APPLICATION NOTE

Exaqua procedure

User methods

Creating and adjusting methods on Exaqua photometer

Exaqua

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INTRODUCTION

Creating user method is one of the features of the Exaqua photometer that allows the user to create their own method for any set of reagents intended for the determination of water parameters. Creating user method can be used to adapt reagents from outside the Exaqua series and to increase the accuracy of methods based on Exaqua range. User methods can be shared and transferred to other Exaqua photometers.

When one analyses results of photometric methods, it is important to always consider potential sources of measurement errors, such as:

- slight differences in photometric systems between units,
- differences in reagent kits depending on the system properties,
- the change of reagent properties over time,
- differences in the conditions under which the measurement is performed.

All these factors contribute to the declared measurement error.

Exaqua®
photometer reinvented

However, the impact of these factors can be minimized by adapting methods to individual conditions, consisting of:

- measurement environment (in particular, temperature),
- the photometric properties of the Exaqua photometer unit,
- a particular batch of reagents,
- the user's habits affecting the way the method is carried out (e.g. the method of dosing and mixing).

Creating the user's method allows to minimize measurement error and obtain better performance in the measurements with Exaqua photometer.

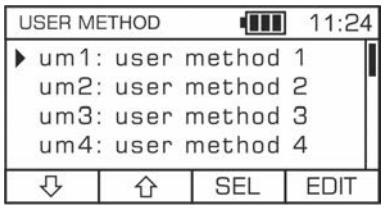
CREATING USER METHODS

REQUIRED REAGENTS AND ACCESSORIES

The key element for the creation of an user method is the preparation of a series of standard solutions of known concentrations of the analyte (the substance being determined in the method), prepared usually from a single stock solution, from which dilutions are made.
In addition, a set of reagents that are used during the test is needed, as well as an additional blank sample representing a solution free of the analyte (usually a sample of demineralized water).

PROCEDURE FOR CREATING A USER METHOD

To create a user method, go to the user methods section by entering **MAIN MENU** → **Methods** → **user methods**. Four user methods are available there, all of which can be edited. Pressing the **EDIT** key opens the method editing screen. In each user method, you can edit the name, unit, wavelength and points of the calibration curve, shown as consecutive values of absorbance (abs0, abs1,...) and concentration (conc0, conc1, ...) of each point. The name and unit of the method are recorder when saving the obtained results to the log and exported to the mobile application.



USER METHOD PARAMETERS

Name

The method name can be up to 19 characters long and consist of upper and lower-case letters, numbers, spaces and symbols.

Unit

The unit in the user method can be any combination of characters composed of upper-case and lower-case letters, numbers, spaces and symbols. The unit name can be up to 10 characters long.

Wavelength

The wavelength at which the absorbance measurement will be performed can be selected from the available measurement wavelengths for the photometer (Exaqua PRO3 model - 470, 520 and 610 nm; Exaqua PRO6 model - 430, 470, 520, 560, 610, 650 nm).

Knowing the optimal wavelength for a given method is crucial for obtaining a calibration curve with the best dynamic, and thus for obtaining a method with the highest accuracy.

DETERMINING OPTIMAL WAVELENGTH

When creating a method based on an **existing Exaqua method**, it is a recommended to choose the same wavelength as the one declared for the method. Information about the declared wavelength can be found in the manual, on the list of method procedures and in the Additional Information section for the respective reagent kit. The manual and the list of method procedures are available on the site exaqua.com/support/downloads, while pages for respective reagent sets on exaqua.com/products/reagents.

Additionally, there is a QR code on the box of each reagent kit that leads directly to that kit's webpage.

If you want to create a method based on **new reagents**, the wavelength can be selected experimentally according to one of the procedures below.

A. FROM THE ABSORBANCE SPECTRUM

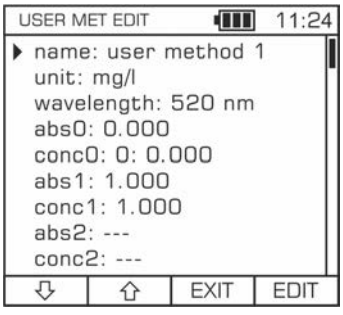
If a spectrophotometric spectrum is available for the solutions with induced colour, a wavelength can be selected that is as close as possible to the absorbance maximum observed in the spectrum.

B. FROM ABSORBANCE MEASUREMENTS ON EXAQUA PHOTOMETER

It is possible to directly measure absorbances of solutions with added reagents, using the absorbance methods available on the photometer (Axxx methods, e.g. A470 for measurements at 470 nm) and select the wavelength at which the highest absorbance readings are obtained. Be aware that the readings should not significantly exceed the value of 3.0 for the highest concentration tested.

C. BY PREDICTING THE RIGHT WAVELENGTH

It is also possible to predict which wavelength will be suitable based on the colour of the solution obtained after adding the reagents. The table 1 shows a range of observed colours and corresponding absorbed colours, along with suggested wavelengths for user method (UM).



User method edit screen
In this section, the user can enter his own parameters for the created method.

TABLE 1
List of colours and the corresponding absorbed colours with suggested wavelengths.

Observed sample colour	Absorbed colour	Absorbed colour wavelength	Suggested UM wavelength [nm]
Yellow-green	Purple	400-435 nm	430
Yellow	Blue	435-480 nm	430; 470
Orange	Blue-green	480-490 nm	470; 520
Red	Cyan	490-500 nm	470; 520
Magenta	Green	500-560 nm	520; 560
Purple	Yellow-green	560-580 nm	560
Blue	Yellow	580-595 nm	560; 610
Blue-green	Orange	595-605 nm	560; 610
Cyan	Red	605-750 nm	610; 650

SELECTION OF CONCENTRATION RANGE

The points chosen to create the calibration curve should cover the entire expected concentration range - from concentration 'zero' (analyte-free water - most often a demineralized water sample) to a concentration greater than the highest expected reading. It is also important that the selected points of the calibration curve are as close as possible to the concentrations expected in the study – for example, if a parameter is expected to hover around a value of 1 ppm, adding a point corresponding to a concentration of 1 ppm to the user's method will increase method's accuracy. If obtained absorbance exceeds the absorbance range covered by points of the calibration curve, the photometer is able to extrapolate the results. The most reliable readings however, are those that are contained within the entered points of the calibration curve.
For methods in which absorbance increases with analyte concentration, it should be born in mind that reliable and stable absorbance readings are limited to a value of about 3.2.

Therefore, the entered points of the standard curve should not exceed this value and, at the same time, the concentration corresponding to this absorbance becomes the maximum concentration included in the created user method.

The chart 1 visually presents an example of a calibration curve and how the inserted points are used to obtain measurement results. The blue points represent the points of the calibration curve (absx, concx). Between each two points, the photometer creates segments of the curve (shown as blue lines). Absorbance values from the measurements are matched to the calibration curve and a concentration value is obtained (yellow points). For absorbance values outside the calibration curve, the photometer extrapolates the last section and matches the concentration to the point (red point on the extrapolated, dashed blue line). Note that the most reliable readings are for results between the entered points of the calibration curve.

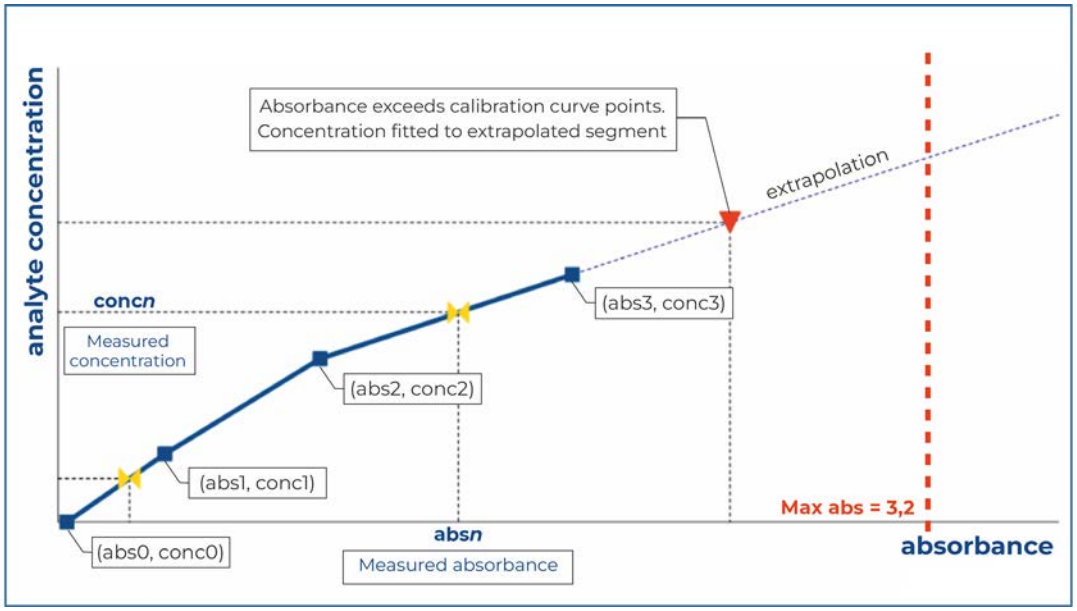


CHART 1
Calibration curve
Setting the range of concentrations based on measurement of solutions with reagents added using absorbance methods available on Exaqua photometer.

CALIBRATION CURVE POINTS

Data entered as abs0, abs1, ... and conc0, conc1, ... are points of the calibration curve that defines the relationship between absorbance and concentration. Up to 10 points can be entered in any order. When leaving the method editing screen, the entered values for the selected user method are automatically sorted in ascending order and then saved.

The absorbance value can be entered manually or through measurement by pressing the **MEAS** key in the edit section after selecting any absx parameter.

For **manual input**:

- absorbance values entered can be in the range of 0.000 to 4.000,
- concentration values entered can be in the range of 0.000 to 999.999,
- acceptable format for entered numbers is XXX.XXX.

When **entering values by measuring absorbance,** follow the procedure below:

1. When the measurement screen appears, insert a vial containing no less than **4 ml** of the test solution sample without added reagents into the vial holder and press the **ZERO** key.
2. Then carry out the adapted method on the test water in the vial, adding reagents and following the procedure of the given method.
3. After completing the method, reinsert the sample into the photometer vial holder and press the **MEAS** key.

Alternatively, the measurement can be made using the absorbance method, obtained results can be noted and manually enter into the method editing menu. In this way, additional points of the calibration curve can be entered.

SHARING AND IMPORTING USER METHODS

EXPORTING USER METHOD TO EXAQUA PHOTOMETER

To export user method you will need an Exaqua photometer with the user method, a micro-USB cable (included with the photometer) and a computer with USB port. Follow the below procedure.

1. Connect Exaqua photometer to a computer using USB cable.
2. To enable USB mode press the **Home** button and go to **MAIN MENU**, then select **USB**. The photometer's internal memory should be opened on the computer screen.
3. Open the folder **User Methods** on the computer and copy text file umX_cfg containing desired user method.

> **NOTE**
> In the umX_cfg name of text file, the 'X' represents a user method number from 1 to 4, e.g. um3_cfg.

> **NOTE**
> Do not change the file name or restore it to its original name umX_cfg before uploading the method to another photometer memory.

4. Press the **EXIT** button on photometer to leave USB mode and disconnect photometer from the computer.
5. The method has been correctly exported and can be shared with other users and imported to any Exaqua photometer.

IMPORTING USER METHOD INTO EXAQUA PHOTOMETER

To import user method you will need an Exaqua photometer to which the method will be imported, a micro-USB cable (included with the photometer), a computer with USB port and the user method in the form of a .txt file. Follow the below procedure:

1. Connect the Exaqua photometer to a PC via USB cable.
2. To enable FW Update mode press the **Home** button and go to **MAIN MENU**, then select **FW Update**. The photometer's internal memory should be opened on the computer screen.
3. Open **User Methods** folder and replace umx_cfg text file with the provided custom method with the same file name.

> **NOTE**
> In the umX_cfg name of text file, the 'X' represents a user method number from 1 to 4, e.g. um_cfg*3*

> **NOTE**
> Do not change the uploaded umX_cfg file name.

4. Press the **EXIT** button to leave update mode. The screen will display *'FW update cancelled'* message.
5. The method has been correctly imported and can be used now.

SELECTING AND USING IMOPRTED USER METHODS

1. Press the **Home** button on the photometer keyboard to enter the **MAIN MENU** section.
2. Choose **Methods** from the displayed list, then **User methods**.
3. Select imported method from the displayed list and press the **SEL** key to confirm**.**

> **NOTE**
> Pressing the middle button on the alphanumeric keyboard (digit **5**) activates edit section of a method as in the case of pressing the **EDIT** key.

Now, it is possible to return to the section with the list of user methods by pressing the **EXIT** key or the **Home** button on the photometer keyboard.

USER METHOD LIMITATIONS

Some kinds of methods cannot be created using user methods. They include:

A. WIDE-RANGE METHODS

Wide-range methods work by analysing the correlations between several wavelengths. This feature is currently not available in user methods.

B. TITRATION METHODS

Titration methods utilize the Exatitr system that performs continuous measurement during titration. This feature is currently not available for user methods.

EXAMPLE PROCEDURES FOR INCREASING METHOD ACCURACY

I. FIRST EXAMPLE

Z410 – METHOD FOR DETERMINING THE CONTENT OF IRON

To demonstrate the benefits of user method adapting existing method to improve its performance, adaptation of exemplary method Z410 method for iron will be presented. The method allows the determination of iron in the range of 0.05 - 10 ppm, in both fresh and salt water. For the purpose of this calibration, a user method operating in the range of 0 - 5 ppm will be made. The wavelength at which absorbance will be measured remains the same as for the original Z410 method – 520 nm.

A standard iron solution of 10 ppm and demineralized water were used to make solutions of known iron concentrations. Solutions of 0, 0.5, 1, 2.5 and 5 ppm iron concentrations were prepared to obtain calibration curve points. The solutions were prepared by adding the appropriate amounts of iron standard and demineralized water to the vials, as shown in the table 2, resulting in 5 ml samples. Ingredients were measured and added using automatic pipettes; if lab equipment is not available, 5 ml and 1 ml graduated syringes will also be suitable for this purpose - a slightly higher error in the dilutions made should then be expected.

TABLE 2
Preparation of solution with the known concentrations

Iron concentration [ppm]	Volume of standard iron solution [ml]	Volume of demineralized water [ml]
0.0	0.00	5.00
0.5	0.25	4.75
1.0	0.50	4.50
2.5	1.25	3.75
5.0	2.50	2.50

For each solution the method is carried out according to the instruction, using reagents from the Z410 method kit. Once the reaction is completed the absorbances are measured using the **A520** method. Absorbance values for the corresponding ion concentration values were entered in the user method edit menu.

Ready method was verified by measuring new iron solutions of 0.25, 0.8, 2 and 4 ppm. Once the reaction is completed, the samples were measured using the Z410 method and the newly developed user method. The results obtained are shown in the table 3. The reading values represent the mean value of 7 measurements. The measurement uncertainty (plus-minus value) represents the standard deviation.

Adaptation of Z410 method using user method allowed a 5-fold reduction in measurement error.

TABLE 3
Comparison of measurement results using the ready-made Z410 method and the optimized user method

	Method Z410		User method	
Fe concentration [ppm]	**Reading** [ppm]	**Reading error**	**Reading** [ppm]	**Reading error**
0.25	0.25 ± 0.02	0.00 ppm (0 %)	0.25 ± 0.01	0.00 ppm (0 %)
0.80	0.89 ± 0.02	0.09 ppm (11 %)	0.82 ± 0.02	0.02 ppm (2 %)
2.00	2.10 ± 0.02	0.10 ppm (5 %)	2.02 ± 0.02	0.02 ppm (1 %)
4.00	4.09 ± 0.02	0.09 ppm (2 %)	3.98 ± 0.02	-0.02 ppm (-0.5 %)

II. SECOND EXAMPLE
Z210 – METHOD FOR DETERMINING THE NITRATE CONTENT

Methods for measuring nitrates Z210L and Z210H are good examples of methods which accuracy can be improved by creating a user method. In nitrate methods, the mixing procedure following the addition of the second reagent (powder reagent) significantly affects the accuracy of obtained results, adjusting the test will allow to greatly reduce the measurement error.
Nitrate determination using the Z210 reagent kit can be performed in the low range (0.5 – 30 ppm) and in the high range (5 – 150 ppm) in both fresh and marine water. In this section, the performance of user method operating in the 0 – 20 ppm range will be presented. The wavelength at which absorbance will be measured remains the same as for the original Z210 method – 520 nm.
A 100 ppm nitrate standard solution and demineralized water were used to make solutions of known nitrate concentrations. Solutions of 0, 5, 10, 15 and 20 ppm of nitrates were prepared to obtain the points for the calibration curve. The solutions were prepared by adding appropriate amounts of nitrate standard and demineralized water to the vials, as shown in the table 4, resulting in 5 ml samples. Constituents were measured and added using automatic pipettes; if lab equipment is not available, 5 ml and 1 ml graduated syringes will also be adequate for this purpose - a slightly higher error in the dilutions made should then be expected.

TABLE 4
Preparation of solution with the known nitrate concentrations

Nitrate concentration [ppm]	Volume of standard nitrate solution [ml]	Volume of demineralized water [ml]
0	0.00	5.00
5	0.25	4.75
10	0.50	4.50
15	0.75	4.25
20	1.00	4.00

For each solution the method was carried out according to the instruction, using reagents from the Z210 method kit. After the reaction was completed, their absorbance values were measured using the A520 method. Absorbance values for the corresponding ion concentration values were entered in the user method edit menu.

Finalized method was verified by measuring new nitrate solutions of 2, 8, 12 and 23 ppm. After reaction was completed, the samples were measured using the Z210L method and the newly developed user method. The results obtained are shown in the table 5. The reading values represent the mean value of 7 measurements. The measurement uncertainty (plus-minus value) represents the standard deviation. For Z210L method, standard deviation values that were lower than the method resolution were replaced with the resolution value of 0.5 ppm.

The adjusted user method produced results with significantly lower reading error.

TABLE 5
Comparison of measurement results using the ready-made Z210 method and the optimized user method

	Method Z210L			User method	
NO_3 Concentration [ppm]	**Reading** [ppm]	**Reading error**	**Allowed measurement error***	**Reading** [ppm]	**Reading error**
2	2.00 ± 0.5	0.00 ppm (0 %)	85 %	2 ± 0.1	0 ppm (0 %)
8	6.50 ± 0.5	-1.50 ppm (-19 %)	30 %	8.2 ± 0.3	+0.19 ppm (2 %)
12	9.63 ± 0.54	-2.38 ppm (-20 %)	23 %	11.8 ± 0.5	-0.18 ppm (-1 %)
23	20.00 ± 0.5	-3.00 ppm (-13 %)	17 %	23.2 ± 0.5	+0.15 ppm (1 %)

* acceptable error was calculated for the expected value of NO_3 concentration based on the method's declared measurement error of ± 1 ppm ± 10 %, including a resolution of ± 0.5 ppm.

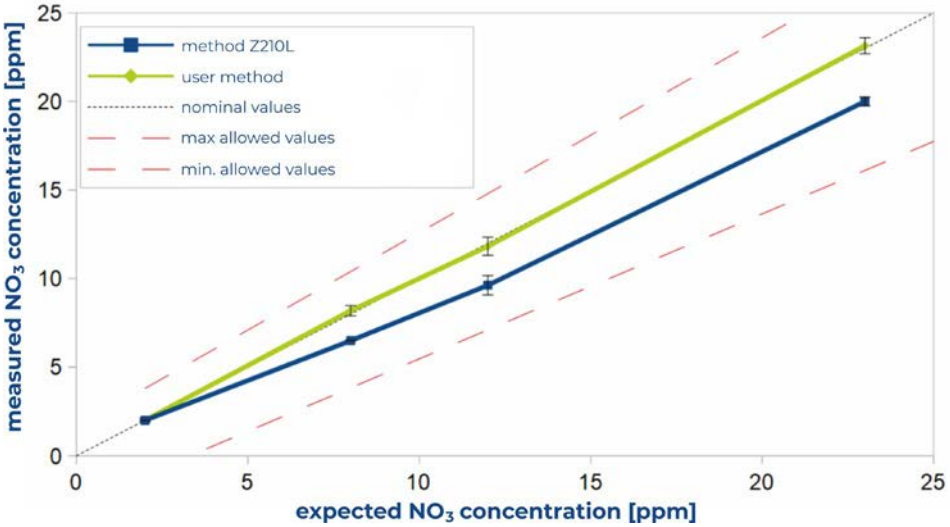


CHART 2
User method verification
Comparison of the results for the measurement of the nitrate content obtained with the standard Exaqua Z210L method and with the user method.