Test method validation of assays in closed tubes

Julien SAGNARD

Introduction

The COD (Chemical Oxygen Demand) expresses the amount of oxygen originating from potassium dichromate that reacts with the oxidizable substances contained in 1 litre of water. A high concentration of organic compound in water provokes asphyxiation of aquatic organisms. In French Guiana rivers, the average COD level is about 20 mgO2.L-1. The water sample is oxidized with a hot sulfuric solution of potassium dichromate with silver sulfate used as a catalyst. Chloride is masked by complexation with mercury sulfate. The concentration of unconsumed yellow Cr2O72- ions is then determined by spectrometric photometry λ=445nm. Because on the use of many toxic compounds, like mercury or potassium dichromate, Hydreco needs to develop some new procedures like closed tubes test in order to limit contact between analyst and toxic compounds. However, the use of new types of methods needs a validation including the determination of the quantification limit with a defined correctness and precision.

Materials and methods

The calibration range used in COD runs from 5 to 250 mgO2.L^{-1}. Solutions are prepared by dissolution of 1.7004 g of KHP (Hydrogen potassium phosphate) in 100 mL of ultra pure water to give a C_1=20000 $mgO_2.L^{-1}$ solution.
To determine the quantification limit, it was necessary to carry out a Student test using 10 repetitions of the low concentration solution 5 $mgO_2.L^{-1}$. The Student test allows showing the correctness method.
Then the precision of the result was defined.
A 5 $mgO_2.L^{-1}$ solution was prepared by two successive dilutions. The first one, 5 mL of C_1 solution at 20000 $mgO_2.L^{-1}$ in 50 mL to obtain a C_2 solution at 100 $mgO_2.L^{-1}$. The second one, 5 mL of C_2 solution at 100 $mgO_2.L^{-1}$ in 100 mL.
Solutions were analyzed just after their preparation because of their instability.

Results and discussion

Solutions	1	2	3	4	5	$\bar{X}$
ST-COD $mgO_2.L^{-1}$	5.67	4.51	4.57	4.98	5.91	**5.19**
Solutions	6	7	8	9	10	**Standard deviation σ**
ST-COD $mgO_2.L^{-1}$	4.98	4.74	5.44	5.21	5.67	**0.47**

Table 1: repetition test, average value and standard deviation.

The Student test conducted with ten repetitions proved the correctness of the method. The value $t_{calculate}$ is less than $t_{Student}$ ($t_{Student}$ depends on repetition number and bilateral risk, here 5%). There was no difference between the reference value and the average value (X)(see table1). The method was correct. Then the fidelity was determined using the Student value (tStudent=2.262).

Correctness:

$$t_{calculate} = \frac{|REF - \bar{X}|}{\sigma/\sqrt{n}} = 1.250$$

Precision:

$$\bar{X} \pm t_{Student} \times \frac{\sigma}{\sqrt{n}}$$

$$t_{Student} \times \frac{\sigma}{\sqrt{n}} = 0.34$$

Conclusion

The determination of the quantification limit was successfully achieved. This part of the COD method validation was important to prove the process performances, so it was possible for the Hydreco engineers to quantify up to 5$mgO_2.L^{-1}$ with a precision determined: 5.00 ± 0.34 $mgO_2.L^{-1}$.

HYDRECO
GUYANE

Environment laboratory of Petit Saut
BP 823
97388 KOUROU Cedex