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A method optimization study for atomic absorption spectrophotometric determination of total zinc in insulin using direct aspiration technique

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Abstract A sensitive, reliable and relative fast method has been developed for the determination of total zinc in insulin by atomic absorption spectrophotometer. This designed study was used to optimize the procedures for the existing methods. Spectrograms of both standard and sample solutions of zinc were recorded by measuring the absorbance at 213.9 nm for determination of total zinc. System suitability parameters were evaluated and were found to be within the limits. Linearity was evaluated through graphical representation of concentration versus absorbance. Repeatability (intra-day) and intermediate precision (inter-day) were assessed by analyzing working standard solutions. Accuracy and robustness were experimented from the standard procedures. The percentage recovery of zinc was found to be 99.8%, relative standard deviation RSD 1.13%, linearity of determination LOD 0.0032 μg/mL, and limit of quantization LOQ 0.0120 μg/mL. This developed and proposed method was then validated in terms of accuracy, precision, linearity and robustness which can be successfully used for the quantization of zinc in insulin.

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1. Introduction

Insulin is a hormone produced by the pancreas, a large leaf like gland that lies in the duodenum. This hormone is necessary for the metabolism of aldose sugars. Diabetes occurs when the pancreas does not make enough insulin to meet human body needs. Insulin helps to keep blood glucose level nearly at normal.[1,2]

Zinc plays its role in all stages of insulin metabolism, from production through secretion to utilization and storage. Zinc protects pancreatic beta cells from destruction and its deficiency affects their ability to produce and secrete insulin. Blood glucose level increases if the pancreas does not produce or secrete enough insulin. Decreased zinc concentration in the body has been implicated in lack of insulin sensitivity. In other words the insulin receptors on the beta cells are being inhibited, that means not enough glucose is entering into the beta cells.[3,4]

The techniques nowadays used to determine zinc in insulin include colorimetry, neutron activation analysis, polarography, X-ray fluorescence, emission spectroscopy, fluorometry and atomic absorption spectrophotometry. Atomic absorption spectrophotometric technique is preferred due to its specificity, sensitivity, precision, simplicity and relatively low cost per analysis.[5–8]

The objective of analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories. 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.[9,10]

The purpose of the present study is to realize validation (system suitability, linearity, accuracy robustness and precision) of an existing analytical procedure[11] for total zinc determination present in human insulin.

2. Materials and methods

2.1. Reagents and materials

All the reagents used were chemically pure, analytical reagent grade and were used without further purification. Triple distilled water was used throughout this study. Zinc standard stock solution (1000 μg Zn/mL), 6 M and 0.01 M hydrochloric acid solutions were prepared according to the standard procedure. Zinc working solution (0.20–1.20 μg/mL of Zn) was freshly prepared by diluting zinc standard solution (1 mg/mL Zn) with 0.01 M hydrochloric acid. Humulin N Vial (5 mL, 100 IU/mL) of Lilly Pharmaceuticals, Egypt was used. Well shaken insulin vial (1.0 mL containing 100 IU of insulin) was dilute to 100.0 mL with 0.01 M hydrochloric acid.

2.2. Instrumentation

The atomic absorption spectrophotometer (Hitachi model A-1800) was used during this study. For simultaneous analysis, it consists of eight turret lamps with a wavelength range of 190–900 nm. For analysis precision, it has D_2 and self reversal background correction with a grating of 1800 gooves/mm. Manufacturer brand Win 2.1 software was used for data integration and processing. The spectroscopic conditions were these; bandwidth 0.4 nm with a 1.0 filter factor and deuterium (D_2) background correction. The integration time was 3.0 s set at 5.0 mA lamp current. The analytes were detected at 213.9 nm.

2.3. Method development

2.3.1. Assay method

With the optimized spectroscopic conditions a steady base line was recorded. Standard and sample solutions of zinc were aspirated and spectrograms were recorded by measuring the absorbance at 213.9 nm using a zinc hollow-cathode lamp as a source of radiation and an air-acetylene flame with fuel flow rate of 1600 mL/min. The concentration of the zinc was calculated using the following formula.

$$\text{Total zinc} \quad (\mu\text{g/mL}) = \frac{A_{\text{Sample}} \times C_{\text{Standard}}}{A_{\text{Standard}}} \times b/a \qquad (1)$$

where A_{Sample} = sample absorbance, A_{Standard} = standard solution absorbance, C_{Standard} = concentration of standard solution measured before sample (μg/mL), a = sample volume pipetted for analysis (mL), and b = final volume of sample solution (mL).

2.4. Method validation

2.4.1. System suitability

Standard solutions (0.20–1.20 μg/mL of Zn) were prepared by using a zinc working standard (1 mg/mL Zn) and aspired into the flame of an atomic absorption spectrophotometer. For each sample at least five readings were noted. System suitability parameters were evaluated and found to be within the limits. The purpose of the system suitability test was to ensure that the complete testing system (including instrument, reagents and analyst) is suitable for the intended application. The % relative standard deviation for absorbance from five readings of each zinc standard is presented in Table 1. This shows the suitability of atomic absorption spectrophotometer for the determination of zinc in insulin.

2.4.2. Linearity

Linearity is evaluated through a graphical representation of 'concentration' versus 'absorbance', the measured absorbance at λ = 213.9 nm depending on total zinc concentration of zinc standard solutions. Table 2 shows the necessary data for obtaining the calibration curve, limit of detection and limit of quantization. Fig. 1 shows the regression line in the prediction interval. The linearity verifies for total Zn determination through flame atomic absorption spectrometry.

2.5. Precision

2.5.1. Repeatability (intra-day)

Repeatability is studied by calculating the relative standard deviation (RSD) for five determinations of the