Enzymatic Analysis of Urea from Swimming Pool Waters

According to J.Wojtowicz (2001), Nitrogen-containing impurities (e.g urea, ammonia, amino-acids, creatinine, uric acid etc.) introduced to swimming pool water by bathers, react with free chlorine to form combined chlorine compounds. It is important to control the level of urea in swimming pool waters because from nitrogen-containing impurities urea is a potential source of hazardous ammonia chloramines, but it is also a potential nutrient for bacteria and algae and therefore poses a hygienic risk.

"Thermo Scientific method is a calculated test based on individual urea and ammonia measurements. Method is most repeatable with higher concentrations, and the method determination limit can be set to as low as 0.064 mg/l."

Kirsti Nikkola, Marita Jokinen, Tuula Jumppanen, Mari Klemm, Juhani Airo & Annu Suoniemi-Kähärä
Thermo Fisher Scientific
CDD Vantaa, Finland
Corresponding author annu.suoniemi-kahara@thermofisher.com

In Finland, urea concentration in the swimming pool waters is guided by Valvira (National Supervisory Authority for Welfare and Health). Based on the current quidelines, urea concentration needs to be lower than ≤ 0.8 mg/l.

Typically urea is measured by the Koroleff method (1983) which is based on persulphate digestion, but also an enzymatic method is used. In the Finnish SYKE (Finnish Environment Institute) swimming pool water report (2013), six of the proficiency test participants used the Koroleff method and three laboratories used the enzymatic method. Based on the study, the Koroleff method results are generally lower than the results obtained from the enzymatic method. The results of the enzymatic method were closer to the the calculated results of the proficiency test samples.

By switching from the Koroleff method to the enzymatic method, the result levels will be more accurate and therefore higher than the results reported before.

Precise enzymatic method can be easily automated with Thermo Scientific Gallery or Aquakem discrete analyzers and typically more than hundred results can be reported in an hour.

Method principles

Enzymatic test is based on urease and glutamate dehydrogenase (GLDH) enzymatic reactions.

Urea + 2H2O ---Urease---> 2NH4+ + 2HCO3-

2-Oxoglutare + NH4+ + NADH ---GLDH---> L-Glutamate + NAD+ + H2O

Method is performed at 37 °C, using 340 nm filter. The difference between ammonia determination with and without enzymatic conversion by urease indicates the value for urea. The reagents are ready-to-use.

The results are calculated automatically by the analyzer using a calibration curve. The measured ammonia expressed as urea includes the amount of free ammonia and ammonia after splitting urea with urease. For differentiation, the free ammonia content should be measured in an additional analysis. Urea is calculated by substraction of the free ammonia content from the total ammonia content.

Experiments

2.1 Materials and methods

2.1.1 Sample preparation

Sample is recommended to be dechlorinated with sodium tiosulphate prior to analysis to remove the chlorine interference.

In the Gallery and Aquakem swimming pool water applications the dechlorinating reagent has been added automatically to the test flow. Therefore no manual declorination steps were needed. Samples were analyzed within 2 days.

2.1.2 Testflow for Aquakem analyzer

Urea (Ammonia) test flow: Application consists of sample dispensing (80 µl) followed by Reagent R4 (dechlorination) addition (3 µl), Reagent R1 addition (40 µl) followed by Reagent R2 (10 µl). Mixture is incubated for 300 seconds and then blanked. This step also eliminates the interference coming from the sample color. Reaction is completed by addition of Reagent R3 (10 µl) and incubating for 900 seconds. Reaction is measured at 340 nm. Ordering code for the Thermo Scientific enzymatic Urea kit is 984321.

Method was calibrated using 6 mg/l stock solution. Calibrator points were automatically diluted by 1+2, 4, 5, 11, 59. Also a zero calibrator was used. Calibration fitting was polynomial.

Ammonia was separately measured by an application designed for low (500 µg/l) ammonia levels. Method is based on salicylate and sodium nitroprusside reaction at alkaline pH. Application consists of 100 µl sample dispensing, Reagent R1 addition (15 µl), Blanking, Reagent R2 addition (15 µl), incubation for 540 s and measurement at 660 nm. Reagent components can be seen on the Thermo Scientific Ammonia (DIC) kit insert, ordering codes for the reagents are 984362, 984363.

Calibration was performed from 2 mg/l stock. Water zero samples and automated dilution of 1+3, 4, 9, 19, 39 and 79 were performed. Calibration fitting was polynomial.

Calculated test for urea: The equation converts ammonia result (µg N/l) to urea (mg/l) and substracts it from urea results.

Urea (mg/l) = Urea (Ammonia) (mg/l) – ((Ammonia as N (µg/l) x 2.144)/1000)

2.1.3 Other methods

Samples were sent to an external laboratory for another enzymatic method analysis (later called Reference enzymatic method). Reference method details are not described in this paper.

The Koroleff method is an accreditated method at Metropolilab. The method details are seen from the Bibliography section (Koroleff, 1983).

3 Results and Discussion

3.1 Method correlation studies

Table 1. Results of Urea analysis with two enzymatic methods

Sample	Thermo Scientific Enzymatic Urea method (mg/l)	Enzymatic Reference method (mg/l)	Koroleff Reference method (mg/l)
1	0.00	< 0.2	0.10
2	0.26	0.30	0.12
3	1.90	1.90	0.92
4	0.27	0.30	< 0.1
5	1.80	1.70	0.59
6	0.28	0.20	< 0.1
7	0.49	0.40	0.21
8	0.49	0.50	0.23
9	2.10	2.00	0.51

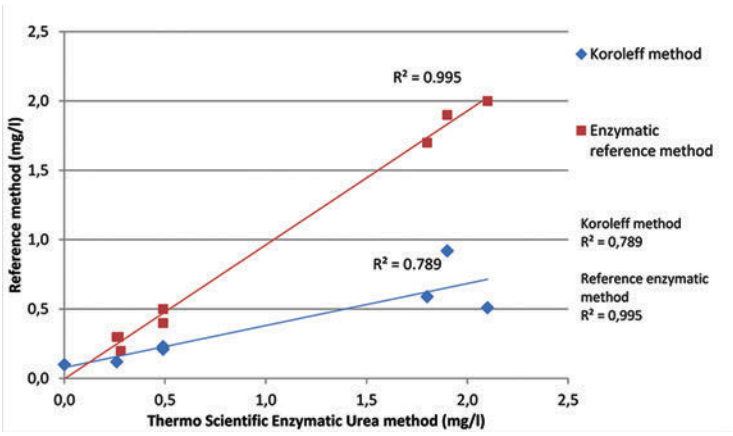

Figure 1: Correlation graph of Thermo Scientific Enzymatic Urea method compared to Koroleff and Enzymatic reference methods.

According to the method correlation studies, both enzymatic methods correlate well with each other (R^2 = 0,995). Koroleff method correlates better with low level samples, but samples with higher concentration seems to have too low recoveries compared to the enzymatic measurement.

3.2 Analysis for errors

Determination limit: A control sample of 0.1 mg/l was measured 25 times and the change between the urea results and theoretical urea value were reported. Results ranged from 0.080 to 0.130 mg/l and calculated determination limit was set to 0.064 mg/l.

Systematic error analysis: Systematic error was analyzed using two concentrations, 1 mg/l and 0.2 mg/l. For the 1 mg/l concentration, the error calculated from the result and the theoretical value was changing from 0 to 18 % error, average being 6.52 %. Samples were analyzed over a 3 months period and they represent many reagent lots and calibrations.

Similar analysis was performed for 0.2 ml/l control sample. Average error from the calculated theoretical concentration was 18.13 %. As described in the introduction section of this paper, Enzymatic Urea is a calculated test from separately measured urea and ammonia. Calculated tests typically create more errors because they are based on two independent chemistries. Ammonia chemistry is also very sensitive for atmosphere contamination. These facts may explain the higher average error of the lower concentration sample.

3.3 Profiency test results

According to the profiency test, Thermo Scientific urea method correlates in an excellent way to the samples tested. This gives also confidence that the enzymatic methods in general are more accurate than the Koroleff method.

Table 2. Results of Finnish Environment Institute (SYKE) profiency test.

Sample	Result (mg/l)	Expected value (mg/l)	Profiency test result status
A1U (synthetic)	0.56	0.54	Excellent
U2U (swimming pool water)	0.995	0.96	Excellent
U3U (swimming pool water)	0.575	0.54	Excellent

4 Conclusion

- In general, the Koroleff method measures higher concentration samples with lower recovery as the enzymatic methods. Changing from the Koroleff method to the enzymatic method improves accuracy with the high concentration samples.
- Two different enzymatic methods correlate together in an excellent way. Thermo Scientific method measured in a profiency test shows also excellent results level.
- Thermo Scientific method is a calculated test based on individual urea and ammonia measurements. Method is most repeatable with higher concentrations, and the method determination limit can be set to as low as 0.064 mg/l.

5 Bibliography

Koroleff, F. 1983. Determination of urea. In Methods of Seawater Analysis (Grasshoff, K., Erhardt, M. & Kremling K., eds.). Verlag Chemie, Weinheim, pp. 158-162.