AN ANALYTICAL METHOD VALIDATION FOR ATOMIC ABSORPTION SPECTROMETRY ANALISYS OF TOTAL ZINC FROM INSULIN

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abstract: An analytical method validation represents a complete investigation of the materials' properties, following certain steps, being necessary for "proving the fit for purpose of an analytical method". The validation procedure demonstrate that a defined validation protocol for an analytical method applied to a specified testing material and a defined concentration of the analyt – named analytic system – fits for a certain analytical purpose. The analytical purpose reflects the obtaining of analytical results with an acceptable degree of accuracy. The scope of the present study is a supplementary validation (linearity, range, accuracy and precision) of flame atomic absorption spectrometry analytical procedure for total zinc analysis from Insulatard Penfill.

key words: Analytical method validation. Traceability, Linearity and range, Precision, Repeatability, Recovery.

Introduction

The results comparability is a key property of chemical measurements. When the results can be directly compared under repeatability conditions, then a general approach it is necessary in order to realise the understanding of the comparison of the results of any measurements performed at different time. This "comparability in all over time and space" is routinely made by binding of the results obtained from individual measurements to some established common references or measurements standards. Thus the measurement results are correlated to the relevant reference. The strategy of binding the results to a reference is named "traceability" [1÷3]. Traceability is the key property in metrology and therefore, the results traceability is specifically required by the international standard ISO/IEC 17025:1994 [4].

According to certain works [1÷5] a measurement represents a set of operations having the object of determining a value of a quantity. The measurements include a set of conditions and an equation of measuring from which is calculated the result using the values of the measured parameters. If the values of all the involved parameters are traceable to stable references the results will be constant. Therefore, this expectation is based on some assumptions: specifically, a functional relationship between the measurand quantity and its response bias free and the absence of any significant effects.

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Analytical method validation answers the following question: "are these assumptions valid?" using the experimental testing of these assumptions [6]. When no other effect is visible, than the method does not explicitly include all the factors known for the traceability requirement. If all involved identified factors are really traceable to adequate references, than is expected the method to produce constant traceable results. When is considered that the method is validated than it can be used without adjustments.

The validation represents the tool which is used for proving the fact that a specific analytical method measures which pretends to measure and, in this way, is fitted for the desired purpose [7]. The classical approach of validation is done based on estimated number of parameters related to the performance of the method. The development stage based on criteria is composed by precision studies and method translation, proof of specificity/selectivity, proof of linearity, robustness studies and an evaluation of the detection and/or quantification limits based on the practical requirements. The goal of validation is to prove that the measurement conditions and the equation used for final result calculation include all the interferences which will affect the final results. The validation measures the different effects in the whole analytical system which influences the result and ensures that there are no other effects which have not been considered.

The purpose of the present study is to realise a supplementary validation (linearity, range, accuracy and precision) of an analytical procedure for total zinc determination from human insulin Insulatard Penfill, manufactured by the pharmaceutical company Novo Nordisk A/S – Denmark, using the flame atomic absorption spectrometry.

The principle of analysis

The principle of analysis is to determine zinc using flame atomic absorption spectrometry (AAS) technique as describes in European Pharmacopoeia [8]. Sample preparation is briefly described below.

A sample of Insulatard Penfill (3 mL, 100 IU/mL) is acidulated with hydrochloric acid 6N. After one hour one mL of sample is diluted with hydrochloric acid 0.01N to a 50 mL. Total zinc is determined using the flame atomic absorption spectrometry technique, based on the following equation:

$$\text{Total zinc } (\mu g \cdot mL^{-1}) = \frac{A_{sample} \cdot C_{std}}{A_{std}} \cdot \frac{b}{a} \quad (1)$$

where: A_{sample} – sample absorbance, A_{std} – standard solution absorbance, C_{std} – concentration of standard solution measured before sample, $\mu g \cdot mL^{-1}$, a – sample volume pipetted for analysis, mL, b – final volume of sample solution, mL.

Materials and equipments

The following materials and equipments have been used during the proposed validation study:

- Materials: Zn standard stock solution (1000 μg Zn/mL), Hydrochloric acid solution 6N, Hydrochloric acid solution 0.01N, Insulatard Penfill cartridges (3 mL, 100 IU/mL), Tridistilated water.
- Equipments: Atomic absorption spectrometer SOLAAR 32 AA, Eppendorf pipettes.

Results and discussions

As previously presented, the objective of the validation study is to prove that the measurement conditions and the equation used for the final result calculation include all the interferences which affect the final result. The validation process measures the different effects in whole analytical system which affects the result and ensures that here are no other effects which have not been considered [1]. For instance, a checking of specificity ensures that the method responds only to the interested analyt and do not respond to others interferences or contaminants. A linearity control checks if the assumptions of the binding between measured signal and the used measurement units can be used. A study of method translation represents the checking of a certified reference material which demonstrates that the method is not significantly affected (translated). The precision and robustness study "covers" the conditions variability effects due to the analyst, equipment and time.

In the proposed validation study the following parameters are considered: linearity, range, precision and accuracy.

Linearity is evaluated through graphical representation of the measured absorbance at λ = 213.9 nm depending on total zinc concentration of zinc standard solutions and depending on linear regression (least square technique) of the obtained calibration data. Table 1 shows the necessary data for obtaining the calibration curve and Fig. 1 shows the regression line in the prediction interval.

Table 1. Data for calibration curve and linearity verification for zinc determination through flame atomic absorption spectrometry

No	Standard solution concentration $\mu g \cdot mL^{-1}$	Absorbance A (UA)				
		Measured value 1	Measured value 2	Measured value 3	Mean value	RSD %
1	0.0500	0.028	0.027	0.029	0.028	1.9
2	0.1000	0.042	0.042	0.042	0.042	0.1
3	0.2000	0.075	0.074	0.076	0.075	0.5
4	0.4000	0.138	0.137	0.137	0.137	0.4
5	0.6000	0.196	0.196	0.196	0.196	0.0
6	0.8000	0.250	0.248	0.250	0.249	0.4
7	1.0000	0.300	0.300	0.298	0.299	0.4

The linear regression data are not sufficient for the evaluation of linearity of an analytical method. Moreover, the residual values must be calibrated. The last ones represent the differences between the true y value and the estimated y value from regression curve, corresponding to each x value. If the calculated residual values through simple linear

regression are randomly distributed around the regression line, the linearity is confirmed but the systematic tendencies indicate non-linearity.

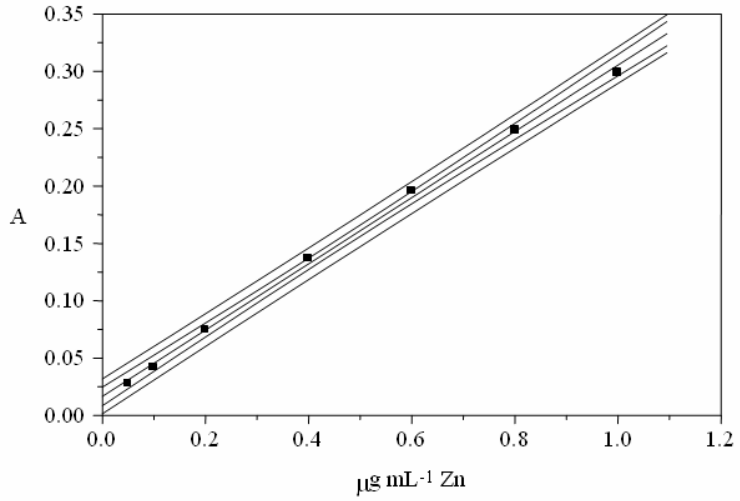

Fig. 1 *The linearity verification for total Zn determination through flame atomic absorption spectrometry*

An alternative approach for establishing the linearity is the ratio of the analyt response to the respective concentrations and the graphical representation of these relative answers depending on concentration on a logarithmic scale. The obtained line must be horizontal on the whole linear range, having a positive deviation at lower concentrations and a negative deviation at higher concentrations [9].

Precision: The total Zn determination from Insulatard Penfill was carried out three times per day, in six different days. Twelve measurements have been obtained in total (see Table 2). In this study the precision will be evaluated as repeatability $\%RSD_{(n-1)}$ and as intermediate precision $\%RSD_{(n-1)}$, as follows:

Repeatability: $$\%RSD_{(n-1)} = \frac{\sigma_{rep}}{\overline{x}} \cdot 100 \quad (2)$$

$$\sigma^2_{rep} = \frac{1}{n(n-1)} \cdot \sum_{j=1}^{k} \sum_{i=1}^{n} \left(x_{ij} - \overline{x}_j \right)^2 \quad (3)$$

where: $\overline{x}$ = the average of the 18 measurements, k = days number, n = the number of performed measurements/day, $\overline{x}_j$ = the average of "n" measurements for "j" days.

Acceptance criteria: $\%RSD_{(n-1)} = \pm 3\%$; Result: $\%RSD_{(n-1)} = 2.37137 \cong 2.37\%$

Intermediate precision: $$\%RSD_{(n-1)} = \frac{\sigma_{p1}}{\overline{x}} \cdot 100 \quad (4)$$

$$\sigma^2_{p1} = \frac{1}{(k-1)} \cdot \sum_{j=1}^{k} \left(\overline{x_j} - \overline{\overline{x}} \right)^2 \quad (5)$$

Acceptance criteria: $\%RSD_{(n-1)} = \pm 3\%$; Result: $\%RSD_{(n-1)} = 2.6877 \cong 2.69\%$

Table 2 – The obtained results in the precision study at total zinc determination from Insulatard Penfill using the flame atomic absorption spectrometry technique

Day	Analyst	Theoretical concentration µg Zn / mL	First value ratio µg Zn / mL	Second value ratio µg Zn / mL	Third value ratio µg Zn / mL	Average µg Zn / mL $\overline{x}_j$
1	Analyst 1	0.80	0.6677	0.6793	0.6809	0.6759
2	Analyst 2	0.80	0.6459	0.6575	0.6383	0.6473
3	Analyst 3	0.80	0.6949	0.6809	0.6888	0.6883
4	Analyst 4	0.80	0.6972	0.6634	0.6612	0.6739
5	Analyst 5	0.80	0.6712	0.6472	0.6597	0.6593
6	Analyst 6	0.80	0.6818	0.6633	0.6635	0.6628
Average 18 values, $\overline{\overline{x}}$				$\overline{\overline{x}} = 0.6679$		

Table 3 – The obtained results in the accuracy (recovery) study at total zinc determination from Insulatard Penfill using the flame atomic absorption spectrometry technique

Working day	1	2	3
The analyst who performed the measurement	Analyst 1	Analyst 2	Analyst 3
µg Zn/mL in sample solution	0.7688	0.7692	0.7690
µg Zn/mL added	0.1748	0.1796	0.1787
µg Zn/mL in strengthened sample	0.9436	0.9488	0.9477
% Recovery	99.42%	100%	100%
µg Zn/mL in the sample solution	0.7688	0.7692	0.7690
µg Zn/mL added	0.5018	0.4997	0.4989
µg Zn/mL in strengthened sample	1.2706	1.2589	1.2679
% Recovery	99.98%	99.59%	100%
µg Zn/mL in the sample solution	0.7688	0.7692	0.7690
µg Zn/mL added	0.6677	0.6793	0.6809
µg Zn/mL in strengthened sample	1.4369	1.4475	1.4499
% Recovery	100.05%	99.85%	100%

Accuracy: In each of the three chosen days the Insulatard Penfill sample is correspondingly diluted to 50 ml. One mL sample solution is strengthened with 0.50 mL, 1.00 mL and 2.0 mL 10 µg Zn/mL reference stock solution. To these volumes hydrochloric acid 0.01 N is added. The strengthened sample solution is analysed according to the analytical procedure and the recovery is calculated according to the following equation:

$$\%\text{Recovery} = \frac{\mu g_{\text{Zn/mL in the stengthened sample}} - \mu g_{\text{Zn/mL in sample}}}{\mu g_{\text{Zn/mL aded}}}$$

Acceptance criteria: $95\% \leq \text{Recovery} \leq 115\%$

Results: $99.42\% \leq \text{Recovery} \leq 100.05\%$

Conclusions

The analytical results and the statistical evaluation of this validation study conducted to the following conclusions:

The presented analytical procedure for total zinc determination from Insulatard Penfill it is compliant and may be used in this purpose in accepted imposed conditions.

The results of the presented validation study are synthetically listed in Table 4.

Table 4 – Overview of the results obtained in the validation study

Tested parameter	Results	Acceptance criteria
Linearity range	1 μg mL^{-1}	
Detection limit	0.01 μg mL^{-1}	
Quantification limit	0.01 μg mL^{-1}	
Accuracy (Recovery)	99.42 ÷ 100.05%	95 ÷ 105 %
Repeatability	RSD_{n-1} = 2.37%	± 3%
Intermediate precision	RSD_{n-1} = 2.69%	± 3%
Linearity	y = 0.01667 + 0.28868 x	
Correlation coefficient	R = 0.9906	0.999
Standard deviation (SD)	SD = 0.00502	

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