$), as shown in Figure 2. The resulting calibration curve of the net absorbance against concentration is shown in Figure 3 and Figure 4 and an excellent R^2-value of 1 was achieved. Due to the high precision of the instrument, the R^2-values of each individual measurement were also very good with R^2-values of 0.9999.

As no real washing samples were available, the washing samples (e.g., swatches) were simulated with three diluted solutions of IEC-A*. Absorbance spectra of all three samples were recorded three times and the LAS concentration was calculated with the help of the calibration curve (Figure 3, Figure 4 and Table 5). As the calibration curve was created using the formula $A_{223} - A_{330}$, the samples are automatically also evaluated using this formula, which helps to avoid time-consuming calculations or even calculation errors.

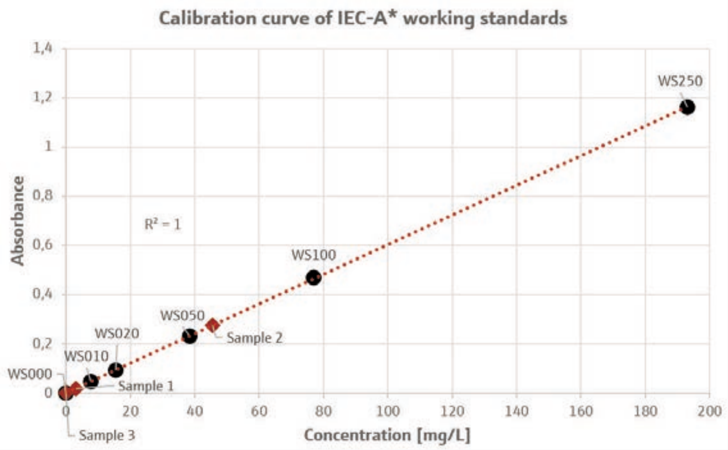


Figure 3: Calibration curve of the IEC-A* working standards WS000 to WS250 (black dots); linear regression with an R^2-value of 1 (red dotted line) and corresponding samples 1–3 (red rhombs)

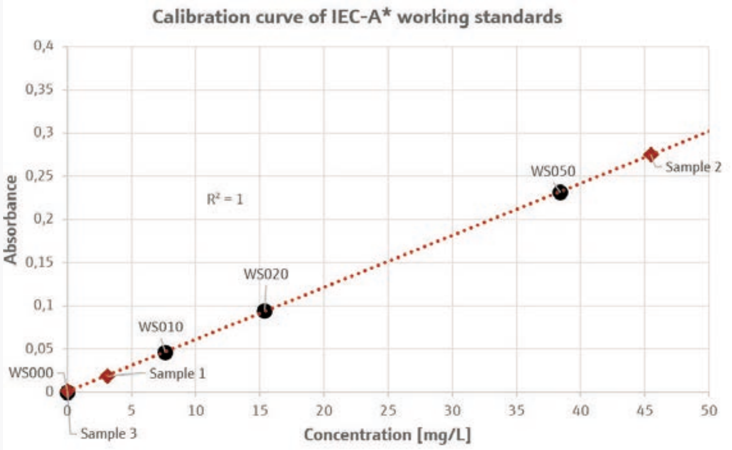


Figure 4: Enlarged view of Figure 3 in the low concentration range (0–50 mg/L)

		Sample 1	Sample 2	Sample 3
Absorbance	1st measurement	0.0215	0.2945	0.0017
	2nd measurement	0.0211	0.2931	0.0014
	3rd measurement	0.0218	0.2956	0.0019
	Average	0.0214	0.2944	0.0016
	Standard deviation	0.0003	0.0010	0.0002
Concentration [mg/L]		3.11	45.51	0.04

Table 5: Calculated concentration of the diluted IEC-A* samples (in average) based on the absorbance measurements

Conclusion

The sample preparation and UV/Vis measurements according to technical specification PD CLC/TS 50677:2019 were performed using the spectrophotometer SPECORD 210 PLUS (Figure 5) and the procedure was evaluated. Several preliminary studies have been performed in order to figure out the best and easiest way to obtain reliable calibration curves with low limits of detection and a high R²-value.
A time-saving method for preliminary tests to check for the cuvette and solvent purity was presented in this application note. Additionally, time-consuming multiple measurements can be avoided when using the SPECORD 210 PLUS instrument. The high accuracy and precision of the spectrophotometer results in good and reliable values even with a single measurement. Furthermore, the software ASpect UV 1.4.4 supports the automatic creation of the calibration curve with a formula, thus reducing time consuming calculations which are also prone to errors.
In addition, the application of the SPECORD 200 PLUS instrument is also possible, as comparison spectra with different spectral bandwidth had been performed and the difference in absorbance for the wavelength in question for different spectral bandwidth is negligible. For large sample throughput, the usage of additional accessory such as cell changers, cell carousel or the autosampler APG is recommended.

The calibration curve of IEC-A* according to technical specification PD CLC/TS 50677:2019 was created and some test samples had been analyzed. Due to the extensive preliminary studies and the high precision of SPECORD 210 PLUS, a very good R²-value of 1, and therefore very reliable values, even after time-saving single measurements, could be achieved.

Figure 5: SPECORD 210 PLUS

References

BSI Standards Publication; Clothes washing machines and washer-dryers for household and similar use – Method for the determination of rinsing effectiveness by measurement of the surfactant content at textile materials. PD CLC/TS 50677:2019.

Microbiology

Automated Nucleic Acid Extraction

Overview

Water is the basis of all life. It is not only essential for our nutrition and daily hygiene, but also serves as a habitat for many plants, animals, and other organisms. In addition, it is widely used in the industrial sector and is one of the most important economic factors.

For a sustainable use and careful handling of the resource water, many different requirements are placed on the physical, chemical, and microbiological quality of water, depending on the intended use. Next to parameters such as TOC (total organic carbon), TN_b (total bound nitrogen), mercury, and other toxic elements, microbiological monitoring of water and wastewater is becoming increasingly important for environmental analyses. The focus here is on protection against infections or contaminations with biosubstances, which can lead to sensitizing toxic effects in humans or the spread of resistance (e.g., to antibiotics). Detection times and specificity often play a crucial role here. With the powerful quantitative real time PCR system qTOWER³ and the corresponding analysis kits, as well as efficient nucleic acid extraction kits and extraction systems, Analytik Jena offers a broad product portfolio for the quick and sensitive determination of smallest amounts of pathogen nucleic acids in varying sample materials. Pathogenic microorganisms (bacteria, fungi, and parasites) and infectious agents (viruses) can be identified and quantified. See for yourself, optimize your analysis workflow, and benefit from absolutely reliable measurement results.

Challenge

Fast and reliable quantification of bacterial load in cooling tower water samples as well as detection of *Legionella spp.* and especially pathogenic *Legionella pneumophila.*

Solution

Efficient automated extraction of bacterial DNA from cooling tower water samples using the InnuPure C16 *touch* and subsequent qPCR analysis by innuDETECT Assays on qTOWER^3.

Extraction and Detection of Legionella DNA From Cooling Tower Water Samples Using InnuPure C16 *touch* and qTOWER^3

Introduction

Bacteria of the genus *Legionella* are the cause of Legionnaires' disease as well as Pontiac fever. A common source of Legionnaires' disease outbreaks are cooling towers. The bacteria are spread via aerosols and infect alveolar macrophages upon inhalation. Legionnaires' disease is most commonly caused by *Legionella pneumophila* (*L. pneumophila*). However, other species of *Legionella* (*Legionella spp.*) can also cause legionellosis.

According to the 42. Bundesimmissionsschutzverordnung (BImSchV), the German Federal Immission Control Ordinance, operators of evaporative cooling systems, cooling towers and wet separators must regularly monitor the parameter *Legionella spp.* to assess the hygienic quality of service water. If specific limit values are exceeded, the additional differentiation into the *L. pneumophila* species is mandatory.
In addition, monitoring the general bacterial load in cooling towers is mandatory as well. Conventional detection methods reliant on bacterial plating and cultivation are challenging due to complex nutritional demands of these bacteria as well as interference by accompanying flora. Cultivation also requires at least a couple of days to generate conclusive results. Detection of general bacterial DNA and/or *Legionella* DNA by means of real-time PCR, however, is a matter of hours.

As little as a 1 mL water sample from a cooling tower is sufficient for extraction of bacterial DNA using InnuPure C16 *touch*. The usage of the innuPREP Bacteria Lysis Booster, an optimized mix of several lytic enzymes, ensures efficient non-mechanical pre-lysis of a wide range of bacteria. The lysis of the bacteria was included in the automated protocol, making external lysis and subsequent manual addition of the lysate to the reagent plate obsolete.
To analyze the extracted DNA for overall bacterial load as well as for *Legionella* including sub-specification, two distinct innuDETECT Water Pathogen Assays were used in combination with the qTOWER^3 real-time cycler. The probe-based *Legionella*-specific assay can differentiate between *Legionella pneumophila* and *Legionella spp.* in one multiplex real-time PCR. The complete procedure starting with the

nucleic acid extraction from cooling water samples to the final result obtained from the qPCR analyses can be done within only a few hours as shown in Figure 1.

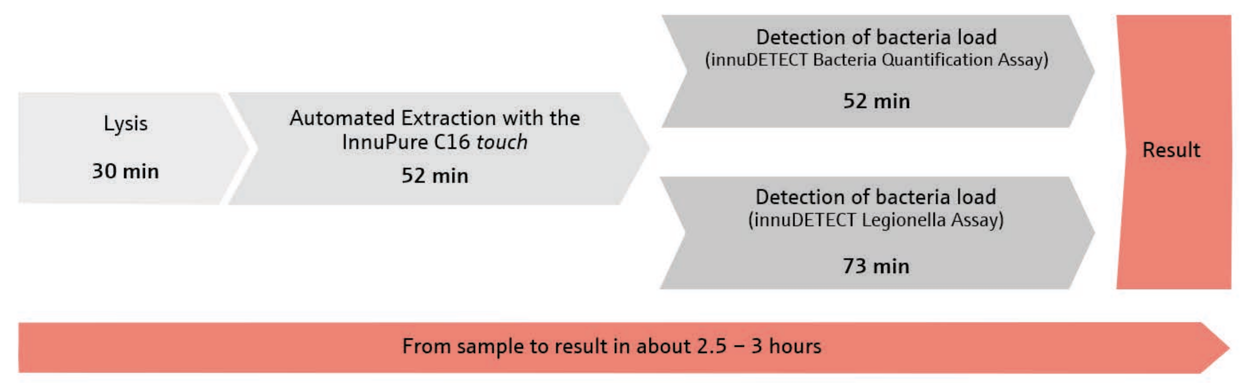


Figure 1: Timeline - overview of the necessary steps to get to the result within a few hours

Materials and Methods

Samples and reagents

- innuPREP Bacteria Lysis Booster (845-KA-1000050)
- innuPREP Bacteria DNA Kit-IPC16 (845-IPS-5516096)
- innuDETECT Bacteria Quantification Assay (845-IDF-0031024)
- innuDETECT Legionella Assay (845-IDF-0033024)
- Legionella pneumoniae DNA (strain: DSM 7513) as positive control
- Cooling tower water samples

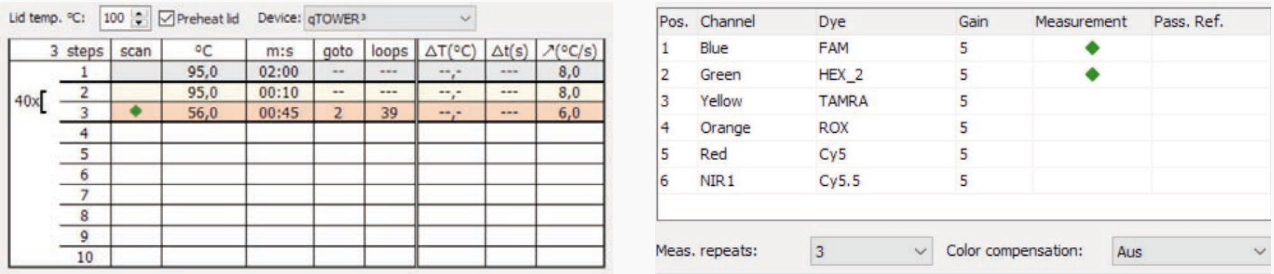
Instrumentation

- Vortex mixer
- Benchtop centrifuge
- Plate centrifuge
- Thermal shaker
- InnuPure C 16 *touch*
- qTOWER^3

Sample preparation

Three 1 mL - samples of cooling tower water were rebuffered in Tris/EDTA buffer and pre-lysed enzymatically by following the instructions of the innuPREP Bacteria Lysis Booster. Subsequently, bacterial DNA was extracted from the samples in a fully automated manner on InnuPure C16 *touch* by using the innuPREP Bacteria DNA Kit-IPC16. Samples were extracted in duplicates (A+B) and subsequently analyzed via real-time PCR. PCR analysis was performed in duplicates as well.

Total bacterial content of the samples was determined by use of the innuDETECT Bacteria Quantification Assay with a temperature time protocol and channel settings as shown in Figure 2. This real-time PCR assay detects the 16S ribosomal RNA gene, a universal bacterial target gene. The assay includes standard DNA with the defined amount of bacterial DNA of 10^7 copies/µL. Amplification of a serial dilution of this DNA allows semi-quantification of the samples by correlating the resulting Ct values.



Lid temp. °C: 100 ☑ Preheat lid Device: qTOWER³

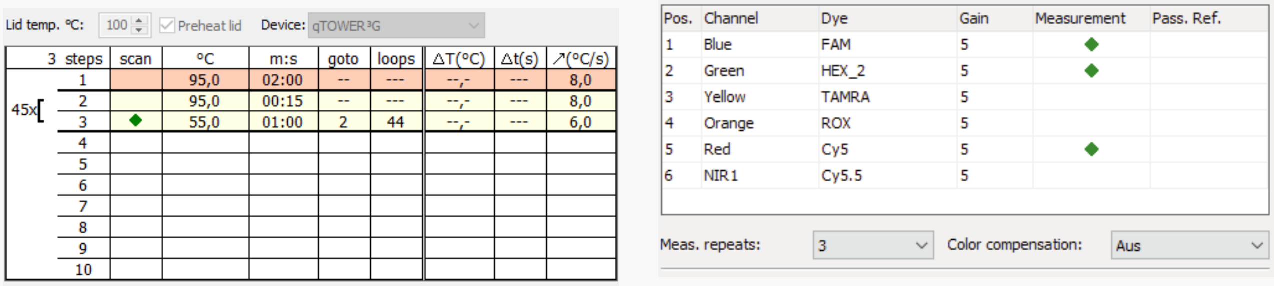
	3 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	02:00	--	---	--,-	---	8,0
40x	2		95,0	00:10	--	---	--,-	---	8,0
	3	◆	56,0	00:45	2	39	--,-	---	6,0
	4								
	5								
	6								
	7								
	8								
	9								
	10								

Pos.	Channel	Dye	Gain	Measurement	Pass. Ref.
1	Blue	FAM	5	◆	
2	Green	HEX_2	5	◆	
3	Yellow	TAMRA	5		
4	Orange	ROX	5		
5	Red	Cy5	5		
6	NIR1	Cy5.5	5		

Meas. repeats: 3 Color compensation: Aus

Figure 2: Overview of the settings for bacterial detection in qPCRsoft for the real-time PCR on the qTOWER^3. The target DNA is detected in the blue channel, while the internal control is detected in the green channel.

Detection of *Legionella* DNA within the extracted samples was done using the innuDETECT Legionella Assay on qTOWER³. The FAM-labelled probe detects the *Legionella pneumophila*-specific *mip* gene, whereas the Cy5-probe detects a universal target present in all species of the genus *Legionella*. The instrument settings are summarized in Figure 3. An amplification control added to the PCR mixture can be detected in the HEX-channel. To control for the presence of contaminating DNA in the PCR set up, a no template control (NTC) is included in each real-time PCR run. The DNA of *Legionella pneumoniae* (strain: DSM 7513) was used as a positive control.



Lid temp. °C: 100 ☑ Preheat lid Device: qTOWER³G

	3 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	02:00	--	---	--,-	---	8,0
45x	2		95,0	00:15	--	---	--,-	---	8,0
	3	◆	55,0	01:00	2	44	--,-	---	6,0
	4								
	5								
	6								
	7								
	8								
	9								
	10								

Pos.	Channel	Dye	Gain	Measurement	Pass. Ref.
1	Blue	FAM	5	◆	
2	Green	HEX_2	5	◆	
3	Yellow	TAMRA	5		
4	Orange	ROX	5		
5	Red	Cy5	5	◆	
6	NIR1	Cy5.5	5		

Meas. repeats: 3 Color compensation: Aus

Figure 3: Overview of the settings for *Legionella* detection in qPCRsoft for the real-time PCR on the qTOWER³. *Legionella pneumophila* and *Legionella spp.* can be detected in the blue and red channel, respectively. In the green channel the internal control can be detected

Results and Discussion

Determination of total bacterial load

The standard DNA dilutions were amplified and detected with innuDETECT Bacteria Quantification Assay (Figure 4). If the PCR efficiency is 100%, dilutions of 1:10 show a difference in Ct values of 3.3. The average Ct shift for the dilutions of the DNA standard is 3.6, corresponding to a PCR efficiency of 89% (Figure 5). Thus, a good correlation between the Ct values and the bacterial DNA content was achieved (Table 1). The NTC shows a Ct value above 35. It should be noted, that the polymerases used in PCR mixtures are usually generated in bacteria, thus containing trace amounts of bacterial DNA. These can also be detected with this assay, making the NTC necessary to account for this background of bacterial DNA. The determination of the abundance of bacterial DNA in the three samples that were extracted in duplicates (1A+B, 2A+B, 3A+B) was done by inference from their respective Ct values (Table 2) compared to Ct values of the standard DNA. As can be seen in Figure 6, the content of bacterial DNA is very high within the extracted samples, attesting to the very high efficiency of extraction and detection using this system. The amplification of the Internal Control DNA (Figure 7) confirms the correct performance of the PCR as well as the detection system.

Bacterial DNA standard

Dilution	Ct value	Copies
1 :10	19.69	1.0 x 10^6
	20.04	
1:100	23.51	1.0 x 10^5
	23.49	
1:1,000	27.17	1.0 x 10^4
	27.27	
1:10,000	30.63	1.0 x 10^3
	30.74	
NTC	36.32	-
	35.18	

Table 1: Ct values of a serial dilution of the bacterial DNA standard detected in the FAM channel

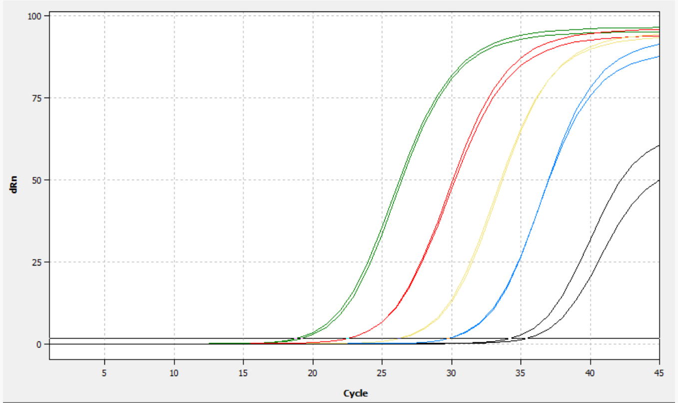
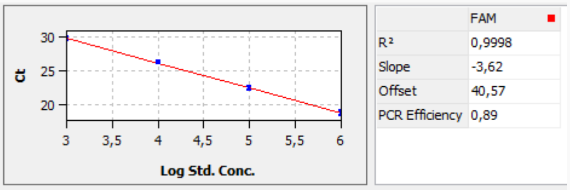


Figure 4: Amplification curves of bacterial DNA standard
Serial dilutions containing 1 x 10^6 (green), 1 x 10^5 (red), 1 x 10^4 (yellow) and 1 x 10^3 (blue) copies of bacterial DNA were recorded in duplicates. The no template controls (NTCs) are shown in black. Signals were detected in the FAM channel on qTOWER³.



	FAM
R²	0,9998
Slope	-3,62
Offset	40,57
PCR Efficiency	0,89

Figure 5: Linear regression of the bacterial DNA standard
The slope of the linear regression when plotting the logarithm of the concentration of the DNA standard against its Ct value, indicates the shift in Ct between the serial dilutions. The efficiency of this PCR is at 89%.

Detection of total bacterial DNA

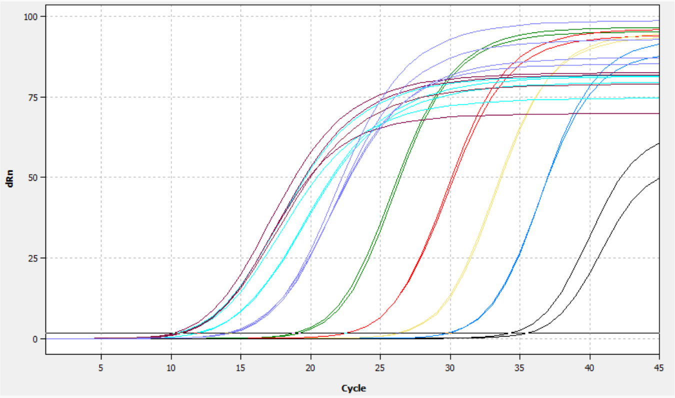


Figure 6: Bacterial DNA in unknown samples
Detection of bacterial DNA via real-time PCR on qTOWER³. Standard curves as shown in Figure 4. Duplicate curves of 3 duplicate samples are shown in cyan (sample 1A+1B), brown (sample 2A+2B), and lilac (sample 3A+3B), the NTCs are shown in black. Ct values and corresponding copy numbers of bacterial DNA in the samples are listed in Table 2.

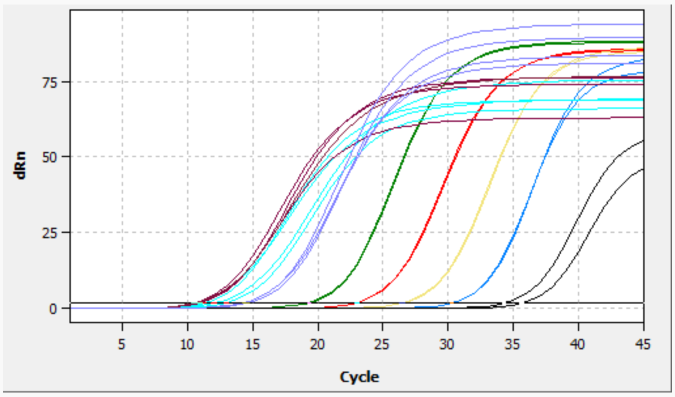


Figure 7: Internal control DNA – Amplification control via the Internal Control (IC) DNA, added to the PCR. The HEX-labeled IC-specific probe can be detected in the green or yellow color module of the qTOWER³. Here, the detection in the green channel is shown.

Sample name	Ct value	Copies
Sample 1A	10.29 10.48	2.2 x 10^8
Sample 1B	11.69 11.80	9.3 x 10^7
Sample 2A	10.01 10.60	2.3 x 10^8
Sample 2B	10.40 10.31	2.3 x 10^8
Sample 3A	13.83 14.15	2.2 x 10^7
Sample 3B	14.08 14.13	2.1 x 10^7

Table 2: Calculation of total bacterial DNA in cooling tower water samples

Legionella detection

The amplification of the gene conserved across the *Legionella* genus (Figure 8) shows that sample 1 is only weakly positive, with one extraction duplicate having a high Ct value above 37, and the second duplicate not showing a Ct. Sample 2 is negative for this gene. Sample 3 shows a positive signal in both extraction duplicates and therefore contains *Legionella* species. The strong signal of the positive control confirms that the real-time PCR set up and run was performed correctly. Whereas the NTC with no Ct values shows that there was no contamination within the PCR reagents.

To ascertain if sample 1 or sample 3 contain *Legionella pneumophila* DNA, the amplification of *mip* (Figure 9) was analyzed. Neither of the samples shows any amplification of this *L. pneumophila*-specific gene (Table 4). The positive control and negative control (NTC) confirm that the PCR results are valid, and reagents were not contaminated.

Conserved *Legionella* detection

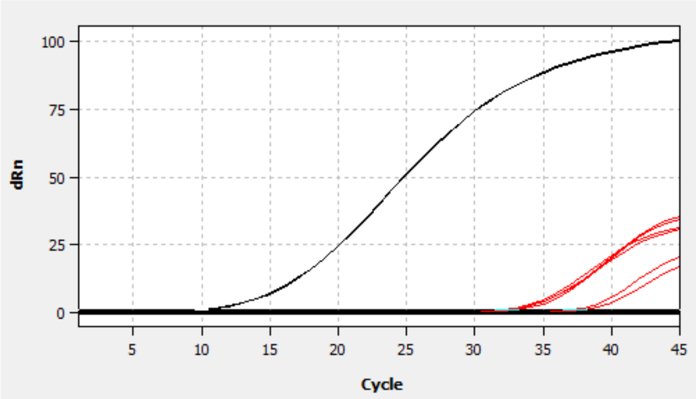


Figure 8: Amplification of the conserved gene
Detection of *Legionella pneumophila* as well as other *Legionella* species (*Legionella spp.*) in the Cy5 channel. The positive control is shown in black; samples are shown in red. Each sample is analyzed in duplicate.

Sample name	Ct value	*Legionella spp.*
Sample 1A	No Ct	(weakly) positive
Sample 1B	37.62	
Sample 2A	No Ct	negative
Sample 2B	No Ct	
Sample 3A	32.70	positive
Sample 3B	32.29	
Positive Control	10.42	positive
NTC	No Ct	negative

Table 3: Detection of an universal *Legionella* gene in the Cy5 channel

L. pneumophila-specific *mip* gene

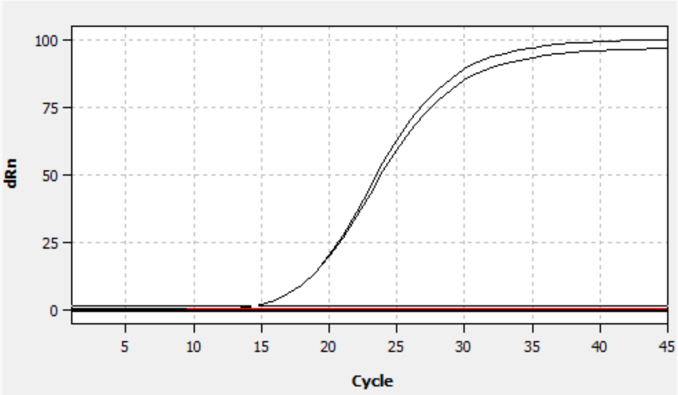


Figure 9: Amplification of *Legionella pneumophila*-specific *mip* gene
Detection of *mip* gene amplification in the FAM channel. The positive control is shown in black; samples are shown in red.

Sample name	Ct value	*Legionella pneumophila.*
Sample 1 A+B	No Ct	negative
Sample 2 A+B	No Ct	negative
Sample 3 A+B	No Ct	negative
Positive Control	15.15	positive
NTC	No Ct	negative

Table 4: Detection of *Legionella pneumophila*-specific *mip* gene in the FAM channel

Conclusion

The standard DNA dilutions were amplified and detected with innuDETECT Bacteria Quantification Assay (Figure 4). If the PCR efficiency is 100%, dilutions of 1:10 show a difference in Ct values of 3.3. The average Ct shift for the dilutions of the DNA standard is 3.6, corresponding to a PCR efficiency of 89% (Figure 5). Thus, a good correlation between the Ct values and the bacterial DNA content was achieved (Table 1). The NTC shows a Ct value above 35. It should be noted, that the polymerases used in PCR mixtures are usually generated in bacteria, thus containing trace amounts of bacterial DNA. These can also be detected with this assay, making the NTC necessary to account for this background of bacterial DNA. The determination of the abundance of bacterial DNA in the three samples that were extracted in duplicates (1A+B, 2A+B, 3A+B) was done by inference from their respective Ct values (Table 2) compared to Ct values of the standard DNA. As can be seen in Figure 6, the content of bacterial DNA is very high within the extracted samples, attesting to the very high efficiency of extraction and detection using this system. The amplification of the Internal Control DNA (Figure 7) confirms the correct performance of the PCR as well as the detection system.

Figure 10: qTOWER^3 real-time thermal cycler

Figure 11: InnuPure C16 *touch*

References

(1) TechNote: Implementation of SARS-CoV-2 (Coronavirus) detection assays from TIB MOLBIOL on qTOWER³ for research use

Literature

Zweiundvierzigste Verordnung zur Durchführung des Bundes-Immissionsschutzgesetzes

Cunha, Burke A et al. “Legionnaires’ disease.” Lancet (London, England) vol. 387,10016 (2016): 376-385. doi:10.1016/S0140-6736(15)60078-2

Paschke, Anne et al. “Legionella transmission through cooling towers: towards better control and research of a neglected pathogen.” The Lancet. Respiratory medicine vol. 7,5 (2019): 378-380. doi:10.1016/S2213-2600(19)30041-4

Elemental Analysis

AAS, ICP-MS, and ICP-OES

The analysis of toxic and ecotoxic elements is an important topic in environmental monitoring. All over the world defined regulations exist for analytical methods for the quantitative detection of lowest element concentrations. Special challenges for environmental monitoring laboratories are high sample throughput and the increasing complexity of the parameters to be monitored, as well as the increasing demands on the detection limits, all this at lowest costs.

Established analytical methods for multi-element analysis are inductively coupled plasma techniques of the PlasmaQuant series for optical emission (ICP-OES with PlasmaQuant 9100) and mass spectrometry (ICP-MS with PlasmaQuant MS) for ultra-trace analysis. The atomic absorption spectrometry (AAS) with the novAA, ZEEnit, and contrAA series offers easy-to-use and robust methods for routine analysis of few elements with moderate sample quantities. Complementary techniques such as dedicated mercury analysis using atomic fluorescence spectrometry (AFS) and AAS with the mercur round up the regulatory laboratory equipment for elemental analysis.

Overview

Application notes

Challenge

Determination of cadmium, lead, nickel, manganese, zinc, and chromium in sewage sludge.

Solution

Reliable routine analysis using flame AAS on the novAA 800 F.

Determination of Toxic and Eco-toxic Elements in Sewage Sludge with Flame AAS

Introduction

A global boom of industrialization and an increasing demand for advanced materials and products is leading to environmental release of harmful and even toxic compounds in many regions of the world. Toxic metals, such as cadmium, lead, or chromium, as well as high concentrations of other potentially harmful elements, e.g., nickel or copper, often pass into eco systems through sewage sludge from industrial sites, as well as from weathering or wearing of pipes, reactors, and other industrial facilities. Hence, a close monitoring of sewage sludge is the key to meeting statutory limits, and to allow targeted intervention in case of potential hazards.

This application note describes a straight-forward and robust flame AAS method for routine analysis of cadmium, lead, nickel, copper, manganese, zinc, and chromium in sewage sludge for industrial QC labs with moderate sample loads.

Materials and Methods

Samples and reagents

- Reference materials for sewage sludge, BCR 143R, and BCR 146R

Two reference materials for sewage sludge with known analyte concentrations were analyzed for method validation.

Sample preparation

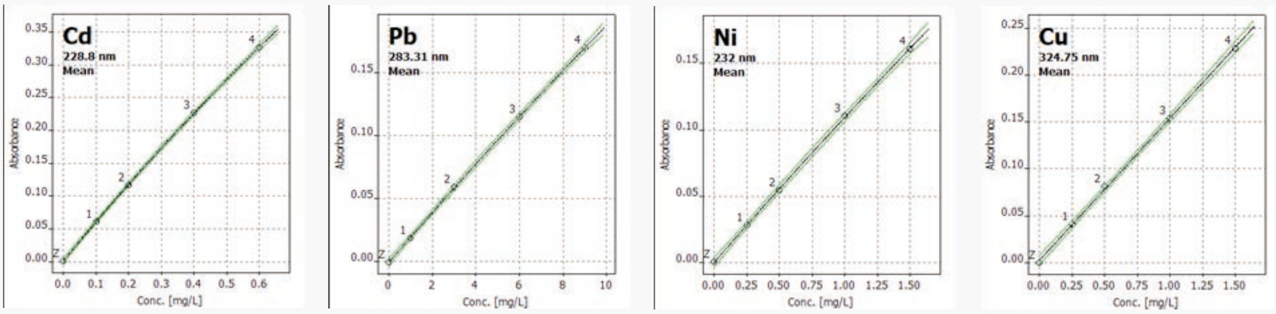
The sample was prepared according to ISO 15587-1 using the microwave system TOPwave (vessel type PM60). Approximately 0.5 g of the reference sample was digested in 6 mL aqua regia as digestion agent, transferred to a graduated flask and filled up to 50 mL with deionized water. If the extraction of potential fumes during the aqua regia digestion cannot be ensured, ISO 15587-2 using nitric acid as digestion agent can be applied as well. Alternatively, the sample can be digested in a beaker on a hot plate. However, silicones or certain organic compounds of the sewage sludge may not be completely digested in this case.

Calibration

A standard calibration was applied and standards were prepared manually using 1 % HCl and 0.1 % $CsCl/LaCl_3$. Alternatively, the standards can be prepared by the autosampler from a stock solution using the automated dilution function. The limit of quantification (LOQ) was calculated from 3 times the limit of detection (LOD). The method-specific limit of detection (LOD) was calculated from the 3-fold standard deviation of the 11-fold measurement of the digestion blank.

Standard	**Concentration [mg/L]**						
	Cd	**Pb**	**Ni**	**Cu**	**Zn**	**Mn**	**Cr**
Cal. 0	0	0	0	0	0	0	0
Cal. Std. 1	0.1	1	0.25	0.5	0.1	0.2	0.5
Cal. Std. 2	0.2	3	0.5	1	0.2	0.5	1
Cal. Std. 3	0.4	6	1	1.5	0.4	1	2
Cal. Std. 4	0.6	9	1.5	2	0.5	1.5	3

Table 1: Concentration of calibration standards



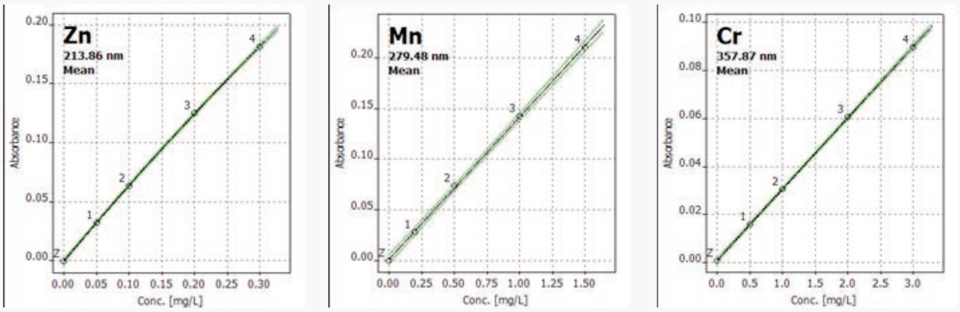
non-linear rational
$R^2 = 0.9999$
LOQ = 0.003 mg/L

non-linear rational
$R^2 = 0.9997$
LOQ = 0.024 mg/L

Linear
$R^2 = 0.9995$
LOQ = 0.094 mg/L

non-linear rational
$R^2 = 0.9991$
LOQ = 0.012 mg/L

Figure 1a–d: Calibration curves



non-linear rational
$R^2 = 0.9999$
LOQ = 0.003 mg/L

non-linear rational
$R^2 = 0.9992$
LOQ = 0.115 mg/L

non-linear rational
$R^2 = 0.9999$
LOQ = 0.102 mg/L

Figure 1e–g: Calibration curves

Instrument settings and method parameters

The measurements were performed using a novAA 800 F for flame AAS, equipped with injection switch SFS 6.0 and an autosampler with automatic dilution function. The analysis was carried out using a 100 mm burner head for air/acetylene flame and a 50 mm burner head for air/nitrous oxide flame.

The use of an automatic burner head cleaner, the Scraper, allows automated removal of deposits from the burner slit at regular intervals when using the nitrous oxide flame.

Figure 2: novAA 800 F

Element	Wavelength [nm]	Slit [nm]	Lamp current [mA]	Burner with [mm]	Burner height [mm]	Flame type	Fuel gas flow [L/h]
Cd	228.8	1.2	2	100	9	C_2H_2/air	40
Pb	283.3	1.2	4	100	7	C_2H_2/air	50
Ni	232.0	0.2	5	100	7	C_2H_2/air	50
Cu	324.7	1.2	5	100	6	C_2H_2/air	45
Zn	213.9	0.8	2	100	5	C_2H_2/air	50
Mn	279.5	0.2	5	100	9	C_2H_2/air	55
Cr	357.9	0.8	4	50	5	C_2H_2/N_2O*	210

* Cr as a refractory metal requires higher atomization temperatures, hence a C_2H_2/N_2O gas mixture and a 50 mm burner head may benefit the Cr analysis

Table 2: Instrument settings and method parameters

Results and Discussion

The analysis results (Table 3) for the two certified reference materials of industrial sewage sludge, BCR 143R and BCR 146R, show very good agreement of 94% to 103% with the certified values. All elements could be quantified by external calibration with aqueous standards in diluted or undiluted samples.

Sample	Element	Dilution factor	Measured concentration [mg/kg]	RSD [%]	Certified concentration [mg/kg]	Revovery rate [%]
Sewage sludge BCR 143 R	Cd	3	71.8	0.6	72.0	100
	Pb	2	174	3.7	174	100
	Ni	3	290	3.4	296	98
	Cu	2	125.7	0.6	128	96
	Zn	50	1061	2.5	1063	100
	Mn	15	848	0.6	858	99
	Cr	4	419	1.2	426	98
Sewage sludge BCR 146 R	Cd	2	18.7	0.8	18.4	102
	Pb	2	554	2.0	583	95
	Ni	1	64.9	2.8	65	100
	Cu	10	817	0.8	831	98
	Zn	200	2975	1.9	3040	98
	Mn	10	299	2.1	298	100
	Cr	2	179	1.9	174	103

Table 3: Measurement results

Conclusion

The novAA 800 F allows fast, simple and highly precise determination of cadmium, lead, nickel, copper, zinc, manganese, and chromium in pre-digested sewage sludge samples. Very good agreement of the results with the values of two certified reference materials (95–103%) as well as low standard deviations prove validity of the analysis procedure including the microwave-assisted digestions and therewith the high method robustness in high matrix samples.

The SFS 6.0 injection switch with continuous rinsing function and segmented sample injection ensures reduced carryover in case of high salt and matrix content while the automatic cleaning of the burner head using the Scraper provides stable analysis conditions for highly reproducible results. Using the autosampler with integrated dilution function enables a high sample throughput even for high matrix samples.

Challenge

Routine analysis of Cd, Cu, Ni, Pb, Zn, and Cr in wastewater and sewage sludge in a treatment plant.

Solution

Fast-sequential analysis using contrAA 800 in flame mode and autosampler AS-FD for full automation.

Fast-sequential Analysis of Toxic Elements in Wastewater and Sewage Sludge by HR-CS AAS

Introduction

In municipal and industrial treatment plants, many different wastewater samples from various inlets, outlets, and pumping stations within the wastewater treatment plant as well as samples from different industrial dischargers must be analyzed regularly. Relevant analytes include many toxic and ecotoxic elements, such as cadmium, copper, nickel, lead, zinc, and chromium. While the concentration of heavy metals in household wastewater is usually rather low, it can be very high in industrial effluents. In addition to wastewater, sewage sludge samples are also important. The heavy metal concentration in the sludge determines whether it can be used as a fertilizer in agriculture, needs to undergo separate treatment, e.g., in biogas plants, or even has to be stored as hazardous waste in special landfills. The high matrix content of the samples, especially due to organic components, usually requires a digestion step. Although this successfully destroys the organic matrix, it also introduces a high content of oxidizing acids, e.g., nitric acid, which can cause spectral interference. Matrix effects can also be caused by high concentrations of other elements, e.g., iron.

To meet all these challenges, the demands on the analysis instrument are high. In this application note, we analyzed wastewater and sewage sludge samples from a municipal sewage treatment plant using the contrAA 800 F, which is a high-resolution continuum source flame atomic absorption spectrometer (HR-CS-AAS). The contrAA 800 is able to process many samples with different matrices with a wide range of analytes. It is robust, easy to operate, and fully automated for daily routine operation using the autosampler AS-FD with automatic dilution function. The xenon short-arc lamp emits a continuous spectrum, so that all analysis lines can be used without changing the lamp. Thereby it allows a fast-sequential analysis of several elements with just one sample aspiration. Thus, the analysis of several elements in one sample hardly takes more time than the analysis of a single element. The high intensity of the lamp at all wavelengths allows very low detection limits. For higher concentrated samples an alternative wavelength with lower sensitivity can be selected, thus avoiding the need to dilute the sample.

Materials and Methods

Samples and reagents

The samples were supplied by the main sewage treatment plant of a midsized city in Germany. The inlet, the outlet, and two different pump stations of the sewage treatment plant were sampled, as well as wastewater from three different industrial dischargers. In addition, two different sewage sludge samples were analyzed.

Reagents:

- Hydrochloric acid, c(HCl) = 32wt-%, ρ(HCl) = 1.16 g/mL
- Nitric acid, c(HNO_3) = 65wt-%, ρ(HNO_3) = 1.39 g/mL
- Hydrogen peroxide solution, c(H_2O_2) = 30 %
- CsCl/$LaCl_3$ buffer (100 g/L each, 5% HCl)
- Certified single element standard solutions of Cd, Cu, Ni, Pb, Zn, and Cr (1000 mg/L each)
- Ultrapure water

Sample preparation

All samples went through a microwave digestion procedure. The wastewater samples were digested with a mixture of nitric acid and hydrogen peroxide, the sewage sludge samples with aqua regia. Before measurement, 0.1wt-% CsCl/$LaCl_3$ buffer (= 1 g/L) was added to each sample. Dilutions were prepared with 1vol-% HCl and 0.1wt-% CsCl/$LaCl_3$ in water.

Instrument settings and method parameters

The analysis was performed with the high-resolution continuum source atomic absorption spectrometer contrAA 800 in flame mode. The instrument was equipped with a 50 mm burner head, the injection switch SFS 6, and an AS-FD autosampler with automatic dilution function. For operation of the nitrous oxide flame, an automatic burner head cleaner, the scraper, was used additionally. The method parameters are shown in Table 1 and the evaluation parameters are shown in Table 2.

Element	Wavelength [nm]	Flame type	Fuel gas flow [L/h]	Burner height
Cd	228.8018	C_2H_2/air	45	7
Cu	324.7540	C_2H_2/air	45	6
Ni	232.0030	C_2H_2/air	45	6
Pb	217.0005	C_2H_2/air	50	8
Zn	213.8570	C_2H_2/air	50	7
Cr	359.3488	C_2H_2/N_2O	185	6

Table 1: Method parameters

Element	Measurement time [s]	Evaluation pixel	Spectral observation width		Background correction mode
			[nm]	[pixel]	
Cd	3	5	0.30	200	IBC
Cu	3	5	0.39	200	IBC
Ni	3	5	0.29	200	IBC + LSBC
Pb	3	5	0.28	200	IBC
Zn	3	5	0.27	200	IBC + LSBC
Cr	3	5	0.49	200	IBC

Table 2: Evaluation parameters

Calibration

All standards were prepared from certified single element standards with a concentration of 1000 mg/L and were diluted with 1 vol-% HCl and 0.1 wt-% $CsCl/LaCl_3$ in water. The calibration curves are shown in Figures 1 to 6 and the concentrations of the calibration standards are given in Table 3.

Standard	Concentration [mg/L]					
	Cd	Cu	Ni	Pb	Zn	Cr
Cal 0	0	0	0	0	0	0
Std. 1	0.025	0.05	0.1	0.25	0.025	0.2
Std. 2	0.05	0.1	0.2	0.5	0.05	0.4
Std. 3	0.1	0.2	0.4	1.0	0.1	0.6
Std. 4	0.2	0.4	0.8	2.0	0.2	0.8

Table 3: Concentrations of the calibration standards

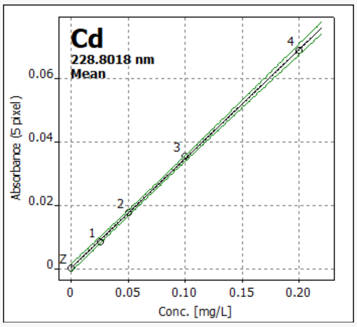


Figure 1: Cd 228.8018 nm
$R^2 = 0.9993$
LOD = 0.0005 mg/L

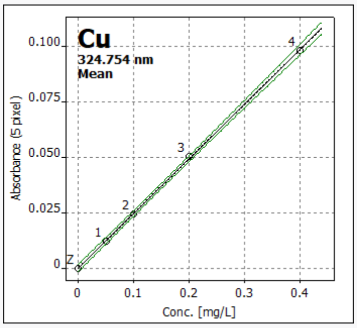


Figure 2: Cu 324.7540 nm
$R^2 = 0.9994$
LOD = 0.001 mg/L

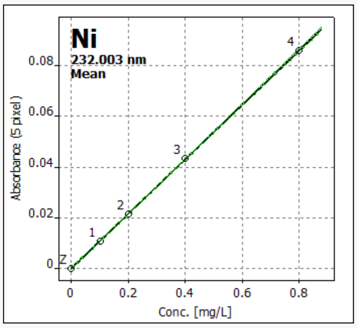


Figure 3: Ni 232.0030 nm
$R^2 = 0.9999$
LOD = 0.002 mg/L

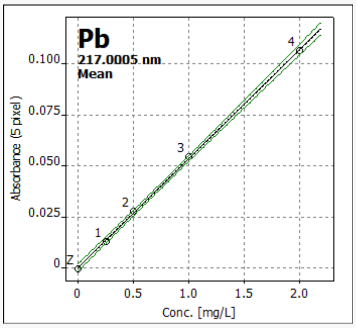


Figure 4: Pb 217.0005 nm
$R^2 = 0.9994$
LOD = 0.005 mg/L

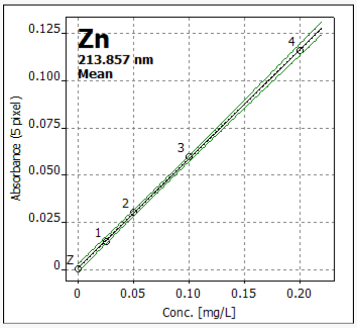


Figure 5: Zn 213.8570 nm
$R^2 = 0.9993$
LOD = 0.001 mg/L

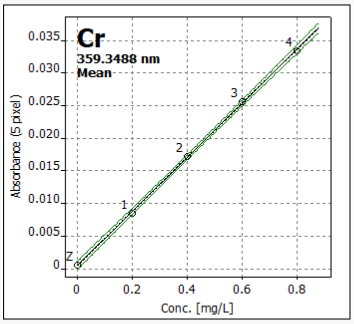


Figure 6: Cr 359.3488 nm
$R^2 = 0.9994$
LOD = 0.007 mg/L

Spectral vicinity and correction

Example spectra of all analytes, recorded during the sample measurements, are shown in Figures 7 to 12. In some spectra, additional lines caused by iron can be seen in the spectral vicinity of the analyte line. However, these lines are well resolved from the analyte line and therefore do not cause spectral interferences. All spectra were corrected automatically with the iterative background correction (IBC) mode. For nickel and zinc, an additional spectral correction using the least squares background correction (LSBC) method was applied to eliminate spectral interferences caused by nitric acid. The correction spectra of nitric acid used for this purpose as well as the original and corrected spectra for zinc are shown as an example in Figures 13 to 15.

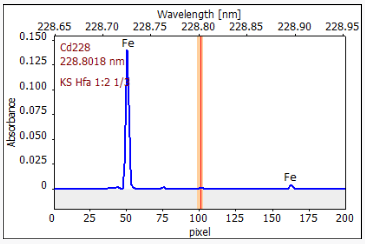


Figure 7: Spectrum of Cd in sewage sludge 1 corrected with IBC

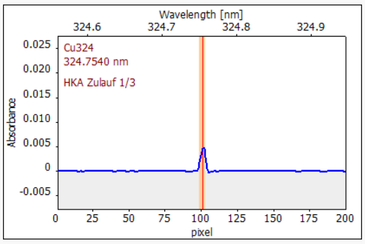


Figure 8: Spectrum of Cu in inlet wastewater corrected with IBC

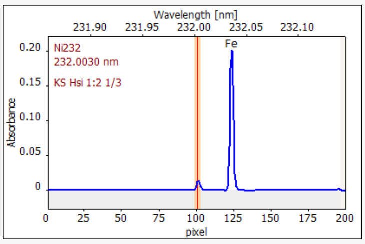


Figure 9: Spectrum of Ni in sewage sludge 2 corrected with IBC

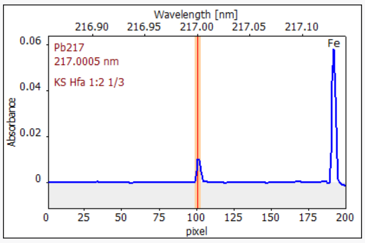


Figure 10: Spectrum of Pb in sewage sludge 1 corrected with IBC

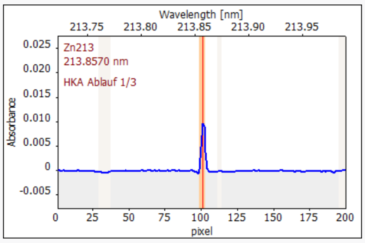


Figure 11: Spectrum of Zn in outlet wastewater corrected with IBC and LSBC

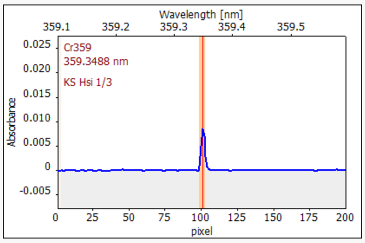


Figure 12: Spectrum of Cr in sewage sludge 2 corrected with IBC

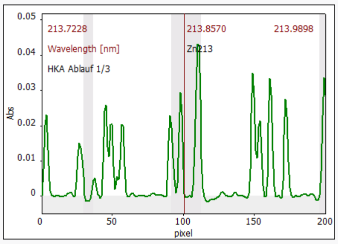


Figure 13: Correction spectrum of NO (diluted HNO_3) used for Zn

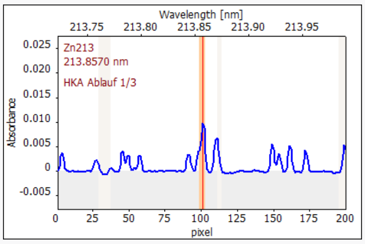


Figure 14: Uncorrected spectrum of Zn in wastewater

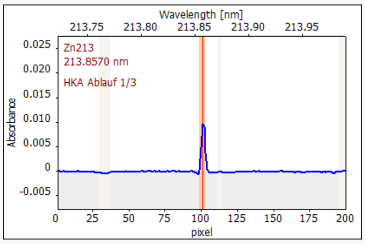


Figure 15: Corrected spectrum of Zn in wastewater with LSBC

Quality control and validation

The limits of detection (LOD) for this method were determined for all elements via the blank value procedure, in which the reagent blank, which contained all reagents used for microwave digestion and dilution, was measured eleven times. For quality control and validation, spiking experiments were conducted for all elements with one wastewater sample and one sewage sludge sample, respectively, and recovery rates were determined.

Results and Discussion

The results for the wastewater samples are shown in Table 4. The concentrations of lead and cadmium were below the detection limit in all samples. For all other elements, small but measurable concentrations from 0.01 mg/L to 0.15 mg/L were determined in all samples. The precision was very high for these low concentrations with RSD values from 0.1% to 5.5%. The inlet, outlet, and pump station samples as well as two of the industrial wastewater samples were measured directly without dilution, while the third industrial sample with a concentration of 0.4 mg/L zinc required a dilution by a factor of 10.

The concentrations in the sewage sludge samples (Table 5) ranged from 0.4 mg/kg for cadmium up to 603 mg/kg for zinc. None of the samples was below the detection limit. For the analysis of copper and zinc, the samples were diluted by a factor of 200, for cadmium, nickel, lead, and chromium by a factor of 2. The precision was excellent with standard deviations from 0.3% to 1.6%.

In the spiking experiments of wastewater and sewage sludge samples (Table 6), recovery rates between 92.5% and 108% were reached. These results show that there were no interfering matrix effects and prove the validity of the method.

Sample	Element	Pre-dilution factor	Concentration in original sample [mg/L]	RSD** [%]
Inlet	Cd	1	< 0.0005*	-
	Cu	1	0.083	0.8
	Ni	1	0.015	2.5
	Pb	1	< 0.005*	-
	Zn	1	0.12	0.4
	Cr	1	0.026	3.8
Outlet	Cd	1	< 0.0005*	-
	Cu	1	0.023	1.5
	Ni	1	0.018	1.5
	Pb	1	< 0.005*	-
	Zn	1	0.046	1.3
	Cr	1	0.033	5.5
Pump station 1	Cd	1	< 0.0005*	-
	Cu	1	0.048	0.4
	Ni	1	0.019	1.5
	Pb	1	< 0.005*	-
	Zn	1	0.069	0.5
	Cr	1	0.043	4.2
Pump station 2	Cd	1	< 0.0005*	-
	Cu	1	0.053	1.1
	Ni	1	0.022	1.0
	Pb	1	< 0.005*	-
	Zn	1	0.063	1.2
	Cr	1	0.039	5.2
Industrial discharger 1	Pb	1	< 0.005*	-
Industrial discharger 2	Cu	1	0.145	1.3
Industrial discharger 3	Zn	10	0.389	0.1

*) Limit of detection (LOD), determined via blank value procedure with eleven measurements of reagent blank

**) RSD = Relative Standard Deviation for three repetition measurements

Table 4: Results for wastewater samples

Sample	Sample weight per 100 mL [g]	Element	Pre-dilution factor	Measured concentration [mg/L]	Concentration in original sample [mg/kg]	RSD [%]
Sewage sludge 1	4.46	Cd	2	0.0095	0.427	1.1
		Cu	200	0.060	270	0.5
		Ni	2	0.320	14.4	0.5
		Pb	2	0.556	25.0	0.7
		Zn	200	0.128	574	0.3
		Cr	2	0.564	25.3	1.6
Sewage sludge 2	4.62	Cd	2	0.011	0.498	1.2
		Cu	200	0.061	265	1.2
		Ni	2	0.362	15.7	0.4
		Pb	2	0.488	21.1	0.9
		Zn	200	0.139	603	0.9
		Cr	2	0.585	25.3	0.9

Table 5: Results for sewage sludge samples

Conclusion

With this method, wastewater, and sewage sludge can be analyzed trouble-free. By using a xenon short-arc lamp as continuum source in HR-CS-AAS, all absorption lines in the spectral range of 185 to 900 nm are available for analytical evaluation. This enables the fast-sequential analysis for the elements cadmium, copper, nickel, lead, and zinc in one method, which reduces the measurement time per sample by factor 3 to 4 compared to individual measurements and thus significantly reduces costs. Spectral background is corrected directly on the analysis line, simultaneously and independently of the wavelength used. The spectral environment of cadmium, nickel, and lead shows further absorption lines caused by iron. However, due to the high spectrometer resolution, the signals are clearly separated from each other so that no spectral interference occurs. When spiking different sample matrices with a defined element concentration as quality control, very good recovery rates of 92.5 to 108% were achieved. This shows the matrix independence of the measurements.
For the determination of chromium, the use of the nitrous oxide flame is recommended, since the higher flame temperature eliminates interferences and thus allows an interference-free analysis without the need to add further reagents. A recommended regular cleaning of the burner head using the additional flame accessory, the scraper, ensures a reliable nitrous oxide flame operation.

Sample	Element	Expected concentration increase [mg/L]	Recovery rate [%]
Inlet	Cd	0.05	93.1
	Cu	0.1	92.5
	Ni	0.2	93.3
	Pb	0.5	95.6
	Zn	0.05	95.2
Sewage sludge 1	Cd	0.05	103
	Cu	0.1	95.8
	Ni	0.2	94.5
	Pb	0.5	105
	Zn	0.05	108
Pump station 2	Cr	0.4	94.1
Sewage sludge 2	Cr	0.4	96.7

Table 6: Recovery rates of the QC stock samples

Figure 16: contrAA 800D and autosampler AS-FD

Challenge

Trace determination of As, Sb, Se, Hg, Pb, and Cd in surface and drinking water.

Solution

Reliable routine analysis with lowest detection limits using AAS in combination with the hydride and HydrEA technique.

Trace Analysis of Toxic Elements in Surface and Drinking Water by Combined AAS Techniques

Introduction

Safe drinking water is essential for health. Even low concentrations of toxic elements can be harmful if contaminated water is consumed regularly over a long period of time. Many groundwater sources supplying public and private wells contain a high concentrations of naturally occurring arsenic. Lead contamination can be caused, for example, by slow dissolution of lead water pipes. In addition, groundwater and surface water can be contaminated with toxic elements through environmental pollution. Therefore, strict regulations apply to the control of drinking water, such as the Drinking Water Directive of the European Commission[1], the U.S. EPA National Primary Drinking Water Regulations (NPDWR)[2], or the German Drinking Water Regulation (TrinkwV)[3].

The limits for the most toxic elements are usually regulated from 1 to 10 µg/L. Atomic absorption spectroscopy with graphite furnace and hydride technology meets the high requirements of drinking water analysis, such as lowest detection limits and high accuracy. It is simple, robust, and suitable for routine use by means of automation. The hydride system HS60 with flow injection allows an automated sample processing with detection limits down to 0.05 µg/L for the hydride forming elements. With the integrated gold-collector, mercury can be enriched and measured down to 0.02 µg/L. For even greater sensitivity, the so-called HydrEA technique can be used. This combination of graphite furnace AAS and hydride technology provides in-situ enrichment of mercury as well as the hydride forming elements arsenic, antimony, and selenium inside the graphite tube. Thus, the technique allows trace element analysis with detection limits as low as 0.005 µg/L.

Materials and Methods

In this application note, mercury and the hydride-forming elements arsenic, antimony, and selenium were analyzed in certified reference materials and drinking water using the HydrEA technique. The hydride system HS60 with continuous flow injection was coupled with an autosampler, allowing a fully automated analysis procedure. Combined with the graphite furnace AAS of the ZEEnit series, sensitive analysis of mercury and the hydride-forming elements was achieved. The analytes were preconcentrated in the graphite tube (wall type), which was coated with gold (for mercury) or iridium (for the hydride-forming elements). Subsequently, they were electrothermally atomized and analyzed. For comparison, a calibration curve for mercury and arsenic using the classical hydride technique with a quartz cell was also performed. In addition, cadmium and lead were determined via graphite furnace AAS with superior Zeeman background correction.

Samples and reagents

- Hydrochloric acid: c(HCl) = 32 (wt-%, ρ(HCl) = 1.16 g/mL
- Nitric acid: c(HNO_3) = 65 wt-%, ρ(HNO_3) = 1.39 g/mL
- Potassium iodide (KI) / ascorbic acid solution: c(KI) = 500 g/L, c(ascorbic acid) = 100 g/L
- $NaBH_4$ solution: 0.3 wt-% $NaBH_4$ and 0.1 wt-% NaOH in deionized water
- Solution for gold coating of the graphite tube: c(Au) = 1 g/L
- Solution for iridium coating of the graphite tube: c(Ir) = 1 g/L
- Pd/Mg$(NO_3)_2$-modifier for GF-AAS: c(Pd) = 1 g/L, c(Mg$(NO_3)_2$) = 0.1 g/L
- Certified reference material for Hg in water: CRM 1641d
- Certified reference materials for As, Sb, Se, Pb, and Cd in water: CRM 1640a and CRM 1643f

Sample Preparation

The analysis of arsenic and antimony was performed according to ISO 17378-2:2014. Since only As(III) and Sb(III) react quickly and quantitatively under the conditions used in hydride technique, As(V) and Sb(V) have to be reduced to As(III) or Sb(III) prior to the step of hydride generation. This was assured by adding 15 mL of hydrochloric acid and 1 mL of KI/ascorbic acid solution to 25 mL of prediluted sample solution. After a reaction time of two hours at room temperature the mixture was filled up to a volume of 50 mL with deionized water. The blank sample and the standard solutions were prereduced in the same way.

The analysis of selenium was performed according to ISO/TS 17379-2:2013. Similar to arsenic and antimony, Se(V) has to be reduced to Se(III) prior to the step of hydride generation. For the prereduction of Se(V) to Se(III), 15 mL of hydrochloric acid were added to 25 mL of prediluted sample solution. The mixture was carefully boiled under reflux for one hour and then made up to a volume of 50 mL with deionized water. The blank sample and the standard solutions were prereduced in the same way.

For the analysis of mercury, the dilutions of the samples and the standards were prepared in 0.6 vol-% HCl and 0.3 vol-% HNO_3. For the analysis of cadmium and lead, 0.5 vol-% HNO_3 was used as diluting solution for samples and standards.

Instrumentation

All measurements were carried out with the ZEEnit 650 P GF-AAS, equipped with an AS-GF autosampler. For mercury and the hydride forming elements, the flow injection hydride system HS60 with HydrEA upgrade kit and the AS-F autosampler were used additionally. The method parameters for the GF-AAS for all elements are shown in Table 1, the parameters for the hydride system in HydrEA mode for mercury, arsenic, antimony, and selenium can be found in Table 2. The method parameters for the hydride system in classical hydride mode with a quartz cell are shown in Table 3 for arsenic and in Table 4 for mercury.

Element	Technique (graphite tube type)	Wave-length [nm]	Slit [nm]	Lamp current [mA]	T_{Drying} [°C]	$T_{Pyrolysis}$ [°C]	$T_{Atomizing}$ [°C]	Ramp [°C/s]	Modifier
Hg	HydrEA (wall)	253.7	1.2	3	65	-	950	1200	Gold coating
As	HydrEA (wall)	193.7	0.8	5	300	-	2100	1000	Iridium coating
Sb	HydrEA (wall)	217.6	0.2	7	300	-	2100	1000	Iridium coating
Se	HydrEA (wall)	196.0	1.2	6	300	-	2150	1000	Iridium coating
Cd*	EA (platform)	228.8	0.8	2	80/90/110	600	1600	1500	5 µL $Pd/Mg(NO_3)_2$
Pb*	EA (platform)	283.3	0.8	2	85/95/110	900	1900	1900	5 µL $Pd/Mg(NO_3)_2$

* Pb and Cd were measured in Zeeman 2-field mode with max. 0.8 T magnetic field strength.

Table 1: Method parameters for GF-AAS for all analytes

Element	Load time [s]	Reaction time [s]	Purge time 1 [s]	Purge time 2 [s]	Transport solution	Reduction solution
Hg	20	20	30	7	3% HCl	0.3% $NaBH_4$ + 0.1% NaOH
As, Sb, Se	20	20	35	5	3% HCl	0.3% $NaBH_4$ + 0.1% NaOH

Table 2: Hydride system parameters for mercury, arsenic, antimony, and selenium in HydrEA mode

Element	Load time [s]	AZ wait time [s]	Reaction time [s]	Purge time 1 [s]	Transport solution	Reduction solution
As	14	20	20	40	3% HCl	0.3% $NaBH_4$ + 0.1% NaOH

Table 3: Hydride system parameters for arsenic in classical hydride mode with quartz cell

Element	Load time [s]	Reaction time [s]	Purge time 1 [s]	Purge time 2 [s]	Purge time 3 [s]	Heat time collector [s]	Cool time collector [s]	Transport solution	Reduction solution
Hg (without enrichment)	14	20	30	15	-	-	-	3% HCl	0.3% $NaBH_4$ + 0.1% NaOH
Hg (with enrichment)	14	20	20	50	0	15	40	3% HCl	0.3% $NaBH_4$ + 0.1% NaOH

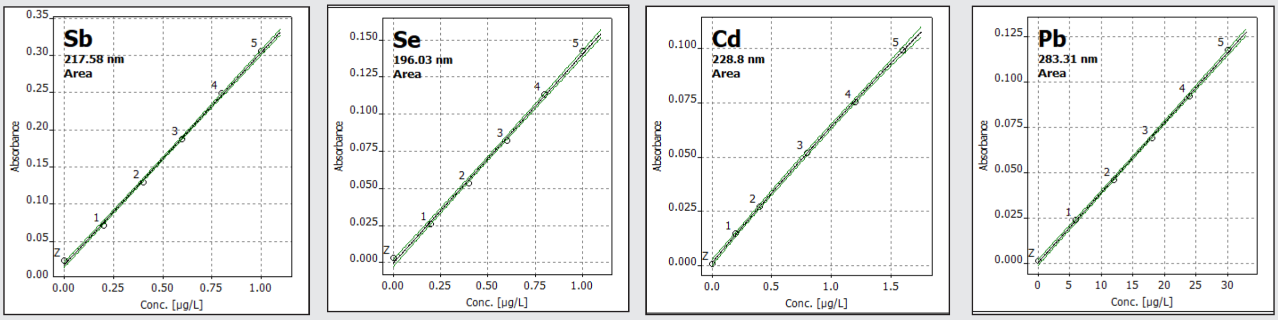
Table 4: Hydride system parameters for mercury with and without enrichment in classical hydride mode with quartz cell

Calibration

An external calibration with aqueous standards was performed for all analytes. The concentrations of the calibration standards are shown in Table 5. Figures 1a–d show the calibration curves with corresponding correlation factors R^2 and limits of detection (LOD) for antimony and selenium obtained by HydrEA technique, as well as for cadmium and lead obtained by graphite furnace AAS. Figures 2a–e shows a comparison of the calibration curves for mercury and arsenic, which were obtained by classical hydride technique with quartz cell and using the HydrEA technique.

Standard	**Concentration [µg/L]**								
	Hg (HydrEA)	**Hg** (hydride w/o enrich.)	**Hg** (hydride w/ enrich.)	**As** (hydride)	**As** (HydrEA)	**Sb** (HydrEA)	**Se** (HydrEA)	**Cd** (GF-AAS)	**Pb** (GF-AAS)
Cal 0	0	0	0	0	0	0	0	0	0
Std. 1	0,1	0.5	0.2	0.5	0.2	0.2	0.2	0.2	6
Std. 2	0.5	1.0	0.4	1.0	0.4	0.4	0.4	0.4	12
Std. 3	1.0	2.5	0.8	2.0	0.6	0.6	0.6	0.8	18
Std. 4	1.5	5.0	1.6	4.0	0.8	0.8	0.8	1.2	24
Std. 5	2.0	-	-	8.0	1.0	1.0	1.0	1.6	30

Table 5: Concentrations of the calibration standards



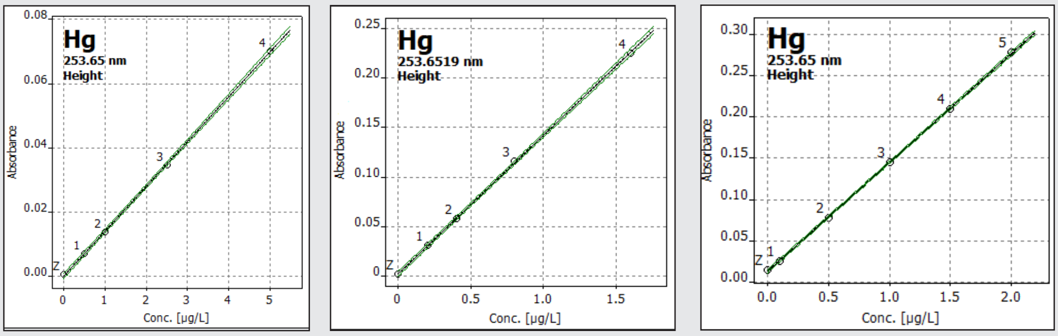
Evaluation by area
Linear, R^2 = 0.9979
LOD = 0.01 µg/L

Evaluation by area
Linear, R^2 = 0.9960
LOD = 0.01 µg/L

Evaluation by area
Non-linear rational, R^2 = 0.9997
LOD = 0.009 µg/L

Evaluation by area
Linear, R^2 = 0.9992
LOD = 0.25 µg/L

Figures 1a–d: Calibration parameters and limits of detection (LOD) for antimony and selenium with HydrEA technique and for cadmium and lead with graphite furnace AAS

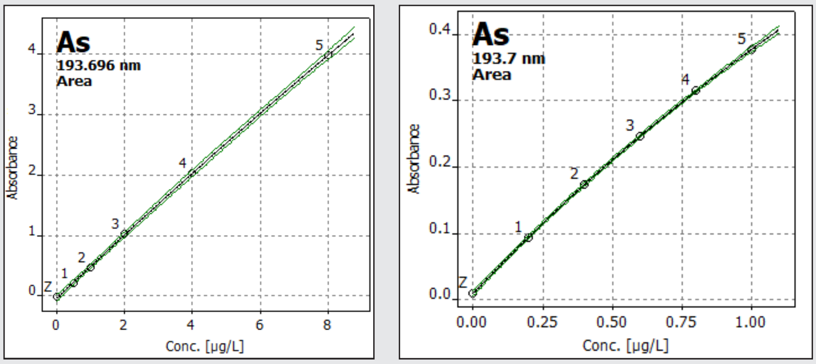


Evaluation by height
Linear, R^2 = 0.9997
LOD = 0.05 µg/L
Classical hydride technique
without enrichment

Evaluation by height
Linear, R^2 = 0.9997
LOD = 0.02 µg/L
Classical hydride technique
with enrichment on gold collector

Evaluation by height
Linear, R^2 = 0.9998
LOD = 0.005 µg/L
HydrEA technique (hydride + GF-AAS)

Figures 2a–c: Calibration parameters and limits of detection (LOD) for mercury with classical hydride technique and HydrEA technique



Evaluation by area
Linear, $R^2 = 0.9997$
LOD = 0.05 µg/L
Classical hydride technique

Evaluation by area
Non-linear rational, $R^2 = 0.9999$
LOD = 0.005 µg/L
HydrEA technique (hydride + GF-AAS)

Figures 2d–e: Calibration parameters and limits of detection (LOD) for arsenic with classical hydride technique and HydrEA technique

Results and Discussion

All samples in this application note were analyzed by HydrEA technique and graphite furnace AAS. For the analysis of certified reference materials, very good recovery rates of the measured concentrations with the certified values could be achieved (93–109%, Table 6). The relative standard deviations for three replicate measurements were usually below 3%. In the drinking water sample, all analyte concentrations were below 1 µg/L. Excellent recovery values of 94–105% for spiked concentrations in the drinking water sample have been achieved (Table 7).

Sample	Element	Dilution factor	Certified concentration [µg/L]	Measured concentration [µg/L]	RSD [%]	Recovery of certified concentration
CRM 1641d	Hg	1000	1568	1520	1.3	97.4
CRM 1640a	As	10	8.075	7.51	0.5	93.4
	Sb	10	5.105	4.81	2.5	94.1
	Se	25	20.13	19.3	1.5	95.9
	Cd	10	3.992	4.37	3.4	109.3
	Pb	1	12.101	11.89	0.4	98.2
CRM 1643f	As	100	57.42	53.4	2.8	92.9
	Sb	100	55.45	53.6	1.5	96.7
	Se	25	11.700	12.2	5.6	104.2
	Cd	10	5.89	5.66	1.8	96.1
	Pb	1	18.488	17.16	0.9	92.8

Table 6: Results for the certified reference materials

Sample	Element	Dilution factor	Measured concentration [µg/L]	RSD of sample [%]	Spiked concentration increase [µg/L]	RSD of spiked sample [%]	Recovery rate [%]
Drinking water	Hg	2	< LOD	-	1.0	0.5	99.4
	As	2	0.77	1.4	0.6	1.2	94.0
	Sb	2	0.11	13.0	0.6	0.8	95.5
	Se	2	0.56	2.2	0.6	1.8	100.2
	Cd	1	< LOD	-	0.6	0.9	105.0
	Pb	1	0.92	23.4	9	0.7	104.5

Table 7: Results for drinking water and recovery rates for spiking experiments

Conclusion

The hydride system HS60 with continuous flow injection in combination with Analytik Jena's AAS instruments are reliable, easy to use, and therefore perfectly suited for analysis of mercury and the hydride forming elements arsenic, selenium, and antimony in the low µg/L range. Coupling the hydride system with an AS-F autosampler allows complete automation of the analysis procedure and makes the application perfect for robust routine analysis. For the precise analysis of trace concentrations below 1 µg/L, as frequently found in drinking water, the combination of the HS60 hydride system (with the autosampler AS-F) and graphite furnace AAS is an excellent tool. This so-called HydrEA technique unites the high atomization efficiency and sensitivity of graphite furnace AAS with the high selectivity of the hydride generation or cold vapor technique due to the complete separation of the interfering matrix. Limits of detection as low as 0.005 µg/L can be reached with this technique. Furthermore, traces of toxic elements such as lead and cadmium can be analyzed even in complex matrices using the graphite furnace AAS of the ZEEnit series with superior Zeeman background correction.

Figure 3: ZEEnit 650P GF-AAS

Figure 4: Hydride system HS60

References:

(1) Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, OJ L 330, 5.12.1998, p. 32–54 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)

(2) National Primary Drinking Water Regulations, United States Environmental Protection Agency

(3) Verordnung über die Qualität von Wasser für den menschlichen Gebrauch (Trinkwasserverordnung - TrinkwV), 2001

Challenge

Analysis of trace element impurities and major components in saline matrices such as brines or sea water with high precision, accuracy, and long-term stability.

Solution

HR ICP-OES with high-resolution optical system and superior sensitivity and matrix tolerance to achieve sub-ppb detection limits for trace element analysis in saline matrices.

Direct Analysis of Saline Matrices by HR ICP-OES

Introduction

Brines play an important role in many industrial processes, e.g., in the production of magnesium and chlorine by electrolysis, as agent for water-injection in oil-wells, or as starting material for the fabrication of high-purity salts and preservatives. In some regions, naturally occurring brines and sea water are also considered to be an attractive candidate for drinking water production. Yet the direct elemental analysis of saline matrices using ICP techniques in routine laboratories is challenging in respect to (a) achieving good signal stability with low signal to noise levels, (b) ionization interferences of alkali and alkaline earth metals that often lead to intensity deviations in the range from ± 5 to 10%, and (c) maintenance issues, memory effects, and short lifetime of glassware and clogging.

Since saline matrices are used for various purposes, a large number of elements with limits of detection as low as possible are of interest. Due to its high sensitivity, ICP-MS is typically the method of choice to achieve limits of detection in the ppt range. However, ICP-MS typically tolerates matrix concentrations not higher than 0.3%, which requires high dilution of the samples. Thus, matrix-specific limits of detection are compromised depending on the applied dilution factor. In general, ICP-OES instrumentation is more matrix tolerant compared to ICP-MS. The comparatively low sensitivity of conventional ICP-OES, however, hampers achieving sufficiently low limits of detection.

The HR ICP-OES PlasmaQuant 9100 Elite exhibits excellent matrix and plasma robustness, being able to tolerate salt concentrations of up to 300 g/L. Additionally, the high-resolution optical system achieves high sensitivity and ensures free line selection. The PlasmaQuant 9100 Elite allows for running undiluted saline samples of almost any salt concentration with the highest sensitivity amongst ICP-OES instrumentation. Matrix specific limits of detection (ppt to ppb range) can be achieved.

Within this study, the performance of the PlasmaQuant 9100 Elite was studied on an exemplary saline matrix of 35 g/L NaCl for concentrations, limits of detection, and long-term stability of B, Ba, Be Br, Ca, Co, Cr, Fe, I, K, Li, Mg, Mn, Mo, Na, P, S, Si, Sr, V, and Zn.

Materials and Methods

Samples and reagents

Two samples with a nominal NaCl content of 35 g/L were submitted as-received to a standard calibration routine using a 100 mg/L aqueous multi-element standard and 1000 mg/L single-element standards from SIGMA ALDRICH in 2% HNO_3.

Calibration

A standard calibration was applied and standards were prepared manually using 1% HCl and 0.1% CsCl/$LaCl_3$. Alternatively, the standards can be prepared by the autosampler from a stock solution using the automated dilution function.

Standard	Unit	Cal.0	Cal.1	Cal.2
B, Sr	mg/L	0	0.1	1
Ba, Be, Co, Cr, Fe, Li, Mn, Mo, V, Zn	µg/L	0	20	50
Br, Ca, I, K, S,	mg/L	0	10	100
Mg	mg/L	0	10	200
Si	mg/L	0	10	500
Na	mg/L	0	100	1000
P	mg/L	0	50	500

Table 1: Concentration of calibration standards

Instrument settings and method parameters

For the analysis, a PlasmaQuant 9100 Elite equipped with Salt-Kit and ASPQ 3300 autosampler was used. The detailed system configuration is given in Table 2.

Parameter	Unit
Power	1400 W
Plasma gas flow	15 L/min
Auxillary gas flow	1.0 L/min
Nebulizer gas flow	0.5 L/min
Nebulizer	Concentric nebulizer for high salt content, borosilicate, 2.0 mL/min
Spray chamber	Cyclonic spray chamber with dip tube[1], 50 mL, borosilicate
Injector	Quartz, inner diameter 2 mm
Outer tube/ Inner tube	Syalon[2] / quartz
Pump tubing	PVC
Sample pump rate	1 mL/min
Rinse/ Read delay	45 s
Auto sampler	ASPQ 3300

1 ... the double-path geometry improves precision for high matrix-loadings
2 ... ceramic outer tube prolongs the life-time of torch in sodium-rich matrices

Table 2: Plasma configuration and set-up of the sample introduction system

Evaluation parameters

Element	Line [nm]	Plasma view	Integration mode	Read time [s]	Evaluation			
					No. of pixel	Baseline fit	Polynomial degree	Correction
B	249.773	axial	peak	3	3	ABC[1]	auto	Y[2]
Ba	455.403	axial	peak	3	3	ABC	auto	Y
Be	313.107	axial	peak	3	3	ABC	auto	Y
Br	163.283	axial	peak	3	3	ABC	auto	Y
Ca	239.856	axial plus	peak	3	3	ABC	auto	Y
Co	228.615	axial	peak	3	3	ABC	auto	Y
Cr	267.716	axial	peak	3	3	ABC	auto	Y
Fe	259.940	axial	peak	3	3	ABC	auto	Y
I	178.218	axial	peak	3	3	ABC	auto	Y
K	766.491	radial plus	peak	3	3	ABC	auto	Y
Li	670.791	radial	peak	3	3	ABC	auto	Y
Mg	285.213	radial	peak	3	3	ABC	auto	Y
Mn	257.610	axial	peak	3	3	ABC	auto	Y
Mo	203.844	axial	peak	3	3	ABC	auto	Y
Na	330.237	radial plus	peak	3	3	ABC	auto	Y
P	177.436	axial	peak	3	3	ABC	auto	Y
S	180.672	axial plus	peak	3	3	ABC	auto	Y
Si	251.611	axial	peak	3	3	ABC	auto	Y
Sr	421.552	radial	peak	3	3	ABC	auto	Y
V	292.464	axial	peak	3	3	ABC	auto	Y
Y	371.030	axial[3]	peak	3	3	ABC	auto	-
Zn	206.200	axial	peak	3	3	ABC	auto	Y

1 … automatic baseline correction (ABC)
2 … correction by internal standard; no mathematical correction of spectral interferences by CSI tool required
3 … in addition line was measured in axial Plus, radial and radial Plus plasma view

Table 3: Overview of method-specific evaluation parameters

Results and Discussion

The detection power of ICP-OES for matrix-rich saline samples is highly dependent on plasma stability. For samples like brines and sea water, effective limits of detection will be best when excellent plasma and signal stability are achieved while sample dilution is kept at a minimum.

The here developed method for undiluted saline samples (35 g/L NaCl) uses the most sensitive lines for all investigated trace elements resulting in matrix specific limits of detection of significantly less than 1 ppb, respectively (e.g., 0.14 µg/L for Cr). From the same run, major contents of Na, Ca, Sr, Br, K, Li, Mg and S could be obtained be employing the Dual View Plus plasma observation modes of the PlasmaQuant 9100 Elite. Long-term stability testing was performed by continuous aspiration of a spiked saline solution. RSD values of less than 3% over a period of 8 hours of aspiration without intermediate cleaning of the glassware (Figure 1) proves the applicability of the used methodology for routine analyses.

Isotope	Expected range		Sample A	RSD[1] [%]	Sample B	RSD [%]	matrix specific LOD
	Value	Unit					
B	4	mg/L	4.60	0.31	4.55	0.63	0.46
Ba	< 10	µg/L	7.78	0.22	7.89	0.63	0.02
Be	< 1	µg/L	0.21	3.31	0.22	2.19	0.02
Br	60	mg/L	54.9	7.98	56.0	6.96	8.65
Ca	400	mg/L	400	0.22	396	0.21	-[2]
Co	< 1	µg/L	< LOD[3]	–	< LOD[3]	–	0.32
Cr	< 1	µg/L	0.78)[4]	5.67	0.55)[4]	9.16	0.14
Fe	< 1	µg/L	< LOD	–	< LOD	–	1.63
I	< 60	mg/L	< LOD	–	< LOD	–	0.02
K	400	mg/L	388	0.31	393	0.16	-[3]
Li	200	µg/L	192	0.95	209	0.42	1.88
Mg	1,300	mg/L	998	0.01	999	0.01	1.40
Mn	< 2	µg/L	0.28	2.69	0.31	4.24	0.03
Mo	< 12	µg/L	10.5	7.06	10.8	4.22	2.16
Na	10,000	mg/L	10,880	2.93	10,960	1.29	-[2]
P	< 50	µg/l	36.0	4.73	39.8	6.4	8.96
S	900	mg/L	919	0.93	894	0.18	9.46
Si	< 500	µg/L	411	0.78	402	1.47	1.66
Sr	8	mg/L	7.36	0.31	7.25	0.38	0.05
V	< 1	µg/L	0.77)[4]	10.8	0.80)[4]	518	0.45
Zn	< 5	µg/L	1.30	4.77	1.42	4.62	0.24

1 … RSD values obtained from three replicate measurements
2 … no values obtained
3 … value below detection limit
4 … value in the range or above of matrix-specific detection limit, hence leading to high RSD values

Table 4: Overview of results of two saline samples

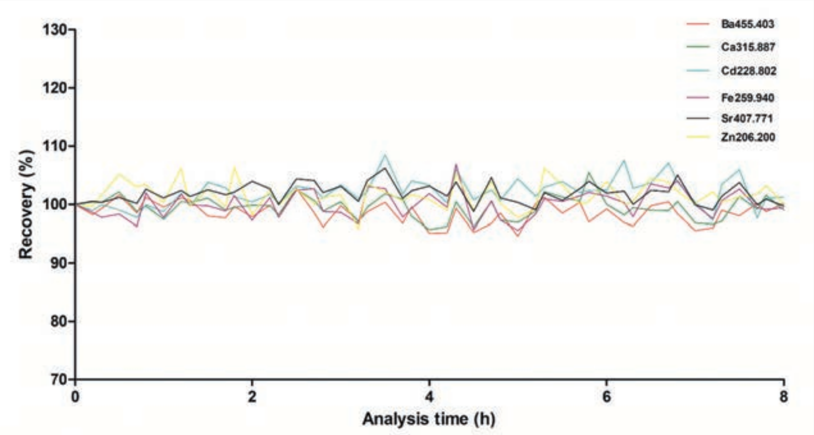


Figure 1: Percentage recoveries of an 8 hour measurement of exemplary elements (100 µg/L) in NaCl matrix (35 g/L)

Conclusion

The analysis of samples such as brines or sea water often demands the quantification of elements in trace or ultratrace levels. At the same time, the salinity of the samples poses a highly demanding challenge, since this salinity has a negative impact on signal stability. For most instrumentation (ICP-OES and ICP-MS), dilution is often the only solution in order to guarantee signal stability, which is crucial if the small analyte signals are to be detected.

In contrast to ICP-MS instrumentation, the PlasmaQuant 9100 Elite HR ICP-OES is able to run undiluted saline samples. Moreover, its optical resolution and sensitivity allows for unmatched matrix specific limits of detection among ICP-OES instrumentation. Since sample dilution is not required, limits of detection comparable to ICP-MS can be achieved.

Due to the highly robust plasma, even medium salt concentrations (e.g. 35 g/L) can be measured by using a simple aqueous calibration. Complex sample and standard preparation procedures such as matrix-matching or standard-additions are not required.

In addition to trace elements, major components can be analyzed from the same run by exploiting the wide working range offered as DualView Plus by the PlasmaQuant 9100 Elite. Its high sensitivity, exceptional plasma robustness, and wide working range make the it the ideal tool for the analysis of highly saline samples.

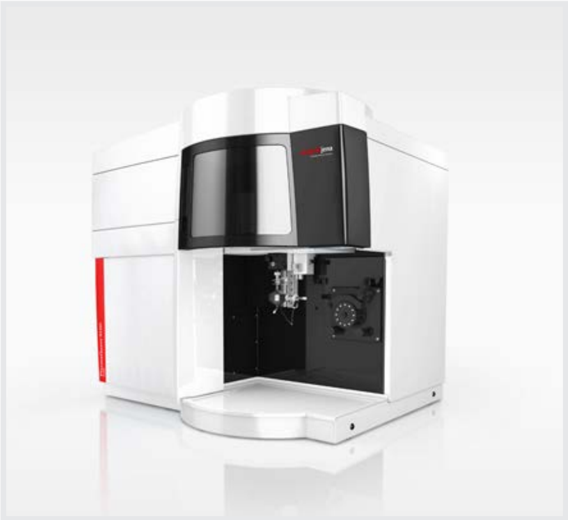


Figure 2: PlasmaQuant 9100

Challenge

Analysis of environmental samples with complex high matrix concentration and quantification of major, minor and trace elements.

Solution

The PlasmaQuant MS provides reliable, precise and accurate results from trace to major elements concentration range.

Analysis of Marine Sediments and Sewage Sludge by ICP-MS

Introduction

To evaluate the potential ecotoxicology of soils, sediments an particularly sewage sludge constant monitoring for heavy metal content is required.The recycling of sewage sludge is closely linked to environmental regulations, so that a precise characterization is necessary. Industrial wastewater and waste are also subject to strict regulations and must be regularly checked and characterized. The determination of harmful and toxic elements in solid materials requires a digestion procedure prior to the analysis. Depending on sample type, matrix composition and the analyte elements of interest, the degree of difficulty of sample preparation varies. In this work, sample preparation was performed by microwave assisted acidic mineralization.

In total, 26 elements from major (percent), minor (ppm) and trace (ppb) levels were determined in three certified reference materials CRMs (two sewage sludge: BCR-144R and CRM055, one marine sediment: PACS-2). The obtained results clearly show excellent agreement with the certified values. Spike recoveries in the range of 90 to 104%, accuracy with Z-score uncertainty values ranging within -2.0 and +2.0 as well as precicion of less than 5% relative standard deviation (RSD) prove the method robustness and applicability to environmental samples.

Materials and Methods

Instrumentation

All analytical work was performed using the PlasmaQuant MS featuring the integrated collision/reaction cell (iCRC) technology to remove polyatomic species formed in the plasma and the ReflexION ion optical system with 90° ion mirror for unique reflection of analyte ions that guarantees high sensitivity for improved precision and accuracy of all analyses. To detect the major, minor and trace element concentrations in a single run, the ADD, all digital detector, was used providing 11 orders of linear dynamic range without changing the detection mode and thus without the need for a frequent cross calibration. The ICP-MS system was coupled to an autosampler ASPQ 3300.

All experiments were carried out in a routine analytical laboratory, and not under 'clean room' conditions. Instrument operating conditions are summarized in Table 1, including the integrated Collision Reaction Cell (iCRC) modes using helium and hydrogen gases to remove problematic spectroscopic interferences on first-row transition metals.

Parameter	Specification
Plasma gas flow	9.0 L/min
Auxiliary gas flow	1.10 L/min
Nebulizer gas flow	1.07 L/min
iCRC gas setting	No Gas for ^{7}Li, ^{9}Be, ^{59}Co, ^{66}Zn, ^{98}Mo, ^{107}Ag, $^{206+207+208}$Pb, ^{238}U He – 130 mL/min for ^{23}Na, ^{31}P, ^{24}Mg, ^{27}Al, ^{39}K, ^{44}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{60}Ni, ^{65}Cu, ^{86}Sr, ^{114}Cd and ^{205}Tl H2 – 120 mL/min for ^{33}S; ^{54}Fe, ^{75}As and ^{78}Se
Plasma RF power	1.20 kW
Dwell time	30 ms
Scan per replicate	25 (peak hopping, 1pt/peak)
No. of replicates	5
Rump rate	20 rpm – black/black PVC pump tubing (<1mL/min)
Torch	Fassel torch with 2.4mm injector
Cones	Ni-sampler and Ni-skimmer
Sampling depth	6.0 mm
Nebulizer type	MicroMis^{tTM} 0.4 mL/min (quartz concentric)
Ion optics	Auto-optimized
Spray chamber type	Quartz glass Scott-type with Peltier chiller
Spray chamber temperature	3 °C
Internal standards	Sc, Y, Ge, Rh, In, Ir, Tb and Bi, 5 µg/L, interpolate correction

Table 1: Instrument settings – PlasmaQuant MS

Samples and reagents

The following high purity reagents were used for all solution preparations:

- Deionized water (>18.2 MΩ*cm, Millipore MiliQ)
- Nitric acid Supra-quality 69% (ROTIPURAN® Supra)
- Hydrochloric acid Supra–quality 35% (ROTIPURAN® Supra)

Sample preparation

All three reference materials were digested in a microwave digestion system using the setting displayed in Table 2. After digestion samples were left to cool to ambient temperature, filled up to 50 mL using deionized water and filtered through a Whatman membrane prior to analysis. Afterward, all samples were diluted 100, 20 and 10 folds. Since all the samples had certified values, Z-score parameter was used to evaluate accuracy besides spike recovery efficiency.

$$Z - score = \frac{(x - \mu)}{\delta}$$

Where x represents the mean value obtained, $\boldsymbol{\mu}$ the reference value and $\boldsymbol{\delta}$ the uncertainty of the reference value.
Note: A Z-score indicates where the score lies on a normal distribution curve. A Z-score of zero describes a result that is exactly the average certified value, while a score of +3.0 describes a value that is much higher than average. Z-score between -2.0 and 2.0 is defined by a normal distribution of 97.72%.

Calibration

The method was calibrated with multi-element standards in the concentration levels of 0.5, 5, 25, 50, 250 and 500 µg/L for the minor elements Li, Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Tl, Pb, and U and 0.5, 1, 2.5, 10, 25, 50 mg/L for P, S, Na, K, Al, Ca, Mg, and Fe. All solutions were prepared in 50 mL plastic tubes from SARSTEDT using 1% HNO_3.
The calibration graphs in Figure 1 show good examples for the performance of the PlasmaQuant MS at defined concentrations levels. All calibrations were measured with correlation coefficients higher than 0.9999 in the range tested.

Evaluation

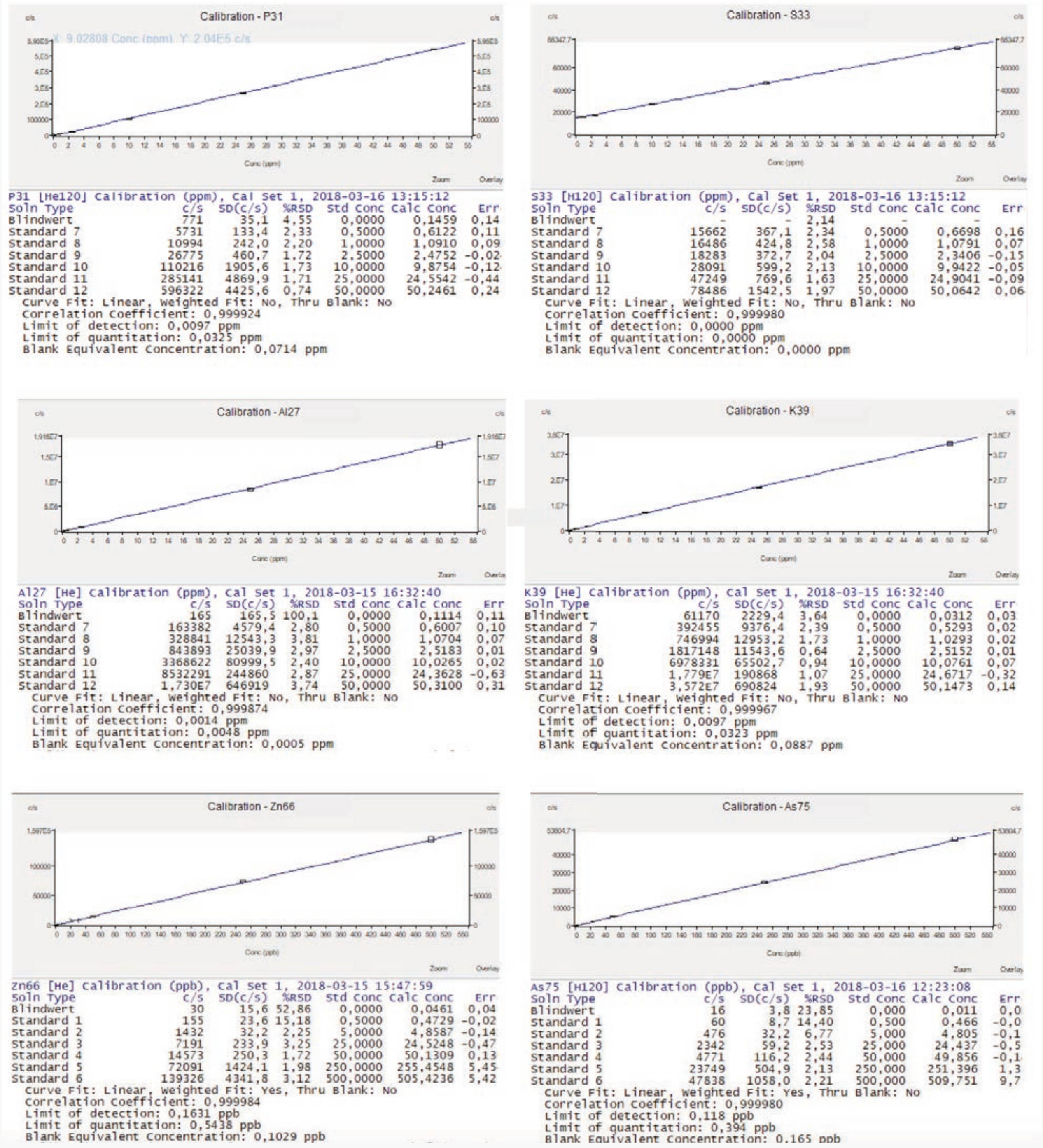
According to the expected interferences on certain mass/charge ratios, different isotopes were measured by using collision gas (He) or a reactive gas (H2) by utilizing the iCRC technology of Analytik Jena. Therefore, for each measurement, three condition sets (Helium, no Gas and Hydrogen modes) were prepared with the respective isotopes in the appropriate set (see Table 3). Within one measurement, all condition sets were executed with switching times of 30 seconds. For data recording, five average values were calculated from 25 scans each, which were used for the calculation of one average value including standard deviation.

Parameter	Specification
Sample amount	0.5 g dried and sieved
H_2O	2 mL
HNO_3	7.5 mL
HCl	2.5 mL
Vessel	PM60
Heating Stage 1 / time	140°C / 5 min
Heating Stage 2 / time	175°C / 5 min
Heating Stage 3 / time	210°C / 20 min
Cooling / time	50°C / 30 min
Final volume	50 mL with ultrapure H_2O filtered through Whatman N°.42 prior to analysis

Table 2: Digestion method parameters used by the microwave digestion system

Isotope	Expected interference	iCRC mode
^{7}Li	-	No gas
^{9}Be	-	No gas
^{23}Na	-	He (due to high concentration/ signal)
^{24}Mg	-	He (due to high concentration/ signal)
^{27}Al	^{11}B^{16}O	He
^{31}P	^{15}N^{16}O	He
^{33}S	^{17}O^{16}O	H_2
^{39}K	^{23}Na^{16}O	He
^{44}Ca	^{28}Si^{16}O	He
^{51}V	^{35}Cl^{16}O	He
^{52}Cr	^{36}S^{16}O; ^{36}Ar^{16}O; ^{40}Ar^{12}C	He
^{54}Fe	^{38}Ar^{16}O	H_2
^{55}Mn	^{39}K^{16}O	He
^{59}Co	-	No gas
^{60}Ni	^{44}Ca^{16}O	He
^{65}Cu	^{49}Ti^{16}O	He
^{66}Zn	-	No gas
^{75}As	^{40}Ar^{35}Cl	H_2
^{78}Se	^{40}Ar^{38}Ar	H_2
^{86}Sr	-	He (due to high concentration/ signal)
^{98}Mo	-	No gas
^{107}Ag	-	No gas
^{114}Cd	^{98}Mo^{16}O	He
^{205}Tl	-	He (due to high concentration/ signal)
$^{206+207+208}$Pb	-	No gas
^{238}U	-	No gas

Table 3: Expected interferences and used iCRC gases



```
P31 [He120] Calibration (ppm), Cal Set 1, 2018-03-16 13:15:12
Soln Type                c/s  SD(c/s)  %RSD  Std Conc Calc Conc   Err
Blindwert                771     35,1  4,55    0,0000    0,1459  0,14
Standard 7              5731    133,4  2,33    0,5000    0,6122  0,11
Standard 8             10994    242,0  2,20    1,0000    1,0910  0,09
Standard 9             26775    460,7  1,72    2,5000    2,4752 -0,02
Standard 10           110216   1905,6  1,73   10,0000    9,8754 -0,12
Standard 11           285141   4869,9  1,71   25,0000   24,5542 -0,44
Standard 12           596322   4425,6  0,74   50,0000   50,2461  0,24
  Curve Fit: Linear, Weighted Fit: No, Thru Blank: No
  Correlation Coefficient: 0,999924
  Limit of detection: 0,0097 ppm
  Limit of quantitation: 0,0325 ppm
  Blank Equivalent Concentration: 0,0714 ppm
```

```
S33 [H120] Calibration (ppm), Cal Set 1, 2018-03-16 13:15:12
Soln Type                c/s  SD(c/s)  %RSD  Std Conc Calc Conc   Err
Blindwert                  -        -  2,14         -         -
Standard 7             15662    367,1  2,34    0,5000    0,6698  0,16
Standard 8             16486    424,8  2,58    1,0000    1,0791  0,07
Standard 9             18283    372,7  2,04    2,5000    2,3406 -0,15
Standard 10            28091    599,2  2,13   10,0000    9,9422 -0,05
Standard 11            47249    769,6  1,63   25,0000   24,9041 -0,09
Standard 12            78486   1542,5  1,97   50,0000   50,0642  0,06
  Curve Fit: Linear, Weighted Fit: No, Thru Blank: No
  Correlation Coefficient: 0,999980
  Limit of detection: 0,0000 ppm
  Limit of quantitation: 0,0000 ppm
  Blank Equivalent Concentration: 0,0000 ppm
```

```
Al27 [He] Calibration (ppm), Cal Set 1, 2018-03-15 16:32:40
Soln Type                c/s  SD(c/s)  %RSD  Std Conc Calc Conc   Err
Blindwert                165    165,5 100,1    0,0000    0,1114  0,11
Standard 7            163382   4579,4  2,80    0,5000    0,6007  0,10
Standard 8            328841  12543,3  3,81    1,0000    1,0704  0,07
Standard 9            843893  25039,9  2,97    2,5000    2,5183  0,01
Standard 10          3368622  80999,5  2,40   10,0000   10,0265  0,02
Standard 11          8532291  244860   2,87   25,0000   24,3628 -0,63
Standard 12          1,730E7  646919   3,74   50,0000   50,3100  0,31
  Curve Fit: Linear, Weighted Fit: No, Thru Blank: No
  Correlation Coefficient: 0,999874
  Limit of detection: 0,0014 ppm
  Limit of quantitation: 0,0048 ppm
  Blank Equivalent Concentration: 0,0005 ppm
```

```
K39 [He] Calibration (ppm), Cal Set 1, 2018-03-15 16:32:40
Soln Type                c/s  SD(c/s)  %RSD  Std Conc Calc Conc   Err
Blindwert              61170   2229,4  3,64    0,0000    0,0312  0,03
Standard 7            392455   9376,4  2,39    0,5000    0,5293  0,02
Standard 8            746994  12953,2  1,73    1,0000    1,0293  0,02
Standard 9           1817148  11543,6  0,64    2,5000    2,5152  0,01
Standard 10          6978331  65502,7  0,94   10,0000   10,0761  0,07
Standard 11          1,779E7  190868   1,07   25,0000   24,6717 -0,32
Standard 12          3,572E7  690824   1,93   50,0000   50,1473  0,14
  Curve Fit: Linear, Weighted Fit: No, Thru Blank: No
  Correlation Coefficient: 0,999967
  Limit of detection: 0,0097 ppm
  Limit of quantitation: 0,0323 ppm
  Blank Equivalent Concentration: 0,0887 ppm
```

```
Zn66 [He] Calibration (ppb), Cal Set 1, 2018-03-15 15:47:59
Soln Type                c/s  SD(c/s)  %RSD  Std Conc Calc Conc   Err
Blindwert                 30     15,6 52,86    0,0000    0,0461  0,04
Standard 1               155     23,6 15,18    0,5000    0,4729 -0,02
Standard 2              1432     32,2  2,25    5,0000    4,8587 -0,14
Standard 3              7191    233,9  3,25   25,0000   24,5248 -0,47
Standard 4             14573    250,3  1,72   50,0000   50,1309  0,13
Standard 5             72091   1424,1  1,98  250,0000  255,4548  5,45
Standard 6            139326   4341,8  3,12  500,0000  505,4236  5,42
  Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
  Correlation Coefficient: 0,999984
  Limit of detection: 0,1631 ppb
  Limit of quantitation: 0,5438 ppb
  Blank Equivalent Concentration: 0,1029 ppb
```

```
As75 [H120] Calibration (ppb), Cal Set 1, 2018-03-16 12:23:08
Soln Type                c/s  SD(c/s)  %RSD  Std Conc Calc Conc   Err
Blindwert                 16      3,8 23,85     0,000     0,011   0,0
Standard 1                60      8,7 14,40     0,500     0,466  -0,0
Standard 2               476     32,2  6,77     5,000     4,805  -0,1
Standard 3              2342     59,2  2,53    25,000    24,437  -0,5
Standard 4              4771    116,2  2,44    50,000    49,856  -0,1
Standard 5             23749    504,9  2,13   250,000   251,396   1,3
Standard 6             47838   1058,0  2,21   500,000   509,751   9,7
  Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
  Correlation Coefficient: 0,999980
  Limit of detection: 0,118 ppb
  Limit of quantitation: 0,394 ppb
  Blank Equivalent Concentration: 0,165 ppb
```

Figure 1: Calibration graphs for some major and minor elements investigated

Results and Discussion

Tables 4, 5, and 6 present results achieved in BRC-144R (sewage sludge), CRM055 (sewage sludge 4) and PACS-2 (marine sediment) respectively. Mean values of the different dilution performed, standard deviation (%RSD), recoveries rates (%REC) and Z-score are listed.

Isotope	Certified value [mg/kg]	Uncertainty [mg/kg]	Mean value [mg/kg]	stdev [mg/kg]	RSD [%]	Recovery [%]	Z-score
^{52}Cr	104	3	98.7	0.7	0.7	95	-1.8
^{55}Mn	208	3	210	2.5	1.2	101	0.7
^{59}Co	15	0.6	14.2	0.7	4.9	95	-1.3
^{60}Ni	47.7	1.1	49.0	0.6	1.1	103	1.2
^{65}Cu	308	7	301	7.1	2.4	98	-1.0
^{66}Zn	932	23	912	23	2.5	98	-0.9
^{114}Cd	1.82	0.10	1.83	0.12	6.6	100	0.0
$^{206+207+208}$Pb	106	1.6	103	4.4	4.3	97	-1.9

Table 4: Results of BCR-144R, sewage sludge

Isotope	Certified value [mg/kg]	Uncertainty [mg/kg]	Mean value [mg/kg]	stdev [mg/kg]	RSD [%]	Recovery [%]	Z-score
^{23}Na	774	198	954	25.5	2.7	123	0.9
^{31}P	22000	6370	21175	1255	5.9	96	-0.1
^{33}S	10900	4030	14785	49.5	0.3	136	1.0
^{24}Mg	9180	2640	11221	165	1.5	122	0.8
^{27}Al	15300	3390	17825	213	1.2	117	0.7
^{39}K	2460	410	2866	31.2	1.1	117	1.0
^{44}Ca	47400	13000	52957	930	1.8	112	0.4
^{52}Cr	288	36.7	336	4.1	1.2	117	1.3
^{54}Fe	19000	9930	24416	163	0.7	129	0.5
^{55}Mn	667	121	685	14.5	2.1	103	0.2
^{59}Co	95.5	7.88	112	3.3	2.9	118	0.8
^{60}Ni	163	16.3	177	4.5	2.5	109	0.9
^{65}Cu	482	59.6	487	16.8	3.4	101	0.1
^{66}Zn	1250	253	1294	36.7	2.8	104	0.2
^{75}As	236	28.1	257	8.5	3.3	109	0.7
^{98}Mo	131	34.9	147	3.6	2.5	113	0.5
^{114}Cd	60.6	4.13	60.0	1.1	1.8	99	-0.1
$^{206+207+208}$Pb	154	14.2	161	2.3	1.4	104	0.5

Table 5: Results of CRM055, sewage sludge 4

Isotope	Certified value [mg/kg]	Uncertainty [mg/kg]	Mean value [mg/kg]	stdev [mg/kg]	RSD [%]	Recovery [%]	Z-score
^{7}Li	32.2	2.0	30.1	2.2	7.3	93	-1.1
^{9}Be	1.0	0.2	0.90	0.1	5.6	90	-0.5
^{51}V	133	5	130	3.5	2.7	98	-0.5
^{52}Cr	90.7	4.6	84.0	1.0	1.2	93	-1.5
^{55}Mn	440	19	421	2.4	0.6	96	-1.0
^{59}Co	11.5	0.3	11.2	0.2	1.8	97	-1.1
^{60}Ni	39.5	2.3	40.2	0.6	1.6	102	0.3
^{65}Cu	310	12	313	0.9	0.3	101	0.2
^{66}Zn	364	23	347	20	5.7	95	-0.7
^{75}As	26.2	1.5	25.5	1.3	5.1	97	-0.5
^{78}Se	0.92	0.22	0.94	0.09	9.3	102	0.1
^{86}Sr	276	30	265	1.5	0.6	96	-0.4
^{107}Ag	1.22	0.14	1.27	0.1	7.8	104	0.4
^{114}Cd	2.11	0.15	2.06	0.12	5.8	97	-0.4
^{205}Tl	0.6	-	0.58	0.04	6.8	97	-
$^{206+207+208}$Pb	183	8	189	1.3	0.7	103	0.7

Table 6: Results of PACS-2, marine sediment

Accuracy

Certified reference materials were measured in order to evaluate the accuracy of the method. The Z-score parameter was used to evaluate accuracy besides spike recovery efficiency. All certified elements were within the certified control ranges (-2.0 < Z-score < 2.0) (Figure 2). It should be mentioned that such accuracy can only be achieved with sensitive and precise measurement. Due to the outstanding sensitivity and robustness the PlasmaQuant MS was able to detect trace and major concentrations in a single method run and is a highly suitable instrument for the determination of trace and major elements in all types of samples.

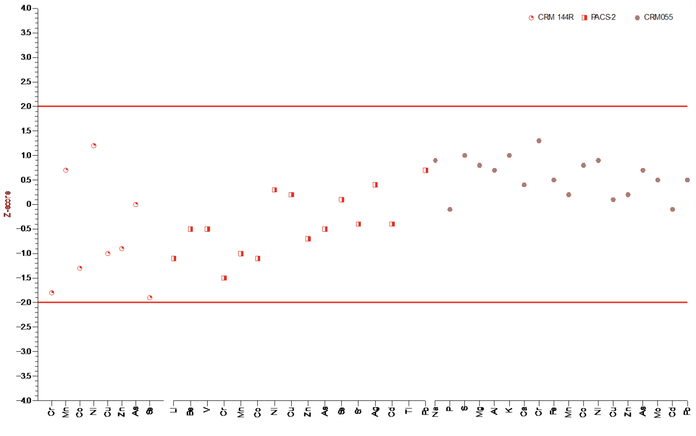


Figure 2: Z-score distribution of all elements measured in three CRMs

Precision

The relative standard deviation (RSD) was used as a parameter to assess the precision of the measurements. On average RSDs of 2.8% were achieved. Precision and accuracy (recoveries rates) of the three CRMs are shown in Figure 3. It can be seen that all recovery rates are within 80-120%, except for Na, S, Mg and Fe in CRM055 due to higher uncertainty of the reference value (Z-score for those elements where <1.0) while the RSDs are < 5%. Except for some elements such as Li, Se and Ag in PACS-2 which are within 8-9%. All results within the range of ppb to % showed great precision and accuracy with the methodology applied during all the measurements.

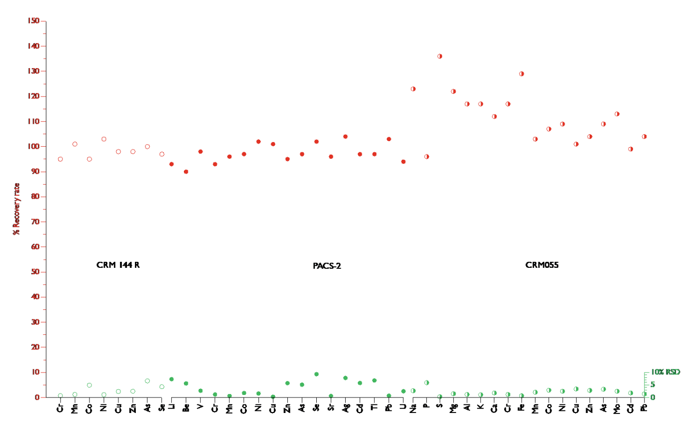


Figure 2: Z-score distribution of all elements measured in three CRMs

Conclusion

The PlasmaQuant MS offers a simple, fast, and cost-efficient method for the analysis of soils and sewage sludge. The instrument behaves as a robust instrument able to run different matrices in the same sequence using the same calibration curve without the need of preparing different methods for different matrices. In summary, the PlasmaQuant MS is a highly suitable instrument for the determination of trace and major elements in complex environmental sample types such as soil, sediment ad sewage sludge. The PlasmaQuant MS includes several innovative technologies (Eco Plasma, ReflexION ion mirror, iCRC, pre-quadrupole, all-digital detector) that provide the following advantages:

- Excellent sensitivity on the whole mass range for the lowest limits of detection.
- Excellent plasma robustness for all matrices with the new RF generator using only half the Ar consumption (<11 L/min).
- The iCRC (integrated Collision Reaction Cell) combines collision and reaction modes to correct efficiently for the spectral interferences.
- Ease of use of the instrument with simplified maintenance operations and the userfriendly software Aspect MS.

Figure 3: PlasmaQuant MS

Challenge

Meeting industry regulations for mercury (Hg) in environmental waters.

Solution

PlasmaQuant MS with high-throughput sample introduction system and sub ng/L detection limit for mercury (Hg).

Ultra-trace Determination of Mercury in Environmental Waters by ICP-MS

Introduction

Mercury is a well-known toxic element and considered as one of the top ten chemicals of public health concern by the World Health Organization. It is a neurotoxin that rapidly bioaccumulates and can cause major health problems, and even death, in small quantities. Chronic exposure at low levels is known to affect the nervous, digestive and immune systems as well as the lungs, kidneys, and eyes with fetuses and breast feeding babies susceptible to developmental effects.

Mercury is a naturally occurring element found in air, water and soil through volcanic activity. Although, human activity contributes significantly through the burning of coal, waste incineration, mining and other industrial uses, finding its way into the environment and eventually, into the food chain. It exists in various forms including the elemental or metallic form, the inorganic form as in mercury salts, and the organic form with methylmercury being the most toxic.

The determination of mercury concentrations in environmental samples such as natural waters and waste waters is important in environmental risk assessment. On the 23rd of October 2000, the “Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy” or, in short, the EU Water Framework Directive was formerly adopted. Mercury and its compounds are part of this directive. Environmental quality standards (EQS) of 2008/105/EC define the maximum allowable concentration (MAC) for mercury as 0.05 µg/L.

Materials and Methods

Instrumentation

- PlasmaQuant MS: Inductively Coupled Plasma – Mass Spectrometer
- ASPQ 3300 autosampler
- ESI oneFAST high-throughput sample introduction system

Method

The system was optimized for lowest detection limits of mercury and minimum oxide formation. A solution of 1 µg/L mercury was used to optimize the ion optics for maximum signal for mercury isotopes listed in Table 1. The sum of the four mercury isotope masses were used for calculations, allowing a higher signal to be recorded for improved precision and detection limits. Given there is the potential for mercury isotopes to be interfered with by the formation of tungsten oxides (WO^+) polyatomics, the plasma conditions were set for minimum oxide formation at <2 % (CeO to Ce ratio). The instrument parameters are listed in Table 1.

Parameter	Specification
Plasma Gas	9.0 L/min
Auxiliary Gas	1.25 L/min
Nebulization Gas	1.00 L/min
Sampling Depth	5.0 mm
RF power	1.4 kW
Pump rate	10 rpm
Measured Hg isotopes	198, 199, 200 and 202
Scan Mode	Peak hopping
Points per Peak	1
Scans per Replicate	30
Replicates	3
Dwell Time	50 ms
Rinse time	30 s

Table 1: Instrument parameters

Samples and reagents

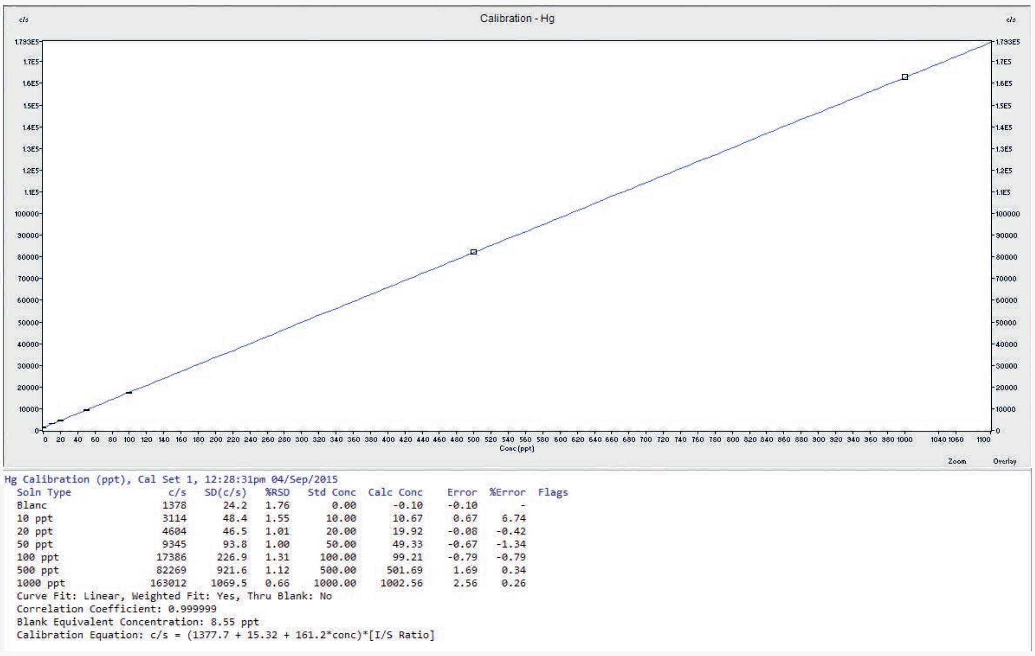
- High purity ICP-MS grade nitric acid (NORMATOM from VWR)
- High purity ICP-MS grade hydrochloric acid (NORMATOM from VWR)
- Potassium bromide (Merck)
- Potassium bromate (Merck)
- Hydroxylammonium chloride (Merck).
- Mono-elemental mercury stock standard solution (100 mg/L, Inorganic Ventures)
- National Research Council Canada ORMS-5 River Water (Certified Reference Material)
- LGC Stanards ERM-CA615 Ground water (Certified Reference Material)

Sample preparation

A combined step of conservation and digestion was carried out. Monovalent- and organomercury compounds of mercury are converted into divalent mercury by oxidation using a mixture of potassium bromide and potassium bromate ($KBr/KBrO_3$) in 1% HCl. The excess of bromide is stabilized with hydroxyl ammonium chloride. Approximately 5.95 g of KBr (Merck) and 1.39 g of $KBrO_3$ (Merck) were dissolved in 500 mL deionized water. Both powders (KBr and $KBrO_3$) were dried overnight at 300 °C prior to dissolution to remove any traces of mercury contamination. The hydroxyl ammonium chloride (NH_4ClOH) solution was prepared by dissolving 6 g in 50 mL of deionized water.

Solutions were prepared by adding 0.5 mL of 1% HCl (v/v) and 1.0 mL of the above $KBr/KBrO_3$ solution into 50 mL of the standard and sample solutions. They were then left at room temperature for 15 minutes to allow the reaction to occur (solutions develop a yellow tint) after which 1.0 mL of NH_4ClOH was added to neutralize the Br_2 formed and producing a clear solution.

Two certified water reference materials and three proficiency test water samples were prepared and analyzed. A mono-elemental mercury stock standard solution from Inorganic Ventures (100 mg/L) was used to make up all calibration solutions, covering the range from 10 ng/L up to 1000 ng/L (Figure 1). ^{193}Ir was used as an internal standard to correct for any possible signal drift or matrix effect.



```
Hg Calibration (ppt), Cal Set 1, 12:28:31pm 04/Sep/2015
  Soln Type               c/s    SD(c/s)   %RSD   Std Conc   Calc Conc     Error  %Error  Flags
  Blanc                  1378       24.2   1.76       0.00       -0.10     -0.10       -
  10 ppt                 3114       48.4   1.55      10.00       10.67      0.67    6.74
  20 ppt                 4604       46.5   1.01      20.00       19.92     -0.08   -0.42
  50 ppt                 9345       93.8   1.00      50.00       49.33     -0.67   -1.34
  100 ppt               17386      226.9   1.31     100.00       99.21     -0.79   -0.79
  500 ppt               82269      921.6   1.12     500.00      501.69      1.69    0.34
  1000 ppt             163012     1069.5   0.66    1000.00     1002.56      2.56    0.26
  Curve Fit: Linear, Weighted Fit: Yes, Thru Blank: No
  Correlation Coefficient: 0.999999
  Blank Equivalent Concentration: 8.55 ppt
  Calibration Equation: c/s = (1377.7 + 15.32 + 161.2*conc)*[I/S Ratio]
```

Figure 1: Calibration curve from the summation of Hg 199-202 isotopes

Results and Discussion

The measured and certified values for mercury in river and ground water reference materials are shown in Table 2. The measured and expected values for natural, drinking and waste waters, along with the Z-score are reported in Table 3. The Z-score indicates how many standard deviations the measured value is from the mean value and was determined to be ≤±1 for this analysis.

Reference material	Certified value (ng/L)	Measured value (ng/L)
ORMS-5 River water	37.0 ± 4.0	36.6 ± 0.8
ERM-CA615 Groundwater	26.2 ± 1.3	26.4 ± 0.8

Table 2: Reference materials results

Proficiency test	Measured value (µg/L)	Assigned value (µg/L)	Z-score
Natural water	1.2	1.1	0.3
Drinking water	2.7	2.5	1.0
Waste water	34.5	35.0	-0.1

Table 3: Proficiency tests results

Linearity tests, limit of quantification and accuracy profiles were evaluated according to the French norm NF T 90210 (2009). A natural water sample collected from a river in Vendée, France, was used to estimate the limit of quantification (LOQ). A final LOQ of 2 ng/L was determined along with a 3σ method limit of detection (MDL) of 0.3 ng/L.

Figure 2 shows the readback of a 2 ng/L spiked water sample measured ten times over the duration of five days. The readback at the LOQ was easily within the ±60 % concentration range as defined by the NF T 90210 norm.

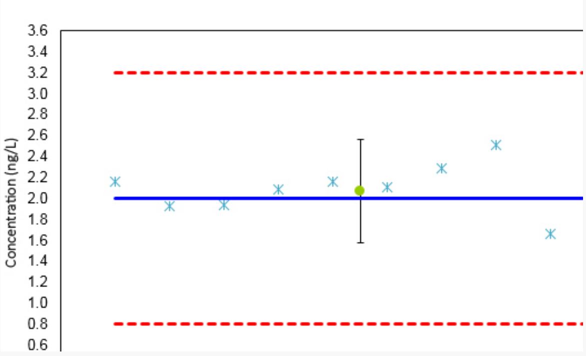


Figure 2: Validation of the LOQ according to the norm NF T 90210

Sample introduction washout test

Mercury is known to exhibit serious memory effects, affecting data quality at ultra-trace concentrations. Sample preparation and instrument setup is important to minimize memory effects. A study was carried out to demonstrate the effectiveness of the sample preparation and the washout efficiency of the sample introduction system.

Conclusion

The superior sensitivity of the PlasmaQuant MS offers reliable ultra-trace determination of mercury in environmental waters. A method LOD of 0.3 ng/L was obtained without the need for pre-concentration techniques (e.g., hydride or cold vapor generation). The ASPQ 3300 autosampler with oneFAST sample introduction system ensures high efficiency and reduced memory effects. Results obtained on reference materials and proficiency tests were in excellent agreement with certified values and well within defined ranges. The modern RF generator design of the PlasmaQuant MS Elite provides very robust plasma conditions for complex wastewater samples containing high total dissolved solids, and with only half the argon gas consumption.

Figure 3: PlasmaQuant MS

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