Development of an internal quality control for the quantification of citric acid in the serum on an automate ABX Pentra 400

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Introduction

Citric acid (2-hydroxypropane-1, 2, 3-tricarboxylic acid) is a tri- acid of formula C6H8O7 (Fig.1) involved in the Krebs cycle. The determination of this component permits the medical supervision of the toxicity during significant blood transfusion especially in case of a dialysis. The analytical method is based on enzymatic colorimetric. The main goal of this work is to create an internal quality control (IQC) for the automatic method

Principle of the enzymatic reaction

Citric acid is changed into oxaloacetate and acetate by Citrate Lyase. (a)

$$\text{(a) Citrate} \xrightarrow{\text{CL}} \text{oxaloacetate + acétate}$$

With Malate-dehydrogenase (MDH) and Lactate-dehydrogenase (LDH),oxaloacetate and pyruvate (decarboxilated product of oxaloacetate), are transformed into L-Malate and L-Lactate, giving oxidization of NADH in NAD^+. (b, c) The formation of NAD^+ causes a diminution of absorbance at 340 nm.

$$\text{(b)} \quad \text{Oxaloacetate + NADH + } H^+ \xrightarrow{\text{L-MDH}} \text{L-malate + } NAD^+ ,$$

$$\text{(c)} \quad \text{Pyruvate + NADH + } H^+ \xrightarrow{\text{L-LDH}} \text{L-lactate + } NAD^+$$

The NADH concentration oxidized is proportional to the citrate concentration.

Test carried out

Pentra ABX 400 is an automatic system based on a spectrophotometer (colorimetric and turbidimetry) and a potentiometer analysis. It has a maximal efficiency of 420 tests by hours and it can analyze up to 55 samples in one series. For the citric acid analysis, it is important that IQC is made of the even matrix as the serum. But if samples remain frozen for a long time, the acid will degrade. So, all samples are going to be freeze-dried to keep their properties. Freeze-dry samples were analyzed by the automatic machine. Then repeatability and reproducibility tests were conducted.

Conclusion

Coefficients of variations are less than 10% (Fig.2 and Fig. 3), so we can say that the analysis method and the method of conservation of IQC are repeatable and reproducible. But these tests were performed on a small number of samples, it would be necessary to carry them out on more samples and for a longer time to see if the creation of IQC is a real solution.

(Fig 1) : Molecular structure of citric acid

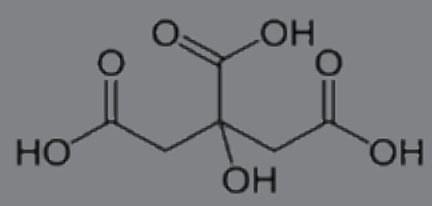

Fig 2) Test of repeatability

Number of passages	Average	Standard Deviation	CV
10	16.51	0.39	2.3 %

(Fig 3): Test of reproducibility

Number of passages	Average	Standard Deviation	CV
10	69.77	3.5	5.0 %

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